Oznaka poročila: ARRS-RPROJ-ZP-2012/12

ZAKLJUČNO POROČILO O REZULTATIH RAZISKOVALNEGA PROJEKTA

A. PODATKI O RAZISKOVALNEM PROJEKTU

1.Osnovni podatki o raziskovalnem projektu

Šifra projekta	Z1-2142
Naslov projekta	Uravnavanje koproteazne aktivnosti proteina RecA v bakterijah
Vodja projekta	24290 Matej Butala
Tip projekta	Zt Podoktorski projekt - temeljni
Obseg raziskovalnih ur	3400
Cenovni razred	B
Trajanje projekta	05.2009 - 04.2011
Nosilna raziskovalna organizacija	481 Univerza v Ljubljani, Biotehniška fakulteta
Raziskovalne organizacije - soizvajalke	
Raziskovalno področje po šifrantu ARRS	1 NARAVOSLOVJE 1.05 Biokemija in molekularna biologija
Družbeno-ekonomski cilj	13.01 Naravoslovne vede - RiR financiran iz drugih virov (ne iz SUF)

2.Raziskovalno področje po šifrantu FOS[1]

Šifra	1.05
- Veda	1 Naravoslovne vede
- Področje	1.05 Vede o zemlji in okolju

B. REZULTATI IN DOSEŽKI RAZISKOVALNEGA PROJEKTA

3.Povzetek projekta[2]

SLO

Odkritje in uporaba antibiotikov je eden največji dosežkov sodobne medicine, kar je omogočilo zdravljenje infektivnih bolezni. Danes je v svetovnem merilu ena največjih groženj zdravju ljudi odpornost patogenih bakterij proti antibiotikom. Potrebujemo nove pristope k zdravljenju infekcij.

Novejše raziskave so razkrile, da številni klinično pomembni antibiotiki v nizkih koncentracijah (v našem telesu lahko prisotna v začetku / koncu terapije z antibiotiki; v določenih delih telesa tekom terapije) poškodujejo DNA v bakterijah in posledično aktivirajo bakterijski odziv SOS. Sistem SOS je regulatorno omrežje genov odgovorno za popravilo poškodovane DNA. Bakterije se prilagodijo na stres, ki ga povzročijo antibiotiki, sprožijo odziv SOS, kar vodi v popravljanje DNA, nastanek točkovnih mutacij in prenosa genov med bakterijami. Z oviranjem sprožitve odziva SOS pri bakterijah, znižamo nastanek odpornosti proti nekaterim antibiotikom ter tako podaljšamo njihovo učinkovitost. Ključni proteini odziva SOS so pomembna tarča za izdelavo učinkovin, ki bi podaljšali učinkovitost obstoječih antibiotikov z znižanjem mutageneze in prenosa genov med bakterijami, kar sproži večina v kliniki uporabljenih antibiotikov!
Odziv SOS je široko razširjen med bakterijami, preučevali smo odziv pri modelni bakteriji *Escherichia coli*. Sistem SOS je uravnavan z dvema proteinoma, LexA je dejavnik transkripcije, ki v pogojih normalne bakterijske rasti zmanjša lastno izražanje in v *E. coli*, izražanje vsaj 43 fizično nepovezanih genov. Protein RecA je induktor, ki se kot odziv na poškodbe DNA veže na enoverižno DNA (ssDNA) in tvori filament. Filament RecA-ssDNA-ATP (RecA*) interagira z LexA in aktivira samocepitveno aktivnost LexA, ianktivacija LexA vodi v sprožitev prepisa genov SOS.
V projektu sem poskusal, v sodelovanju z ostalimi raziskovalci, pojasniti ključne mehanizme sprožitve odziva SOS. Razumevanje teh mehanizmov nam je omogočilo začetek razvoja učinkovine, s katero želimo zamrzniti zaznavanje stresa pri bakterijah, njihovo prilagoditev na antibiotike. Za izvedbo projekta sem vzpostavil sodelovanja z raziskovalci iz Biotehniške Fakultete (UL), Fakultete za Farmacijo (UL), Kemijskega inštituta, Univerze v Birminghamu (Anglija) ter Univerze v Osnabrücku (Nemčija).

ANG

One of the most serious health care problems worldwide is bacterial resistance to antibiotics. Although revolutionizing the treatment of infectious diseases, have antibiotics also rapidly selected for the emergence of resistant pathogens. Today, resistance has rendered most of the original antibiotics obsolete for many infections, typically by acquiring chromosomal mutations. Traditional methods of antibiotic discovery have failed to keep pace with the evolution of the resistance, which suggests that new strategies to combating the emerging threat of antibiotic resistant bacteria are needed.
It has recently been shown that numerous clinically significant antibiotics can in bacteria induce the production of single stranded DNA and thus activate the SOS response. The SOS response induces the expression of a set of genes in response to DNA damage, leading to the arrest of cell division and induction of DNA repair and prophages and concommitant mutagenesis. The SOS system is a programmed DNA repair regulatory network, which results in mutations and genetic exchange, presumably to facilitate bacterial evolution in times of stress. Recent studies have shown that the antibiotic induced SOS response can modulate the evolution and spread of drug resistance as well as virulence factors.
The SOS response is wide-spread among bacteria. Thus, key regulators of this system are important targets for the development of substances that would prolong the efficiency of the currently used antibiotics and act as antibiotic adjuvants.
We studied the molecular mechnaism of the induction of the SOS response in a model bacterial organism, *Escherichia coli*. The SOS system is controlled by the interplay of 2 key regulatory proteins which alternate between on and off states. These are a repressor, LexA, which, during normal bacterial growth downregulates its own expression and, in *E. coli*, the expression of at least of 43 unlinked genes. The RecA protein is the inducer, which, in response to DNA damage, binds to single-stranded DNA (ssDNA) to form a filament. The RecA-ssDNA-ATP (RecA*) filament interacts with LexA and activates a self-cleaving activity in LexA, leading to induction of the SOS genes.
In collaboration with other researchers, I tried to determine the key steps in the induction of the SOS response. The insights into this mechanism enabled us to set us a system for developing a drug that would disable bacteria to sense the antibiotic stress and adapt to antibiotics. To acomplish this project I continued with the previously estabiliset, or newly set up a colaboration with researchers from the Biotechnical faculty and the Faculty of Pharmacy, University of Ljubljana, Chemical institute (Slovenia), University of Birmingham (UK) and from the University of Osnabrück (Germany).

4.Poročilo o realizaciji predloženega programa dela na raziskovalnem projektu[3]

1. Ali aktivni filament RecA sproži cepitev proteina LexA, ko je represor specifično vezan na tarčna mesta DNA?

Hipoteza: aktivni filament RecA sproži cepitev na DNA specifično vezanega represorja LexA.

Dokazali smo, da aktivni filament RecA (RecA*), bakterij *Escherichia coli*, ne sproži cepitve transkripcijskega faktorja LexA, ko je represor LexA specifično vezan na tarčna mesta DNA. Prikažemo, da konformacijska sprememba v proteinu LexA omogoči programiran prepis genov bakterijskega stresnega odziva na poškodbe DNA. LexA je homo-dimeren protein, C-terminalna domena (CTD) služi za dimerizacijo, N-terminalna (NTD) za vezavo na DNA. Pripravili smo >95% očiščene proteine bakterije *E. coli*: LexA, necepljivo različico (LexASA119), različico, ki se boljše veže na DNA (LexAEK71), različice z uvedenim aminokislinskim ostankom cistein na NTD ali CTD (LexA54, LexA29, LexA191 ter LexA29-191) ter protein RecA.
V sodelovanju s skupino prof. H.J. Steinhoffa, Nemčija, smo z elektronsko paramagnetno resonanco (EPR) dokazali, da sta NTD LexA prosto gibljivi, ko protein ni vezan na DNA, a v specifični konformaciji, ko je protein vezan na tarčno DNA. V nasprotju, ob vezavi na DNA, ni velike konformacijske spremembe v CTD.
Dokazali smo, da aktivni filament RecA (RecA*) sproži inaktivacijo ene podenote prostega LexA in ob ponovni interakciji med proteinoma, cepitev še preostale podenote.
Razložili smo mehanizem sinhronizirane sprožitve bakterijskega odziva na poškodbe DNA: S površinsko plazmonsko resonanco (SPR) smo razjasnili, da v specifični, na DNA vezani konformaciji LexA ne interagira z RecA*. Nadalje, s SPR smo dokazali različne hitrosti sproščanja represorja iz različnih tarčnih zaporedij DNA *E. coli*. Posledično: na mestu poškodovane DNA se tvori RecA*, slednji sproži samo-cepitev prostega represorja LexA, znižanje koncentracije na DNA nevezanega/nespecifično vezanega LexA v celici. Slednje povzroči programiran prepis genov SOS, saj imajo zgodnji geni v odzivu (produkti, ki omogočijo natančno popravljanje poškodb) promotorska področja s tarčnim zaporedjem LexA, nizko afiniteto do represorja. Obratno, ob dolgotrajni poškodbi se prepišejo pozni geni SOS, ki imajo visoko afiniteto do LexA (mutageneza, sineteza toksinov).
Pridobljeno temeljno zananje sem uporabil v primeru nastanka bakterij tolerantnih na antibiotike (dormantnih, perzisterskih celic). Nastanek slednjih je uravnano v odzivu SOS in predstavlja veliko težavo v zdravstvu. Dokazal sem, da z uravnavanjem funkcij LexA vplivamo na nastanek bakterij tolerantnih na antibiotike. Patentna prijava je v postopku: EPO, #10005558.1-2405, popravilo pomanjkljivosti. Članek je bil sprejet v reviji Nucleic Acids Research (točka 6.1). Raziskava je plod vzpostavitve/nadljevanje sodelovanja raziskovalcev iz Slovenije, Anglije in Nemčije.

2. Preučili smo zakaj LexA v DNA vezani konformaciji ne interagira z RecA*. Strukturni vpogled v interakcijo RecA* ter LexA ni poznan. Iz biokemijskih raziskav se predvideva, da le CTD LexA interagira z RecA*. Izdelali smo mutante proteina LexA v različnih konformacijah (LexAQM – LexA v cepitvi zmožni konformaciji, LexA13-91 – represor v cepitvi nezmožni konformaciji, LexA54 – represor v konformaciji nevezani na DNA, LexA24 – represor v konformaciji vezani na DNA). Pripravili smo tudi CTD ter NTD različic. S SPR smo dokazali, da poleg CTD tudi DNA vezavne domene LexA (NTD) interagirajo direktno z RecA*. Dokazali smo, da RecA* sproži cepitev mutante LexA24 (LexA v na DNA vezani konformaciji). posledično smo dokazali, da specifična DNA sterično ovira interakcijo RecA* z LexA. Iz rezultatov smo izdelali represor LexA v konformaciji, ki stabilno interagira z RecA*, z namenom kristalizacije RecA*-LexA.

3. Hipoteza: Neidentificirani proteini uravnavajo izražanje genov SOS, vplivajo na srostitev represorja LexA iz DNA.
Preučilo smo ali obstajajo proteini, ki interagirajo z DNA vezanim represorjem LexA ter vplivajo na pozen prepis nekaterih genov odziva SOS. Kolicini so toksini bakterije *E. coli*, ki toksično učinkujejo na bakterije iste ali sorodne vrste ter vplivajo na raznovstnost bakterij v prebavilih sesalcev. Kolicini so uravnani z LexA in prepisani zadnji v odzivu SOS, saj se sprostijo ob lizi producentske bakterije. Predvidevali smo, da obstaja protein, ki stabilizira represor LexA na promotorskem področju gena za kolicin K (*cka*).
Izvedli smo in vitro različico nedavno razvite metode ˝DNA sampling˝ (Butala et al, 2009, NAR). Z masno spektormetrijo smo prepoznali 6 DNA vezavnih proteinov (H-NS, DeoR, IscR, GlcC, UlrR, MqsA), z morebitnm vplivom na LexA pri prepisu *cka*. Z določevanjem aktivnosti promotorja smo dokazali, da protein IscR omogoči zakasneli prepis *cka* (2h lag fazo po nastanku poškodb DNA), najverjetneje stabilizira protein LexA na DNA. S SPR smo dokazali vezavno mesto za IscR na promotorskem področju *cka*, vezavno mesto prekriva element -35 promotorja. Kot prvi smo dokazali, da je prepis nekaterih genov za kolicine uravnan z dvema transkripcijskima faktorjema in se odzove na dva signala iz okolja. Dokazali smo, da nivo železa in dostopnost hranil vplivata na

koncentracijo proteina IscR v celici ter na vezavne lastnosti proteina na tarčno zaporedje kolicina K. Med ~50 z LexA uravnanimi geni, je to drugi primer, da pri izražanju gena SOS, poleg LexA, sodeluje še dodaten dejavnik transkripcije.
Dokazali smo fiziološki pomen tega skrbno uravnanega prepisa, ki privede do lize producentskih celic. V *iscR*⁻ sevu, se kolicin K prepiše med prvimi geni odziva SOS, posledično bakterije ne morejo vključiti popravljalnih mehanizmov zaradi prezgodnje lize bakterij. Z difuzijskimi antibiogrami smo dokazali, da IscR vpliva na prepis ter posledično sintezo kolicina K in preživetje producentskih bakterij. Rezultati so bili poslani v revijo Molecular Microbiology, pregledani s strani urednika in treh recezentov, popravki bodo poslani v revijo sredi marca, 2012. Raziskava je plod sodelovanja raziskovalcev iz Biotehniške fakultete (UL), Kemijskega inštituta (Ljubljana) ter raziskovalcev iz Univerze v Birminghamu (Anglija).

4. Izdelava učinkovine, ki repreči inaktivacijo LexA, inhibicijo sprožitve odziva SOS. Kot navedeno zgoraj, smo z bazičnimi raziskavami ugotovli v kakšni konformaciji bi bilo najustrezneje zamrzniti protein LexA v celici, da bi preprečili porajanje odpornosti proti antibiotikom med baterijami. Uporabili smo knjižnice peptidnopredstavitvenih fagnih klonov, a neuspešno. Nadajle smo izvedli in silico iskanje učinkovine, ki bi mimikrirala cepitevno regijo LexA ter inhibirala samo-inaktivacijo LexA (preprečila sprožitev odziva SOS) v bakteriji *E. coli*. Izbrali in pridobili smo 30 učinkovin, izdelali hiter (in vitro) test inhibitornega učinka substanc na LexA. Rezultati raziskave nakazujejo potencial nekaterih učinkovin na inhibicijo inaktivacije represorja LexA. Ob izteku financiranja projekta, nisem uspel pridobit sredstev, ki bi nam omogočale nadaljevanje razvoja učinkovine. Raziskave so bile opravljene v sodelovanju s skupino prof. S. Gobca, Fakulteta za farmacijo, UL.

5.Ocena stopnje realizacije programa dela na raziskovalnem in zastavljenih raziskovalnih ciljev[4]

Projekt je bil uspešno realiziran, kar je razvidno iz objavjenih rezultatov točke 7, 8, 9. Zaradi aktualnosti določenih tem je bilo v primerjavi s prvotno načrtovanim projektom izvedenih nekaj sprememb.
Hipotezo 1, da aktivni filament RecA sproži cepitev na DNA specifično vezanega represorja LexA, smo ovrgli, tako razjasnili programiran odziv SOS. Naši rezultati razložijo zakaj se nekateri geni odziva SOS prepišejo pred drugimi. Hipoteze 2, da ima protein RecA preferenčna vezavna mesta za vezavo in tvorbo aktivnega filamenta na genomu bakterije *E. coli*, še proučujemo. Vzrok zakasnitve je zaradi visokih stroškov analize - raziskave smo prilagodili finančnim zmožnostim projekta. Hipotezo 3, da dodatni proteini (poleg osmih poznanih proteinov) interagirajo z RecA* filamentom in uravnavajo sprožitev cepitve represorja LexA ne morem povsem zavreči. Rezultati kažejo na vlogo YdjM proteina pri uravnavanju funkcij RecA*. Hipotezo 4, da obstajajo proteini, ki z vezavo na DNA stabilizirajo interakcijo LexA z DNA in preprečijo prepis genov SOS smo potrdili, ob uporabi ˝DNA sampling˝ metode *in vitro*. IscR je prvi opisani protein, ki vpliva na stabilnost vezave LexA-DNA. V kombinaciji z LexA omogoči pozni prepis gena *cka*. Hipoteza 5, identificirati peptid, ki se veže na LexA in prepreči cepitveno aktivnost represorja LexA: knjižnice peptidnopredstavitvenih fagnih klonov se niso izkazale za uporabne v primeru LexA. Posledično, z mimikrijo cepitvene regije LexA in silico smo pridobili več kot 30 učinkovin. Uporabili smo znanje pridobljeno tekom izvedbe projekta, uporabili kot protimikrobno tarčo protein LexA v konformaciji vezani na specifično DNA. Analizo delovanja učinkovin na preprečitev z RecA* sprožene inaktivacije LexA smo torej uspešno začeli, izdelali hiter test za identifikacijo učinkovine in upam, da bomo ustrezno učinkovino tudi izdelali.

6.Utemeljitev morebitnih sprememb programa raziskovalnega projekta oziroma sprememb, povečanja ali zmanjšanja sestave projektne skupine[5]

Dokazali smo da RecA* (aktivator odziva SOS) ne interagira z LexA (represorjem odziva) vezanim na DNA. Slednji rezultati nakazujejo, da lokacija nastanka poškodb DNA v genomu bakterij ni ključna za uravnavanje sinhronizirane sprožitve prepisa genov SOS. Torej ni nujno potrebna za izdelavo inhibitorja odziva SOS. Posledično smo analizo mesta tvorbe filamenta RecA* v genomu bakterije *E. coli* tekom normalne rasti bakterij oziroma, tekom z antibiotiki sproženega odziva SOS začeli izvjati v zaključnih mesecih raziskovalnega projekta, ki pa je še nismo uspeli zaključiti. Sodelujemo s skupino dr. David C. Graingerja, Univerza v Birminghamu. Obratno, ob pisanju predloga projekta nisem predvidel poglobljene študije dinamike strukture represorja LexA,

izdelava mutant LexA, študije EPR/SPR, v kar je privedla aktualnost naših rezultatov iz prvega sklopa. Poznavanje pridobljenih rezultatov je bil predpogoj za snovanje načina inhibicije inaktivacije LexA. Sprememba načina iskanja inhbitorja LexA (in silico mimikrija) je bila razumna, saj uporaba knjižnice peptidnopredstavitvenih fagnih klonov ni bila uspešna.

7.Najpomembnejši znanstveni rezultati projektne skupine[6]

	Znanstveni dosežek			
1.	COBISS ID		2368847	Vir: COBISS.SI
	Naslov	*SLO*	Pretvorba LexA iz DNA nevezane v DNA vezano konformacijo orkestrira bakterijski odziv SOS.	
		ANG	Interconversion between bound and free conformations of LexA orchestrates the bacterial SOS response	
	Opis	*SLO*	RecA* ne sproži inaktivacije LexA ko je ta specifično vezan na DNA. Z meritvami EPR dokažemo, da so DNA vezavne domene nevezanega LexA gibljive, a protein v specifični konformaciji ko je vezan na DNA. V slednji konformaciji je interakcija RecA* z LexA-DNA preprečena. Disociacija LexA iz različnih operatorjev poteka z različno hitrostjo, kar sinhronizira prepis genov SOS. S spreminjanjem aktivnoti LexA smo uravnali nastanek perzisterskih celic v bakterijski populaciji.	
		ANG	We show that self cleavage of LexA repressor is prevented by binding to specific DNA operator targets, depends on LexA dissociation from the targets and, hence, this controls the SOS response. Distance measurements using EPR spectroscopy reveal that in unbound LexA the DNA binding domains sample different conformations, one of which is captured when bound to operator targets, precluding RecA interaction. Modulation of LexA activity changes the occurrence of persister cells in bacterial populations.	
	Objavljeno v		Oxford University Press; Nucleic acids research; 2011; Vol. 39, issue 15; str. 6546-6557; Impact Factor: 7.836;Srednja vrednost revije / Medium Category Impact Factor: 3.787; A': 1; WoS: CQ; Avtorji / Authors: Butala Matej*, Klose Daniel, Hodnik Vesna, Rems Ana, Podlesek Zdravko, Klare Johann P., Anderluh Gregor, Busby Steve J. W., Steinhoff Heinz-Jürgen, Žgur-Bertok Darja	
	Tipologija		1.01 Izvirni znanstveni članek	
2.	COBISS ID		1	Vir: vpis v poročilo
	Naslov	*SLO*	Regulatorni sistem LexA	
		ANG	The LexA regulatory system	
	Opis	*SLO*	Na povabilo dr. Nancy L. Craig (Howard Hughes Medical Institute) smo za drugo izdajo "Encyclopedia of Biological Chemistry" spisali poglavje o stresnem odzivu bakterij na poškodovano DNA, izdaja Elsevier.	
		ANG	The chapter named The LexA regulatory system for the second edition of "Encyclopedia of Biological Chemistry" published by Elsevier. An invitation from Nancy L. Craig (Howard Hughes Medical Institute).	
	Objavljeno v		Butala, M.*, Zgur-Bertok, D., and Busby, S.J.W. (2012) The LexA Regulatory System. In Lennarz, W.J., and Lane, M.D. (eds.), Encyclopedia of Biological Chemistry, 2nd Edition, Elsevier, in press.	
	Tipologija		1.16 Samostojni znanstveni sestavek ali poglavje v monografski publikaciji	
3.	COBISS ID		2	Vir: vpis v poročilo
	Naslov	*SLO*	Dvojno zklenjen promotor gena za kolicin K, z dvema represorjema, prepreči prezgodnjo lizo bakterij po poškodbi DNA	

		ANG	Double-locking of the Escherichia coli colicin K gene promoter by two repressors prevents premature cell lysis after DNA damage
	Opis	*SLO*	Sinteza kolicinov bakterije E. coli je letalna za producentsko bakterijo. Izražanje kolicinov je zato tekom normalne bakterijske rasti močno utišano z represorjem LexA. Ob poškodbi DNA, se prvi prepišejo geni za popravilo DNA in z zamikom geni za kolcine. Ni bilo poznano, kaj omogoči zakasnjen prepis genov za kolicine. Dokazali smo, da globalni dejavnik transkripcije IscR, omogoči zakasneli prepis nekaterih genov za kolicine, tekom sproženega odziva SOS. Idfentificirali smo DNA vezavno mesto za IscR. Razložimo molekularni mehanizem, kako lahko bakterije omogočijo prepis gena za kolicine le ko so bakterije močno poškodovane in ne morejo vzdrževati integritete DNA.
		ANG	The synthesis of Eschericha coli colicins is lethal to the producing cell and is repressed during normal growth by the LexA transcription factor, which is the master repressor of the SOS system for repair of DNA damage. Following DNA damage, LexA is inactivated and SOS repair genes are induced immediately, but colicin production is delayed and induced only in terminally damaged cells. The cause of this delay is unknown. Here we identify the global transcription repressor, IscR, as being directly responsible for the delay in colicin K expression during the SOS response and identify the DNA target for IscR at the colicin K operon promoter. Hence, this promoter is 'double locked' to ensure that suicidal colicin K production is switched on only as a last resort.
	Objavljeno v		Revision, due March 2012. Matej Butala*, Douglas F. Browning, Silva Sonjak, Milan Hodošček, Darja Žgur Bertok, Stephen J. W. Busby. Molecular Microbiology; Impact Factor: 4.819; Srednja vrednost revije / Medium Category Impact Factor: 3.787; A': 1; WoS: CQ;
	Tipologija		1.01 Izvirni znanstveni članek

8.Najpomembnejši družbeno-ekonomsko relevantni rezultati projektne skupine[7]

	Družbenoekonomsko relevantni dosežki			
1.	COBISS ID		3639160	Vir: COBISS.SI
	Naslov	*SLO*	Interakcija represorja LexA in rekombinaze RecA	
		ANG	Interaction of repressor LexA with recombinase RecA	
	Opis	*SLO*	Komentor pri diplomskem delu	
		ANG	Co-menthor, graduation thesis	
	Šifra		D.10 Pedagoško delo	
	Objavljeno v		[A. Rems]; 2009; X, 47 f.; Avtorji / Authors: Rems Ana	
	Tipologija		2.11 Diplomsko delo	
2.	COBISS ID		3782008	Vir: COBISS.SI
	Naslov	*SLO*	Identifikacija nepoznanih proteinov, ki uravnavajo odziv SOS bakterije Escherichia coli	
		ANG	Identification of the unknown proteins taht regulate the induction of the bacterial SOS response	
	Opis	*SLO*	Komentor pri diplomskem delu	
		ANG	Co-menthor, graduation thesis	
	Šifra		D.10 Pedagoško delo	
	Objavljeno v		[T. Đapa]; 2010; X, 66 f.; Avtorji / Authors: Đapa Tanja	
			2.11	

	Tipologija		Diplomsko delo
3.	COBISS ID		Vir: vpis v poročilo
	Naslov	*SLO*	PathoGenoMics PhD award 2009
		ANG	PathoGenoMics PhD award 2009
	Opis	*SLO*	Doktorat dr. Mateja Butale je bil ocenjen kot eden izmed treh najboljših doktoratov s področja genetike človeku patogenih mikroorganizmov. Doktorsko delo je bilo predstavljeno v obliki kratkega predavanja na tretjem evropskem kongresu mikrobiologov: 3rd FEMS Congress of European Microbiologists 2009, Göteborg, Švedska.
		ANG	Matej Btala`s PhD thesis were selected as one of teh best three thesis from the field of genetics on the research on disease-causing microorganisms by a review board of internationally renowned experts in the field of microbial research. Work was presented in a short lecture at 3rd FEMS Congress of European Microbiologists 2009, Göteborg, Sweden.
	Šifra		E.02 Mednarodne nagrade
	Objavljeno v		http://www.pathogenomics-era.net/index.php?index=322
	Tipologija		1.08 Objavljeni znanstveni prispevek na konferenci

9.Drugi pomembni rezultati projetne skupine[8]

Vložena je patentna prijava: Controlling antibiotic tolerance, persister formation in a bacterial cell population by modulating LexA repressor functions (5/2010, patentna prijava, številka: 10005558.1, European Patent Office, München, Germany).

2.05 Drugo učno gradivo
AMBROŽIČ, Jerneja, BUTALA, Matej, STARČIČ ERJAVEC, Marjanca. Učno gradivo za program iz biologije genov : laboratorijske vaje in delavnice. Ljubljana, 2010: [S.n.]. 47 f., ilustr., graf.prikazi. [COBISS.SI-ID 26962393]

10.Pomen raziskovalnih rezultatov projektne skupine[9]

10.1.Pomen za razvoj znanosti[10]

SLO

Bakterijski odziv SOS je ključen za vzdrževanje integritete genoma, a tudi za porajanje odpornosti proti antibiotikom. Rezultati raziskave so pomembni za razumevanje kompleksnega bakterijskega odziva na poškodbe DNA kot je odziv SOS, tvorijo temelj za nadaljne raziskave oziroma, izhodišče za razvoj učinkovin ali ko-učinkovin katere bomo lahko uporabljali skupaj z že obstoječimi antibiotiki.

Za izvedbo projekta sem vzpostavil mednarodno sodelovanje s skupino prof. Heinz-Juergen Steinhoffa v Nemčiji. Z raziskovalcema Danielom Klose ter dr. Johannom Klare smo preučili konformacijske spremembe proteina LexA. Nadaljevali smo sodelovanje s skupino prof. Steva Busby-a ter se povezali tudi s skupino dr. Davida Graingerja v Veliki Britaniji. Vpetost projekta v Sloveniji: meritve izvedene v infrastrukturnem centru SPR v sodelovanju s prof. Gregorjem Anderluhom ter Vesno Hodnik; razvoj anti-LexA učinkovine, sodelovanje z dr. Mojco Lunder in s skupino prof. Stanislava Gobca, Fakulteta za Farmacijo. Nadaljevali smo s sodelovanjem s dr. Milanom Hodoščkom, Kemijski inštitut, Ljubljana. Povezave so razvidne iz skupnih publikacij.
S projektom smo poglobili razumevanje kako bakterije uvnavajo izražanja genov v stresnih razmerah, kot je poškodba DNA, kar je lahko povod za razvoj odpornost proti antibiotikom. Poglavitna dodana vrednost rezultatov projekta je, povezovanje Evropskih inšitutov pri preučevanju teh pomembnih vprašanj. Področje raziskav molekularnih mehanizmov porajanja odpornosti proti protimikrobnim učinkovinam je visoko kompetitativno po svetu. Centri raziskav s tega področja so v ZDA, Japonskem in na Kitajskem. Posledično so vzpostavitev sodelovanj

tekom tega projekta in pridobljena dognanja pomembna za odličnost Evropskih raziskav na področju odziva SOS ter porajanja odpornosti. Z zgoraj omenjenimi raziskovalci nadaljujemo z raziskavami na odzivu SOS.

ANG

The bacterial SOS response is essential for the maintenance of genomes, but also modulates antibiotic resistance. Our results provide insights into the mechanisms underlying SOS response and are prerequisite to understand the mechanism behind programmed expression of the LexA regulon genes. Hence, this work sets a novel platform for drug discovery to treat bacterial pathogens and offers an approach to control bacterial survival of antibiotic therapy.

I have established international colaborations in order to carry out this project. I have collaborated with prof. Steinhoff`s group from Germany. We have applied EPR methods to LexA. We continued collaboration with the group of prof. Steve Busby and established collaboration with dr. David Grainger from the UK. Collaborations established in Slovenia: Infrastractural centre for surface plasmon resonance, measurements performed in collaboration with prof. Gregor Anderluh and Vesna Hodnik; for development of anti-LexA compounds I collaborated with dr. Mojca Lunder and the group of prof. Stanislav Gobec, Faculty of Pharmacy, UL. We continued collaboration with dr. Milan Hodošček, Chemical institute, Ljubljana. International colaboration in this project can be observed from the joint publications.
This research project focused on deepening and broadening the understanding of bacterial gene regulation due to stress response in bacteria and their influence on phenomena of antibiotic resistance. One of the main added values to the European research community lies in increasing the potential of Slovenia as a centre for fundamental research in molecular microbiology. The area of antimicrobial stress response is highly competitive internationally. There are rapidly developing centres of excellence in this research area within Japan, China and US. This project established the international community in this field and promoted its general ability to make high impact research contributions to further European Excellence. Thus, after this project is finished we will continue collaborating on the SOS response with the above mentioned research groups.

10.2.Pomen za razvoj Slovenije[11]

SLO

Rezultati projekta prispevajo k razumevanju molekulskega mehanizma, ki omogoči bakterijam da se odzovejo na stres in predstavlja učno gradivo za študente. Naši rezulati pripomorejo k prepoznavnosti Slovenske zananosti v svetu, saj so/bodo rezultati projekta objavljeni v revijah z visokim faktojem citiranosti ter v encilopediji.
Iz vsebine projekta sta diplomirali Ana Rems uni. dipl. mikrobiol., ki nadaljuje s podiplomskim študijem na Danskem ter Tanje Đapa uni. dipl. mikrobiol., ki je trenutno doktorantka v Novartisu, Siena. Projekt je torej omogočil razvoj dveh odličnih mladih Slovenskih znanstvenic.

Potreben je nov pristop k zdravljenju bakterijskih okužb. Rezultati projekta so ogrodje za nadaljne raziskave v tej smeri. Odkritje in uporaba spojin, ki inhibirajo mehanizme razvoja odpornosti proti antibiotikom, kot je odziv SOS, bo omogočilo učinkovitejše zdravljenje z že obstoječimi antibiotiki. Vložena je patentna prijava (Točka 9).
Razvoj učinkovitega inhibitorja odziva SOS, bo lahko omogočil farmacevtskim družbam ohranitev proizvodnje obstoječih klinično pomembnih antibiotikov, kar je izjemnega pomena za Slovensko gospodarstvo.

Sredstva, ki so bila vložena v projekt so bila ustrezno porabljena! Verajmem, da je projekt Uravnavanje koproteazne aktivnosti proteina RecA v bakterijah, le eden od mnogih projektov mlajših raziskovalcev, ki so bili uspešno ralizirani. Pomen izvedenega projekta za Slovenijo je torej tudi, da se zavedamo, da je koristno (in nujno) omogočiti čim večjemu številu mlajšim raziskovalcem sredstva za izvedbo/razjasnitev svojih idej in razvoja lastnega potenciala!

ANG

Results obtained from this project elucidate how bacteria respond to the environmental stress, promote bacterial evolution, which is important for further studies on the SOS response and presents a model for textbooks for the students. The results from this project will benifit to the recognition of Slovenian science abroad as the results are /will be published in a high impact

journals and in the encyclopedia.
Part of this project was performed by Ana Rems uni. dipl. microbiol. (currently a PhD student at Technical University of Denmark), Tanje Đapa uni. dipl. microbiol. (currently a PhD student in Novartis, Siena), results from this project were used for their graduation thesis. Thus, this project established two talented Slovanian young scientist.

As the treatments to treat bacterial pathogens are narrowing, new methods are needed.
The set up collaborations and the obtained results enabled us to elucidate the important insights into the molecular mechanism of the bacterial response to antibiotics. Thus, development of an efficient inhibitor that will block SOS response and prevent development and spread of antibiotic resistance genes among bacteria, will hopefuly allow pharmaceutical companies to maintain production of clinically significant antibiotics, which is of great importance for the Slovenian economy.

11. Samo za aplikativne projekte!
Označite, katerega od navedenih ciljev ste si zastavili pri aplikativnem projektu, katere konkretne rezultate ste dosegli in v kakšni meri so doseženi rezultati uporabljeni

Cilj		
F.01	**Pridobitev novih praktičnih znanj, informacij in veščin**	
	Zastavljen cilj	○ DA ○ NE
	Rezultat	
	Uporaba rezultatov	
F.02	**Pridobitev novih znanstvenih spoznanj**	
	Zastavljen cilj	○ DA ○ NE
	Rezultat	
	Uporaba rezultatov	
F.03	**Večja usposobljenost raziskovalno-razvojnega osebja**	
	Zastavljen cilj	○ DA ○ NE
	Rezultat	
	Uporaba rezultatov	
F.04	**Dvig tehnološke ravni**	
	Zastavljen cilj	○ DA ○ NE
	Rezultat	
	Uporaba rezultatov	
F.05	**Sposobnost za začetek novega tehnološkega razvoja**	
	Zastavljen cilj	○ DA ○ NE
	Rezultat	
	Uporaba rezultatov	
F.06	**Razvoj novega izdelka**	
	Zastavljen cilj	○ DA ○ NE
	Rezultat	
	Uporaba rezultatov	
F.07	**Izboljšanje obstoječega izdelka**	
	Zastavljen cilj	○ DA ○ NE

	Rezultat	
	Uporaba rezultatov	
F.08	**Razvoj in izdelava prototipa**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.09	**Razvoj novega tehnološkega procesa oz. tehnologije**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.10	**Izboljšanje obstoječega tehnološkega procesa oz. tehnologije**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.11	**Razvoj nove storitve**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.12	**Izboljšanje obstoječe storitve**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.13	**Razvoj novih proizvodnih metod in instrumentov oz. proizvodnih procesov**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.14	**Izboljšanje obstoječih proizvodnih metod in instrumentov oz. proizvodnih procesov**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.15	**Razvoj novega informacijskega sistema/podatkovnih baz**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.16	**Izboljšanje obstoječega informacijskega sistema/podatkovnih baz**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	

F.17	**Prenos obstoječih tehnologij, znanj, metod in postopkov v prakso**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.18	**Posredovanje novih znanj neposrednim uporabnikom (seminarji, forumi, konference)**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.19	**Znanje, ki vodi k ustanovitvi novega podjetja ("spin off")**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.20	**Ustanovitev novega podjetja ("spin off")**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.21	**Razvoj novih zdravstvenih/diagnostičnih metod/postopkov**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.22	**Izboljšanje obstoječih zdravstvenih/diagnostičnih metod/postopkov**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.23	**Razvoj novih sistemskih, normativnih, programskih in metodoloških rešitev**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.24	**Izboljšanje obstoječih sistemskih, normativnih, programskih in metodoloških rešitev**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.25	**Razvoj novih organizacijskih in upravljavskih rešitev**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.26	**Izboljšanje obstoječih organizacijskih in upravljavskih rešitev**	

	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.27	**Prispevek k ohranjanju/varovanje naravne in kulturne dediščine**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.28	**Priprava/organizacija razstave**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.29	**Prispevek k razvoju nacionalne kulturne identitete**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.30	**Strokovna ocena stanja**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.31	**Razvoj standardov**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.32	**Mednarodni patent**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.33	**Patent v Sloveniji**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.34	**Svetovalna dejavnost**	
	Zastavljen cilj	DA NE
	Rezultat	
	Uporaba rezultatov	
F.35	**Drugo**	
	Zastavljen cilj	DA NE
	Rezultat	

	Uporaba rezultatov	

Komentar

12.Samo za aplikativne projekte!
Označite potencialne vplive oziroma učinke vaših rezultatov na navedena področja

	Vpliv	Ni vpliva	Majhen vpliv	Srednji vpliv	Velik vpliv	
G.01	**Razvoj visoko-šolskega izobraževanja**					
G.01.01.	Razvoj dodiplomskega izobraževanja	○	○	○	○	
G.01.02.	Razvoj podiplomskega izobraževanja	○	○	○	○	
G.01.03.	Drugo:	○	○	○	○	
G.02	**Gospodarski razvoj**					
G.02.01	Razširitev ponudbe novih izdelkov/storitev na trgu	○	○	○	○	
G.02.02.	Širitev obstoječih trgov	○	○	○	○	
G.02.03.	Znižanje stroškov proizvodnje	○	○	○	○	
G.02.04.	Zmanjšanje porabe materialov in energije	○	○	○	○	
G.02.05.	Razširitev področja dejavnosti	○	○	○	○	
G.02.06.	Večja konkurenčna sposobnost	○	○	○	○	
G.02.07.	Večji delež izvoza	○	○	○	○	
G.02.08.	Povečanje dobička	○	○	○	○	
G.02.09.	Nova delovna mesta	○	○	○	○	
G.02.10.	Dvig izobrazbene strukture zaposlenih	○	○	○	○	
G.02.11.	Nov investicijski zagon	○	○	○	○	
G.02.12.	Drugo:	○	○	○	○	
G.03	**Tehnološki razvoj**					
G.03.01.	Tehnološka razširitev/posodobitev dejavnosti	○	○	○	○	
G.03.02.	Tehnološko prestrukturiranje dejavnosti	○	○	○	○	
G.03.03.	Uvajanje novih tehnologij	○	○	○	○	
G.03.04.	Drugo:	○	○	○	○	
G.04	**Družbeni razvoj**					
G.04.01	Dvig kvalitete življenja	○	○	○	○	
G.04.02.	Izboljšanje vodenja in upravljanja	○	○	○	○	
G.04.03.	Izboljšanje delovanja administracije in javne uprave	○	○	○	○	
G.04.04.	Razvoj socialnih dejavnosti	○	○	○	○	
G.04.05.	Razvoj civilne družbe	○	○	○	○	
G.04.06.	Drugo:	○	○	○	○	
	Ohranjanje in razvoj nacionalne					

G.05.	**naravne in kulturne dediščine in identitete**	○	○	○	○	
G.06.	**Varovanje okolja in trajnostni razvoj**	○	○	○	○	
G.07	**Razvoj družbene infrastrukture**					
G.07.01.	Informacijsko-komunikacijska infrastruktura	○	○	○	○	
G.07.02.	Prometna infrastruktura	○	○	○	○	
G.07.03.	Energetska infrastruktura	○	○	○	○	
G.07.04.	Drugo:	○	○	○	○	
G.08.	**Varovanje zdravja in razvoj zdravstvenega varstva**	○	○	○	○	
G.09.	**Drugo:**	○	○	○	○	

Komentar

13.Pomen raziskovanja za sofinancerje[12]

	Sofinancer			
1.	Naziv			
	Naslov			
	Vrednost sofinanciranja za celotno obdobje trajanja projekta je znašala:			EUR
	Odstotek od utemeljenih stroškov projekta:			%
	Najpomembnejši rezultati raziskovanja za sofinancerja			Šifra
		1.		
		2.		
		3.		
		4.		
		5.		
	Komentar			
	Ocena			

C. IZJAVE

Podpisani izjavljam/o, da:

- so vsi podatki, ki jih navajamo v poročilu, resnični in točni
- se strinjamo z obdelavo podatkov v skladu z zakonodajo o varstvu osebnih podatkov za potrebe ocenjevanja ter obdelavo teh podatkov za evidence ARRS
- so vsi podatki v obrazcu v elektronski obliki identični podatkom v obrazcu v pisni obliki
- so z vsebino zaključnega poročila seznanjeni in se strinjajo vsi soizvajalci projekta

Podpisi:

zastopnik oz. pooblaščena oseba raziskovalne organizacije: in *vodja raziskovalnega projekta:*

Univerza v Ljubljani, Biotehniška fakulteta

Matej Butala

ŽIG

Kraj in datum:	Ljubljana	5.3.2012

Oznaka prijave: ARRS-RPROJ-ZP-2012/12

---

[1] Zaradi spremembe klasifikacije je potrebno v poročilu opredeliti raziskovalno področje po novi klasifikaciji FOS 2007 (Fields of Science). Prevajalna tabela med raziskovalnimi področji po klasifikaciji ARRS ter po klasifikaciji FOS 2007 (Fields of Science) s kategorijami WOS (Web of Science) kot podpodročji je dostopna na spletni strani agencije (http://www.arrs.gov.si/sl/gradivo/sifranti/preslik-vpp-fos-wos.asp). Nazaj

[2] Napišite povzetek raziskovalnega projekta (največ 3.000 znakov v slovenskem in angleškem jeziku) Nazaj

[3] Napišite kratko vsebinsko poročilo, kjer boste predstavili raziskovalno hipotezo in opis raziskovanja. Navedite ključne ugotovitve, znanstvena spoznanja, rezultate in učinke raziskovalnega projekta in njihovo uporabo ter sodelovanje s tujimi partnerji. Največ 12.000 znakov vključno s presledki (približno dve strani, velikosti pisave 11). Nazaj

[4] Realizacija raziskovalne hipoteze. Največ 3.000 znakov vključno s presledki (približno pol strani, velikosti pisave 11) Nazaj

[5] V primeru bistvenih odstopanj in sprememb od predvidenega programa raziskovalnega projekta, kot je bil zapisan v predlogu raziskovalnega projekta oziroma v primeru sprememb, povečanja ali zmanjšanja sestave projektne skupine v zadnjem letu izvajanja projekta (obrazložitev). V primeru, da sprememb ni bilo, to navedite. Največ 6.000 znakov vključno s presledki (približno ena stran, velikosti pisave 11). Nazaj

[6] Znanstveni in družbeno-ekonomski dosežki v programu in projektu so lahko enaki, saj se projekna vsebina praviloma nanaša na širšo problematiko raziskovalnega programa, zato pričakujemo, da bo večina izjemnih dosežkov raziskovalnih programov dokumentirana tudi med izjemnimi dosežki različnih raziskovalnih projektov.

Raziskovalni dosežek iz obdobja izvajanja projekta (do oddaje zaključnega poročila) vpišete tako, da izpolnite COBISS kodo dosežka – sistem nato sam izpolni naslov objave, naziv, IF in srednjo vrednost revije, naziv FOS področja ter podatek, ali je dosežek uvrščen v A'' ali A'. Nazaj

[7] Znanstveni in družbeno-ekonomski dosežki v programu in projektu so lahko enaki, saj se projekna vsebina praviloma nanaša na širšo problematiko raziskovalnega programa, zato pričakujemo, da bo večina izjemnih dosežkov raziskovalnih programov dokumentirana tudi med izjemnimi dosežki različnih raziskovalnih projektov.

Družbeno-ekonomski rezultat iz obdobja izvajanja projekta (do oddaje zaključnega poročila) vpišete tako, da izpolnite COBISS kodo dosežka – sistem nato sam izpolni naslov objave, naziv, IF in srednjo vrednost revije, naziv FOS področja ter podatek, ali je dosežek uvrščen v A'' ali A'.

Družbenoekonomski dosežek je po svoji strukturi drugačen, kot znanstveni dosežek. Povzetek znanstvenega dosežka je praviloma povzetek bibliografske enote (članka, knjige), v kateri je dosežek objavljen.

Povzetek družbeno ekonomsko relevantnega dosežka praviloma ni povzetek bibliografske enote, ki ta dosežek dokumentira, ker je dosežek sklop več rezultatov raziskovanja, ki je lahko dokumentiran v različnih bibliografskih enotah. COBISS ID zato ni enoznačen izjemoma pa ga lahko tudi ni (npr. v preteklem letu vodja meni, da je izjemen dosežek to, da sta se dva mlajša sodelavca zaposlila v gospodarstvu na pomembnih raziskovalnih nalogah, ali ustanovila svoje podjetje, ki je rezultat prejšnjega dela … - v obeh primerih ni COBISS ID). Nazaj

[8] Navedite rezultate raziskovalnega projekta iz obdobja izvajanja projekta (do oddaje zaključnega poročila) v primeru, da katerega od rezultatov ni mogoče navesti v točkah 7 in 8 (npr. ker se ga v sistemu COBISS ne vodi). Največ 2.000 znakov vključno s presledki. Nazaj

[9] Pomen raziskovalnih rezultatov za razvoj znanosti in za razvoj Slovenije bo objavljen na spletni strani: http://sicris.izum.si/ za posamezen projekt, ki je predmet poročanja Nazaj

[10] Največ 4.000 znakov vključno s presledki Nazaj

[11] Največ 4.000 znakov vključno s presledki Nazaj

[12] Rubrike izpolnite / prepišite skladno z obrazcem "izjava sofinancerja" http://www.arrs.gov.si/sl/progproj/rproj/gradivo/, ki ga mora izpolniti sofinancer. Podpisan obrazec "Izjava sofinancerja" pridobi in hrani nosilna raziskovalna organizacija – izvajalka projekta. Nazaj

Obrazec: ARRS-RPROJ-ZP/2012 v1.00
89-EB-82-9E-E5-84-81-CD-EE-98-E1-0B-13-D8-90-5B-C9-C4-31-BF

6546–6557 *Nucleic Acids Research, 2011, Vol. 39, No. 15* *Published online 16 May 2011*
doi:10.1093/nar/gkr265

Interconversion between bound and free conformations of LexA orchestrates the bacterial SOS response

Matej Butala[1,*], Daniel Klose[2], Vesna Hodnik[1], Ana Rems[1], Zdravko Podlesek[1], Johann P. Klare[2], Gregor Anderluh[1], Stephen J. W. Busby[3], Heinz-Jürgen Steinhoff[2] and Darja Žgur-Bertok[1]

[1]Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia, [2]Department of Physics, University of Osnabrück, Barbarastrasse 7, D-49076 Osnabrück, Germany and [3]School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK

Received November 23, 2010; Revised and Accepted April 6, 2011

ABSTRACT

The bacterial SOS response is essential for the maintenance of genomes, and also modulates antibiotic resistance and controls multidrug tolerance in subpopulations of cells known as persisters. In *Escherichia coli*, the SOS system is controlled by the interplay of the dimeric LexA transcriptional repressor with an inducer, the active RecA filament, which forms at sites of DNA damage and activates LexA for self-cleavage. Our aim was to understand how RecA filament formation at any chromosomal location can induce the SOS system, which could explain the mechanism for precise timing of induction of SOS genes. Here, we show that stimulated self-cleavage of the LexA repressor is prevented by binding to specific DNA operator targets. Distance measurements using pulse electron paramagnetic resonance spectroscopy reveal that in unbound LexA, the DNA-binding domains sample different conformations. One of these conformations is captured when LexA is bound to operator targets and this precludes interaction by RecA. Hence, the conformational flexibility of unbound LexA is the key element in establishing a co-ordinated SOS response. We show that, while LexA exhibits diverse dissociation rates from operators, it interacts extremely rapidly with DNA target sites. Modulation of LexA activity changes the occurrence of persister cells in bacterial populations.

INTRODUCTION

In unstressed, growing *Escherichia coli* cells, the SOS system is shut off due to repression by LexA of ~50 promoters that control expression of the SOS regulon (1,2). Under these conditions, *E. coli* is thought to contain ~1300 molecules of LexA (3). Most LexA is DNA bound, but ~20% is thought to be free. LexA is a homodimeric protein (4) that likely locates its target sites by multiple dissociation–reassociation events within the same DNA molecule (5). Around each landing site, the repressor is thought to diffuse along non-specific DNA and to undergo rotation-coupled sliding to facilitate the search for specific binding sites (6).

The majority of *E. coli* SOS promoters are regulated by LexA alone (7). LexA activity is modulated by the active form of RecA (RecA*), that stimulates self-cleavage of a scissile peptide bond between Ala84 and Gly85, thereby de-activating LexA (8), lowering LexA's affinity for the DNA and exposing residues that target LexA for ClpXP and Lon protease degradation (9). As a result, the cellular concentration of LexA drops from ~2 to ~0.2 μM, thereby de-repressing SOS genes (3).

A key characteristic of the SOS response is the orchestrated induction of individual SOS genes. Thus, initially, genes with low-affinity SOS boxes are expressed, enabling protection and maintenance of the structural integrity of the replisome, while genes with high-affinity operators are expressed late in the SOS response (1). To circumvent unrepaired DNA damage, even after high-fidelity nucleotide excision, and recombinational repair, low fidelity DNA damage tolerance pathways are induced, presumably to increase bacterial mutation rates

*To whom correspondence should be addressed. Tel: +386 1 320 3405; Fax: +386 1 257 3390; Email: matej.butala@bf.uni-lj.si

and survival in times of stress (10). As DNA damage is repaired, LexA accumulates and the system is reset. Alternatively, if cells are severely damaged and may not survive, the sensing of long-lived-inducing signal triggers the synthesis of bacteriocins and prophages, resulting in cell lysis (11). Thus, RecA* also catalyzes self-cleavage of lambdoid phage repressors (12) whose catalytic, carboxy-terminal domains (CTDs) exhibit homology with the LexA CTD (13).

Similarly to LexA inactivation, cleavage of phage repressors leads to destruction of the protein's abilities to firmly bind DNA, enabling a switch from the latent or lysogenic to replicative and lytic phase. Interestingly, the λ cI repressor is cleaved only when monomeric (14), while the cI repressor of the temperate 434 bacteriophage is inactivated preferably when bound to specific DNA (15). LexA is predominately dimeric in the cell (4) and repressor dimers can undergo RecA*-mediated self-cleavage when off the DNA (16). Therefore, the mechanisms of repressor inactivation among various biological systems related to SOS functions vary from one system to another.

Even though many studies have investigated the SOS response, it is still unclear how diversity within SOS boxes co-ordinates temporal induction of the different SOS genes. In addition, it is not known how RecA* induces self-cleavage of LexA and which are the structural determinants required for RecA*-mediated cleavage of LexA (16,17). Here, we present the first report describing LexA repressor with defects in LexA–RecA* interaction. We demonstrate that, the unbound LexA structure is highly flexible in contrast to the rigid DNA-bound state, in which interaction with RecA* is precluded. Thus, we show that RecA* indirectly activates the SOS system, by mediating a decrease in the intracellular pool of unbound LexA provoking dissociation of the operator-bound repressor and concomitantly inducing the LexA regulon genes. Our data further imply that two sequential interactions of the unbound LexA with RecA* are required for inactivation of both subunits of the LexA repressor dimer.

MATERIALS AND METHODS

Cloning, expression and isolation of the proteins

The *lexA*, *recA* and *oxyR* genes were amplified by polymerase chain reaction (PCR) from the *E. coli* K-12 strain RW118 (18) using oligonucleotide primers LexA_u, LexA_d; RecA_u, RecA_d or OxyR_u, OxyR_d, respectively (Supplementary Table S1). The PCR products were subsequently cut with BamHI and MluI and cloned into an expression vector (19) to prepare plasmids pAna1, pAna2 and pOxyR. The LexA and RecA proteins overexpressed from the pAna1 or pAna2 plasmids, respectively, were constructed as His_6 fusion proteins with an N-terminal hexa-histidine tag and a thrombin cleavage site ($(H)_6$SSLVPRGS). A variant of the pAna1 expression plasmids, pLexA29, pLexA54, pLexA71, pLexA119, pLexA71-119 and pLexA191 were constructed employing the QuickChange® Site-directed Mutagenesis kit manual (Stratagene) and pairs of oligonucleotides 29AC_1, 29AC_2 and 54GC_1, 54GC_2; 71EK_1, 71EK_2; 119SA_1, 119SA_2 or 191LC_1, 191LC_2 (Supplementary Tables S1 and S2), respectively. Proteins LexA, LexA29, LexA54, LexA71, LexA119, LexA191 and RecA were expressed with a His-tag present on the N-terminus in the *E. coli* BL21 (DE3) strain and purified from the bacterial cytoplasm by Ni-chelate chromatography and gel-filtration chromatography (20). Purified proteins were stored at −80°C in 20 mM NaH_2PO_4 (pH 7.3), 200 mM NaCl except for LexA, LexA71 and RecA which were stored in buffer containing 20 mM Tris−HCl (pH 7.3), 200 mM NaCl. Protein concentrations were determined using NanoDrop1000 (Thermo SCIENTIFIC) (4). Three LexA cysteine mutants (LexA29, LexA54, LexA191) were used for the electron paramagnetic resonance (EPR) analysis. The LexA71 repressor variant exhibits enhanced DNA-binding affinity, but the mechanism for the improved DNA binding is unknown (21). The LexA119 is a non-cleavable repressor derivative with modified Ser119 in the active center to Ala; this mutation does not affect the ability of LexA to bind RecA* (13,16). Thus, the LexA119 variant was used to prevent repressor self-cleavage during the study of the LexA−RecA* interaction.

Operator-containing DNA fragments

The 88 bp *recA* and the 114 bp *tisB* operator-containing DNA fragments were PCR amplified. The colicin K encoding plasmid pKCT1 and its derivatives with altered SOS boxes pKCT3-UP1, pKCT3-UP3 (22) were used to amplify the 121 bp *cka*, *cka*-UP1 and the *cka*-UP3 fragment, respectively. Centered on the generated DNA fragments were none, single or double LexA-binding sites presented in Figure 1. One strand of the amplified PCR products was biotinylated at the 5′-end, and primers RecA_1, RecA_2; TisB_1, TisB_2 were used to amplify DNA fragments with *recA* or *tisB* operators and primers Cka_1, Cka_2 to amplify DNA fragments harboring *cka*, *cka*-UP1 and *cka*-UP3 operators, respectively (Supplementary Table S1). The PCR generated fragments were gel purified (QIAquick kit, Qiagen).

LexA repressor cleavage assays

Activation of the RecA filament (10 μM), carried out on ice for 2 h, and the RecA*-induced (2 μM) cleavage of LexA (1.8 μM) at 37°C interacting with specific or non-specific DNA (~1.5 μM) were performed as described previously for the unbound LexA repressor (16). The LexA dimer to operator/modified operator ratio was 1:2. The LexA repressor was preincubated with specific and non-specific DNA or for the titration reactions with increasing concentrations of DNA for 10 min at 37°C in a DNA-binding buffer (23). The reaction time course was initiated with the addition of the RecA*. The proteolytic cleavage reactions (20 μl) were stopped by adding 4xNuPAGE LDS sample buffer (Invitrogen). Samples were analyzed on 12% NuPAGE gels (Invitrogen) and stained by Page blue protein stain (Fermentas). The experiments were conducted at least three times and representative gels are shown. The resolved bands were quantified using a G:Box (Syngene). The integrated optical

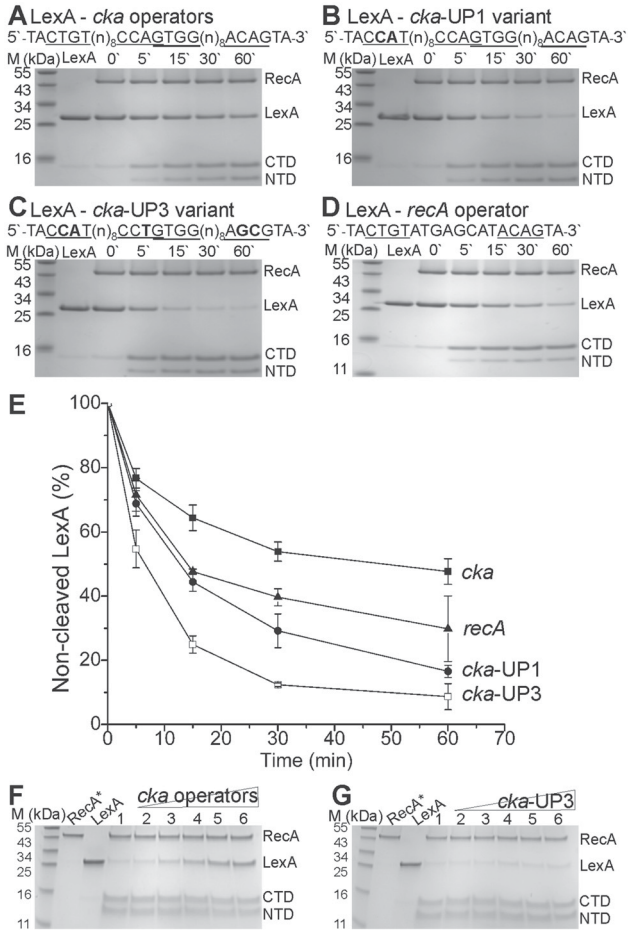


Figure 1. RecA* cannot induce self-cleavage of specifically bound LexA. (**A–D**) Time course (min) of RecA*-induced LexA proteolysis showing inhibition of cleavage due to operator DNAs compared with non-specific DNA (*cka*–UP3). Operator sequences used are presented with SOS boxes underlined and mutated nucleotides in bold typeface. (**E**) Quantitations of the LexA self-cleavage presented are averages with the standard deviation of at least triplicate reactions. (**F**) LexA was pre-incubated with operators or (**G**) non-specific DNA in a ratio 1:0.2; 0.7; 1.2; 1.6; 2.1 (mol:mol) for lanes from 2 to 6, or without DNA for lane 1. The RecA*-activated self-cleavage of LexA was stopped after 15 min. RecA protein, LexA repressor and its cleaved products are marked by the CTD or NTD for the dimerization or the DNA-binding domain, respectively.

density of the intact LexA monomer was normalized to that determined for the RecA protein to account for lane-dependent artifacts. The ratio of LexA cleavage was calculated as the ratio of the normalized density value for the intact LexA relative to the normalized value of LexA exposed to RecA*.

Cross-linking of LexA repressor

Glutaraldehyde cross-linking: at the indicated time, RecA*-mediated LexA (both at the final concentration of 5.6 μM) proteolytic cleavage reactions conducted as stated above were stopped with 16 mM glutaraldehyde for 30 s before adding glycine to 60 mM (16).

Covalent cross-linking reactions: the LexA54 variant was reduced with 20 mM dithiothreitol (DTT) or oxidized with a mixture of 0.1 mM $CuSO_4$ and 0.5 mM 1,10-phenantroline for 30 min at room temperature. At the indicated time, RecA*-mediated proteolytic cleavage reactions of the oxidized LexA54 (at the final concentration of 4 and 5.6 μM for the LexA54 and RecA, respectively) conducted as stated above were stopped by adding 4xNuPAGE LDS sample buffer (Invitrogen). Presence of oxidant in the reactions did not affect RecA*-stimulated LexA self-cleavage, as determined by oxidation of wild-type LexA and implementation of self-cleavage reaction (data not shown).

Samples were analyzed as described above. We resolved the various repressor forms: dimers, monomers, CTDs, N-terminal domains (NTDs) and combinations of intact LexA protein and its cleavage products, by analysis of protein molar masses in comparison with the PageRuler prestained protein ladder (Fermentas) and by comparing our data with earlier results (16).

Spin labeling of LexA mutants

For spin labeling, purified single cysteine mutants (~10 mg) of *E. coli* LexA (Supplementary Table S2) were pretreated with DTT at 15 mM final concentration in buffer containing 20 mM NaH_2PO_4 (pH 7.3), 500 mM NaCl (4 h, 4°C). DTT was removed by exchanging the buffer two times with the use of PD-10 desalting column (GE Healthcare) and after removal protein solutions were incubated with 1 mM MTSSL (1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-methyl) methanethiosulfonate spin label (Toronto Research, Alexis), for 16 h (8°C). Excess MTSSL was removed by exchanging the buffer two times with 20 mM NaH_2PO_4 (pH 7.3), 200 mM NaCl with a PD 10 desalting column. The spin-labeled proteins were concentrated to ~100 μM and buffer exchanged by buffer of the same composition containing deuterated water (Acros Organics) by the use of Amicon centrifugal filters (Millipore). Labeling efficiencies have been determined to be ~80% for LexA54 and >95% for LexA29 and LexA191.

EPR measurements

Distance measurements between nitroxide spin labels attached to the LexA variants (~100 μM) were carried out either unbound or bound to the 24 bp *tisB* operator-containing DNA fragment (5′-TTTACTGTAT AAATAAACAGTAAT-3′, marked are the SOS boxes) composed of oligonucleotide primers Tis_1b, Tis_2b (Supplementary Table S1). Cw EPR spectra for interspin distance determination in the range from ~0.8 to 2.0 nm were obtained on a homebuilt cw X-band EPR spectrometer equipped with a Super High Sensitivity Probehead (Bruker Biospin GmbH, Rheinstetten, Germany). The magnetic field was measured with a B-NM 12 B-field meter (Bruker Biospin). A continuous flow cryostat Oxford ESR9 (Oxford Instruments, Oxfordshire, UK) was used in combination with an Intelligent Temperature Controller (ITC 4; Oxford Instruments) to stabilize the sample temperature to 160 K. The microwave power was

set to 0.2 mW and the B-field modulation amplitude to 0.25 mT. EPR quartz capillaries (3 mm inner diameter) were filled with sample volumes of 40 µl. Fitting of simulated dipolar broadened EPR powder spectra to the experimental ones was carried out using the program *WinDipFit* (24).

Double electron–electron resonance (DEER)/PELDOR EPR experiments were performed at X-band frequencies (9.3–9.4 GHz) on a Bruker Elexsys 580 spectrometer equipped with a Bruker Flexline split-ring resonator ER 4118X-MS3. Temperature was stabilized to 50 K using a continuous flow helium cryostat (ESR900; Oxford Instruments) controlled by an Oxford Intelligent Temperature Controller ITC 503 S. EPR quartz capillaries (2.4 mm inner diameter) were filled with sample volumes of 40 µl.

All measurements were performed using the four-pulse DEER sequence with two microwave frequencies: $\pi/2(\upsilon_{obs}) - \tau_1 - \pi\ (\upsilon_{obs}) - t' - \pi\ (\upsilon_{pump}) - (\tau_1+\tau_2 - t') - \pi\ (\upsilon_{obs}) - \tau_2 -$ echo (25,26). A two-step phase cycling (+<x>, − <x>) was performed on $\pi/2(\upsilon_{obs})$. Time t' is varied, whereas τ_1 and τ_2 are kept constant. The dipolar evolution time is given by $t = t' - \tau_1$. Data were analyzed only for $t > 0$. The resonator was overcoupled and the pump frequency υ_{pump} was set to the center of the resonator dip (coinciding with the maximum of the nitroxide EPR spectrum) whereas the observer frequency υ_{obs} was 65 MHz higher (low-field local maximum of the spectrum). All measurements were performed at a temperature of 50 K with observer pulse lengths of 16 ns for $\pi/2$ and 32 ns for π pulses and a pump pulse length of 12 ns. Proton modulation was averaged by adding traces at eight different τ_1 values, starting at $\tau_{1,0} = 200$ ns and incrementing by $\Delta\tau_1 = 8$ ns. For proteins in D_2O buffer with deuterated glycerol, used for its effect on the phase relaxation, corresponding values were $\tau_{1,0} = 400$ ns and $\Delta\tau_1 = 56$ ns. Data points were collected in 8 ns time steps or, if the absence of fractions in the distance distribution below an appropriate threshold was checked experimentally, in 16 ns time steps. The total measurement time for each sample was 4–24 h. Analysis of the data was performed with DeerAnalysis 2009 (27).

Rotamer library analysis

The canonical ensemble of spin label side-chain (R1) conformations is modeled by a discrete set of 210 precalculated rotamers (28). From the rotamer library analysis, a conformational distribution of R1 at a specific position in the otherwise fixed protein structure can be determined. Briefly, the superposition of R1′s backbone atoms onto the protein backbone at the respective position provides the orientation of R1 with respect to the protein structure. The resulting energy for the R1–protein interaction is then calculated from the Lennard Jones potential using the MD force field CHARMM27 (29). Subsequent Boltzmann weighting and normalization by the partition function gives a probability for each rotamer which is then multiplied by the probability of R1 to exhibit this conformation, resulting in the final rotamer probability distribution at the site of interest. Between two such probability distributions a distance distribution is calculated as the histogram of all pairwise interspin distances weighted by the product of their respective probabilities. Structural aspects of LexA were generated using VMD software (30).

Functional properties Of LexA mutants

For EPR analysis, we selected LexA residues that are surface exposed and do not impair repressor functions when modified (31). *Esherichia coli* strain DM936 (*lexA41*) was transformed with plasmid pLexA29, pLexA54, pLexA191 to complement the temperature-sensitive LexA mutation. As a control strain DM936 expressing the wild-type *lexA* (pAna1) or expressing the repressor OxyR (pOxyR) was used. To verify the *in vivo* ability of the LexA mutants to regulate the SOS system and to repress the *sulA* gene, preventing induction of filamentous growth, strains were grown in Luria–Bertani (LB) ampicillin (Ap, 100 µg/ml) media at 28.0°C or at 42.5°C and in stationary phase cell counts were determined (20). Surface plasmon resonance (SPR) analysis and RecA*–mediated cleavage experiments were conducted as described in this chapter.

SPR assays

SPR RecA*–LexA interaction measurements were performed on a Biacore X (GE Healthcare) at 25°C. The streptavidin sensor chip was equilibrated with SPR_2 buffer containing 20 mM NaH_2PO_4 (pH 7.4), 150 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT, 1 mM ATP (Sigma–Aldrich), 0.005% surfactant P20 (GE Healthcare). Approximately 200 response units (RU) of 5′-biotinylated 30-mer (32) was immobilized on the flow cell 2. Subsequently, RecA protein (2.1 µM) was passed in the SPR_2 buffer at 2 µl/min to create RecA*. The LexA119 repressor variant interacting with the 24 bp *tisB* operator (annealed primers Tis_1b, Tis_2b, Supplementary Table S1) or the 24-bp non-specific DNA (annealed primers Tis_1nb, Tis_2nb), free LexA119 or the DNA fragments, were injected across the immobilized RecA* (1000 RU) at 10 µl/min for 60 s, to study the interaction. The sensor chip with bound RecA* was regenerated by injection of 500 mM NaCl. A 0.05% SDS was used to additionally regenerate flow cell 1.

SPR LexA–operator interaction measurements were performed on a Biacore T100 at 25°C. The 88 bp *recA*, 114 bp *tisB*, 121 bp *cka* operator-containing DNA fragments and the *cka*-UP3 DNA fragment were PCR amplified and gel purified as described above. The resulting fragments were 5′-end biotinylated. The streptavidin sensor chip was equilibrated with SPR_1 buffer containing 20 mM Tris–HCl (pH 7.4), 150 mM NaCl, 0.005% surfactant P20 (GE Healthcare). The biotinylated DNA in SPR buffer was immobilized to approximately 20 RUs. An empty flow cell was used as a control. The interaction between LexA and chip-immobilized DNA was studied by injecting various concentrations of LexA or LexA71 in SPR buffer. The sensor chip with bound DNA was regenerated by injection of SPR buffer containing 500 mM NaCl. We noted that the interaction of both LexA and LexA71 with DNA was extremely rapid and

use of standard assays revealed that it is heavily influenced by the mass transfer effect (33). However, the dissociation of the proteins from the DNA was not influenced by the flow rate of the SPR buffer. For the final determination of dissociation rates, proteins were injected across the surface chip at a saturating concentration (40 nM) for 30 s and dissociation was followed for 20 min at a flow rate of 100 µl/min. The dissociation of LexA71 from *cka* operator was extremely slow; therefore, we followed dissociation for 40 min. The data were doubly referenced and fitted to a 1:1 binding model to obtain the dissociation rates constants. Three to six independent experiments were performed.

Persistence of *lexA* defective strain complemented by LexA and its variants

For the persistence assay, strain RW542 (*thr-1 araD139* Δ(*gpt-proA*)62 *lacY1 tsx-33 supE44 galK2 hisG4 rpsL31 xyl-5 mtl-1 argE3 thi-1 sulA211 lexA51*), encoding a defective LexA protein that cannot bind to target DNA sites due to impaired dimerization (18) was used. The λDE3 prophage, encoding the T7 RNA polymerase, was integrated into the RW542 chromosome according to instructions (λDE3 Lysogenization kit, Novagen). The λDE3 lysogenic RW542 strain, designated MB542, exhibited basal-level T7 RNA polymerase expression without addition of isopropyl beta-D-1-thiogalactopyranoside as determined according to the manufacturer's instructions. Subsequently, strain MB542 was transformed with plasmid harboring T7 promoter controlled wild-type *lexA*, mutant *lexA119* or the double-mutant *lexA71-119*. The minimum inhibitory concentration (MIC) for mitomycin C (Sigma) was determined by the broth dilution method (34). The MIC for the strain MB542 *lexA*(Def) was 3.2 µg/ml, for the strain harboring the plasmid encoding wild-type repressor 4.0 and 1.8 µg/ml for the strains with *lexA119* or the double *lexA71-119* mutant. The 2.5 MIC of mitomycin C was used for the persister assay. The isogenic strain RW118 expressing chromosomally encoded *lexA* exhibited identical mitomycin C MIC as the strain MB542 complemented with the plasmid encoding wild-type repressor. Thus, data indicate that the SOS system of the *lexA* complemented strain MB542 pAna1 functioned similarly as the wild-type strain. Experiments were conducted at 37°C essentially as described previously (35) except that transformed strains were grown (180 rpm) in 10 ml LB medium supplemented with 100 µg/ml Ap and cell counts determined by plating on LB or LBAp agar plates. No difference in cell count was detected when cells were plated on LB or LBAp media, indicating that plasmid loss did not occur during the experiments (data not shown). The percentage of survival was determined as the ratio of colony forming units (cfu) before to cfu following exposure to mitomycin C and plotted as a function of time.

Trypsin cleavage of LexA repressor bound to operator

The LexA repressor (2.4 µM) was bound to the *recA* or *cka* operator-containing fragments or to the *cka* variant fragments *cka*-UP1 or *cka*-UP3. The LexA dimer to operator/modified operator ratio was 1:2. DPPC-treated Trypsin (Sigma–Aldrich) digestions were conducted at 25°C in DNA-binding buffer at a LexA concentration of 2.4 µM with a protease to repressor ratio of 1:50 (m:m). The reaction time course was initiated with the addition of the protease. Bands were resolved as described above.

Western blotting

Thrombin (Novagen) digestion of 3.4 µM LexA was carried out at 20°C for 2 h in 20 mM Tris (pH 7.3), 200 mM NaCl with a protease to repressor weight ratio of 1:2000. LexA–DNA complex was formed by 10 min incubation of 3.4 µM LexA and DNA fragment-containing *recA* operator in the LexA dimer toward operator ratio 1:2 at 37°C in DNA-binding buffer prior to trypsin digestion carried out for 30 min as described above. Samples were resolved on a 12% acrylamide gel. Blotting and detection was done as described before (36). Primarily, the proteins were stained with mouse anti-hexahistidine tag antibody (Quiagen) and secondary antibodies conjugated by horseradish peroxidase. The same membrane was re-stained by primary LexA rabbit polyclonal antibody (Upstate) and same secondary antibodies. Antibodies were used at a concentration of 0.5 µg/ml.

Agarose gel mobility shift assays

The LexA repressor was, immediately before use, serially diluted from 2.4 µM to 2.0 nM. The 10 µl reaction mixtures contained ~50 mM *recA*, *tisB* or ~25 mM *cka* operator-containing DNA or its variants *cka*-UP1 or *cka*-UP3, interacting with LexA in the DNA-binding buffer. Protein–DNA complexes were resolved on 2.5% agarose gels (20) after incubation at room temperature for 10 min in 20 mM Tris (pH 7.5), 200 mM NaCl, 1 mM EDTA, 12% glycerol.

RESULTS AND DISCUSSION

DNA is an allosteric effector of bacterial LexA protein

It was previously suggested that SOS box-containing DNA fragments can inhibit RecA*-mediated LexA self-cleavage (37). In contrast, recently published LexA–DNA crystal structures indicate that LexA–operator interaction exerts minimal interference with RecA*-induced self-cleavage (38).

Most of the *E. coli* SOS genes possess a single SOS box, but the number of operators can range up to 3 (7). We have measured rates of RecA*-stimulated self-inactivation of purified LexA interacting with either tandem (colicin K gene, *cka*) or modified, lower LexA affinity tandem operator (*cka*-UP1) or single (*recA*) operator-containing DNA fragment in comparison with the non-specific DNA (*cka*-UP3) (Supplementary Figures S1 and S2). The results shown in Figure 1A–E indicate that RecA* cannot induce self-cleavage in LexA that is bound to target DNA operator sites. This was confirmed by measuring LexA inactivation in reactions with a range of concentrations of specific (*cka* operators) or non-specific DNA. Non-specific DNA had little inhibitory effect on LexA

induced inactivation, in comparison with the operator-containing DNA (Figure 1F and G).

It has been suggested that it is not possible for both subunits of a LexA dimer to simultaneously make contact with the deep helical groove of RecA*, and that separate docking events are required to cleave both LexA subunits (38). Thus, we used glutaraldehyde cross-linking to follow the kinetics of RecA*-mediated cleavage of unbound LexA repressor and found that self-cleavage proceeds primarily via one subunit of a dimer (Figure 2A). The reaction reached completion by 20 min (Supplementary Figure S3). Data indicate that RecA* predominately induces self-cleavage in one monomer of the LexA dimer and that the resulting LexA–LexA/CTD heterodimer is an inactive intermediate, exhibiting weaker DNA binding (31).

The LexA repressor is mostly dimeric at the concentration used for the glutaraldehyde cross-linking experiment (4); however, complete cross-linking of the dimers could not be achieved. Thus, a cysteine cross-linking experiment was exploited. Structural data of the unbound LexA dimer suggest that residues Gly54 positioned in the DNA-binding NTDs could come in close proximity (13). Data show that the oxidized repressor derivative LexA54, with Gly 54 replaced by Cys, forms covalently bound dimers (Figure 2B). Hence, to complement the glutaraldehyde cross-linking data, RecA*-induced self-cleavage of oxidized LexA54 was determined. The kinetics of appearance of a singly cleaved LexA dimer in the time course of the cleavage reaction indicate that, the LexA heterodimer is an intermediate on the pathway that leads to the fully cleaved dimer (Figure 2). Thus, two successive dockings with RecA* are necessary for the inactivation of both repressor subunits.

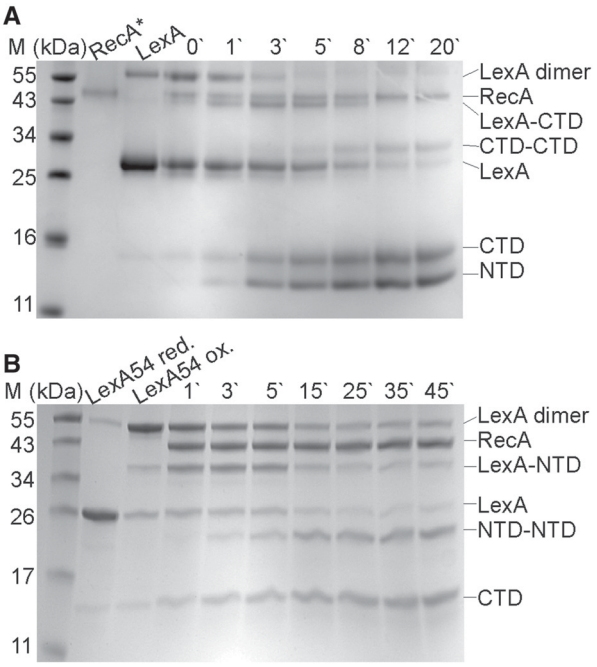


Figure 2. RecA*-induced LexA self-cleavage proceeds primarily by one subunit. (**A**) Cleavage of unbound LexA was induced by addition of RecA*, and samples were cross-linked by glutaraldehyde at different time points (min) and analyzed by gel electrophoresis. RecA and LexA markers were also cross-linked as indicated. Homodimer (LexA dimer), LexA monomer cross-linked to the C-terminal fragment (LexA–CTD), cross-linked C-terminal fragments (CTD–CTD), monomer (LexA) and cleavage forms of LexA (CTD, NTD) are marked. (**B**) The LexA54 derivate with residue Gly54 replaced by Cys in the DNA-binding domain was reduced (LexA54 red.) or oxidized (LexA54 ox.) to show that the repressor can be covalently bound at residue 54. Cleavage of oxidized LexA54 was induced by addition of RecA* and samples taken at different time points (min) and analyzed by SDS–PAGE electrophoresis. Homodimer (LexA dimer), LexA monomer cross-linked to the N-terminal fragment (LexA–NTD), monomer (LexA), cross-linked N-terminal fragments (NTD–NTD), and C-terminal fragment (CTD) are marked.

Intracellularly, almost all LexA is dimeric (4) and pre-existing repressors dissociate slowly to monomers (16). Thus, the source of monomers is supposedly newly synthesized LexA. We propose that, following DNA damage repair and disappearance of the SOS-inducing signal, both newly synthesized LexA as well as heterodimers could provide a source of monomers for resetting repression and for fine-tuning of the SOS response.

LexA conformational dynamics

A recent report of the structure of LexA–operator complexes suggested that flexibility in bound LexA could facilitate interaction with RecA*, leading to LexA self-cleavage, provoking separation of the DNA-binding domain from the rest of the operator–bound dimer and inactivation (38). To test this directly, we used site-directed spin labeling EPR (39) in combination with DEER (25,26) spectroscopy. Interactions between the paramagnetic centers attached to the two subunits of the LexA dimer were measured in order to investigate the mobility of both the N-terminal DNA-binding domain and the C-terminal, regulatory domain, in free and DNA-bound LexA. LexA derivatives with single cysteines substituting residues Ala29 or Gly54 in the DNA-binding domain or residue Leu191 in the dimerization domain were spin labeled (Figure 3A and B, Supplementary Table S2 and Figure S4).

Measurements of the interaction between the spin-label side chains (denoted R1) reveal high-conformational flexibility of the DNA-binding domains in the unbound repressor (apo), but a defined conformation when bound to a specific DNA target. For spin labels at positions 29 (A29R1) or 54 (G54R1) in the apo state broad, multimodal interspin distance distributions are revealed ranging from 30 to 65 Å and from 15 to 50 Å, respectively (Figure 3C, solid lines, inset and Supplementary Figures S5 and S6). Remarkably, for A29R1 and G54R1 in the apo state the DEER traces (Supplementary Figure S5) exhibit significantly smaller modulation depths, compared with the DNA bound state. For A29R1, this observation can be explained by the presence of a significant fraction of the protein molecules with interspin distances beyond the range accessible to DEER experiments (>70 Å). For G54R1, the reduced modulation depth in the apo state is caused by the contributions of molecules with interspin distances <15 Å which do not contribute to the DEER signal as revealed by cw EPR data. Thus, high

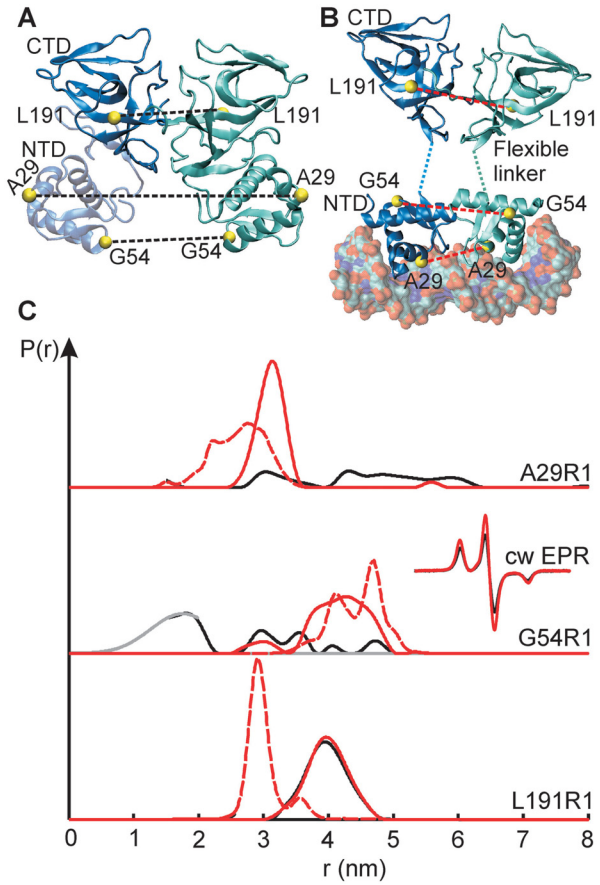


Figure 3. Conformational dynamics of the LexA binding to the *tisB* operator. (**A**) Structure of unbound LexA dimer [pdb ID:1JHH (13)] with modeled (20) undetermined residues (transparent) and (**B**), operator-bound LexA [pdb ID:3JSO (38)]. Individual subunits are colored blue and cyan, residues changed to cysteines and spin labeled are presented as yellow beads. Interspin distances were determined for spin-label pairs connected by dashed lines. (**C**) Experimental interspin distance distributions measured by DEER (solid lines) and simulations based on LexA crystal structures (dashed lines) for the DNA bound (red) and apo states (black). For G54R1 in the apo state, the distribution for interspin distances <2 nm (gray) was determined from the dipolar broadened cw EPR spectra (Supplementary Data). Results are shown as normalized probability distributions.

flexibility of the DNA-binding domains is obvious as they sample conformations leading to interspin distances ranging from 25 to >70 Å for A29R1 and <15 to 50 Å for G54R1. In contrast, in the operator-bound state both mutants show single population maxima centered at 31 Å (±3 Å) for A29R1, and at 43 Å (±5 Å) for G54R1. Remarkably, the distance distributions of both constructs indicate that the conformations LexA samples in the apo state cover also the DNA bound structure. Measurements with labeled LexA191 (L191R1) revealed that interspin distance distributions were very similar in both the unbound and DNA bound states, with a clear maximum at a distance of 40 Å (Figure 3C). Hence, the C-terminal regulatory domains of each subunit in the LexA dimer function as a rigid scaffold for the DNA-binding NTDs. In the unbound state, these are flexible and can adapt the conformation in which the RecA*-induced attack of the scissile A84–G85 bond by the active-site Ser119 is facilitated. On the contrary, in the rigid operator-bound state of the LexA dimer, this conformation cannot be accessed and RecA*-induced inactivation of LexA is prevented.

Again, an interesting observation concerns the modulation depths of the DEER traces, which is significantly lower for A29R1 and G54R1 in the NTDs compared with L191R1 in the CTD (Supplementary Figure S5). Although a lower labeling efficiency of ~80% has been obtained for G54R1 (A29R1 and L191R1: >95%), this does not explain the observed differences in the modulation depths. Instead, this observation is in line with the fact that unbound LexA has been shown to undergo the process of self-cleavage (13), leading to LexA–LexA/CTD heterodimer formation. Such heterodimers contain two spin labels in the CTD, but only one spin-labeled NTD is present, thus explaining the lower modulation depth for A29R1 and G54R1.

A comparison of the experimental interspin distances for LexA-A29R1, G54R1 and L191R1 in the DNA bound state with values predicted from the LexA–DNA crystal structure (pdb ID:3JSO) using the rotamer library approach (Figure 3C, dashed lines) shows reasonable agreement for the two positions located in the NTDs (A29R1 and G54R1) indicating that, the arrangement found in the crystal structures seems to reflect the state in solution well. On the contrary, the data for L191R1 indicate that the conformation of the LexA dimerization domain in solution might slightly differ from that observed in crystals, most probably due to crystal packing effects. Nevertheless, it cannot be excluded that limitations in the accuracy of the rotamer library approach account for the observed differences.

Repressor's dissociation from operators orchestrates SOS response

SPR analysis was subsequently performed to determine the mechanism of operator-bound repressor interference with RecA*-induced autoproteolysis. Active RecA filament was formed on single-stranded DNA bound to the surface of the sensor chip (Figure 4A). Non-cleavable repressor variant LexA119 (S119A) interacted with chip-immobilized RecA* in a concentration-dependent manner (Figure 4B). The presence of *tisB* operator interfered with the ability of LexA119 to bind to RecA* (Figure 4C). We show that binding of operator induces LexA in a particular conformation in which interaction with RecA* is precluded (Figure 4D), revealing why RecA*-induced inactivation of specifically bound LexA is unfeasible.

The LexA CTD provides the determinants for dimerization and self-cleavage activity, thus the interface interacting with RecA* (13). In the crystal structure of the unbound LexA mutant dimer (pdb ID: 1JHH) one subunit is well ordered throughout and in a non-cleavable state, whereas the second subunit, while disordered in the NTD, adopts the cleavable state in the CTD (13). The structure of the intact monomer also exhibits LexA intramolecular contacts between the DNA-binding NTD and

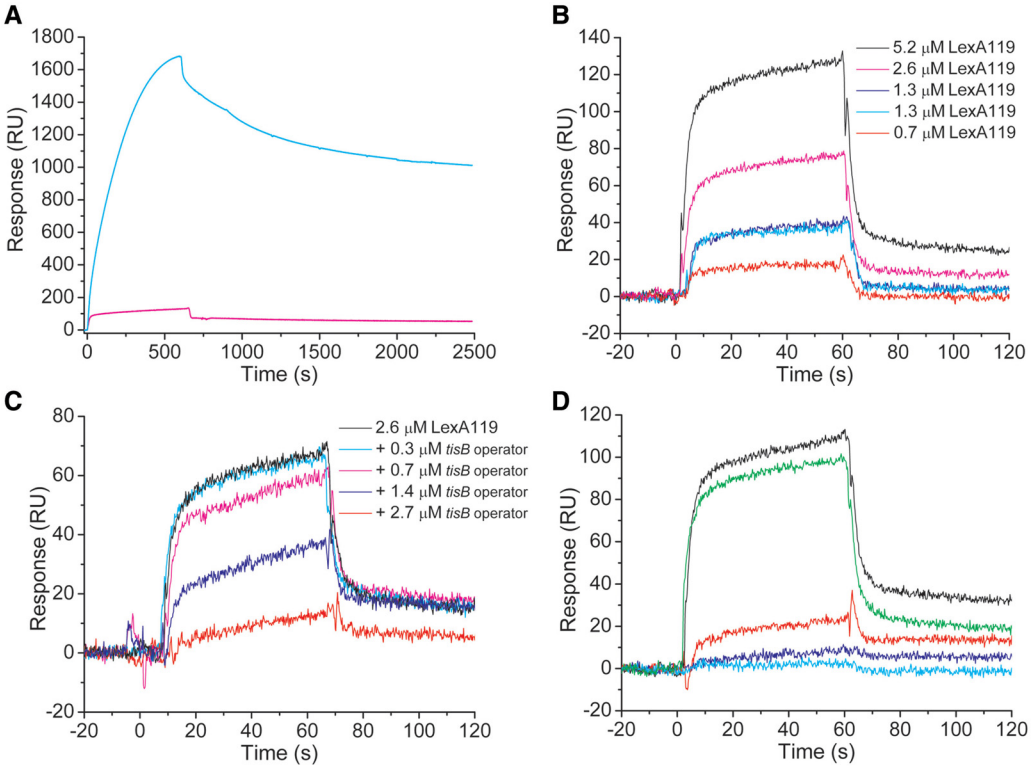


Figure 4. Interaction of unbound or specifically bound LexA119 with RecA*. (**A**) SPR sensorgrams of the binding of the 2.1 μM RecA to the flow cell 1 (red) or to the flow cell 2 with immobilized *tisB*-operator DNA (cyan). (**B**) Unbound LexA119 repressor in concentration range from 0.7 to 5.2 μM or (**C**) LexA119 interacting with 24-bp *tisB* operator DNA in concentration range from 0.3 to 2.7 μM were injected across the chip-immobilized RecA* for 60 s at 10 μl/min. The used DNA to repressor ratio (mol:mol) was approximately 0.1:1, 0.3:1, 0.5:1, 1:1, 2:1, respectively. (**D**) Sensorgrams of the 2.6 μM repressor variant LexA119 (black), the 24 bp DNA fragments (2.7 μM) consisting of the *tisB* operator (violet) or the non-specific DNA (cyan), *tisB* operator bound LexA119 (red) or LexA119 mixed with the non-specific DNA (green), interacting with the chip-immobilized RecA*. The used DNA to repressor ratio was ~2:1 (mol:mol).

the cleavage site loop lying just within the CTD. This is most likely not an artifact due to crystal packing (13) as cleavage site region–NTD interactions were also confirmed by experiments exploiting cysteine cross-linking (20). Thus, orientation of NTDs might affect the position of the cleavage loop containing the scissile peptide bond. Our EPR results indicate that a five residue hydrophilic linker that connects the NTD of LexA to its catalytic core domain does not impede movement of the NTDs, as suggested previously (20). Thus, although LexA is a homodimeric protein, variable positions of its NTDs in the dimer might modulate the position of the cleavage-site regions in the CTDs.

The repressor recognizes its targets as a dimer (4) and the dimer does not exert stringency requirement on the binding domain (38). In the operator-bound LexA, an extensive dimer interface is observed between the DNA-binding NTDs, formed of residues which are solvent exposed in the unbound LexA (13). Interactions between the two DNA-binding domains are acting synergistic with DNA binding, thus increasing LexA dimer stability by 1000-fold (4,38). In contrast to the alternating conformations of the cleavage loops in the unbound LexA dimers, both scissile peptide bonds in the operator-bound mutant dimers are displaced or docked in the active center (38). The results of this investigation show that the operator is an alosteric effector of the LexA repressor indicating that, a specific orientation of the DNA-binding NTDs sets the repressor in a conformation in which interaction with RecA* and a subsequent self-cleavage reaction is precluded. Interestingly, mutations in LexA that specifically impair RecA*-dependent cleavage, but do not alter catalysis have not been identified (16). Therefore, further studies will be employed to elucidate how diverse positions of the LexA cleavage loop and orientation of the NTDs modulate interaction with the RecA*.

Our results imply that LexA dissociation from operators coordinates expression of the SOS genes. This is in agreement with previous reports, showing that the timing of induction of LexA-regulated genes correlates with the binding affinity of the SOS boxes (1). However, previously LexA operator affinity was ranked by quantitative gel retardation and DNase I footprinting experiments and by calculating the relatedness of an operator sequence to that of the consensus sequence derived from the known LexA targets (18,23). To provide further details, we used SPR to measure LexA–operator interactions under near physiological salt and pH conditions in real time. We used DNA fragments that contained *recA*, *tisB*, *cka* operators

or non-specific DNA *cka*-UP3. Binding to operators was concentration dependent (data not shown), but LexA did not bind to the control DNA (Figure 5). The association of LexA with the SOS operators was extremely rapid, and it was therefore not possible to determine accurately the association rate constants due to the mass transfer effect. Control experiments showed that dissociation of LexA from the surface of the chip was not dependent on the flow rate (data not shown), therefore it was possible to determine the rates of dissociation. In spite of rapid LexA association with all the tested operators, the repressor exhibited diverse dissociation rates. Dissociation was similar for *recA* and *tisB*, but significantly slower from the *cka* operator. This explains, for example, why *recA* is one of the first transcribed genes in the SOS response, while expression of the *cka* gene is delayed, limited to conditions of extensive, long-lived DNA damage (1,11). We conclude that differences between LexA operators affect repressor dissociation and influence the timing of expression of SOS genes.

Decreasing persister formation by modulating LexA functions

The insights into LexA functions presented here may provide new directions in the battle against the emergence and spread of drug resistance. It has recently been shown that persisters form during the SOS response and depend on the LexA-regulated TisB toxin (40). Hence, bacterial killing by antibiotics can be enhanced by dislabeling the SOS response, either by deleting the *recA* gene (41) or overexpression of non-cleavable *lexA* variants (42,43). We used the LexA71 (E71K) repressor variant (21) that exhibits three to nine times slower dissociation from operators compared with wild-type LexA repressor (Figure 5). We then measured persister formation in an *E. coli* strain defective for *lexA*, complemented with wild-type LexA or its non-cleavable mutants, exhibiting either normal or enhanced DNA binding, treated with 2.5 times MIC of mitomycin C. Our results (Figure 6) show that the occurrence of persister cells in bacterial populations triggered by DNA damage can be altered by changing LexA activity. Notably, when cells expressed the non-cleavable and enhanced operator-binding LexA repressor variant, no persisters were detected 1 h after induced DNA damage. LexA homologs are found in prokaryotes (31), but to date there are no known orthologs in eukaryotes. Hence, this work sets a novel platform for drug discovery to treat bacterial pathogens and offers an approach to control bacterial survival of antibiotic therapy.

CONCLUSIONS

In the present paper, we show that RecA*-mediated LexA repressor self-cleavage cannot be induced in LexA specifically bound to target DNA. Our results contradict the observation that the LexA operator bound conformation allows docking to RecA* and subsequent LexA

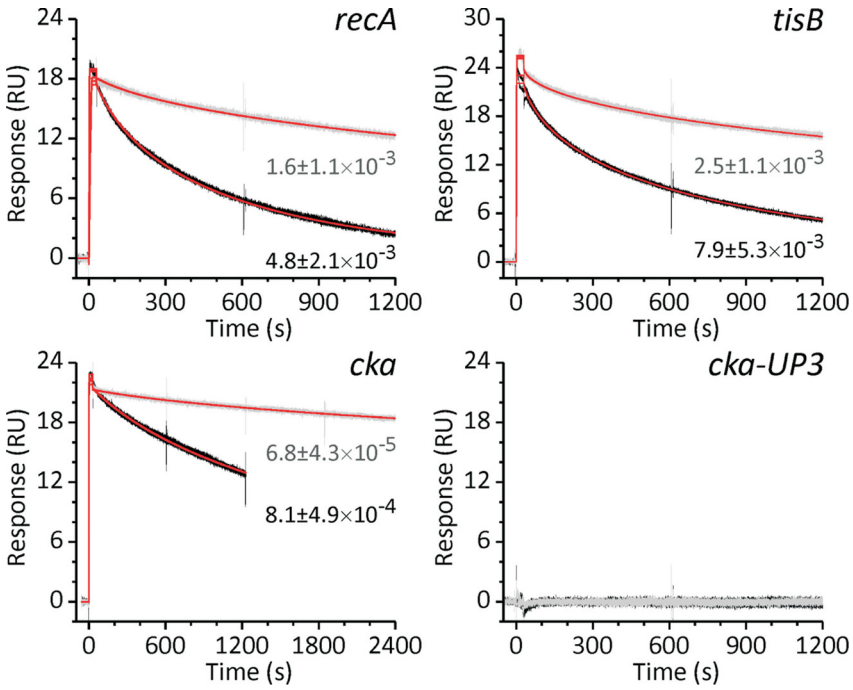


Figure 5. Interaction of LexA and LexA71 with various promoter regions. SPR was used to assess the interaction of LexA (black) or LexA71 (gray) with various operators as indicated. Biotinylated DNA fragments were immobilized on the surface of the streptavidin sensor chip. Purified protein at saturating concentration was injected across the chip for 30 s and dissociation followed as shown on the graphs. The sensorgrams were doubly referenced and fitted to a 1:1 binding model. Data shown are triplicate injections of the protein and overlaid with fits (red). Calculated dissociation rate constants (average ± standard deviation) are shown for each condition.

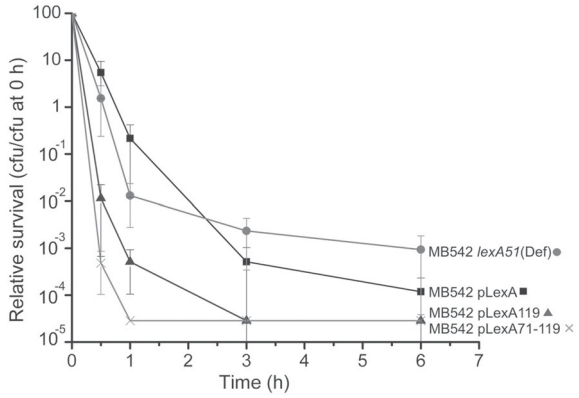


Figure 6. Mitomycin C survival of the *E. coli lexA*$^{-}$ strain complemented with LexA repressor variants. MB542 (*lexA51*) strain complemented with wild-type LexA (pLexA) or its non-cleavable mutants exhibiting either normal (pLexA119) or enhanced DNA (pLexA71–119) binding was grown to exponential phase (~10^8 cfu/ml), when exposed to 2.5 times MIC of mitomycin C. At 0, 0.5, 1, 3, 6 h after addition, viable cell number was determined (cfu/ml). As a control, strain MB542 was used. The data points are averages of at least four independent experiments and error bars indicate the standard error.

inactivation (38). Thus, diverse LexA conformations enable either repression of SOS genes by specific DNA binding or repressor cleavage in response to DNA damage. Data presented here imply that mobility of the LexA NTDs affects the repressor's interaction with the RecA*. Our results indicate that RecA*-mediated inactivation of unbound LexA must decrease the intracellular pool of free LexA which provokes dissociation of the functional repressor from its DNA targets (Figure 7). Taken together, our results indicate how the signal from DNA damage at a particular chromosomal location is transduced into the induction of the SOS genes, co-ordinated by the distinct LexA repressor conformations. In addition, we show that, upon DNA damage, separate interactions between the two key SOS players are required to cleave both subunits of the LexA dimer. Therefore, when the inducing signal disappears, the remaining self-cleavage intermediates, inactive heterodimers, can provide a source of subunits which dimerize into the functional repressor to accelerate resetting of the system.

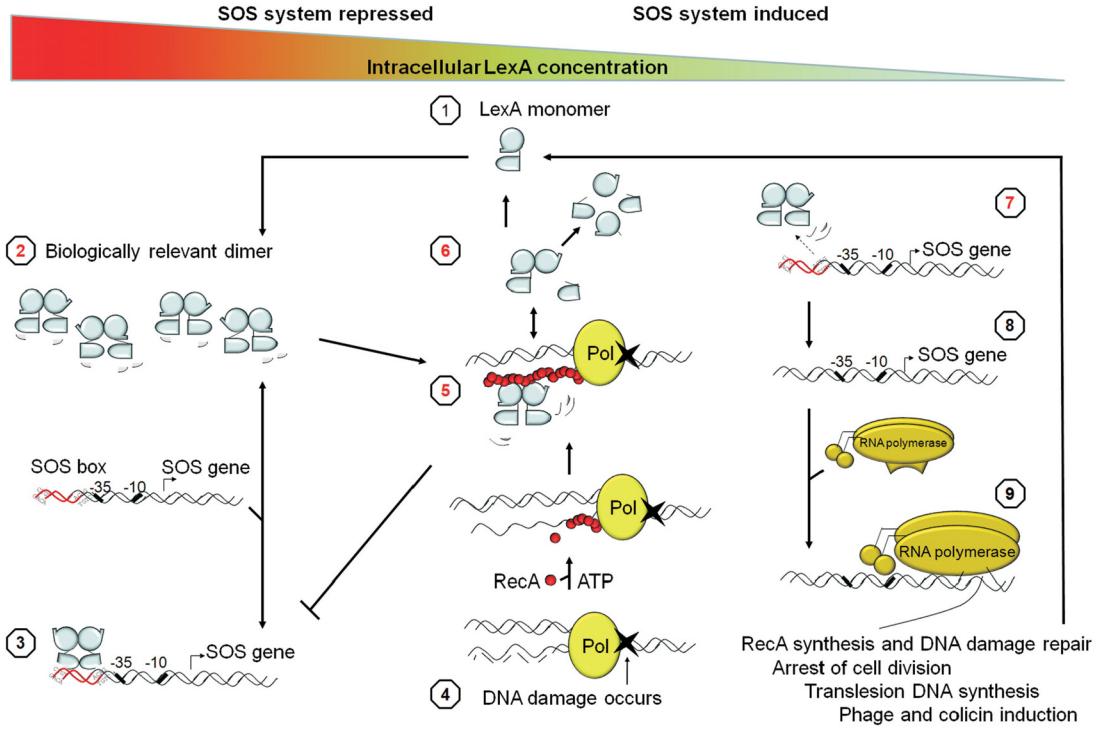


Figure 7. An overview of the SOS response in *E. coli*. (1) Concentration of LexA monomers increases. (2) LexA monomers in solution form biologically relevant dimers. DNA-binding domains of the unbound LexA are highly mobile and can move freely to one another. (3) Repression of the SOS system occurs when LexA dimers bind specifically to SOS boxes located at the promoter regions of SOS genes and sterically precludes their transcription. (4) The polymerase III holoenzyme (Pol) carries out DNA replication. At the site of DNA damage PolIII arrests, and single-stranded DNA (ssDNA) accumulates. RecA binds to ssDNA in the presence of ATP, forming active RecA–ssDNA–ATP filaments (RecA*). (5) RecA* induces self-cleavage in the unbound LexA but cannot stimulate inactivation of LexA specifically bound to target DNA. (6) In the unbound repressor dimer, one monomer is preferentially inactivated and the uncleaved monomer could affect resetting of the system. Cleaved LexA products are rapidly degraded by the ClpXP and Lon proteases (44). (7) Due to induced unbound LexA self-cleavage, intracellular LexA pool decreases. Specifically bound LexA repressor dissociates from operators, (8) leading to co-ordinated de-repression of SOS genes. (9) The rate of LexA dissociation from target sites is influenced by operator sequences and acts in orchestrating the response. Subsequently, as DNA damage is repaired, SOS induction is reversed. Numbers in red indicate novel insights into the system.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

FUNDING

Slovenian Research Agency (Z1–2142 to M.B., J4–2111 to D.Ž.B.). Funding for open access charge: Slovenian Research Agency.

Conflict of interest statement. None declared.

REFERENCES

1. Courcelle,J., Khodursky,A., Peter,B., Brown,P.O. and Hanawalt,P.C. (2001) Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient Escherichia coli. *Genetics*, **158**, 41–64.
2. Wade,J.T., Reppas,N.B., Church,G.M. and Struhl,K. (2005) Genomic analysis of LexA binding reveals the permissive nature of the Escherichia coli genome and identifies unconventional target sites. *Genes Dev.*, **19**, 2619–2630.
3. Sassanfar,M. and Roberts,J.W. (1990) Nature of the SOS-inducing signal in Escherichia coli. The involvement of DNA replication. *J. Mol. Biol.*, **212**, 79–96.
4. Mohana-Borges,R., Pacheco,A.B., Sousa,F.J., Foguel,D., Almeida,D.F. and Silva,J.L. (2000) LexA repressor forms stable dimers in solution. The role of specific dna in tightening protein-protein interactions. *J. Biol. Chem.*, **275**, 4708–4712.
5. Gowers,D.M. and Halford,S.E. (2003) Protein motion from non-specific to specific DNA by three-dimensional routes aided by supercoiling. *EMBO J.*, **22**, 1410–1418.
6. Blainey,P.C., Luo,G., Kou,S.C., Mangel,W.F., Verdine,G.L., Bagchi,B. and Xie,X.S. (2009) Nonspecifically bound proteins spin while diffusing along DNA. *Nat. Struct. Mol. Biol.*, **16**, 1224–1229.
7. Keseler,I.M., Bonavides-Martinez,C., Collado-Vides,J., Gama-Castro,S., Gunsalus,R.P., Johnson,D.A., Krummenacker,M., Nolan,L.M., Paley,S., Paulsen,I.T. *et al.* (2009) EcoCyc: a comprehensive view of Escherichia coli biology. *Nucleic Acids Res.*, **37**, D464–470.
8. Little,J.W. (1991) Mechanism of specific LexA cleavage: autodigestion and the role of RecA coprotease. *Biochimie*, **73**, 411–421.
9. Neher,S.B., Flynn,J.M., Sauer,R.T. and Baker,T.A. (2003) Latent ClpX-recognition signals ensure LexA destruction after DNA damage. *Genes Dev.*, **17**, 1084–1089.
10. Napolitano,R., Janel-Bintz,R., Wagner,J. and Fuchs,R.P. (2000) All three SOS-inducible DNA polymerases (Pol II, Pol IV and Pol V) are involved in induced mutagenesis. *Embo J.*, **19**, 6259–6265.
11. Cascales,E., Buchanan,S.K., Duche,D., Kleanthous,C., Lloubes,R., Postle,K., Riley,M., Slatin,S. and Cavard,D. (2007) Colicin biology. *Microbiol. Mol. Biol. Rev.*, **71**, 158–229.
12. Galkin,V.E., Yu,X., Bielnicki,J., Ndjonka,D., Bell,C.E. and Egelman,E.H. (2009) Cleavage of bacteriophage lambda cI repressor involves the RecA C-terminal domain. *J. Mol. Biol.*, **385**, 779–787.
13. Luo,Y., Pfuetzner,R.A., Mosimann,S., Paetzel,M., Frey,E.A., Cherney,M., Kim,B., Little,J.W. and Strynadka,N.C. (2001) Crystal structure of LexA: a conformational switch for regulation of self-cleavage. *Cell*, **106**, 585–594.
14. Cohen,S., Knoll,B.J., Little,J.W. and Mount,D.W. (1981) Preferential cleavage of phage lambda repressor monomers by recA protease. *Nature*, **294**, 182–184.
15. Pawlowski,D.R. and Koudelka,G.B. (2004) The preferred substrate for RecA-mediated cleavage of bacteriophage 434 repressor is the DNA-bound dimer. *J. Bacteriol.*, **186**, 1–7.
16. Giese,K.C., Michalowski,C.B. and Little,J.W. (2008) RecA-dependent cleavage of LexA dimers. *J. Mol. Biol.*, **377**, 148–161.
17. Mustard,J.A. and Little,J.W. (2000) Analysis of Escherichia coli RecA interactions with LexA, lambda CI, and UmuD by site-directed mutagenesis of recA. *J. Bacteriol.*, **182**, 1659–1670.
18. Fernandez De Henestrosa,A.R., Ogi,T., Aoyagi,S., Chafin,D., Hayes,J.J., Ohmori,H. and Woodgate,R. (2000) Identification of additional genes belonging to the LexA regulon in Escherichia coli. *Mol. Microbiol.*, **35**, 1560–1572.
19. Kristan,K., Viero,G., Macek,P., Dalla Serra,M. and Anderluh,G. (2007) The equinatoxin N-terminus is transferred across planar lipid membranes and helps to stabilize the transmembrane pore. *FEBS J.*, **274**, 539–550.
20. Butala,M., Hodoscek,M., Anderluh,G., Podlesek,Z. and Zgur-Bertok,D. (2007) Intradomain LexA rotation is a prerequisite for DNA binding specificity. *FEBS Lett.*, **581**, 4816–4820.
21. Oertel-Buchheit,P., Porte,D., Schnarr,M. and Granger-Schnarr,M. (1992) Isolation and characterization of LexA mutant repressors with enhanced DNA binding affinity. *J. Mol. Biol.*, **225**, 609–620.
22. Mrak,P., Podlesek,Z., van Putten,J.P. and Zgur-Bertok,D. (2007) Heterogeneity in expression of the Escherichia coli colicin K activity gene cka is controlled by the SOS system and stochastic factors. *Mol. Genet. Genomics*, **277**, 391–401.
23. Lewis,L.K., Harlow,G.R., Gregg-Jolly,L.A. and Mount,D.W. (1994) Identification of high affinity binding sites for LexA which define new DNA damage-inducible genes in Escherichia coli. *J. Mol. Biol.*, **241**, 507–523.
24. Steinhoff,H.J., Radzwill,N., Thevis,W., Lenz,V., Brandenburg,D., Antson,A., Dodson,G. and Wollmer,A. (1997) Determination of interspin distances between spin labels attached to insulin: comparison of electron paramagnetic resonance data with the X-ray structure. *Biophys. J.*, **73**, 3287–3298.
25. Martin,R.E., Pannier,M., Diederich,F., Gramlich,V., Hubrich,M. and Spiess,H.W. (1998) Determination of end-to-end distances in a series of TEMPO diradicals of up to 2.8 nm length with a new four-pulse double electron electron resonance experiment. *Angew. Chem. Int. Edit.*, **37**, 2834–2837.
26. Pannier,M., Veit,S., Godt,A., Jeschke,G. and Spiess,H.W. (2000) Dead-time free measurement of dipole-dipole interactions between electron spins. *J. Magn. Reson.*, **142**, 331–340.
27. Jeschke,G., Chechik,V., Ionita,P., Godt,A., Zimmermann,H., Banham,J., Timmel,C.R., Hilger,D. and Jung,H. (2006) DeerAnalysis2006 - a comprehensive software package for analyzing pulsed ELDOR data. *Appl. Magn. Reson.*, **30**, 473–498.
28. Polyhach,Y., Bordignon,E. and Jeschke,G. (2011) Rotamer libraries of spin labelled cysteines for protein studies. *Phys. Chem. Chem. Phys.*, **13**, 2356–2366.
29. Mackerell,A.D. Jr., Feig,M. and Brooks,C.L. 3rd (2004) Extending the treatment of backbone energetics in protein force fields: limitations of gas-phase quantum mechanics in reproducing protein conformational distributions in molecular dynamics simulations. *J. Comput. Chem.*, **25**, 1400–1415.
30. Humphrey,W., Dalke,A. and Schulten,K. (1996) VMD: visual molecular dynamics. *J. Mol. Graph.*, **14**, 33–38, 27–38.
31. Butala,M., Zgur-Bertok,D. and Busby,S.J. (2009) The bacterial LexA transcriptional repressor. *Cell Mol. Life Sci.*, **66**, 82–93.
32. Jiang,Q., Karata,K., Woodgate,R., Cox,M.M. and Goodman,M.F. (2009) The active form of DNA polymerase V is UmuD'(2)C-RecA-ATP. *Nature*, **460**, 359–363.
33. Rich,R.L. and Myszka,D.G. (2010) Grading the commercial optical biosensor literature-Class of 2008: 'The Mighty Binders'. *J. Mol. Recognit.*, **23**, 1–64.
34. Miller,J.H. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Press, Cold Spring Harbor, New York, USA.
35. Dorr,T., Lewis,K. and Vulic,M. (2009) SOS response induces persistence to fluoroquinolones in Escherichia coli. *PLoS Genet.*, **5**, e1000760.
36. Kristan,K., Podlesek,Z., Hojnik,V., Gutierrez-Aguirre,I., Guncar,G., Turk,D., Gonzalez-Manas,J.M., Lakey,J.H., Macek,P. and Anderluh,G. (2004) Pore formation by equinatoxin, a eukaryotic pore-forming toxin, requires a flexible N-terminal region and a stable beta-sandwich. *J. Biol. Chem.*, **279**, 46509–46517.

37. Little,J.W. and Mount,D.W. (1982) The SOS regulatory system of Escherichia coli. *Cell*, **29**, 11–22.
38. Zhang,A.P., Pigli,Y.Z. and Rice,P.A. (2010) Structure of the LexA-DNA complex and implications for SOS box measurement. *Nature*, **466**, 883–886.
39. Klare,J.P. and Steinhoff,H.J. (2009) Spin labeling EPR. *Photosynth. Res.*, **102**, 377–390.
40. Dorr,T., Vulic,M. and Lewis,K. (2010) Ciprofloxacin causes persister formation by inducing the TisB toxin in Escherichia coli. *PLoS Biol.*, **8**, e1000317.
41. Kohanski,M.A., Dwyer,D.J., Hayete,B., Lawrence,C.A. and Collins,J.J. (2007) A common mechanism of cellular death induced by bactericidal antibiotics. *Cell*, **130**, 797–810.
42. Cirz,R.T., Chin,J.K., Andes,D.R., de Crecy-Lagard,V., Craig,W.A. and Romesberg,F.E. (2005) Inhibition of mutation and combating the evolution of antibiotic resistance. *PLoS Biol.*, **3**, e176.
43. Lu,T.K. and Collins,J.J. (2009) Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy. *Proc. Natl Acad. Sci. USA*, **106**, 4629–4634.
44. Pruteanu,M. and Baker,T.A. (2009) Proteolysis in the SOS response and metal homeostasis in Escherichia coli. *Res. Microbiol.*, **160**, 677–683.

Encyclopedia of Biological Chemistry - CONTRIBUTORS' INSTRUCTIONS

PROOFREADING

The text content for your contribution is in final form when you receive proofs. Read proofs for accuracy and clarity, as well as for typographical errors, but please DO NOT REWRITE.

Titles and headings should be checked carefully for spelling and capitalization. Please be sure that the correct typeface and size have been used to indicate the proper level of heading. Review numbered items for proper order – e.g., tables, figures, footnotes, and lists. Proofread the captions and credit lines of illustrations and tables. Ensure that any material requiring permissions has the required credit line and that we have the relevant permission letters.

Your name and affiliation will appear at the beginning of the article and also in a List of Contributors. Your full postal address appears on the non-print items page and will be used to keep our records up-to-date (it will not appear in the published work. Please check that they are both correct.

Keywords are shown for indexing purposes ONLY and will not appear in the published work.

Any copy-editor questions are presented in an accompanying Author Query list at the beginning of the proof document. Please address these questions as necessary. While it is appreciated that some articles will require updating/revising, please try to keep any alterations to a minimum. Excessive alterations may be charged to the contributors.

Note that these proofs may not resemble the image quality of the final printed version of the work, and are for content checking only. Artwork will have been redrawn/relabelled as necessary, and is represented at the final size.

DESPATCH OF CORRECTIONS

PLEASE KEEP A COPY OF ANY CORRECTIONS YOU MAKE.

Proof corrections should be returned in one communication to Justin Taylor (**bch2proofs@elsevier.com**), by 15-Apr-2011 using one of the following methods:

1. **PREFERRED**: Corrections should be listed in an e-mail and sent to Justin Taylor in the Elsevier MRW Production Department at **bch2proofs@elsevier.com**.

The e-mail should state the article code number in the subject line. Corrections should be consecutively numbered and should state the paragraph number, line number within that paragraph, and the correction to be made.

2. If corrections are substantial, send the amended hardcopy by courier to **Justin Taylor, Elsevier MRW Production Department, The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, UK.** If it is not possible to courier your corrections, please fax the relevant marked pages to the Elsevier MRW Production Department **(fax number: +44 (0)1865 843974)** with a covering note clearly stating the article code number and title.

Note that a delay in the return of proofs could mean a delay in publication. Should we not receive corrected proofs within 7 days, Elsevier may proceed without your corrections.

CHECKLIST

Author queries addressed/answered?	❑
Affiliations, names and addresses checked and verified?	❑
Permissions details checked and completed?	❑
Outstanding permissions letters attached/enclosed?	❑
Figures and tables checked?	❑

If you have any questions regarding these proofs please contact the Elsevier MRW Production Department at: **bch2proofs@elsevier.com**

BCH2: 00278

Non-Print Items

Abstract:

Organisms have evolved gene regulatory systems to cope with stress. To maintain the structural and functional integrity of their genomes after damage due to environmental or metabolic assaults, bacteria mount a program of gene expression known as the 'SOS response'. Induction of this response requires a repressor, the LexA protein, and an inducer, the recombinase A (RecA) protein. In *Escherichia coli*, upon DNA damage, RecA stimulates cleavage of the LexA repressor, inducing expression of approximately 1% of the genes. The coordinated expression of these genes orchestrates a complex program of DNA repair, which can also result in mutations and genetic exchange that facilitate bacterial evolution. In some bacteria, the SOS response also modulates the expression of virulence factor genes and can induce the formation of dormant cells that are highly tolerant to antibiotics.

Keywords: Antibiotic resistance; Bacteriophage induction; Cell-cycle control; DNA damage; DNA repair; Gene activation; Induction of gene expression; LexA regulon; Transcription responses; Virulence factor regulation

Author and Co-author Contact Information: Au1

Matej Butala
Department of Biology
Biotechnical Faculty
University of Ljubljana
Večna pot 111
Ljubljana
Slovenia
Tel: +368 1 423 3388
Fax: 386 1 257 3390
E-mail: Matej.Butala@bf.uni-lj.si

Darja Žgur-Bertok
Department of Biology
Biotechnical Faculty
University of Ljubljana
Večna pot 111
Ljubljana
Slovenia
Tel: +368 1 423 3388
Fax: 386 1 257 3390
E-mail: Darja.Zgur.Bertok@bf.uni-lj.si

Stephen J W Busby
School of Biosciences
The University of Birmingham
Edgbaston
Birmingham B15 2TT
UK
Tel: +44 (0)121 41 45439
Fax: +44 (0)121 41 45925
E-mail: s.j.w.busby@bham.ac.uk

Biographical Sketch

Matej Butala obtained his PhD from the Medical Faculty, University of Ljubljana, Slovenia. Upon graduation he studied regulation of expression of the SOS genes encoding colicins with Dr. D Žgur-Bertok, and became interested in the LexA biochemical processes. For his PhD thesis he was awarded a 2009 PathoGenoMics PhD award. He did his postdoctoral work in Dr. SJW Busby's lab in Birmingham, UK. He was a teaching assistant for molecular biology at the Biotechnical Faculty in Ljubljana, where he is currently a postdoctoral researcher. He is studying the dynamics of the interaction between the LexA repressor and the RecA filament.

Darja Žgur-Bertok obtained her PhD from the Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia. She is a professor at the Biotechnical Faculty, University of Ljubljana. She has worked on regulation of bacteriocin synthesis in *Escherichia coli* and their antimicrobial activity. She has also worked on plasmids and regulation of plasmid conjugative transfer. Darja Žgur-Bertok is also involved in teaching undergraduate courses in microbial genetics and microbial pathogenesis.

Steve Busby became interested in transcriptional regulation in bacteria when he was a postdoctoral scientist at the Institut Pasteur, Paris. He subsequently joined the academic staff at the University of Birmingham, UK, and is currently professor of biochemistry in the School of Biosciences. He has worked on many different bacterial transcription factors but, recently, his work has focused on how different signals are integrated at promoters, and the application of novel genomic methods to study the global regulation of transcription.

BCH2: 00278

Author Query Form

Book: Encyclopedia of Biological Chemistry
Article No.: 00278

Dear Author,

During the preparation of your manuscript for typesetting some questions have arisen. These are listed below. Please check your typeset proof carefully and mark any corrections in the margin of the proof or compile them as a separate list. Your responses to these questions should be returned within seven days, by email, to MRW Production, email: BCH2proofs@elsevier.com

Query	Details Required	Author's response
AU1	Please check the long affiliations for accuracy. These are for Elsevier's records and will not appear in the printed work.	
AU2	Please provide in-text citations for Figures 1–4.	
AU3	Please provide the place of publication of the reference 'Foti et al. (2010)'.	
AU4	Do Figures 1–4 require permission? If yes, please provide the relevant correspondence granting permission. [If you have already provided this information, please ignore this query.]	

The LexA Regulatory System

M Butala and D Žgur-Bertok, University of Ljubljana, Ljubljana, Slovenia
S J W Busby, The University of Birmingham, Birmingham, UK

This article is a revision of the previous edition article by Veronica G. Godoy, Penny J. Beuning, and Graham C. Walker, volume 2, pp. 546–550, © 2004, Elsevier Inc.

Glossary

Autoregulation A gene product regulates expression of its own gene.

Chromatin immunoprecipitation Technique used to precipitate a protein antigen using specific antibody to identify protein–DNA interactions at the genome level.

DNA microarrays A surface carrying an array of probes, DNA oligonucleotides corresponding to genes of interest, which are hybridized with cDNA from RNA isolated from cells under a given condition.

Operator Specific DNA site where transcription factor binds and modulates initiation of gene transcription.

Promoter Sequence located upstream of a gene to which RNA polymerase binds to initiate transcription.

Regulon Group of genes whose expression is regulated by a common regulator(s).

Repressor Protein that inhibits gene expression by sterically interfering with binding of RNA polymerase or by binding to RNA.

Introduction

The *Escherichia coli* LexA regulon is a regulatory network, encompassing at least 57 genes whose products govern a coordinated bacterial response to DNA damage. The induced LexA regulatory system has also been designated the SOS response to emphasize its role in the cellular response to distress. The expressed SOS functions not only repair DNA damage but also enhance adaptation through mutagenesis and genetic exchange. The SOS response thus plays a broad role, modulating evolution and dissemination of drug resistance and virulence factor genes, as well as the synthesis and secretion of virulence factors. In addition, the SOS system controls persistence and multidrug tolerance in a subpopulation of bacterial cells. The SOS system is widespread among bacteria but exhibits considerable variation with regard to its components and regulation. This article outlines regulation by LexA in *E. coli*, which is the best-understood SOS system and has been studied most extensively.

The *E. coli* LexA Regulatory System

Control of gene expression in response to environmental assaults, and the maintenance of the structural and functional integrity of the genome are essential for cell survival. The bacterial SOS system is an inducible DNA repair and damage-tolerance response triggered either by extrinsic treatments that elicit DNA damage or by intrinsic events that disrupt DNA replication.

A comprehensive response to DNA lesions was first described in detail in *E. coli*. Evelyn M. Witkin postulated that cellular filamentation and phage induction are regulated by a common repressor, which is inactivated in response to DNA damage. In the 1970s, Miroslav Radman proposed that a coordinated cellular response controlled by the interplay of two key proteins, a repressor and an inducer, is mounted upon DNA damage. The product of the *lexA* gene (locus for X-ray sensitivity A) is the repressor of the regulon while recombinase A (RecA) is involved in sensing DNA damage and induces inactivation of the LexA repressor. During normal bacterial growth, LexA downregulates expression of its own gene and, in *E. coli*, the expression of more than 50 unlinked genes. In response to DNA damage, RecA (bound to adenosine triphosphate (ATP)) polymerizes onto single-stranded DNA (ssDNA) exposed upon repair or replication of damaged DNA, creating a helical nucleoprotein filament. The active ssDNA–ATP–RecA filament (RecA*) interacts with LexA and activates its latent self-cleaving activity. Cleavage inactivates LexA, instigating repressor dissociation from its DNA targets (SOS boxes) and induction of the LexA regulon. Subsequently, as DNA damage is repaired or bypassed, the level of ssDNA, the SOS-inducing signal, decreases and the co-protease activity of RecA filaments disappears (note, RecA* does not participate directly in the proteolysis reaction but instead stimulates LexA cleavage and is thus termed a 'co-protease'). Functional LexA rapidly re-accumulates, returning the system to its repressed state.

Defining the LexA Regulon

Genes of the SOS regulon are characterized by (1) basal-level expression during normal bacterial growth and induction following DNA damage; (2) absence of induction in the *lexA* (*ind*$^-$) mutant strain with noncleavable LexA protein; (3) constitutive induction in strains carrying the *lexA* (*def*) allele, due to impaired repressor dimerization and unstable DNA association; and (4) promoter regions that carry DNA targets that resemble the conserved LexA operator sequence.

The first investigations to show that the SOS response is a global genomic response to DNA damage were performed in

Graham C Walker's laboratory. Through random insertion of a *lacZ* reporter gene into the *E. coli* chromosome, they identified genes whose expression was induced following DNA damage. Characterization of genes upregulated in a *recA*/*lexA*-dependent manner revealed a 20-base-pair consensus LexA-binding site in promoter regions of SOS genes. Whole genome technologies that use microarrays to analyze transcriptome or chromatin immunoprecipitation experiments have now identified the full catalog of genes regulated by LexA. While the roles of most of the newly identified LexA-regulated genes are still unknown, unraveling their particular functions will yield insight into the molecular mechanisms underlying the SOS response. Several gene transcripts are decreased following DNA damage and some, while exhibiting a similar expression profile as genes of the LexA regulon, are not directly regulated by LexA. It thus seems that the SOS response is part of a larger, coordinated response network.

s0025 The LexA Regulatory System in the Repressed State

p0035 LexA exerts repression by binding to target sites located near promoters of SOS genes, blocking access of RNA polymerase. The C-terminal domain (CTD) of LexA is involved in dimerization and the N-terminal domain (NTD) in DNA binding. Intact LexA dimerizes by the CTD, and binds to DNA via a helix–turn–helix in its NTD.

p0040 LexA binding motifs are conserved in many Gram-negative bacteria. The consensus DNA target in *E. coli* is a palindromic dyad taCTGT-$(at)_4$-ACAGta and is designated the LexA box or SOS box. Functional LexA repressor is a homodimer while intracellular monomer levels are very low. Each of the two symmetrically inverted DNA-binding elements accommodates one LexA subunit. For stabile and specific DNA binding, a conformational change in LexA must occur. Binding to consensus targets with dyad symmetry requires LexA subunit–subunit interactions that enable high specificity and stabilizes interactions with both halves of the DNA duplex.

p0045 The LexA box exhibits considerable diversity; thus, no two sequences are alike and LexA binds with different affinities to the various variants enabling differential induction of the LexA regulon genes. The location of SOS boxes at promoters varies with respect to the transcription start site; some are positioned between the − 35 and − 10 elements, some overlap with the promoter elements, while others are adjacent to the target promoter. Although most *E. coli* LexA regulon genes possess a single LexA operator site, the number can range up to three SOS boxes. For example, the promoter region of the *lexA* gene carries separated tandem operators. LexA autoregulation sets a control of its own intracellular level via a feedback mechanism, enabling a rapid response to even small amounts of the inducing signal.

s0030 Triggers of the SOS Response

p0050 SOS genes can be induced by diverse exogenous treatments such as irradiation or chemicals, and can also be induced by DNA damage, caused by metabolic intermediates within the cell, by stalled replication forks, or by defects following recombination or chromosome segregation. Physical stress, such as high pressure that induces activity of the type IV restriction endonuclease, and even certain antibiotics, most notably fluoroquinolones such as ciprofloxacin, are also known to induce the SOS response. Note that the SOS-inducing signal is persistent regions of ssDNA that are generated when growing cells attempt to replicate damaged DNA. Depending upon the nature of the inducing signal, either the RecBCD or the RecFOR complex expose ssDNA to RecA.

p0055 The SOS response can also be triggered independently of RecA at low intracellular pH when LexA forms aggregates, which results in induction of LexA-repressed genes. Transient failure of pH homeostasis occurs in *E. coli* upon shifts of extracellular pH or in mutants with improper intracellular pH regulation. Presumably, this is a bacterial survival strategy when crossing the gastric acid barrier.

s0035 Sensing the Signal and Inducing LexA Inactivation

p0060 The major SOS-inducing signal is the accumulation of ssDNA. During normal growth a limited amount of ssDNA is tolerated; however, above this threshold, the SOS system is induced in a LexA-dependent manner. Long-lived ssDNA is protected and stabilized by the ssDNA-binding (SSB) protein. Tetrameric SSB migrates along ssDNA, transiently melting short DNA hairpins and stimulating RecA filament elongation on DNA. Association of ATP-liganded RecA protomers constitutes an activated nucleoprotein filament (RecA*). RecA-mediated SOS induction requires an extended filament conformation but no ATP hydrolysis (note that RecA protein besides working as a co-protease and activator of the DNA polymerase V plays a central role in recombination and is involved in a surprising range of other reactions in *E. coli*).

p0065 LexA is recognized by proteases only following self-cleavage, when otherwise latent protease recognition signals are exposed in the cleaved fragments. The self-cleavage of LexA results generates LexA N- and C-terminal fragments of 83 and 118 amino acids, respectively. The fragments are rapidly degraded by the ClpXP protease and the degradation of the cleaved C-terminal fragment is facilitated by the Lon protease. Proteolysis ensures proper regulation of induction of the SOS response, since the LexA N-terminal fragment, that contains the DNA binding domain, still retains some repressor function.

s0040 Insights into the Key Step in the SOS Response

p0070 The LexA repressor is stable in normal growing cells, with a half-life of nearly 1 h. *E. coli* contains approximately 1300 LexA molecules. Repressor self-cleavage commences approximately 1 min after exposure to UV and, after 5 min, the level of LexA falls 10-fold. Self-cleavage takes place only after LexA has dissociated from its target, since dimers that are bound at specific operator targets cannot be inactivated.

p0075 Upon LexA interaction with the deep helical groove of RecA*, intramolecular cleavage of the repressor occurs. LexA is specifically cleaved at its Ala84–Gly85 bond. John W Little and colleagues proposed a Ser–Lys dyad mechanism for LexA autodigestion. The uncharged form of Lys156 helps remove a

proton from the Ser119 hydroxyl group, which then acts as a nucleophile to attack the Ala84–Gly85 bond. *In vivo* cleavage requires RecA but, *in vitro*, it can proceed independently of RecA at alkaline pH (a reaction termed autocleavage).

p0080 Crystal structures of LexA mutants revealed that the cleavage site can adopt two conformations. In the cleavable state, the cleavage site is located adjacent to the catalytic center, the Ser119–Lys156 dyad, while in the noncleavable conformation it is ~20 Å away from the active site. It has been suggested that interaction with RecA* induces a conformational change in LexA and deprotonation of Lys156. It was also suggested that RecA* may preferentially interact with and stabilize the LexA cleavable state. However, recent evidence suggests that RecA* can bind to LexA in both the cleavable and noncleavable states. Residue Lys156 is solvent exposed and likely protonated in the LexA noncleavable conformation. The energetic cost of burying the charged group of Lys156, which is required for cleavage, provides another layer of regulation of LexA cleavage and helps to prevent autodigestion. Thus, by acting as a co-protease, RecA inactivates LexA, thereby inducing its expression, together with more than 50 other SOS gene products.

s0045 DNA Damage Repair

p0085 The level, timing, and duration of expression of each individual LexA regulon genes differ significantly. Most genes of the LexA regulon, including *recA*, are, in the absence of induction, expressed at a basal level. Specifically bound LexA molecules cannot be inactivated, which accounts for the precise timing of expression of the SOS genes following induction. Genes with high-affinity SOS boxes are expressed late in the SOS response due to a persistent decrease in the intracellular LexA pool. On the contrary, selective derepression of SOS genes with weaker operators occurs in response to minor inducing signals.

p0090 The SOS response is characterized by temporal control. Initially, SOS products (*recA*, *ssb*) sense DNA damage to protect and maintain the structural integrity of the replication fork. The LexA repressor is also induced immediately. Active RecA* initially signals the upregulation of SOS genes involved in high-fidelity DNA repair. Early induced genes include nucleotide excision repair genes *uvrA*, *uvrB*, *uvrD* that enable single-strand repair catalyzed by the UvrABCD proteins. To facilitate the resumption of processive replication, genes *recA*, *recN*, *ruvAB* of recombinational repair are induced. In order to circumvent lesions that inhibit DNA replication even after enhanced recombinational repair, low-fidelity DNA damage tolerance pathways are induced and DNA polymerases, PolIII (*polB*), PolIV (*dinB*), PolV (*umuC*, *umuD*) that operate in a poorly processive and error-prone manner are synthesized. Their ability to perform translesion DNA synthesis, allows a lethal event to be bypassed and replication to recover. These polymerases are the main contributors to SOS mutagenesis, which is an active process.

p0095 Precise temporal modulation of SOS gene expression is coordinated with DNA repair processes and influences many other cellular processes. Damage inflicted on bacterial DNA leads to fast and massive intracellular coaggregation of RecA and DNA into a lateral macroscopic assembly. These intracellular assemblies are the functional target for DNA repair and are responsible for protection of the cell's DNA heritage.

Cell-Cycle Checkpoints s0050

The expression of SOS genes is turned on in a pattern of discrete activation pulses; therefore, the system is not simply induced and turned off when DNA damage is repaired. To prevent the overlap of cell-cycle processes, the SOS system regulates DNA damage and cell division checkpoints. p0100

E. coli cell-cycle checkpoints are regulated by the *umuDC* and *sulA* gene products. Uncleaved $UmuD_2$ in complex with UmuC activates a DNA damage replication checkpoint. $UmuD_2C$ inhibits DNA synthesis directly by associating with the DNA replication complex. If high-fidelity repair is insufficient, the $UmuD'_2C$ complex, PolV polymerase, is formed. Following SOS induction, dimeric UmuD is converted to functionally active UmuD′ by RecA*-induced self-cleavage that is similar to inactivation of LexA. However, RecA*-mediated self-cleavage of UmuD is much slower than self-cleavage of LexA, providing time for accurate repair prior to recovery of replication by translesion DNA synthesis. The $UmuD'_2C$ complex is activated by interacting with a single RecA–ATP transferred from the RecA* filament. Translesion DNA synthesis by the PolV polymerase enables replication over any remaining DNA lesions. p0105

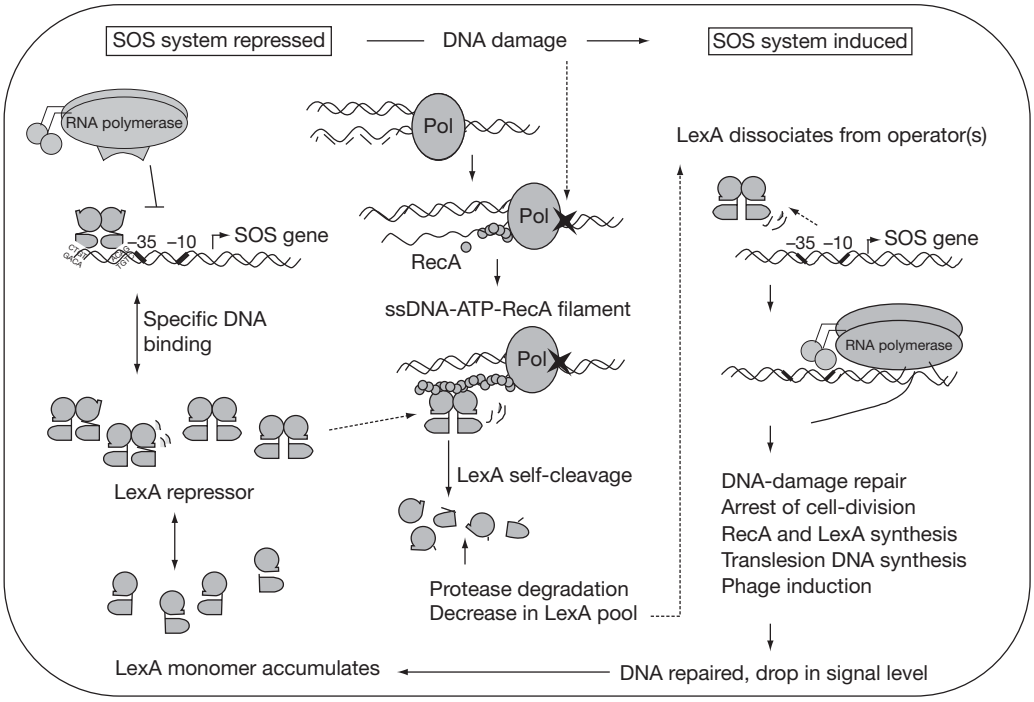
During the DNA repair process, cell division is inhibited which leads to the formation of cellular filaments. Notably, upon damage to the genome, the LexA-regulated *sulA* gene product is highly expressed and interacts with the FtsZ protein, involved in septum formation prior to cell division. Most likely, this checkpoint serves to delay cell division until DNA damage has been repaired. In addition, by inhibiting cell division the two daughter chromosomes are not separated enabling recombinational repair. p0110

Turning Off the SOS Response s0055

Once DNA damage is repaired and replication resumed, the co-protease activity of RecA disappears resulting in re-accumulation of LexA and repression of the SOS genes. Intracellular proteolysis of SOS gene products is also triggered to control and restrict their activity during the repair and recovery phases of the SOS response respectively. p0115

Members of the LexA Super-Family s0060

Jeffrey W Roberts and colleagues demonstrated that exposure of lysogens containing bacteriophage λ to DNA-damaging treatments results in RecA-mediated cleavage of the λCI repressor. SOS regulation enables temperate λ-like bacteriophages to sense the physiological condition of the host cell and switch the phage from lysogenic to lytic growth. LexA, UmuD, and several λCI-like repressors, exhibit CTD homology and undergo completely parallel cleavage reactions in helical groove of the RecA* filament. Self-cleavage of LexA is intramolecular while UmuD is cleaved in an intermolecular reaction. Note that upon self-cleavage, dimeric UmuD is converted to the functionally active UmuD′, in contrast to repressors that are inactivated by cleavage. Remarkably, compared to LexA, RecA* catalyzes slow self-cleavage of the CI repressor and UmuD; hence, prophage induction and mutagenesis are induced only when DNA is severely damaged. p0120

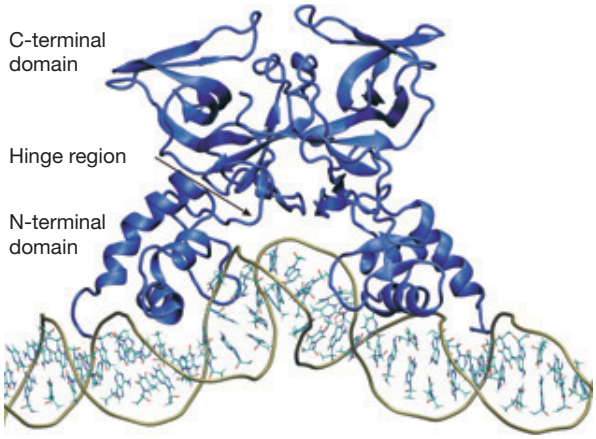


f0010 **Figure 1** An overview of the SOS response in *E. coli*. In the uninduced state, LexA repressor binds to the promoter regions of SOS genes and sterically precludes their transcription. The polymerase III holoenzyme (Pol) carries out DNA replication. In the induced state, at the site of DNA damage PolIII arrests, ssDNA accumulates and active RecA filament is formed. Due to induced LexA self-cleavage, specifically bound LexA repressor dissociates from operators, leading to de-repression of SOS genes. Subsequently, as DNA damage is repaired, SOS induction is reversed. Adapted from Butala M, Žgur-Bertok D, and Busby SJW (2009) The bacterial LexA transcriptional repressor. *Cellular and Molecular Life Sciences* 66: 82–93.

s0065 Plasmid-Encoded Genes of the LexA Regulon

p0125 Some plasmid-encoded genes, with broader functions than defense against DNA damage and adaptation through mutagenesis, are also part of the LexA regulon. For example, colicins are plasmid-encoded bacteriocins, synthesized by and active against *E. coli* strains and its close relatives. Colicins are released into the environment only after lysis of the host cell. Expression of operons encoding colicin functions are always strongly repressed by LexA, and slow dissociation from the operators may account for the late induction of colicin genes during the SOS response. RecA-mediated production of bacteriocins thus resembles prophage induction, leading to cell lysis upon persistent, high level DNA damage. Many colicins can promote genetic diversity in *E. coli* populations pointing to a role in evolution.

p0130 The *qnr* genes, which encode fluoroquinolone-resistance determinants, provide another example of plasmid-borne LexA-repressed genes. These are widespread in Enterobacteriaceae and are all directly regulated by LexA. Since fluoroquinolones induce self-cleavage of LexA, this is the first example of SOS-dependent regulation of an antibiotic-resistance mechanism in response to the antibiotic itself.



f0015 **Figure 2** Model of the *E. coli* LexA repressor bound to the operator DNA site. LexA dimerises by the carboxy-terminal domain, and interacts with DNA by the amino-terminal domain. The two domains are linked by a flexible hinge region. Adapted from Butala M, Žgur-Bertok D, and Busby SJW (2009). The bacterial LexA transcriptional repressor. *Cellular and Molecular Life Sciences* 66: 82–93.

s0070 Bacterial LexA Regulon Diversity

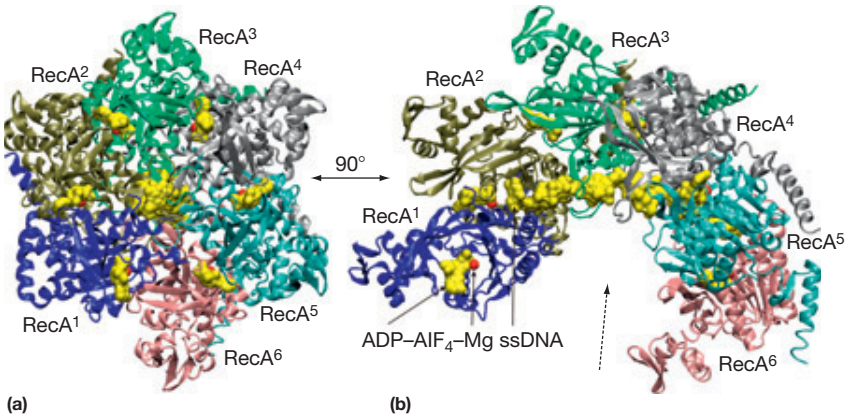
p0135 Although the SOS system is highly conserved among bacteria, the genes controlled by LexA, their regulation and consensus LexA-binding sites differ significantly. In *Bacillus subtilis* LexA regulates 26 operons encompassing 63 genes (note that the *B. subtilis* LexA protein is also designated DinR). In comparison, the *E. coli* LexA regulon comprises 57 genes and has only eight orthologs in *B. subtilis*. To further illustrate the diversity

found in SOS networks, in both *Rhodobacter sphaeroides* and the cyanobacterium *Synechocystis* sp., the LexA paralogue can function both to repress and to activate transcription.

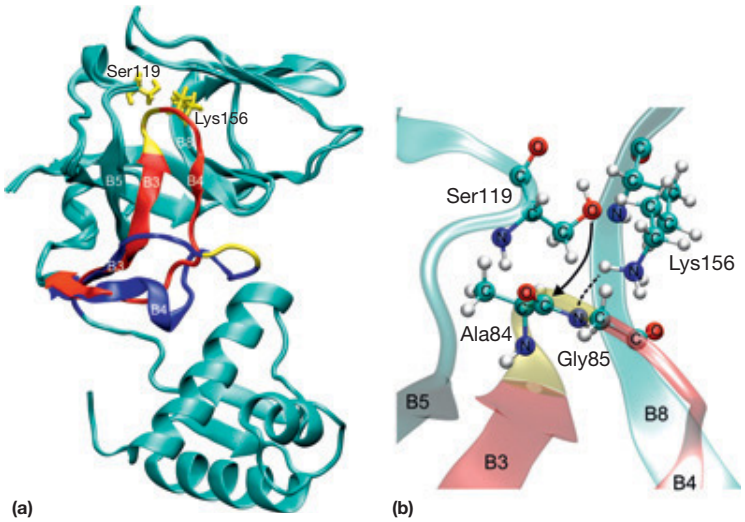
s0075 ## The Virulent Side of the SOS Response

p0140 Besides high-fidelity repair pathways, SOS genes encode low-fidelity translesion DNA polymerases (in *E. coli*, PolII [*polB*], PolIV [*dinB*], and PolV [*umuC*, *umuD*]) that enable bacteria to increase their mutation rate in times of stress. Studies employing therapeutic drugs showed that low or subinhibitory concentrations of certain antibiotics, that interfere with DNA replication as well as cell wall synthesis, can trigger the SOS response. Hence, antibiotics can accelerate evolution by, for example, the acquisition of point mutations that result in inactivation or efflux of the drug.

p0145 SOS-inducing antibiotics also affect virulence in several pathogenic bacteria. Antibiotics that activate RecA*-mediated inactivation of LexA also trigger self-cleavage of phage repressors of resident prophages in *E. coli*, *Vibrio cholerae*, and *Staphylococcus aureus*. Consequently, certain antibiotics promote the horizontal spread of temperate phage and associated pathogenicity islands. In addition, the lateral transfer of integrating



f0020 **Figure 3** Crystal structure of the active *E. coli* RecA filament (pdb ID: 3CMU), the front (a) and the side view (b). The six RecA protomer monomers (numbered) form a filament on the 18 nt ssDNA (nucleotides are in yellow). ADP–aluminum fluoride–Mg (ADP–AF_4–Mg) is a nonhydrolyzable ATP analog. ADP–AlF_4–Mg is sandwiched between two adjacent RecA protomers (ADP in yellow, Mg in red). Dotted arrow indicates deep helical groove. Figure prepared with visual molecular dynamics (VMD). From Humphrey W, Dalke A, and Schulten K (1996) VMD: Visual molecular dynamics. *Journal of Molecular Graphics* 14: 33–38.



f0025 **Figure 4** Two distinct conformations of the LexA cleavage site region and a detailed view of the active site. (a) Cleavage site region in the noncleavable state (pdb ID: 1jhh, chain A) is presented in blue and the CTD (pdb ID: 1jhe, chain A) in the cleavable state in red. The catalytic dyad, Ser119 and Lys156, is presented as a sick model and cleavage site Ala84–Aly85 as a ribbon presentation in yellow. (b) Model of the LexA self-cleavage mechanism. Neutral base Lys156 activates the nucleophile LexA119. Hydroxyl group of the activated nucleophile attacks the carbonyl carbon of the scissile peptide bond (arrow), followed by the transfer of the proton to the newly generated amino group (dotted line). The figure was generated by VMD and adapted from Butala M, Žgur-Bertok D, and Busby SJW (2009) The bacterial LexA transcriptional repressor. *Cellular and Molecular Life Sciences* 66: 82–93.

conjugative elements, for example, the *V. colerae* SXT element encoding antibiotic resistance, can be induced. Thus, SOS-induced mobilization and high-frequency horizontal transfer of DNA elements accelerate the spread of virulence factors and drug resistance genes. In *E. coli*, induction of the LexA regulon has been shown to be required for the acquisition of resistance to ciprofloxacin and rifampicin. In addition, recombination of integrons, genetic elements capable of incorporating and expressing promoterless genes, was shown to be controlled by the SOS response.

p0150 Cells in a bacterial population can survive antibiotic stress by forming dormant cells, designated as persisters that are highly tolerant to antibiotics. Persisters are not mutants but rather phenotypic variants of sensitive cells. Recently, a small membrane-acting peptide encoded by the LexA-regulated gene, *tisB*, was suggested to control persister formation.

p0155 Distinct from drug-induced mobilization of DNA elements, the SOS system also induces chromosomal virulence gene expression. For example, prophage encode the *E. coli* Shiga toxin. In enteropathogenic *E. coli*, SOS regulates a type III secretion system responsible for secretion of virulence-associated factors into host cells. Interestingly, in some *S. aureus* strains, a LexA-regulated gene encodes the fibronectin binding protein (FnbB) that mediates tissue attachment and the establishment of infection.

See also: 00419; 00233; 00238; 00253; 00486.

Further Reading

Butala M, Žgur-Bertok D, and Busby SJW (2009) The bacterial LexA transcriptional repressor. *Cellular and Molecular Life Sciences* 66: 82–93.

Courcelle J, Khodursky A, Peter B, Brown PO, and Hanawalt PC (2001) Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*. *Genetics* 158: 41–64.

Erill I, Campoy S, and Barbe J (2007) Aeons of distress: An evolutionary perspective on the bacterial SOS response. *FEMS Microbiology Reviews* 31: 637–656.

Foti JJ, Simmons LA, Beuning PJ, and Walker GC (2010) Signal transduction in the *Escherichia coli* SOS response. In: Bradshaw RA and Dennis EA (eds.) *Handbook of Cell Signaling*, vol. 3, pp. 2127–2136. Elsevier. Au3

Kelley WL (2006) Lex marks the spot: The virulent side of SOS and a closer look at the LexA regulon. *Molecular Microbiology* 62: 1228–1238.

Little JW (1991) Mechanism of specific LexA cleavage: Autodigestion and the role of RecA coprotease. *Biochimie* 73: 411–421.

Luo Y, Pfuetzner RA, Mosimann S, et al. (2001) Crystal structure of LexA: A conformational switch for regulation of self-cleavage. *Cell* 106: 585–594.

Sassanfar M and Roberts JW (1990) Nature of the SOS-inducing signal in *Escherichia coli*. The involvement of DNA replication. *Journal of Molecular Biology* 212: 79–96.

MS code	Article	Section entry	Author	Section Editor
1	Allosteric Regulation	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
2	Aminopeptidases	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
3	Aspartic Proteases	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
4	B12-Containing Enzymes	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
5	Biotin	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
6	Chemiluminescence and Bioluminescence	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
7	Coenzyme A	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
8	Collagenases	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
9	Cysteine Proteases	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
10	Disulfide Bond Formation	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
11	Enzyme Inhibitors	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
12	Enzyme Kinetics	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
13	Enzyme Reaction Mechanisms: Stereochemistry	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
14	Flavins	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
15	Heme Proteins	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
16	Kinetic Isotope Effects	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
17	Low Barrier Hydrogen Bonds	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
18	Metalloproteases	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
19	Peptide Amidation	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
20	Proteases in Blood Clotting	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister

MS code	Article	Section entry	Author	Section Editor
21	Protein N-Myristoylation	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
22	Protein Palmitoylation	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
23	Pteridines	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
24	Pyridoxal Phosphate	Protein/Enzyme Structure Function and Degradation	Michael Toney	Wolfgang Baumeister
25	Selenoprotein Synthesis	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
26	Substrate Binding Catalysis and Product Release	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
27	Zinc Fingers	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
28	Pentose Phosphate (Hexose Mono Phosphate) Pathway	Metabolism Vitamins and Hormones	NO REVISION	M. Daniel Lane
29	Amino Acid Metabolism	Metabolism Vitamins and Hormones	Luc Cynober	M. Daniel Lane
31	Bile Salts and their Metabolism	Metabolism Vitamins and Hormones	Ulrich Beuers	M. Daniel Lane
32	The Chemistry of Alzheimer Disease	Metabolism Vitamins and Hormones	George H. Sack Jr.	M. Daniel Lane
33	Carbohydrate responsive element binding protein	Metabolism Vitamins and Hormones	Kosaku Uyeda	M. Daniel Lane
34	Coenzyme A	Metabolism Vitamins and Hormones	M. Daniel Lane	M. Daniel Lane
35	Diabetes	Metabolism Vitamins and Hormones	David W. Cooke	M. Daniel Lane
36	Fat Mobilization: Perilipin and Hormone-Sensitive Lipase	Metabolism Vitamins and Hormones	Alan Kimmel	M. Daniel Lane
37	Fatty Acid Metabolism and Cancer	Metabolism Vitamins and Hormones	F Kuhajda	M. Daniel Lane
38	Fatty Acid Synthesis and its Regulation	Metabolism Vitamins and Hormones	Steven D. Clarke	M. Daniel Lane
39	Folate & Vit B12	Metabolism Vitamins and Hormones	B. Shane	M. Daniel Lane
40	Gluconeogenesis	Metabolism Vitamins and Hormones	Richard W. Hanson	M. Daniel Lane
41	Glucose/Sugar Transport in Mammals	Metabolism Vitamins and Hormones	Jeffrey Pessin	M. Daniel Lane

MS code	Article	Section entry	Author	Section Editor
42	Glycogen Metabolism	Metabolism Vitamins and Hormones	Peter J. Roach	M. Daniel Lane
43	Glycogen Storage Diseases	Metabolism Vitamins and Hormones	George H. Sack Jr.	M. Daniel Lane
44	Glycolysis Overview	Metabolism Vitamins and Hormones	Robert A. Harris	M. Daniel Lane
45	Gut orex-anorex NPs	Metabolism Vitamins and Hormones	T. Moran	M. Daniel Lane
46	Insulin- and Glucagon-Secreting Cells of the Pancreas	Metabolism Vitamins and Hormones	Franz M. Matschinsky	M. Daniel Lane
47	Metab/orexigenic & anorex neuropeptides	Metabolism Vitamins and Hormones	G Morton	M. Daniel Lane
48	Metabolomic profiling	Metabolism Vitamins and Hormones	C. Newgard	M. Daniel Lane
49	Photosynthesis	Metabolism Vitamins and Hormones	Richard C. Leegood	M. Daniel Lane
50	Photosynthetic Carbon Dioxide Fixation	Metabolism Vitamins and Hormones	Matthew J. Paul	M. Daniel Lane
51	Phosphofructokinase-2/Fructose Bisphosphatase-2	Metabolism Vitamins and Hormones	Daniel M. Raben	M. Daniel Lane
52	Porphyrin Metabolism	Metabolism Vitamins and Hormones	Harry A. Dailey	M. Daniel Lane
53	Pyruvate Kinase	Metabolism Vitamins and Hormones	Kosaku Uyeda	M. Daniel Lane
54	Regulation of Gene Transcription by Hypoxia-Inducible Factor 1	Metabolism Vitamins and Hormones	Greg Semenza	M. Daniel Lane
55	Role of Aquaporins	Metabolism Vitamins and Hormones	Peter Agre	M. Daniel Lane
56	Vitamin A (Retinoids)	Metabolism Vitamins and Hormones	Joseph L. Napoli	M. Daniel Lane
57	AAA-ATPases	Protein/Enzyme Structure Function and Degradation	Andrei Lupas	Wolfgang Baumeister
58	Calpain	Protein/Enzyme Structure Function and Degradation	Hiroyuki Sorimachi	Wolfgang Baumeister
59	HIV Protease	Protein/Enzyme Structure Function and Degradation	Ben M. Dunn	Wolfgang Baumeister
60	Lipid Modification of Proteins: Targeting to Membranes	Protein/Enzyme Structure Function and Degradation	Marilyn D. Resh	Wolfgang Baumeister
61	Phage Display for Protein Binding	Protein/Enzyme Structure Function and Degradation	Henry B. Lowman	Wolfgang Baumeister

MS code	Article	Section entry	Author	Section Editor
62	Protein Carboxyl Esterification	Protein/Enzyme Structure Function and Degradation	Jeffry B. Stock	Wolfgang Baumeister
63	Protein Degradation	Protein/Enzyme Structure Function and Degradation	Alfred L. Goldberg	Wolfgang Baumeister
64	Protein Folding and Assembly	Protein/Enzyme Structure Function and Degradation	David P. Goldenberg	Wolfgang Baumeister
65	Regulated Intramembrane Proteolysis (Rip)	Protein/Enzyme Structure Function and Degradation	Jin Ye	Wolfgang Baumeister
66	Two-Hybrid Protein–Protein Interactions	Protein/Enzyme Structure Function and Degradation	Ilya Serebriiskii	Wolfgang Baumeister
67	Tyrosine Sulfation	Protein/Enzyme Structure Function and Degradation	Denis Corbeil	Wolfgang Baumeister
68	Ubiquitin-Like Proteins	Protein/Enzyme Structure Function and Degradation	Edward T. H. Yeh	Wolfgang Baumeister
69	Protein Data Resources	Protein/Enzyme Structure Function and Degradation	Janet Thornton	Wolfgang Baumeister
70	Cholesterol Synthesis	Metabolism Vitamins and Hormones	P Espenshade	M. Daniel Lane
71	Fatty Acid Oxidation	Metabolism Vitamins and Hormones	NO REVISION	M. Daniel Lane
72	Branched-Chain amino acids	Metabolism Vitamins and Hormones	David T. Chuang	M. Daniel Lane
73	Hexokinases/Glucokinases	Metabolism Vitamins and Hormones	Emile Van Schaftingen	M. Daniel Lane
75	Metabolic Control during Ischemia of the Heart	Metabolism Vitamins and Hormones	Garry Lopaschuk	M. Daniel Lane
76	Carbohydrate Metabolism in the Central Nervous System	Metabolism Vitamins and Hormones	I Simpson	M. Daniel Lane
77	Regulation by Fatty Acids/Malonyl-CoA in the brain	Metabolism Vitamins and Hormones	M Wolfgang	M. Daniel Lane
78	Role of the micro RNAs in Metabolism	Metabolism Vitamins and Hormones	G. Wong	M. Daniel Lane
79	Structure and Regulation of Pyruvate Dehydrogenase Complex	Metabolism Vitamins and Hormones	J. Milne	M. Daniel Lane
80	Chaperonins	Protein/Enzyme Structure Function and Degradation	Ulrich Hartl	Wolfgang Baumeister
81	Mass Spec of Native Complexes	Protein/Enzyme Structure Function and Degradation	Albert Heck	Wolfgang Baumeister
82	Mass spec and proteomics	Protein/Enzyme Structure Function and Degradation	Matthias Mann	Wolfgang Baumeister

MS code	Article	Section entry	Author	Section Editor
83	Sphingolipid Metabolism and Disease	Metabolism Vitamins and Hormones	Roscoe O. Brady	M. Daniel Lane
84	Biochem of liver regeneration	Metabolism Vitamins and Hormones	A-M Diehl	M. Daniel Lane
85	T Cell Receptor Signaling to NF-kappaB	Metabolism Vitamins and Hormones	Joel Pomerantz	M. Daniel Lane
86	Biliary Cirrhosis Primary	Metabolism Vitamins and Hormones	Marshall M. Kaplan	M. Daniel Lane
87	Starvation	Metabolism Vitamins and Hormones	Richard W. Hanson	M. Daniel Lane
88	Biochem of hematopoiesis	Metabolism Vitamins and Hormones	Alan Friedman	M. Daniel Lane
89	Adipogenesis	Metabolism Vitamins and Hormones	M. Daniel Lane	M. Daniel Lane
90	Biochemistry of muscle contraction	Metabolism Vitamins and Hormones	DD Thomas	M. Daniel Lane
91	Biochemistry of development: Muscle	Metabolism Vitamins and Hormones	Rhonda Bassel-Duby	M. Daniel Lane
92	Vitamin C	Metabolism Vitamins and Hormones	Francene Steinberg	M. Daniel Lane
93	Insect metabolism/hormones	Metabolism Vitamins and Hormones	RL Miesfeld	M. Daniel Lane
94	Biochem of neurogenesis	Metabolism Vitamins and Hormones	H Song	M. Daniel Lane
95	Vitamin K: Biochemistry Metabolism and Nutritional Aspects	Metabolism Vitamins and Hormones	J.W Suttie	M. Daniel Lane
96	Adiponectin: metabolic role	Metabolism Vitamins and Hormones	PE Scherer	M. Daniel Lane
97	Vitamin D	Metabolism Vitamins and Hormones	H DeLuca	M. Daniel Lane
98	Color Vision / Biochem of vision	Metabolism Vitamins and Hormones	Gerald Jacobs	M. Daniel Lane
99	Ketogenesis	Metabolism Vitamins and Hormones	Charles Hoppel	M. Daniel Lane
100	The Fatty Acyl-CoA Synthetases	Metabolism Vitamins and Hormones	P. Watkins	M. Daniel Lane
101	Urea cycle: Disease Aspects	Metabolism Vitamins and Hormones	Marc Yudkoff	M. Daniel Lane
102	Biochemistry: thiamine/thiamine-PP	Metabolism Vitamins and Hormones	L.Bettendorff	M. Daniel Lane

MS code	Article	Section entry	Author	Section Editor
103	Biochemistry: Niacin/NAD(P)	Metabolism Vitamins and Hormones	C Brenner	M. Daniel Lane
104	Peroximsomes: Metabolic Role	Metabolism Vitamins and Hormones	Steve Gould	M. Daniel Lane
105	Riboflavin: flavoproteins-FAD/FMN	Metabolism Vitamins and Hormones	Andrea Mattevi	M. Daniel Lane
106	Gastrointestinal digestion And Absorbtion	Metabolism Vitamins and Hormones	J Keller	M. Daniel Lane
107	Mucins in Embryo Implantation	Lipids Carbohydrates Membranes and Membrane Proteins	Dan Carson	William Lennarz
108	Glycosylation Congenital Disorders of	Lipids Carbohydrates Membranes and Membrane Proteins	Hudson Freeze	William Lennarz
109	Glycoprotein-Mediated Cell Interactions O-Linked	Lipids Carbohydrates Membranes and Membrane Proteins	Robert Haltiwanger	William Lennarz
110	Glycoprotein Folding and Processing Reactions	Lipids Carbohydrates Membranes and Membrane Proteins	Armando Parodi	William Lennarz
111	GlcNAc Biosynthesis and Function O-Linked	Lipids Carbohydrates Membranes and Membrane Proteins	Kaoru Sakabe	William Lennarz
112	Prions Overview	Protein/Enzyme Structure Function and Degradation	Detlev Riesner	Wolfgang Baumeister
113	Proteoglycans	Lipids Carbohydrates Membranes and Membrane Proteins	Jeffrey D. Esko	William Lennarz
114	Lipid Bilayer Structure	Lipids Carbohydrates Membranes and Membrane Proteins	Erwin London	William Lennarz
115	Glycoproteins N-Linked	Lipids Carbohydrates Membranes and Membrane Proteins	Mark Lehrman	William Lennarz
116	Insulin: Mech/Metab actions	Metabolism Vitamins and Hormones	Derek LeRoith	M. Daniel Lane
117	Glycolipid-Dependent Adhesion Processes	Lipids Carbohydrates Membranes and Membrane Proteins	NO REVISION	William Lennarz
118	Lipases	Lipids Carbohydrates Membranes and Membrane Proteins	NO REVISION	William Lennarz
119	Sugar Nucleotide Transporters	Lipids Carbohydrates Membranes and Membrane Proteins	Carlos Hirschberg	William Lennarz
120	Glycation	Lipids Carbohydrates Membranes and Membrane Proteins	John Baynes	William Lennarz
121	Endocytosis	Lipids Carbohydrates Membranes and Membrane Proteins	Julie Donaldson	William Lennarz
122	Luft's Disease	Bioenergetics	NO REVISION	Ernesto Carafoli

MS code	Article	Section entry	Author	Section Editor
123	Calcium Biological Fitness of?????	Bioenergetics	NO REVISION	Ernesto Carafoli
124	Spectrophotometric Assays	Bioenergetics	NO REVISION	Ernesto Carafoli
125	Membrane Transport General Concepts	Bioenergetics	NO REVISION	Ernesto Carafoli
126	Mitochondrial DNA	Bioenergetics	NO REVISION	Ernesto Carafoli
127	Oxygenases	Bioenergetics	NO REVISION	Ernesto Carafoli
128	V-ATPases	Bioenergetics	Michael Forgac	Ernesto Carafoli
129	Superoxide Dismutase	Bioenergetics	Irwin Fridovich	Ernesto Carafoli
130	Cell–Matrix Interactions	Lipids Carbohydrates Membranes and Membrane Proteins	Janet Askari	William Lennarz
131	Cytochrome Oxidases Bacterial	Bioenergetics	Peter Brzezinski	Ernesto Carafoli
132	Membrane Transporters:Na+/Ca2+ Exchangers	Bioenergetics	Jonathan Lytton	Ernesto Carafoli
133	Ion Channel Protein Superfamily	Lipids Carbohydrates Membranes and Membrane Proteins	William A. Catterall	William Lennarz
134	Chlorophylls and Carotenoids	Bioenergetics	Hugo Scheer	Ernesto Carafoli
135	ATP Synthesis in Plant Mitochondria: Substrates Inhibitors Uncouplers	Bioenergetics	Kathleen Soole	Ernesto Carafoli
136	Nicotinamide Nucleotide Transhydrogenase	Bioenergetics	Jan Rydstrom	Ernesto Carafoli
137	Plastocyanin	Bioenergetics	NO REVISION	Ernesto Carafoli
138	Neuronal Calcium Signal	Bioenergetics	Hilmar Bading	Ernesto Carafoli
139	Calcium-Modulated Proteins (EF-Hand)	Bioenergetics	Robert H. Kretsinger	Ernesto Carafoli
140	Calcium Sensing Receptor	Bioenergetics	Edward M. Brown	Ernesto Carafoli
141	Chloroplasts	Bioenergetics	Nicoletta Rascio	Ernesto Carafoli
142	Respiratory Chain Complex II and Succinate: Quinone Oxidoreductases	Bioenergetics	Roy Lancaster	Ernesto Carafoli

MS code	Article	Section entry	Author	Section Editor
143	Mitochondrial Membranes Structural Organization	Bioenergetics	Carmen A. Mannella	Ernesto Carafoli
144	Voltage-Dependent K+ Channels	Bioenergetics	Ramon Latorre	Ernesto Carafoli
145	Heme Synthesis	Bioenergetics	Gloria C. Ferreira	Ernesto Carafoli
146	ER/SR Calcium Pump: Structure	Bioenergetics	Chikashi Toyoshima	Ernesto Carafoli
147	Calcium Buffering Proteins: ER Luminal Proteins	Bioenergetics	Marek Michalak	Ernesto Carafoli
148	Purple Bacteria: Photosynthetic Reaction Centers	Bioenergetics	Roy Lancaster	Ernesto Carafoli
149	Mitochondrial Metabolite Transporter Family	Bioenergetics	Ferdinando Palmieri	Ernesto Carafoli
150	IP3 Receptors	Bioenergetics	Katsuhiko Mikoshiba	Ernesto Carafoli
151	The mitochondrial permeability transition pore	Bioenergetics	Paolo Bernardi	Ernesto Carafoli
152	Chloroplast Redox Poise and Signaling	Bioenergetics	Jean-David Rochaix	Ernesto Carafoli
153	Calcium Oscillations	Bioenergetics	Ole Petersen	Ernesto Carafoli
154	Trp channels	Bioenergetics	Indu S. Ambudkar	Ernesto Carafoli
155	Respiratory Chain Complex I	Bioenergetics	Ulrich Brandt	Ernesto Carafoli
156	Mitochondrial calcium transport : historical aspects	Bioenergetics	Ernesto Carafoli	Ernesto Carafoli
157	Structure of P-type ATPases	Bioenergetics	Poul Nissen	Ernesto Carafoli
158	Cytochrome b6f Complex	Bioenergetics	William Cramer	Ernesto Carafoli
159	Green Sulfur Bacteria: Reaction Center and Electron Transport	Bioenergetics	Donald A. Bryant	Ernesto Carafoli
160	P-Type Pumps: H+/K+ Pump	Bioenergetics	Jai M Shin	Ernesto Carafoli
161	Mitochondrial Outer Membrane and the VDAC Channel	Bioenergetics	Marco Colombini	Ernesto Carafoli
162	Uncoupling Proteins	Bioenergetics	Daniel Ricquier	Ernesto Carafoli

MS code	Article	Section entry	Author	Section Editor
163	Nuclear Genes in Mitochondrial Function and Biogenesis	Bioenergetics	Alexander Tzagoloff	Ernesto Carafoli
164	Cytochrome bc1 Complex (Respiratory Chain Complex III)	Bioenergetics	NO REVISION	Ernesto Carafoli
165	Photosystem II Light Harvesting System: Dynamic Behavior	Bioenergetics	Peter Horton	Ernesto Carafoli
166	Ubiquitin System	Protein/Enzyme Structure Function and Degradation	Aaron Ciechanover	Wolfgang Baumeister
167	Amyloid	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
168	Biotinylation of Proteins	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
169	Collagens	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
170	Elastin	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
171	Proteasome Overview	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
172	Secretases	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
173	Affinity Tags for Protein Purification	Protein/Enzyme Structure Function and Degradation	NO REVISION	Wolfgang Baumeister
174	Calcium Signaling: Calmodulin-Dependent Phosphatase	Bioenergetics	Claude Klee	Ernesto Carafoli
175	Calcium in the regulation of the gene expression	Bioenergetics	Jose Ramon Naranjo	Ernesto Carafoli
176	Ferredoxin	Bioenergetics	Giuliana Zanetti	Ernesto Carafoli
177	Ferredoxin-NADP+ Reductase	Bioenergetics	Giuliana Zanetti	Ernesto Carafoli
178	Pyrimidine Biosynthesis	Bioenergetics	Monika Löffler	Ernesto Carafoli
179	Peroxidase catalysis and redox signaling	Bioenergetics	Alberto Bindoli	Ernesto Carafoli
180	Chemiosmotic Theory	Bioenergetics	Keith Garlid	Ernesto Carafoli
181	Green Bacteria: Secondary Electron Donor (Cytochromes)	Bioenergetics	Hirozo Oh-oka	Ernesto Carafoli
182	Amine Oxidases	Bioenergetics	Giovanni Floris	Ernesto Carafoli

MS code	Article	Section entry	Author	Section Editor
183	Voltage-Sensitive Ca2+ Channels	Bioenergetics	Harald Reuter	Ernesto Carafoli
184	Photosystem I: FX FA and FB Iron–Sulfur Clusters	Bioenergetics	John H. Golbeck	Ernesto Carafoli
185	Lipid Rafts	Lipids Carbohydrates Membranes and Membrane Proteins	NO REVISION	William Lennarz
186	Neoglycoproteins	Lipids Carbohydrates Membranes and Membrane Proteins	NO REVISION	William Lennarz
187	Store operated calcium channels. 2 : ORAI 1	Bioenergetics	Anjana Rao	Ernesto Carafoli
188	P-Type Pumps: Copper Pump	Bioenergetics	Svetlana Lutsenko	Ernesto Carafoli
189	Intracellular Calcium Channels: cADPR-Modulated (Ryanodine Receptors)	Bioenergetics	Gerhard Meissner	Ernesto Carafoli
190	Giant Mitochondria (Megamitochondria)	Bioenergetics	Bernard Tandler	Ernesto Carafoli
192	Mitochondrial Genes and their Expression: Yeast	Bioenergetics	Giovanna Carignani	Ernesto Carafoli
193	Calcium-Binding Proteins: Cytosolic (Annexins Gelsolins C2-Domain Proteins)	Bioenergetics	Joachim Krebs	Ernesto Carafoli
194	P-Type Pumps: Plasma-Membrane H+ Pump	Bioenergetics	Carolyn W. Slayman	Ernesto Carafoli
195	Troponin	Bioenergetics	Iwao Ohtsuki	Ernesto Carafoli
196	The Arachidonic Acid Regulated Calcium Channel	Bioenergetics	Trevor Shuttleworth	Ernesto Carafoli
197	Plasma-Membrane Calcium Pump: Structure and Function	Bioenergetics	Marisa Brini	Ernesto Carafoli
198	P-Type Pumps: Na+/K+ Pump	Bioenergetics	Steve Karlish	Ernesto Carafoli
199	ES/SR Calcium Pump: Function	Bioenergetics	Giuseppe Inesi	Ernesto Carafoli
200	Mitochondrial Auto-Antibodies	Bioenergetics	Harold Baum	Ernesto Carafoli
201	Cytochrome P-450	Bioenergetics	Rita Bernhardt	Ernesto Carafoli
202	Respiratory Processes in Anoxygenic and Oxygenic Phototrophs	Bioenergetics	Roberto Borghese	Ernesto Carafoli
203	Protein Import into Mitochondria	Bioenergetics	Walter Nfeupert	Ernesto Carafoli

MS code	Article	Section entry	Author	Section Editor
204	Quinones	Bioenergetics	Giorgio Lenaz	Ernesto Carafoli
205	Hydrogenase structure and function	Bioenergetics	Wolfgang Lubitz	Ernesto Carafoli
206	Calcium Signaling: NO Synthase	Bioenergetics	Dennis Stuehr	Ernesto Carafoli
207	Membrane-Associated Energy Transduction in Bacteria and Archaea	Bioenergetics	Guenter Schaefer	Ernesto Carafoli
208	Sodium Channels	Bioenergetics	William A. Catterall	Ernesto Carafoli
209	Lipid signaling and ion channels	Bioenergetics	Bertil Hille	Ernesto Carafoli
210	Calcium Transport in Mitochondria	Bioenergetics	Rosario Rizzuto	Ernesto Carafoli
211	Glycosylphosphatidylinositol (GPI) Anchors	Lipids Carbohydrates Membranes and Membrane Proteins	Anant Menon	William Lennarz
212	Carbohydrate Chains: Enzymatic and Chemical Synthesis	Lipids Carbohydrates Membranes and Membrane Proteins	Chi-Huey Wong	William Lennarz
213	Bioenergetics: General Definition of Principles	Bioenergetics	NO REVISION	Ernesto Carafoli
214	Respiratory Chain and ATP Synthase	Bioenergetics	Anthony Moore	Ernesto Carafoli
215	MDR Membrane Proteins	Lipids Carbohydrates Membranes and Membrane Proteins	Nathan C. Rockwell	William Lennarz
216	Mitochondrial dynamics	Bioenergetics	Luca Scorrano	Ernesto Carafoli
217	Lectins	Lipids Carbohydrates Membranes and Membrane Proteins	Nathan Sharon	William Lennarz
218	Periplasmic Electron Transport Systems in Bacteria	Bioenergetics	David Richardson	Ernesto Carafoli
219	Chemolithotrophy??	Bioenergetics	Alan Hooper	Ernesto Carafoli
220	Cyclic ADP ribose and NAADP in calcium signaling	Bioenergetics	Luigia Santella	Ernesto Carafoli
221	Excitation-contraction coupling	Bioenergetics	Donald Bers	Ernesto Carafoli
222	Iron–Sulfur Proteins	Bioenergetics	Richard Cammack	Ernesto Carafoli
223	Vitamin E	Metabolism Vitamins and Hormones	Jeffrey Atkinson	M. Daniel Lane

MS code	Article	Section entry	Author	Section Editor
224	ABC Transporters	Bioenergetics	André Goffeau	Ernesto Carafoli
225	Phosphatidylinositol-3-Phosphate	Bioenergetics	Michael Czech	Ernesto Carafoli
226	Free Radicals Sources and Targets of: Mitochondria	Bioenergetics	Alberto Aboveris	Ernesto Carafoli
227	Photosystem II: Assembly and Turnover of the D1 Protein	Bioenergetics	Eva-Mari Aro	Ernesto Carafoli
228	Calcium Buffering Proteins: Calbindin	Bioenergetics	Sylvia Christakos	Ernesto Carafoli
229	Photoinhibition and photoprotection in plants, algae, and cyanobacteria	Bioenergetics	Giorgio Giacometti	Ernesto Carafoli
230	The sodium/calcium exchanger : structural aspects	Bioenergetics	Kenneth Philipson	Ernesto Carafoli
231	Chromatin: Methyl-CpG-DNA binding proteins	Molecular Biology	David G. Skalnik	Nancy L. Craig
232	Chromatin: Nucleosome positioning - the GAL Promoter	Molecular Biology	Dennis Lohr	Nancy L. Craig
233	DNA Damage: Alkylation	Molecular Biology	John Tainer	Nancy L. Craig
235	DNA Methyltransferases Structural Themes	Molecular Biology	Xiaodong Cheng	Nancy L. Craig
236	DNA Methyltransferases: Eubacterial GATC	Molecular Biology	Martin G. Marinus	Nancy L. Craig
237	DNA Mismatch Repair and Homologous Recombination	Molecular Biology	Ivan Matic	Nancy L. Craig
238	DNA Mismatch Repair and the DNA Damage Response	Molecular Biology	Guo-Min Li	Nancy L. Craig
239	DNA Mismatch Repair in Bacteria	Molecular Biology	A-Lien Lu	Nancy L. Craig
240	DNA Oxidation	Molecular Biology	Dmitry Zharkov	Nancy L. Craig
241	DNA Polymerase β Eukaryotic	Molecular Biology	Samuel H. Wilson	Nancy L. Craig
242	DNA Replication Fork Eukaryotic	Molecular Biology	Zvi Kelman	Nancy L. Craig
243	DNA Restriction and Modification: Type III Enzymes	Molecular Biology	Desirazu N. Rao	Nancy L. Craig
244	DNA Supercoiling	Molecular Biology	Tao-shih Hsieh	Nancy L. Craig

MS code	Article	Section entry	Author	Section Editor
245	DNA Topoisomerases: Type I	Molecular Biology	James J. Champoux	Nancy L. Craig
246	DNA Topoisomerases: Type II	Molecular Biology	Neil Osheroff	Nancy L. Craig
247	HIV-1 Reverse Transcriptase Structure	Molecular Biology	Steven Hughes	Nancy L. Craig
248	Homologous Recombination in Meiosis	Molecular Biology	Nancy M. Hollingsworth	Nancy L. Craig
249	lac Operon	Molecular Biology	Kathleen Matthews	Nancy L. Craig
250	Nonhomologous recombination: DNA transposons	Molecular Biology	Michael Chandler	Nancy L. Craig
251	Nuclear Organization Chromatin Structure and Gene Silencing	Molecular Biology	Lori L. Wallrath	Nancy L. Craig
252	Nucleolus Overview	Molecular Biology	Thoru Pederson	Nancy L. Craig
253	Nucleotide Excision Repair Bacterial: The UvrABCD System	Molecular Biology	Bennett Van Houten	Nancy L. Craig
254	Nucleotide Excision Repair: Biology	Molecular Biology	Errol C. Friedberg	Nancy L. Craig
256	Prions and Epigenetic Inheritance	Molecular Biology	Reed B. Wickner	Nancy L. Craig
257	Recombination-Dependent DNA Replication	Molecular Biology	Kenneth N. Kreuzer	Nancy L. Craig
258	Reverse Transcriptase, Integrase and Retroviral Replication	Molecular Biology	Simon Litvak	Nancy L. Craig
259	Ribosome Assembly	Molecular Biology	John L. Woolford	Nancy L. Craig
260	Riboswitches	Molecular Biology	Adrian R. Ferré-D'Amaré	Nancy L. Craig
261	Ribozymes and Evolution	Molecular Biology	Niles Lehman	Nancy L. Craig
262	RNA Editing	Molecular Biology	Charles E. Samuel	Nancy L. Craig
263	RNA Polymerase I and RNA Polymerase III in Eukaryotes	Molecular Biology	Robert J. White	Nancy L. Craig
264	RNA Polymerase II Structure in Eukaryotes	Molecular Biology	Patrick Cramer	Nancy L. Craig
265	RNA Polymerase Structure Bacterial	Molecular Biology	Sergei Borukhov	Nancy L. Craig

MS code	Article	Section entry	Author	Section Editor
266	Sigma Factors	Molecular Biology	John D. Helmann	Nancy L. Craig
267	T7 RNA Polymerase	Molecular Biology	Rui Sousa	Nancy L. Craig
268	Telomeres: Maintenance and Replication	Molecular Biology	David Shore	Nancy L. Craig
269	Translation Initiation in Bacteria: Factors and Mechanisms	Molecular Biology	Claudio Gualerzi	Nancy L. Craig
270	trp Operon and Attenuation	Molecular Biology	Paul Gollnick	Nancy L. Craig
271	XPV DNA Polymerase and Ultraviolet Damage Bypass	Molecular Biology	Alan R. Lehmann	Nancy L. Craig
272	Non-Homologous End Joining in Eukaryotes	Molecular Biology	David J. Chen	Nancy L. Craig
273	Ligand-Operated Membrane Channels: GABA	Bioenergetics	Erwin Sigel	Ernesto Carafoli
274	DNA Sequence Recognition by Proteins	Molecular Biology	Greg van Duyne	Nancy L. Craig
275	Glycoproteins Plant	Lipids Carbohydrates Membranes and Membrane Proteins	NO REVISION	William Lennarz
276	RecQ Helicase Systems	Molecular Biology	Ian Hickson	Nancy L. Craig
277	Pre-tRNA and Pre-rRNA Processing in Bacteria	Molecular Biology	Zhongwei Li	Nancy L. Craig
278	LexA Regulatory System	Molecular Biology	Matej Butala	Nancy L. Craig
279	DNA Glycosylases: Mechanisms	Molecular Biology	Alex Drohat	Nancy L. Craig
280	Transcription-Coupled DNA Repair Overview	Molecular Biology	Silvia Tornaletti	Nancy L. Craig
281	Messenger RNA Degradation in Bacteria	Molecular Biology	David Bechhofer	Nancy L. Craig
282	Energy Transduction in Anaerobic Prokaryotes	Bioenergetics	Gottfried Unden	Ernesto Carafoli
283	Metabolite Channeling: Creatine Kinase Microcompartments	Bioenergetics	Uwe Schlattner	Ernesto Carafoli
284	Calcium/Calmodulin-Dependent Protein Kinase II	Bioenergetics	Howard Schulman	Ernesto Carafoli
285	ATP Synthesis: Mitochondrial Cyanide-Resistant Terminal Oxidases	Bioenergetics	Jim Siedow	Ernesto Carafoli

MS code	Article	Section entry	Author	Section Editor
286	Photosystem II: Water Oxidation Overview	Bioenergetics	Fabrice Rappaport	Ernesto Carafoli
287	Photosystem I Structure and Function	Bioenergetics	Petra Fromme	Ernesto Carafoli
288	Ligand-Operated Membrane Channels: Calcium (Glutamate)	Bioenergetics	Elias K. Michaelis	Ernesto Carafoli
289	Mitochondrial Channels	Bioenergetics	M. Catia Sorgato	Ernesto Carafoli
290	Light-Harvesting Complex (LHC) I and II: Pigments and Proteins	Bioenergetics	Stefan Jansson	Ernesto Carafoli
291	Mitochondrial Genome Evolution, Inheritance	Bioenergetics	Douglas C. Wallace	Ernesto Carafoli
292	Intracellular Calcium Waves	Bioenergetics	Luigia Santella	Ernesto Carafoli
293	Extracellular Calcium Waves	Bioenergetics	Michael Sanderson	Ernesto Carafoli
294	F1–F0 ATP Synthase	Bioenergetics	John Walker	Ernesto Carafoli
295	Respiratory Chain Complex IV	Bioenergetics	Hartmut Michel	Ernesto Carafoli
296	Mitochondria in myocardial ischemia	Bioenergetics	Fabio Di Lisa	Ernesto Carafoli
297	Complex I of the mitochondrial respiratpory chain	Bioenergetics	Leonid A. Sazanov	Ernesto Carafoli
298	DNA Replication Fork Bacterial	Molecular Biology	Stephen J. Benkovic	Nancy L. Craig
299	Spastic Paraplegia	Bioenergetics	Elena Rugarli	Ernesto Carafoli
300	Green Bacteria: The Light-Harvesting Chlorosome	Bioenergetics	Mette Miller	Ernesto Carafoli
301	Store operated calcium channels . 1 : STIM1	Bioenergetics	Michael Cahalan	Ernesto Carafoli
302	Renewable Hydrogen Energy from Biomass	Bioenergetics	Mike Seibert	Ernesto Carafoli
303	DNA Ligases: Mechanism and Functions	Molecular Biology	Alan Tomkinson	Nancy L. Craig
304	DNA mismatch repair in disease and ageing	Molecular Biology	Peggy Hsieh	Nancy L. Craig
305	DNA Polymerase δ Eukaryotic	Molecular Biology	Peter Burgers	Nancy L. Craig

MS code	Article	Section entry	Author	Section Editor
306	DNA Polymerase I Bacterial	Molecular Biology	Catherine Joyce	Nancy L. Craig
307	DNA Mismatch Repair: E. coli Vsr and Eukaryotic G–T Systems	Molecular Biology	Peggy Lieb	Nancy L. Craig
308	Ribozyme Structural Elements: Group I Introns	Molecular Biology	Barbara Golden	Nancy L. Craig
309	DNA Restriction and Modification: Type I Enzymes	Molecular Biology	David T. F. Dryden	Nancy L. Craig
310	DNA Polymerase III Bacterial	Molecular Biology	Hisaji Maki	Nancy L. Craig
311	Organization of the Bacterial Necleoid	Molecular Biology	Charles Dorman	Nancy L. Craig
312	DNA Polymerases: Kinetics and Mechanism	Molecular Biology	Kenneth A. Johnson	Nancy L. Craig
313	DNA Replication: Initiation in Bacteria	Molecular Biology	Jon M. Kaguni	Nancy L. Craig
314	Inositol-tris-phosphate in calcium signaling	Bioenergetics	Michael Berridge	Ernesto Carafoli
315	DNA Mismatch Repair in Mammals	Molecular Biology	Eric Alani	Nancy L. Craig
316	Alternative Splicing: Regulation of Sex Determination in Drosophila melanogaster	Molecular Biology	Paul Schedl	Nancy L. Craig
317	Peroxisome Proliferator-Activated Receptors	Signaling	Mary C Sugden	Joel Moss
318	G12/G13 Family	Signaling	Stefan Offermanns	Joel Moss
319	Processivity Clamps in DNA Replication: Clamp Loading	Molecular Biology	Michael O'Donnell	Nancy L. Craig
320	DNA Restriction and Modification: Type II Enzymes	Molecular Biology	Stephen E. Halford	Nancy L. Craig
321	Alternative Splicing	Molecular Biology	Kristen Lynch	Nancy L. Craig
322	Ras Family	Signaling	Lawrence A. Quilliam	Joel Moss
323	Nitric Oxide Signaling	Signaling	Michael A. Marletta	Joel Moss
324	Thyroid-Stimulating Hormone/Luteinizing Hormone/Follicle-Stimulating Hormone Receptors	Signaling	Deborah L. Segaloff	Joel Moss
325	B-Cell Antigen Receptor	Signaling	Thomas M. Yankee	Joel Moss

MS code	Article	Section entry	Author	Section Editor
326	Dopamine Receptors	Signaling	Kim A. Neve	Joel Moss
327	Src Family of Protein Tyrosine Kinases	Signaling	NO REVISION	Joel Moss
328	Calcitonin Receptor	Signaling	Samia I. Girgis	Joel Moss
329	G Protein-Coupled Receptor Kinases and Arrestins	Signaling	Jeffrey L. Benovic	Joel Moss
330	Photoreceptors	Signaling	King-Wai Yau	Joel Moss
331	Platelet-Activating Factor Receptor	Signaling	Katherine M. Howard	Joel Moss
332	FAK Family	Signaling	Steven K. Hanks	Joel Moss
333	Von Hippel-Lindau (VHL) Protein	Signaling	Ronald C. Conaway	Joel Moss
334	Adrenergic Receptors	Signaling	David B. Bylund	Joel Moss
335	Nuclear Factor kappaB	Signaling	Thomas D. Gilmore	Joel Moss
336	Muscarinic Acetylcholine Receptors	Signaling	Neil M. Nathanson	Joel Moss
337	Glutamate Receptors Metabotropic	Signaling	P. Jeffrey Conn	Joel Moss
338	Protein Kinase C Family	Signaling	Alexandra C. Newton	Joel Moss
339	GABAA Receptor	Signaling	Richard W. Olsen	Joel Moss
340	Serotonin Receptor Signaling	Signaling	Paul J. Gresch	Joel Moss
341	Parathyroid Hormone/Parathyroid Hormone-Related Protein Receptor	Signaling	Thomas J. Gardella	Joel Moss
342	Chemotactic Peptide/Complement Receptors	Signaling	Eric R. Prossnitz	Joel Moss
343	Cyclic GMP Phosphodiesterases	Signaling	Sharron H. Francis	Joel Moss
344	Cyclic Nucleotide-Dependent Protein Kinases	Signaling	Sharron H. Francis	Joel Moss
345	Neurotransmitter Transporters	Signaling	Kevin Erreger	Joel Moss

MS code	Article	Section entry	Author	Section Editor
346	Phospholipase C	Signaling	Fujio Sekiya	Joel Moss
347	Opioid Receptors	Signaling	P. Y. Law	Joel Moss
348	c-fes Proto-Oncogene	Signaling	Thomas E. Smithgall	Joel Moss
349	Endocannabinoids	Signaling	Daniele Piomelli	Joel Moss
350	P2Y Purinergic Receptors	Signaling	George R. Dubyak	Joel Moss
351	Emerging Concepts of Leptin	Signaling	Heike Muenzberg-Gruening	Joel Moss
352	Inositol Phosphate Kinases and Phosphatases	Signaling	Stephen B. Shears	Joel Moss
353	Cyclic Nucleotide Phosphodiesterases	Signaling	Vincent C. Manganiello	Joel Moss
354	ARF Family	Signaling	Gustavo Pacheco-Rodriguez	Joel Moss
355	Brassinosteroids	Signaling	Steven D. Clouse	Joel Moss
356	BMP signaling and Vascular Disease	Signaling	Mark de Caestecker	Joel Moss
357	DNA Replication Mitochondrial	Molecular Biology	David A. Clayton	Nancy L. Craig
358	Tumor Necrosis Factor Receptors	Signaling	Carl F. Ware	Joel Moss
359	Vascular Endothelial Growth Factor Receptors	Signaling	Kenneth A. Thomas	Joel Moss
360	Phospholipase D	Signaling	Michael A. Frohman	Joel Moss
361	Ran GTPase	Signaling	UNDER INVITE	Joel Moss
362	Mitogen-Activated Protein Kinase Family	Signaling	Silvio Gutkind	Joel Moss
363	Calcitonin Gene-Related Peptide and Adrenomedullin Receptors	Signaling	Debbie L. Hay	Joel Moss
364	Tachykinin/Substance P Receptors	Signaling	Madan M Kwatra	Joel Moss
365	Small GTPases	Signaling	Channing Der	Joel Moss

MS code	Article	Section entry	Author	Section Editor
366	Protein Tyrosine Phosphatases	Signaling	Jack Dixon	Joel Moss
367	Hematopoietin Receptors	Signaling	Barbara A. Miller	Joel Moss
369	Interferon Receptors	Signaling	NO REVISION	Joel Moss
370	p53 Protein	Signaling	Jennifer Pietenpol	Joel Moss
371	Purple Bacteria: Electron Acceptors and Donors	Bioenergetics	Roberto De Philippis	Ernesto Carafoli
372	ABC transporters : structure	Bioenergetics	André Goffeau	Ernesto Carafoli
373	Cell Death by Apoptosis and Necrosis	Bioenergetics	Pierluigi Nicotera	Ernesto Carafoli
374	Cytochrome c	Bioenergetics	NO REVISION	Ernesto Carafoli
375	Monoamine oxidase	Bioenergetics	Andrea Mattevi	Ernesto Carafoli
376	Plasma membrane sodium/calcium exchanger . 2 : structural aspects	Bioenergetics	Ken Philipson	Ernesto Carafoli
379	Intracellular Calcium Channels: NAADP+-Modulated	Bioenergetics	Luigia Santella	Ernesto Carafoli
380	Mitochondria and the NO radical	Bioenergetics	Dr Brown	Ernesto Carafoli
381	Photosystem II: Protein Components	Bioenergetics	James Barber	Ernesto Carafoli
382	Proton Pumping in the Respiratory Chain	Bioenergetics	Marten Wikstrom	Ernesto Carafoli
383	Glutathione Peroxidases	Bioenergetics	Fulvio Ursini	Ernesto Carafoli
384	Conservative site-specific recombination	Molecular Biology	Maggie Smith	Nancy L. Craig
385	Recombination: Helicases and Nucleases	Molecular Biology	Grzegorz Ira	Nancy L. Craig
386	Gi Family of Heterotrimeric G Proteins	Signaling	Maurine E. Linder	Joel Moss
387	mTOR and its downstream targets	Signaling	Christopher G. Proud	Joel Moss
388	Chromatin: Physical Organization	Molecular Biology	Christopher L. Woodcock	Nancy L. Craig

MS code	Article	Section entry	Author	Section Editor
389	Chemokine Receptors	Signaling	Ann Richmond	Joel Moss
390	Control of RNA Polymerase II Elongation in Eukaryotes	Molecular Biology	David Price	Nancy L. Craig
391	Somatostatin Receptors	Signaling	Agnes Schonbrunn	Joel Moss
392	Steroid/Thyroid Hormone Receptors	Signaling	Nancy L. Weigel	Joel Moss
393	DNA Helicases: Dimeric Enzyme Action	Molecular Biology	Timothy M. Lohman	Nancy L. Craig
394	DNA Helicases: HexamericEnzyme Action	Molecular Biology	Smita Patel	Nancy L. Craig
395	Anaplerosis	Bioenergetics	Raymond R. Russell III	Ernesto Carafoli
396	Vitamin D Receptor	Signaling	Diane R. Dowd	Joel Moss
397	Taste Receptors (possibly better title)	Signaling	John Boughter	Joel Moss
398	Proteinase-Activated Receptors	Signaling	Morley D. Hollenberg	Joel Moss
399	T-Cell Antigen Receptor	Signaling	Dario Vignali	Joel Moss
400	Ribosome Structure	Molecular Biology	Brian Wimberly	Nancy L. Craig
401	Adenylyl Cyclases	Signaling	Ron Taussig	Joel Moss
402	Natriuretic Peptides and their Receptors	Signaling	Lincoln Potter	Joel Moss
403	Fibroblast Growth Factor Receptors and Cancer-Associated Perturbations	Signaling	Marko Kornmann	Joel Moss
404	Rab Family	Signaling	Mary McCaffrey	Joel Moss
405	Neurotrophin Receptor Signaling	Signaling	Bruce Carter	Joel Moss
406	Phosphatidylinositol Bisphosphate and Trisphosphate	Signaling	NO REVISION	Joel Moss
407	Non-Homologous End Joining in Bacteria	Molecular Biology	Aidan Doherty	Nancy L. Craig
408	DNA Polymerase α Eukaryotic	Molecular Biology	Bik Tye	Nancy L. Craig

MS code	Article	Section entry	Author	Section Editor
409	Diacylglycerol Kinases and Phosphatidic Acid Phosphatases	Signaling	Matthew K. Topham	Joel Moss
410	Ribozyme Structural Elements: Groups II Introns and the Spliceosome	Molecular Biology	Christina Waldsich	Nancy L. Craig
411	Olfactory Receptors	Signaling	Sigrun Korsching	Joel Moss
411	DNA Ligases: Structures	Molecular Biology	John Pascal	Nancy L. Craig
412	Eicosanoid Receptors	Signaling	Richard M. Breyer	Joel Moss
413	Cyclic AMP Receptors of Dictyostelium	Signaling	Dale Hereld	Joel Moss
414	Reactive Oxygen and Nitrogen Species and Their Interactions With Mitochondria	Bioenergetics	Victor Darley-Usmar	Ernesto Carafoli
415	Actin-Capping and -Severing Proteins	Cell Architecture and Function	James Bamburg	P. Coulombe + C. Parent
416	Autophagy in Fungi and Mammals	Cell Architecture and Function	Dan Klionsky	P. Coulombe + C. Parent
417	Cell Cycle Controls in G1 and G0	Cell Architecture and Function	Steve Dowdy	P. Coulombe + C. Parent
418	Cell Cycle: Control of Entry and Progression Through S Phase	Cell Architecture and Function	Susan L Forsburg	P. Coulombe + C. Parent
419	Cell Cycle: DNA Damage Checkpoints	Cell Architecture and Function	Jean Wang	P. Coulombe + C. Parent
420	Cell Migration	Cell Architecture and Function	John Victor Small	P. Coulombe + C. Parent
421	Chemotaxis	Cell Architecture and Function	Carole Parent	P. Coulombe + C. Parent
422	Chromosome Organization and Structure Overview	Cell Architecture and Function	Sarah Elgin	P. Coulombe + C. Parent
423	Dynactin	Cell Architecture and Function	Trina A. Schroer	P. Coulombe + C. Parent
424	Dynein	Cell Architecture and Function	Kenneth K. Pfister	P. Coulombe + C. Parent
425	Endoplasmic Reticulum-Associated Protein Degradation	Cell Architecture and Function	Maurizio Molinari	P. Coulombe + C. Parent
426	Exosomes	Cell Architecture and Function	Stephen Gould	P. Coulombe + C. Parent
427	Heat/Stress Responses	Cell Architecture and Function	Davis Ng	P. Coulombe + C. Parent

MS code	Article	Section entry	Author	Section Editor
428	Intermediate Filament Linker Proteins: Plectin and BPAG1	Cell Architecture and Function	Gerhard Wiche	P. Coulombe + C. Parent
429	Intermediate Filaments	Cell Architecture and Function	Pierre Coulombe	P. Coulombe + C. Parent
430	Keratins and the Skin	Cell Architecture and Function	Pierre Coulombe	P. Coulombe + C. Parent
431	Kinesin Superfamily Proteins	Cell Architecture and Function	Nobutaka Hirokawa	P. Coulombe + C. Parent
432	Live Imaging of Nuclear Dynamics	Cell Architecture and Function	Karen Reddy	P. Coulombe + C. Parent
433	Major Sperm Protein and Sperm Locomotion	Cell Architecture and Function	Tom Roberts	P. Coulombe + C. Parent
434	Microtubule-Associated Proteins	Cell Architecture and Function	Nobutaka Hirokawa	P. Coulombe + C. Parent
435	Myosin Motors	Cell Architecture and Function	Roy Edward Larson	P. Coulombe + C. Parent
436	Neuronal Intermediate Filaments	Cell Architecture and Function	Ron Liem	P. Coulombe + C. Parent
437	Nuclear Pores and Nuclear Import/Export	Cell Architecture and Function	Anita Corbett	P. Coulombe + C. Parent
438	Phagocytosis and Pinocytosis	Cell Architecture and Function	Chris Janetopoulos	P. Coulombe + C. Parent
439	Rho GTPases and Actin Cytoskeleton Dynamics	Cell Architecture and Function	Anne Ridley	P. Coulombe + C. Parent
440	Tight Junctions	Cell Architecture and Function	Sachiko Tsukita	P. Coulombe + C. Parent
441	Vacuoles	Cell Architecture and Function	Scott D. Emr	P. Coulombe + C. Parent
442	Sliding Clamps in DNA Replication: E. coli β-Clamp and PCNA Structure	Molecular Biology	Linda Bloom	Nancy L. Craig
443	Friedreich's Ataxia	Bioenergetics	Anthony Schapira	Ernesto Carafoli
444	Indicators of intracellular calcium	Bioenergetics	Tullio Pozzan	Ernesto Carafoli
445	Ribosome regulation by EF-G and EF-Tu	Molecular Biology	Steven Gregory	Nancy L. Craig
446	Hydrogen production	Bioenergetics	Maria L. Ghirardi	Ernesto Carafoli
447	Integrin Signaling	Signaling	Larry Goldfinger	Joel Moss

MS code	Article	Section entry	Author	Section Editor
448	Chromatin Remodeling	Molecular Biology	Erica Hong	Nancy L. Craig
449	GABAB Receptor	Signaling	S. J. Enna	Joel Moss
451	Retinoic Acid Receptors	Signaling	Martin Petkovich	Joel Moss
452	Serine/Threonine Phosphatases	Signaling	Tom Ingebritsen	Joel Moss
453	Pheromone Receptors (Yeast)	Signaling	James Konopka	Joel Moss
454	Gq Family	Signaling	Wanling Yang	Joel Moss
455	Cyclic Nucleotide-Regulated Cation Channels	Signaling	Martin Biel	Joel Moss
456	Cytokinin	Signaling	Thomas Schmulling	Joel Moss
457	G Protein Signaling Regulators	Signaling	No Revision	Joel Moss
458	Vasopressin/Oxytocin Receptor Family	Signaling	Mike Brownstein	Joel Moss
459	Glycogen Synthase Kinase-3	Signaling	Jim Woodgett	Joel Moss
460	Retinoblastoma Protein (pRB)	Signaling	Nick Dyson	Joel Moss
461	Cadherin Signaling	Signaling	David B. Sacks	Joel Moss
462	Sphingolipid Catabolism	Lipids Carbohydrates Membranes and Membrane Proteins	Jim Shayman	William Lennarz
463	Glycine Receptors	Signaling	Bodo Laube	Joel Moss
464	Immunoglobulin (Fc) Receptors	Signaling	P. Mark Hogarth	Joel Moss
465	Lysophospholipid Receptors	Signaling	Gabor Tigyi	Joel Moss
466	26S Proteasome Structure and Function	Cell Architecture and Function	Friedrich Förster	P. Coulombe + C. Parent
467	Actin Organization	Cell Architecture and Function	Tatyana Svitkina	P. Coulombe + C. Parent
468	Actin-Related Proteins	Cell Architecture and Function	Dyche Mullins	P. Coulombe + C. Parent

MS code	Article	Section entry	Author	Section Editor
469	Bax and Bcl2 Cell Death Enhancers and Inhibitors	Cell Architecture and Function	David Vaux	P. Coulombe + C. Parent
470	Cell Cycle: Mitotic Checkpoint	Cell Architecture and Function	Tim Yen	P. Coulombe + C. Parent
471	Centromeres	Cell Architecture and Function	Beth Sullivan	P. Coulombe + C. Parent
472	Desmosomes and Hemidesmosomes	Cell Architecture and Function	Kathleen Green	P. Coulombe + C. Parent
473	Focal Adhesions	Cell Architecture and Function	Benny Geiger	P. Coulombe + C. Parent
474	Meiosis	Cell Architecture and Function	Neil Hunter	P. Coulombe + C. Parent
475	Metalloproteinases Matrix	Cell Architecture and Function	Gillian Murphy	P. Coulombe + C. Parent
476	Nuclear Compartmentalization	Cell Architecture and Function	Jeanne Lawrence	P. Coulombe + C. Parent
477	Nuclear Envelope and Lamins	Cell Architecture and Function	Bryce M. Paschal	P. Coulombe + C. Parent
478	Septins and Cytokinesis	Cell Architecture and Function	Christine Field	P. Coulombe + C. Parent
479	Tubulin and its Isoforms	Cell Architecture and Function	Eva Nogales	P. Coulombe + C. Parent
480	Unfolded Protein Responses	Cell Architecture and Function	David Ron	P. Coulombe + C. Parent
481	Translation Elongation in Bacteria	Molecular Biology	Scott C. Blanchard	Nancy L. Craig
482	Translation Initiation in Eukaryotes: Factors and Mechanisms	Molecular Biology	Christopher Hellen	Nancy L. Craig
483	Siglecs	Lipids Carbohydrates Membranes and Membrane Proteins	Ajit Varki	William Lennarz
484	Prostaglandins and Leukotrienes	Lipids Carbohydrates Membranes and Membrane Proteins	William Smith	William Lennarz
485	Flippases	Lipids Carbohydrates Membranes and Membrane Proteins	Charles Waechter	William Lennarz
486	UmuC D Lesion Bypass DNA Polymerase V	Molecular Biology	Penny Beuning	Nancy L. Craig
487	RNA Polymerase II and Basal Transcription Factors in Eukaryotes	Molecular Biology	Jeff Corden	Nancy L. Craig
488	Melanocortin System	Signaling	Roger D. Cone	Joel Moss

MS code	Article	Section entry	Author	Section Editor
489	Angiotensin Receptors	Signaling	NO REVISION	Joel Moss
490	Bradykinin Receptors	Signaling	Ronald Burch	Joel Moss
491	Nucleotide Excision Repair in Eukaryotes	Molecular Biology	Dr. Goosen	Nancy L. Craig
492	Phosphoinositide 4- and 5-Kinases and Phosphatases	Signaling	Shawn F. Bairstow	Joel Moss
493	Calcium/Calmodulin-Dependent Protein Kinases	Signaling	Alfred Robison	Joel Moss
494	Platelet-Derived Growth Factor Receptor Family	Signaling	No Revision	Joel Moss
495	3D Migration	Cell Architecture and Function	Patricia Keely	P. Coulombe + C. Parent
496	Actin Assembly/Disassembly	Cell Architecture and Function	Henry N. Higgs	P. Coulombe + C. Parent
497	Cadherin-Mediated Cell–Cell Adhesion	Cell Architecture and Function	W. James Nelson	P. Coulombe + C. Parent
498	Caspases and Cell Death	Cell Architecture and Function	Gerry Melino	P. Coulombe + C. Parent
499	Cytokinesis	Cell Architecture and Function	Douglas Robinson	P. Coulombe + C. Parent
500	Cytoskeletal motors: general principles	Cell Architecture and Function	Ronald S. Rock, Jr.	P. Coulombe + C. Parent
501	GAP Junctions	Cell Architecture and Function	Bruce J Nicholson	P. Coulombe + C. Parent
502	Mitosis	Cell Architecture and Function	Pat Wadsworth	P. Coulombe + C. Parent
503	Peroxisomes	Cell Architecture and Function	Suresh Subramani	P. Coulombe + C. Parent
504	Toll-Like Receptors	Signaling	Himanshu Kumar	Joel Moss
505	Kinesins as Microtubule Disassembly Enzymes	Cell Architecture and Function	Ryoma Ohi	P. Coulombe + C. Parent
506	Phosphoinositide-Dependent Protein Kinases	Signaling	No Revision	Joel Moss
507	Secretory Pathway	Lipids Carbohydrates Membranes and Membrane Proteins	Karen Colley	William Lennarz
508	Nonhomologous Recombination: Retrotransposons	Molecular Biology	Suzanne Sandmeyer	Nancy L. Craig

MS code	Article	Section entry	Author	Section Editor
509	Recombination: Strand Transferases	Molecular Biology	Wolf-Dietrich Heyer	Nancy L. Craig
510	Phospholipid Metabolism in Mammals	Lipids Carbohydrates Membranes and Membrane Proteins	Dennis R. Voelker	William Lennarz
511	N-Linked Glycan Processing Glucosidases and Mannosidases	Lipids Carbohydrates Membranes and Membrane Proteins	Tadashi Suzuki	William Lennarz
512	Oligosaccharide Chains: Free N-Linked O-Linked	Lipids Carbohydrates Membranes and Membrane Proteins	Tadashi Suzuki	William Lennarz
513	Phospholipid Synthesis in Yeast	Lipids Carbohydrates Membranes and Membrane Proteins	George Carman	William Lennarz
514	Protein Glycosylation Inhibitors	Lipids Carbohydrates Membranes and Membrane Proteins	UNDER INVITE	William Lennarz
515	Sphingolipid Biosynthesis	Lipids Carbohydrates Membranes and Membrane Proteins	Alfred Merrill	William Lennarz
517	tRNA Synthetases	Molecular Biology	Rebecca Alexander	Nancy L. Craig
518	DNA Replication: Eukaryotic Origins and the Origin Recognition Complex	Molecular Biology	Igor Chesnokov	Nancy L. Craig
519	A-Kinase Anchoring Proteins	Signaling	UNDER INVITE	Joel Moss
520	Abscisic Acid (ABA)	Signaling	UNDER INVITE	Joel Moss
521	Adenosine Receptors	Signaling	UNDER INVITE	Joel Moss
522	Cytokines	Signaling	UNDER INVITE	Joel Moss
523	Epidermal Growth Factor Receptor Family	Signaling	UNDER INVITE	Joel Moss
524	Fatty Acid Receptors	Signaling	UNDER INVITE	Joel Moss
525	Glucagon Family of Peptides and their Receptors	Signaling	UNDER INVITE	Joel Moss
526	Glutamate Receptors Ionotropic	Signaling	UNDER INVITE	Joel Moss
527	Gs Family of Heterotrimeric G Proteins	Signaling	UNDER INVITE	Joel Moss
528	Hepatocyte Growth Factor/Scatter Factor Receptor	Signaling	UNDER INVITE	Joel Moss
529	Histamine Receptors	Signaling	UNDER INVITE	Joel Moss

MS code	Article	Section entry	Author	Section Editor
530	Insulin Receptor Family	Signaling	UNDER INVITE	Joel Moss
531	JAK-STAT Signaling Paradigm	Signaling	UNDER INVITE	Joel Moss
532	Neuropeptide Y Receptors	Signaling	UNDER INVITE	Joel Moss
533	Neurotensin Receptors	Signaling	UNDER INVITE	Joel Moss
534	Nicotinic Acetylcholine Receptors	Signaling	UNDER INVITE	Joel Moss
535	P2X Purinergic Receptors	Signaling	UNDER INVITE	Joel Moss
536	Phosphoinositide 3-Kinase	Signaling	UNDER INVITE	Joel Moss
537	Phospholipase A2	Signaling	UNDER INVITE	Joel Moss
538	Plant Signaling Peptides	Signaling	UNDER INVITE	Joel Moss
539	Protein Kinase B	Signaling	UNDER INVITE	Joel Moss
540	Syk Family of Protein Tyrosine Kinases	Signaling	UNDER INVITE	Joel Moss
541	Tec/Btk Family Tyrosine Kinases	Signaling	UNDER INVITE	Joel Moss
548	Biochemistry of bone formation/turnover	Metabolism Vitamins and Hormones	UNDER INVITE	M. Daniel Lane
549	Biochemistry of development: Bone	Metabolism Vitamins and Hormones	UNDER INVITE	M. Daniel Lane
571	Graves disease	Metabolism Vitamins and Hormones	UNDER INVITE	M. Daniel Lane
580	Glucose/Sugar Transport in Bacteria	Metabolism Vitamins and Hormones	Ronald Kaback	M. Daniel Lane
608	Tricarboxylic Acid Cycle	Metabolism Vitamins and Hormones	NO REVISION	M. Daniel Lane
610	Urea Cycle Inborn Defects of	Metabolism Vitamins and Hormones	UNDER INVITE	M. Daniel Lane
612	Vitamin K: Blood Coagulation and Use in Therapy	Metabolism Vitamins and Hormones	UNDER INVITE	M. Daniel Lane
618	DNA Base Excision Repair	Molecular Biology	Bruce Demple	Nancy L. Craig

MS code	Article	Section entry	Author	Section Editor
621	DNA Secondary Structure	Molecular Biology	Albino Bacolla	Nancy L. Craig
623	MicroRNA's in Eukaryotes	Molecular Biology	UNDER INVITE	Nancy L. Craig
624	mRNA Polyadenylation in Eukaryotes	Molecular Biology	UNDER INVITE	Nancy L. Craig
627	RNA Processing in Eukaryotes	Molecular Biology	Jo Ann Wise	Nancy L. Craig
628	Micro RNA's	Molecular Biology	UNDER INVITE	Nancy L. Craig
629	RNA Polymerase Reaction in Bacteria	Molecular Biology	UNDER INVITE	Nancy L. Craig
630	RNA splicing	Molecular Biology	UNDER INVITE	Nancy L. Craig
631	Small RNAs in Bacteria	Molecular Biology	John van der Oost	Nancy L. Craig
632	Transcription Termination	Molecular Biology	Tom Santangelo	Nancy L. Craig
633	Eukaryotic Protein Biosynthesis: The Elongation Cycle	Molecular Biology	Anton A. Komar	Nancy L. Craig
634	Genome-Wide Analysis of Gene Expression	Molecular Biology	UNDER INVITE	Nancy L. Craig
638	Lipoproteins HDL/LDL	Lipids Carbohydrates Membranes and Membrane Proteins	UNDER INVITE	William Lennarz
639	Membrane Fusion	Lipids Carbohydrates Membranes and Membrane Proteins	UNDER INVITE	William Lennarz
670	Mucin Family of Glycoproteins	Lipids Carbohydrates Membranes and Membrane Proteins	Tony Hollingsworth	William Lennarz
671	Polysialic Acid	Lipids Carbohydrates Membranes and Membrane Proteins	UNDER INVITE	William Lennarz
672	Detergent Properties	Lipids Carbohydrates Membranes and Membrane Proteins	Darrell McCaslin	William Lennarz
673	Golgi Complex	Cell Architecture and Function	Mark Stamnes	P. Coulombe + C. Parent

19-Dec-2011

Dear Dr. Butala,

Re: Manuscript MMI-2011-11926

Thank you again for submitting your manuscript "Double-locking of the Escherichia coli colicin K gene promoter by two repressors prevents premature cell lysis after DNA damage" for publication in Molecular Microbiology.

The reviewers appreciate the topic, and they generally feel convinced that IscR is a regulator. It is less clear to them (and to me) that IscR is solely responsible for the colK transcription delay. I am also left wondering how IscR levels are being controlled, since your model points to those levels as being the ultimate determinant of expression. Please see the comments from the reviewers and myself, which are appended below.

If you can respond to all of the referees' points - by making the requested changes or by providing a compelling argument why a change cannot or should not be made - then I encourage you to submit a revised manuscript. Please note that multiple revisions are rarely permitted and acceptance of your revised manuscript is not guaranteed. In general, revised manuscripts should be returned within three months. If you anticipate that significantly more time will be needed, please let me know.

To resubmit, log into Molecular Microbiology's Electronic Editorial Office, enter the Author Centre, enter Manuscripts with Decisions, click on the manuscript link, and upload the following:

1. A Supplemental File in which you have copy-pasted and responded to the editor's and referees' comments point-by-point. This file must be in Word format.

2. A single file containing the revised Text, Figure legends, and Tables. This file should not contain any Figures. This file must be in Word format.

3. Single, high-resolution files for each Figure. These files must be in TIFF or EPS format.

4. (optional) Supplementary material for online presentation in a single PDF file. Movies and other material that cannot be converted to PDF should be in separate files. Please indicate in the text where the supplementary material is cited (Fig. S1, Table S1, etc.). There are no color charges for supplementary material.

5. (optional) Authors are encouraged to submit a proposed journal front cover illustration. Submissions should be high resolution (600 dpi) image files (e.g. TIFF) and should be accompanied by a short description of up to 30 words. Authors who provide an image chosen for the cover will be eligible for free colour art work in a subsequent research paper.

IT IS IMPORTANT TO SUBMIT THESE MATERIALS IN THE REQUIRED FORMATS. FAILURE TO COMPLY WILL DELAY THE PROCESSING OF YOUR MANUSCRIPT.

Citation of a personal communication must be pre-authorized by the concerned party. Use or adaptation of a previously published figure must be pre-authorized by the copyright holder.

If substantial linguistic changes were recommended, you may wish to consult a language service (http://www.blackwellpublishing.com/bauthor/english_language.asp). If you use a language service, please send them the final version of the manuscript and avoid making further changes thereafter.

Please let me know if you have any questions or if I can assist you in any way. We look forward to hearing from you again as soon as possible.

Best wishes,

Jim Imlay

[Editor's comments]
1. Even in the iscR mutant one sees a delay in colK expression (e.g., Fig. 2B). If lexA were the sole remaining regulator, why isn't the gene induced in the manner of sulA (Fig. 1)?
2. Is the deactivation of IscR control driven specifically by something that nalidixic acid does to IscR level or activity, or is its deactivation simply driven by a decline of cellular nutritional status or growth rate? In your experiments the induction of colK occurred roughly commensurate with entry into stationary phase. It is not clear whether this reflects a cause-effect relationship or whether the timing was adventitious. To check: Add Nal, but maintain the cells in a nutritionally rich environment by periodic subculturing (e.g., not allowing cell density to exceed 0.4 OD). Is colK induction affected? Does IscR continue to repress?

This is an important point, because the overarching notion that is articulated in the Abstract is that IscR will stop repressing if the DNA damage is overwhelming. Yet the body of the paper seems to imply that IscR status reflects how well-fed the cell is, not whether DNA damage is irreparable.

Conversely, one might ask whether nutritional starvation by itself depletes IscR titer enough that colK expression becomes somewhat activated even in the absence of DNA damage. Indeed, one might make that case from the no-NAL control in Fig. 1. Use of a lexA3 mutation might enable one to verify that the low-but-significant induction is not due to DNA damage.
3. I would flatly assert that the holo- and apo-IscR overproduction experiments do not demonstrate that both forms of the protein can repress transcription. When you overexpress any Fe/S protein, a substantial fraction exists in the apo-protein form, both because there is necessarily a delay between translation and Fe/S insertion, and because overproduction can overtax the Isc system. On top of that, overproduction of IscR has the additional effect of shutting down the transcription of the genes that encode the Isc assembly system--so that accumulation of apo-protein is inevitable. Therefore, while the genetic experiment does demonstrate that apo-IscR can repress the gene, it does not demonstrate that holo-IscR can do the same thing. One could approach this question by measuring binding constants in vitro, as reviewer 3 suggests. To do so one must build Fe/S clusters in IscR (which is not hard, using purified IscS--we could provide reagent enzyme if you want to attempt this).

I think this uncertainty shines a light on an important point: Why was IscR chosen to control colK expression? The most obvious possibility is to

link expression to Fe/S status in some way. It seems less likely that the system is built to detect a modest (3-fold) decline in IscR as a way of sensing a slow-down in protein synthesis. Do you have any thoughts about this?

Comments to Author from Referees:
Referee: 1
REPORT FOR TRANSMISSION TO AUTHORS
In contrast to many LexA-controlled SOS genes such as sulA which is induced immediately after treatment with DNA-damaging antibiotics (eg, nalidixic acid), another LexA-controlled cka gene that encodes colicin K is delayed in induction. In this work, the authors found that an additional regulator IscR represses the cka gene, and proposes that the decrease in the amount of IscR is the reason for delayed induction.

To prove repression by IscR through direct binding, they showed that mutation of putative IscR binding site caused similar effect as iscR deletion in elevating cka-lacZ expression. Through SPR analysis in vitro, the authors showed that the IscR-binding affinity decreased more than 10-fold by the binding site mutations. The IscR binding was proposed to be independent of the presence of Fe-S, on the basis of similar repression effect between the wild type and the constitutive apo-mutant. They observed decrease in the amount of IscR protein when cells entered stationary phase, and proposed that this is the mechanism behind the delayed induction of cka gene after nalidixic acid treatment.

This is an interesting finding that adds a new function to IscR, which induces its target genes at later phases of growth, possibly through reduction in its amount. It is convincing that IscR functions as a repressor in controlling the cka gene. However, there are several observations that are not well explained and hence needs to be better resolved.

Major points.

1. The mechanism behind delayed induction.
The behavior of DiscR in derepressing cka-lacZ expression upon SOS induction (Fig. 2B) is puzzling, since it still shows some delay in induction as in the wild type. If the amount of IscR is all that matters to enhance cka gene expression when LexA is inactivated rapidly (by nalidixic acid), why is the full induction of cka has to wait until the stationary phase in the absence of IscR? It appears that there still exists another controlling factor that depends on the growth phase. The delayed induction is again observed when the cis-acting binding site mutants were examined (Fig. 3C, p-44G, p-28C mutants). This phenomenon has to be explained and investigated.

It has been previously reported that the stationary phase induction of cka depends on ppGpp and IHF (Kuhar and Zfur-Bertok, 1999). What would the relationship between ppGpp and IscR regulation? What about IHF?

2. Dependence of IscR system on SOS induction.
Even though IscR was fished out by using LexA-bound DNA, it seems to function independently of SOS response. What would be the induction pattern of cka in DiscR mutant in normal growth without nalidixic acid treatment? How would the expression profile look like in comparison with the SOS-induction data in Fig. 2B?

2. Effect of p-12C (“-10” promoter element mutant) in Fig. 5.
First of all, it is not clear why the authors used p-12C mutant as a genetic background in all constructs examined. This needs be explained.
When -10 promoter box is mutated, would the transcription initiation site be changed?
What would the effect of nalidixic acid in p-12C background?
Explanation for UP3 mutation is lacking.

Has +1 site ever been determined for cka gene even in the wild type? If not, it is better to be determined experimentally, to verify that the promoter elements and their mutations mean as they are called.

3. Considering many factors that affect cka gene expression, the two repressor model for SOS induction appears too simple. Since IscR repression seems independent of LexA repression, incorporation of IscR in the model for cka gene regulation needs not necessarily be confined in the context of SOS response. The model pathway in Fig. 6B needs be elaborated by including other factors that affect cka gene regulation.

Minor points
Fig. 6. How many experiments were done to get the average numbers?
Fig. 7. (B) The method for quantifying the increased amount of colicin in DiscR mutant needs be explained.
Page 4, line 4, and page 16, Fig. 1; trigerring -- triggering?

Referee: 2
REPORT FOR TRANSMISSION TO AUTHORS
In the manuscript entitled “Double-locking of the E. coli colicin K gene promoter by two repressors prevents premature cell lysis after DNA damage” enlightens the colicin K expression control, describing its regulation by the IscR regulator. Moreover the authors also describe the presence of the IscR binding sites in the promoter region of other colicins, showing that it may be a widespread control mechanism to delay the colicin expression after SOS induction. The results described in the manuscript are interesting and enhance the knowledge about the SOS response and its relationship with other genetic networks and regulators that permit to adjust precisely the gene expression. Nevertheless I have some concerns about the results showed in this manuscript, some controls are missing and sometimes there are discordances between the results presented by the authors. So I think that all these problems must be solved.

Major concerns:
1. The authors detect the proteins that are involved in colicin C regulation using the cka promoter region attached to streptavidin Dynabeads. After crude extract addition and washing, the authors compare the bands observed using beads without DNA with those containing the Pcka – LexA promorter-protein complex.
Why do the authors use the Pcka associated with LexA protein? Will the same bands appear if LexA was not already associated to the promoter? May the presence of LexA interfere with the attachment of other proteins by competence? In fact, the authors added SOS induced crude extract, so RecA* was present and would activate the auto-hydrolysis of LexA, also those that were

bound to the Pcka promoter. Why do they use the Pcka associated with LexA protein?
On the other hand, in the text, the authors say that they ignored the proteins with less than 20% identity but also “the ones that were previously shown not to regulate pcka”, but the references that support this idea are not stated either in the text or in supplementary material. In the list there are some hypothetical proteins that may be regulators and they are not studied. Why do the authors choose some and some other not? If previous works discard those proteins they must be cited.
2. In Fig. 4 it is shown that the presence of an “empty” plasmid it generates great differences with respect the same strain without the plasmid. Have the authors any explanation of this fact? On the other hand, the Fig. 4 results showed that the strain with the “empty” plasmid has not only a decrease in its expression level but also a delay on it. So, is it really comparable the expression of the sulA fusion and the cka fusion in Fig 1? Actually, the sulA::lacZ fusion is not in a plasmid as cka, but in the chromosome of the E. coli strain. I’m not questioning the delay of the colicin induction (that is fully described), but perhaps the experiment performed here is not the more appropriated to show the delay since the strains used are not isogenic and do not contain the same copies of the lacZ fusion. For instance, quantitative RT-PCR experiments measuring sulA and cka mRNA levels may be suitable in this case to determine the induction moment of each promoter after inducer addition.

3. A major concern is the discordance between results showed in the manuscript. Apparently there are some lacZ fusions that are used in different experiments. For instance, the wtpRW50cka is used in the experiments that are shown in Fig 1, 2 and 3. The beta-galactosidase assays are performed in these three experiments following the same strategy: the SOS inducer was added when the cultures grew up to OD 0.2-0.3, and the betagalactosidase activity was measured several times after the induction. In all case the same amount of inducer was used (37uM NAL). And also in all cases the results are shown with ±SEM. But when one looks carefully to each Figure realizes there are great differences between the results obtained in each experiment. See below:

Betagalactosidase Enzimatic units for wt pRW50cka.

Fig1: 2h post-induction : 200 U
3h PI: about 1400U
4h PI: about 1800U

Fig 2: 2h post-induction : less than 100 U
3h PI: about 750U
4h PI: about 750U

Fig 3B: 2h post-induction : less than 100 U
3h PI: about 400U
4h PI: about 400U

Fig 3C: 2h post-induction : about 200 U
3h PI: about 1500U
4h PI: about 1700U

The less betagalactosidase activity registered in Fig. 3B may be caused by the addition of arabinose. But apparently Fig 1, 2 and 3C are exactly the same experiment using different mutants. Differences between Fig 1 and Fig2 wt

pRW50cka results could be attributed to the different strains used (JCB387 pRW50cka and BW25113 pRW50cka, respectively), but in Fig 3C results are similar to Fig 1 and the strain used in this case was BW25113 the same that is used in Fig 2 so the problem must not be the strain. How the authors can explain that? Why this difference is not seen in the SEM that represents 3 different experiments? Why the authors change the strain between the experiments? Are the other fusion results also so variable? The differences are not negligible since in most cases they would reduce the differences observed in the analyzed mutants.

4. It would be interesting the relationship between IscR and LexA protein. Are both proteins bind together to the Pcka? Is there a competence for the Pcka Promoter region? Could an excess of IscR avoid the LexA binding?

5. The authors describe that either apo-IscR or holo-IscR are able to block the Pcka since no induction of Pcka expression is observed when iscR or iscR-CTM complement the DiscR mutation. Nevertheless the iscR expression levels in the complemented strain have to be high, since they are controlled by PBAD promoter, so great amount of each protein are present, more than in a wild type strain producing IscR. Do the apo-IscR and holo-IscR proteins present the same affinity for the promoter region of cka? EMSAs or SPR analysis will be suitable to determine this.

6. Finally, the authors describe a model for the delayed expression of Pcka: Basically, when SOS system is induced, the IscR retains the cka expression. If the DNA damage is released, then LexA blocks again the cka expression even when the cell is on stationary phase. If the DNA damage persists, when the nutrients decrease, the levels of IscR will go down and so, the cka expression will be no longer blocked and the cell will die. What has it happen if a sulA strain was used in these experiments? It is described that the OD increase in a cell with activated SOS response is due to filamentation that is responsible of sulA gene, which product interacts with FtsZ protein avoiding the cell division. Are cells with an activated SOS response in stationary metabolic state?

Minor concerns:

1. At the end of the results, there is an incomplete sentence: “ In contrast, only a small difference in colicinA production was detected, which could be due to additional posttranscriptional”, the reviewer assumes that the authors do mean, posttranscriptional control.
2. I think it will be easier for the reader that the graphics where performed using post induction time.
3. In M&M, the secondary antibody of the western blot, once anti-RecA is added as primary antibody is missing.
4. Legend of Fig.6. It is not MG1655 the strain that is used in this experiment, it is PK10016, isn’t it?
5. Table S1 must be cited just after “…delay in induction of the cka gene promoter (pcka)” not at the end of the sentence since Table S1 has not expression results, only contains the description of the promoters.
6. MG1655 is not cited in the Table S1.

Referee: 3
REPORT FOR TRANSMISSION TO AUTHORS

The current dogma surrounding the release of colicins involves induction of the SOS response in response to DNA damage that causes the RecA-mediated cleavage of LexA that de-represses colicin transcription leading to the synthesis of colicin. Colicins are released into the environment through induction of a lysis gene leading to the production of a phospholipase that permeabilizes the outer membrane, culminating in cell death. Induction of the lysis gene is often coupled to that of the colicin gene and synthesis of both occurs concurrently but previous studies have shown that this induction may be delayed following SOS induction.

The work of Butala et al in this paper has reinvestigated the SOS induction of colicin K, a pore forming colicin, and reported a second repressor called IscR binding to a region upstream of the SOS promoter that is involved in delayed expression of Colicin K following DNA damage. They highlight a region of the promoter that has palindromic symmetry that is involved in binding of IscR leading to a 'double locking' of the colK promoter that is responsible for the delayed expression of the colicin following DNA damage.

The experimental approach is logical and largely convincing. The inferences are novel and despite some sloppy spelling mistakes the paper is well written, and should be considered for publication in Molecular Microbiology.

However, I have some issues that the authors should consider for revision:

1) Colicin release in response to DNA damage is dependent on the lysis gene. The authors recognize the role of cell lysis in colicin release but do not associate this with the induction of the lysis gene. There is a range of data published in the 80's that report on the organization of the colicin operons, and provide evidence on the role of the lysis gene in colicin release in different systems. Depending on the organization of the ColK operon (ie. relationships between cka and ckl) and despite the data in Fig. 1, would the authors consider that repression of cell lysis by IscR might be repression of ckl, and that the newly identified binding region be a promoter for induction of ckl? There is evidence that cells expressing colicin Ia produce large amounts of colicin that is only released on cell lysis.

2) Both holo and apo IscR appear to regulate cka. This is somewhat surprising as it might seem that loss of the Fe-S cluster might affect folding of IscR and be important for binding the DNA. The authors should repeat their SPR experiments using apo-enzyme to show that loss of the Fe-S cluster does not affect binding to the ligand, or check the relative protein structures using CD spectroscopy.

3) The SPR experiments appear convincing but the response units are arbitrary and do not provide any indication as to the strength of the interaction. I would have liked to have seen an affinity binding constant (Kd) to allow a proper comparison of the binding of wt fragment with mutated DNA fragments. Also the data for wt DNA in Fig. 4C is identical to the 1 mM IscR sample in Fig. 4B. Was the data in Figs. 4B and C obtained from the same experiment?

4) The predicted target for IscR binding has homology to the consensus sequences and the authors showed the importance of two residues within this region by b-gal assays and SPR. I was surprised that mutating just a single residue had such a dramatic effect on IscR binding, but they obtained similar results for both p-44G and p-28C. I would have mutated one or two more residues over the remainder of the consensus, and indeed one outside to confirm the effect. Alternatively they could consider adding the IscR repressor binding site to the promoter of the sulA-lacZ fusion reporter in ENZ1257 to confirm that there is sufficient delay of expression of b-galactosidase by this construct.

5) Colicin is expressed spontaneously in a small percentage <5% of naturally occurring colicin producing cells. Does IscR have any role in the

production of colicin by these colicinogenic cells when grown in the absence of an SOS inducing agent?
6) I would be a little reluctant to state similar inferences between ColN and colicins K and E1 when discussing the induction of these colicins by NA. Colicin production in wt and deletion iscR in response to NA is not that dissimilar in ColN unlike the same data with ColK and E1 induction!
7) There is no effect of IscR on ColA induction despite there being a strong candidate IscR binding site with palindromic symmetry similar to ColK. Is there any difference in the ColA operon that differs from ColK and allows any speculation on these differences?
Minor:
Pg 3 Remove 'Recall that…'
Numerous spelling mistakes:
Pg 5 upstream
Pg 6 line 7, fragment; line 13 repression; line 22 below
Pg7 line 10 sentence not completed, 'additional posttranscriptional….'? Factors?
Pg 8 line21, maintain
Pg11, diluted
Pg11, line21, Is 'injected' the correct word here, would aliquoted or added be more suitable?
Pg13 line 1 throughout; line2, harvested not harvested Pg 16 fig 1 legend 'triggering'
Supplementary information
Fig. S2., dilution
Pg 10 line 5. Built by model….? Sentence in complete?
Pg10 line 17 collection, line24, sub-inhibitory

molecular microbiology

Double-locking of the *Escherichia coli* colicin K gene promoter by two repressors prevents premature cell lysis after DNA damage

Journal:	*Molecular Microbiology*
Manuscript ID:	MMI-2011-11926
Manuscript Type:	Research Article
Date Submitted by the Author:	10-Nov-2011
Complete List of Authors:	Butala, Matej; University of Ljubljana, Department of Biology Browning, Douglas; UB, Biosciences Sonjak, Silva; University of Ljubljana, Department of Biology Hodošček, Milan; National Institute of Chemistry, Laboratory for Molecular Modeling Žgur-Bertok, Darja; University of Ljubljana, Department of Biology Busby, Steve; University Of Birmingham, School Of Biosciences
Key Words:	Colicins, DNA damage, Induction of gene expression, LexA regulon, Transcription factor IscR

for Molecular Microbiology *14th November, 2011*

Double-locking of the *Escherichia coli* colicin K gene promoter by two repressors prevents premature cell lysis after DNA damage

Matej Butala[1*], Douglas F. Browning[2], Silva Sonjak[1], Milan Hodošček[3], Darja Žgur-Bertok[1], Stephen J. W. Busby[2]

[1]Department of Biology, Biotechnical Faculty, University of Ljubljana, 1000 Ljubljana, Slovenia,

[2]School of Biosciences, University of Birmingham, Birmingham B15 2TT, U.K.,

[3]National Institute of Chemistry, 1000 Ljubljana, Slovenia

*Corresponding author:

Matej Butala:

Phone: +386 1 320 3397;

Fax: +386 1 257 33 90;

e-mail: matej.butala@bf.uni-lj.si

RUNNING TITLE

IscR mediates delayed induction of colicin genes

SUMMARY

The synthesis of *Eschericha coli* colicins is lethal to the producing cell and is repressed during normal growth by the LexA transcription factor, which is the master repressor of the SOS system for repair of DNA damage. Following DNA damage, LexA is inactivated and SOS repair genes are induced immediately, but colicin production is delayed and induced only in terminally damaged cells. The cause of this delay is unknown. Here we identify the global transcription repressor, IscR, as being directly responsible for the delay in colicin K expression during the SOS response and identify the DNA target for IscR at the colicin K operon promoter. Hence, this promoter is 'double locked' to ensure that suicidal colicin K production is switched on only as a last resort.

KEYWORDS

Colicins; DNA damage / Induction of gene expression / LexA regulon / Transcription factor IscR

INTRODUCTION

The bacterial SOS response enables cells to deal with DNA damage and associated stresses. The response is controlled by the LexA global transcription factor that represses transcription of dozens of SOS genes that are involved in coping with and repairing DNA damage (Courcelle *et al.*, 2001, Wade *et al.*, 2005). In response to DNA damage, RecA polymerizes onto exposed single-stranded DNA, creating the active helical nucleoprotein filament (RecA*), which mediates cleavage of LexA (Little, 1991), and instigates repressor dissociation from its DNA targets and induction of the LexA regulon (Butala *et al.*, 2011).

In *Escherichia coli* and related bacteria, where the SOS response has been most studied, it has been found that the LexA regulon includes many genes encoding colicins (Ebina *et al.*, 1982, Lloubes *et al.*, 1986). Recall that colicins are toxic suicide proteins that kill other bacteria by a single-hit mode of action, targeting either cell walls, DNA or RNA (Kleanthous, 2010, Cascales *et al.*, 2007). In *Escherichia coli*, most colicins are encoded by plasmids and transcribed from strong promoters whose activity is firmly repressed by LexA, and hence colicin expression is triggered by agents that induce the SOS response (Cascales *et al.*, 2007, Ebina *et al.*, 1982). Most LexA-repressed promoters are induced immediately upon DNA damage (Courcelle *et al.*, 2001) but induction of the majority of colicin genes is delayed and triggered only upon severe and persistent DNA damage (Salles *et al.*, 1987, Herschman and Helinski, 1967). This makes sense as colicins play no role in DNA repair, but rather, the producer cell dies as they are released, and their role appears to be to assist surviving cells by killing potential competitors (Majeed *et al.*, 2011). It has been postulated that the lag period in colicin production after SOS induction provides cells with time for damage repair before induction of the lethal colicin (Salles *et al.*, 1987), but the cause of the delay is unknown.

In previous work, we established how LexA represses the promoter of the *E. coli cka* gene that encodes colicin K, a pore-forming toxin that kills susceptible cells by collapsing the membrane potential (Jerman *et al.*, 2005, Kuhar and Zgur-Bertok, 1999, Mulec *et al.*, 2003). Here, we have studied the timing of *cka* transcription after SOS induction and we report that the IscR global transcription repressor is directly responsible for delaying *cka* expression. We show that the *cka* promoter is 'double locked' to ensure tight and timed regulation of colicin K expression and that induction is triggered by the decrease in IscR levels that occurs as cell growth slows.

RESULTS

Delayed induction of the colicin K gene during the SOS response

When growing *E. coli* cells are treated with DNA damaging agents, initially, LexA regulon genes are induced that relieve DNA damage, arrest cell-division and enhance adaptation through mutagenesis (Courcelle *et al.*, 2001). Consistent with several published studies of colicin induction (Salles *et al.*, 1987, Herschman and Helinski, 1967), after trigerring the SOS response with nalidixic acid, we observed a pronounced delay in induction of the the *cka* gene promoter (p*cka*), compared with expression of the *sulA* LexA-regulon gene (Fig. 1, Table S1). We previously showed that LexA represses p*cka* by binding to tandem DNA sites for LexA located downstream from the -10 promoter element (Mrak *et al.*, 2007). Results in Fig. S1 show that LexA can both block RNA polymerase binding at p*cka* and displace pre-bound polymerase, but this cannot explain the observed kinetics of p*cka* induction. Thus, we searched for another regulator by using affinity chromatography methods, using a DNA fragment containing p*cka* in complex with LexA as bait and cleared SOS-induced cell extracts (see Experimental Procedures). After elution of bound proteins and analysis by SDS-polyacrylamide gel electophoresis (Fig. 2A) and mass spectroscopy, we identified the nucleoid associated factor H-NS and the transcription regulators NsrR, Lrp, GlcC, UlaR, DeoR, IscR, and LexA as factors that had associated with the bait (Table S2). Since LexA was expected, and H-NS, NsrR, UlaR and Lrp were previously shown not to be involved in the *cka* regulation (Kuhar and Zgur-Bertok, 1999; unpublished observations), we focussed on GlcC, DeoR and IscR and assayed *cka* promoter activity following SOS induction from a p*cka-lacZ* fusion in the corresponding deletion mutant strains from the Keio collection (Baba *et al.*, 2006). The results show little effects of the *glcC* and *deoR* deletions, but disruption of

iscR resulted in induced p*cka* activity immediately after addition of sub-inhibitory concentration of nalidixic acid (Fig. 2B), indicating that IscR represses expression from p*cka*.

IscR regulates *cka* expression

The IscR (iron-sulfur cluster regulator) protein, was originally identified as a transcription repressor that regulates genes involved in the formation and the repair of iron-sulfur clusters in proteins (Schwartz *et al.*, 2001). It has homologues in eukaryotes which sustain fundamental life processes (Lill and Muhlenhoff, 2005), IscR exists in two forms, holo IscR that contains an Fe-S cluster, and apo IscR, which is formed upon destruction of the Fe-S cluster, for example, in response to oxidative stress. It is now known that certain targets require holo IscR for repression, whilst the majority of targets are repressed by both forms (Nesbit *et al.*, 2009).

To determine directly whether IscR can bind to the *cka* regulatory region (Fig. 3A) and restore repression of p*cka* in the Δ*iscR* strain, we complemented the latter strain with a plasmid encoding an arabinose-inducible IscR or an IscR mutant locked in the apo- form due to alanine substitutions of the cysteine Fe-S cluster ligands (IscR-CTM) (Wu and Outten, 2009). With the highest concentration of L-arabinose that had a minimal effect on cell growth, both wild-type IscR and IscR-CTM complemented the *iscR* deletion and strongly repressed p*cka* in spite of DNA damage (Fig. 3B). Thus we conclude that both apo- and holo-IscR can repress p*cka*, and inspection of the base sequence identified a perfect palindrome, overlapping the -35 promoter element (Fig. 3A), that corresponds well to the established consensus sequence (Nesbit *et al.*, 2009). To dissect the nucleotides required for the IscR-dependent repression, we modified the two most critical nucleotides in the predicted site (Fig. 3A): the base at position 44 upsteram of the p*cka* transcript start (p-44C to G) and the symmetric modification at position 28 (p-28G to C). Results illustrated in Fig. 3C show that the

mutations have similar effects on the expression of p*cka* as the *iscR* deletion, strongly suggesting that the palindrome is the target for IscR binding.

Next we purified IscR protein and performed surface plasmon resonance (SPR) analysis directly to measure IscR binding at p*cka* using the DNA fragments illustrated in Fig. 4A. Our results show that IscR interacts with the chip-immobilized DNA fragment in a concentration dependent manner (Fig. 4B). Association of IscR with the DNA fragment harbouring mutation p-44G was decreased by ~10-fold in comparison to the wild-type *cka* fregment, and the affinity of IscR for the DNA fragment harbouring both the p-44G and the p-28C mutations was neglible (Fig. 4C).

To measure the effects of oxidative stress on IscR-dependent repression of p*cka in vivo*, we used a *cka* promoter variant with mutated LexA operators (pRW50UP3) unable to bind LexA specifically (Mrak *et al.*, 2007) and the p-12C substitution in the promoter -10 element (Fig. 3A). Results illustrated in Fig. 5 show that IscR represses p*cka* and that this represion is unaffected by oxidative stress from hydrogen peroxide.

Since our data indicate that p*cka* is repressed by both holo- and apo-IscR, we considered that relief of IscR-dependent repression could be due to changes in IscR levels. Thus, we used western blotting to determine intracellular concentrations of IscR during normal growth or during the SOS response in *E. coli* MG1655 strain expressing the FLAG-tagged IscR from the native *iscR* promoter. A 3-fold decrease of the IscR level was observed when cells entered into the late exponential phase and early stationary phase after early exponential growth (Fig. 6A). This suggest that *cka* transcription in SOS induced cells is induced when concentrations of IscR fall bellow a threshold level (Fig. 6B).

IscR controls the expression of different colicins

To investigate the effects of IscR on the expression of other colicins, we introduced the Δ*iscR* allele into strains that produce the pore forming colicins K, E1, A and N. Following SOS induction of the colicinogenic cultures, cell growth and colicin production was compared in the starting strains and the Δ*iscR* mutants. We observed that IscR confers viability to the most of the tested strains (Fig. 7A). Crude cell extracts were prepared from cultures before and after SOS induction and colicin levels were compared by bioassays (Fig. 7B) or by SDS-PAGE (Fig. 7C). The results show that nalidixic acid induces an immediate increase in colicin K, E1 and N levels in the Δ*iscR* strains in comparison to the delayed colicin production in the wild-type strains. In contrast, only a small difference in colicin A production was detected, which coud be due to additional posttranscriptional (Yang *et al.*, 2010). Colicin promoter regions were sequenced and alignment of these sequences (Fig. 7D) revealed SOS boxes and IscR binding sites present in the same organisation and location.

DISCUSSION

Many *E. coli* strains carry plasmids which encode colicins that are expressed in response to extreme stress conditions (Cascales *et al.*, 2007). Colicin production by a bacterial cell is suicidal and it is thought that this is an example of bacterial altruism (Majeed *et al.*, 2011). Thus, in response to extreme stress, a small proportion of the population of a strain sacrifice themselves and produce colicin toxins that kill susceptible competitor strains. Clearly then, colicin synthesis needs to be tightly regulated and it is well known that transcription of most *E. coli* colicins is repressed by the LexA global repressor that coordinates the SOS response to DNA damage. This is understandable since colicins have evolved as a last resort emergency response, but this creates the problem of how to uncouple the induction of colicin expression from temporal induction of the SOS response to deal with repairable DNA damage. Our work

with p*cka* shows that the solution to this is a second repressor, IscR, that binds to a target that overlaps the -35 element. Hence p*cka* is double locked. Interestingly, such double locking of promoters is rare in *E. coli* and appears to be reserved for gene products whose ectopic expression would be harmful, the best characterised examples being the silencing of certain plasmid-encoded genes (Bingle and Thomas, 2001).

Previous studies identified IscR as a regulator of the expression of gene products involved in the synthesis or repair of Fe-S proteins (Tokumoto and Takahashi, 2001, Schwartz *et al.*, 2001). IscR exists in two states apo-IscR and holo-IscR which contains an Fe-S cluster (Schwartz *et al.*, 2001). For some targets, the ability of IscR to repress is dependent on the Fe-S cluster. This is the case for the *iscR* promoter itself and hence IscR levels vary greatly depending on the oxidation status of the cell (Nesbit *et al.*, 2009). For most targets, both apo- and holo-IscR bind and repress transcription, and regulation appears to be due to changes in the cellular concentration of IscR. Our data suggest that this is the case for IscR binding at the *cka* promoter. It was previously shown that *cka* and colcin E1 gene are induced due to lack of nutrients and not by an inducer released from the surrounding cells (Eraso *et al.*, 1996, Kuhar and Zgur-Bertok, 1999). Thus, IscR levels remain high until nutrients become depleted upon entry into stationary phase, and hence, in metabolically active cells in the absence of DNA damage, colicin K synthesis is carefully locked by the IscR and LexA. However, following a prolonged SOS response, when nutrients are depleted and metabolism slows, colicin synthesis is turned on and defective cells are eradicated. This may be in order to donate nutrients to related neighbors or to maintenan a low mutation rate in a microbial community.

To conclude, here we have shown that IscR has a role in programmed bacterial cell death, which is part of the developmental process in a number of bacterial species (Lewis, 2000).

Our data show that IscR affects the expression of many colicin operons by carefully orchestrating colicin gene induction folloing the SOS response.

EXPERIMENTAL PROCEDURES

The following materials and methods are described in the Supplementary Experimental Procedures: plasmids and promoter constructs, computer modeling, β-galactosidase assay and electromobility shift assays.

Proteins

E. coli RNA polymerase holoenzyme containing σ^{70} was purchased from Epicentre Technologies (Madison). The LexA protein was overexpressed and purified as described (Butala *et al.*, 2011). The MH1 strain and the pQ-ORF2-95 plasmid to overexpress the IscR protein were donated by Yonesaki T. The IscR protein was expressed as described (Otsuka *et al.*, 2010) and isolated to >95% purity by the Ni-NTA affinity cromatography and stored at -20°C in 20 mM Tris-HCl (pH 8.0), 0.1 mM NaCl, 0.5 mM EDTA, 40% glycerol, 0.2% Triton-X. Concentrations of the LexA and IscR repressor were determined using NanoDrop1000 (Thermo SCIENTIFIC) and the extinction coefficients of 6990 M^{-1} cm^{-1} and of 9970 M^{-1} cm^{-1} at 280 nm, respectively.

DNA affinity purification

E. coli JCB387 harboring the pRW50*cka* plasmid (0.5 l) were induced with 8.5 μg/ml nalidixic acid when the OD_{600} reached 0.5, and after 45 min, cells were harvested and cell extracts prepared as described (Butala *et al.*, 2009). Biotinylated ~180 bp *cka* promoter fragments were generated by PCR using primers Pull_F, Pull_R and pRW50*cka* as a template and purified by GeneJET PCR purification kit (Fermentas), was attached to 2.5 mg of M-280

streptavidin Dynabeads (Invitrogen) according to the manufacter's instructions. In binding buffer (20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA) 60 µg LexA repressor was bound to 50 µg of DNA immobilized to the magnetic beads and excess LexA was washed off in wash buffer (20 mM Hepes-Na (pH 7.4), 100 mM NaCl, 0.1% (v/v) Tween 20). Binding reactions were performed in binding buffer (20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA) containing: beads either with or without the immobilized *cka* promoter-LexA complex. Dynabeads were collected with a magnet and washed four times with wash buffer. Proteins were eluted from the DNA with buffer containing 800 mM NaCl, and concentrated by TCA precipitation. Proteins were resolved on a 12% SDS-PAGE gel (Invitrogen), 1 mm gel slices were excised and analysed by the Birmingham Functional Genomics and Proteomics Unit (http://www.genomics.bham.ac.uk/) using a Thermo-Finnigan LTQ Orbitrap mass spectrometer. Three protein bands specific for the *cka* promoter-LexA complex were recovered from the high stringency 0.8 M NaCl eluate. These bands, that corresponded to molecular weights of approximately 15 kDa, 19 kDa and 35 kDa (Fig. 2A), were recovered and analysed. We ignored candidate proteins with less than 20% identity and selected those that exhibited DNA binding properties but ignored the ones that were previously shown not to regulate p*cka* (Table S2).

Surface plasmon resonance assays

SPR measurements were performed on a Biacore X (GE Healthcare) at 25°C. The streptavidin (SA) sensor chip (GE Healthcare) was equilibrated with buffer containing SPR_1 buffer containing 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 0.005% surfactant P20 (GE Healthcare). Approximately 100 response units (RU) of 3`-biotinylated S1 primer was immobilized on the flow cells of the SA chip. To prepare double stranded DNA with the predicted IscR operator or its two mutant derivates, complementary primers IscR_F and

IscR_R or IscRm_F and IscRm_R or IscR2m_F and IscR2m_R (Table S1) in 20 mM Tris-HCl (pH 7.5), 0.1 mM NaCl were mixed in 1:1.5 (mol:mol) ratio, respectively. Primers were annealed in temperature gradient from 94°C to room temperature (~1.5 h) in PCR machine (Eppendorf). So prepared 31-bp duplex DNA with a 15 nucleotide overhang complementary to the streptavidin chip-immobilized S1 primer was passed for 2 min at 2 µl/min across the flow cell 1 to immobilize ~90 RU of either IscR operator DNA fragment or its derivates. The interaction between the IscR repressor and the chip-immobilized DNAs was studied by injecting solutions of the desired concentration of the IscR in 20 mM Tris-HCl (pH 8.0), 50 mM KCl, 4 mM $MgCl_2$, 0.5 mM EDTA, 5 mM DTT, 0.005% surfactant P20 at 100 µl/min for 1 min. Dissociation was followed for 2 min. The DNA-sensor chip surface was regenerated by injecting buffer containing 20 mM Tris-HCl (pH 8.0), 500 mM NaCl and the hibridised DNA fragments separated by 50 mM NaOH. SPR experiments were performed at the Infrastructural Centre for Surface Plasmon Resonance, University of Ljubljana.

Colicin production assays

Colicin synthesis was monitored in the wild-type or the Δ*iscR* strain harboring naturally occuring colicinogenic plasmids by a colicin production assay as described previously (Jerman *et al.*, 2005). Cultures of colicin-producing strains were grown in LB broth supplemented with ampicillin (100 µg/ml) with aeration at 37°C. Samples were collected just before nalidixic acid treatment at an OD_{600} of 0.3 and 1, 2 and 3 hours after induction. Cells were dilluted in LB broth to the OD_{600} of 0.3 to make a milliliter of the sample and crude colicin extracts were prepared by sonication (Sonics VCX750) at 40% power for 30 second on ice. Subsequently, 100 µl of the crude extracts were injected into wells in an LBTc plate overlayed with 4 ml of soft agar with 40 µl of the indicator strain DH5α harbouring pBR322 (laboratory stock). For an estimation of colicin production ratio among the strains, a tenfold

dilution series of crude colicin extracts were prepared and 5 µl samples were spotted on the LBAp plate overlayed with the indicator strain. To determine the the ratio of colicin production in wt or Δ*iscR* strain, the sizes of the colicin produced lysis zones were compared and dillution of the cell lysates were taken into account. The remaining crude colicin K extracts were TCA precipitated and protein bands resolved on the 12% SDS-PAGE gel (Invitrogen) and visualized as described above. Experiments were performed in duplicate. Colicin promoter regions were sequenced with primers used previously (Kamensek *et al.*, 2010).

Western blot analysis

The PK10016 strain (*iscR*-FLAG) harbouring the pRW50*cka* was grown in LB broth supplemented with Tc (12.5 µg/ml) with aeration at 37°C. Samples were collected at an OD_{600} of 0.3 and after 0.5, 1.0, 1.5, 2.5 and 4.0 hours of growth in normal or SOS induced conditions. DNA damage was elicted with 8.5 µg/ml nalidixic acid at an OD_{600} of 0.3, where relevant. Samples were equilibrated to an OD_{600} of 0.6 to detect protein levels in equal number of cells during bacterial growth. Cell pellets were resuspended in 10 µl NuPAGE LDS sample buffer, 10 µl of DTT and 20 µl of dH_2O and heated (95°C, 5min) before loading equal ammount of the samples on a 12% SDS-PAGE gel (Invitrogen). For blotting, proteins were transferred to polyvinylidene difluoride membranes (Millipore), blocked in 4% bovine serum albumin at room temperature. Primarly the proteins were stained with monoclonal mouse anti-flag M2 antibody (Sigma-Aldrich) and secondary antibodies conjugated by horseradish peroxidase. The same membrane was re-stained by primary anti-RecA antibody (Anti-RAD51 polyclonal antibody, Thermo Scientific). Antibodies were used at a concentration of 0.5 µg/ml. Bands were stained using 4-chloro-1-naphtol/H_2O_2. The resolved bands were quantified using a G:Box (Syngene). The integrated optical densities of the

IscR-FLAG or the RecA protein were determined. The IscR levels throught the growth were compared and are presented as the ratio of the density value for the sample harested at time indicated as 0h relative to the density value obtained from the samples harvested later in the bacterial growth. Experiments were performed in duplicate.

ACKNOWLEDGMENTS

We would like to thank Dr. Tetsuro Yonesaki for the MH1 strain and the pQ-ORF2-95 plasmid, Dr. F. Wayne Outten and Yun Wu for the plasmids pFWO, p*iscR* and p*iscR*-CTM and Dr. Patricia J. Kiley and Erin Mettert for the *iscR*-FLAG strain. This work was supported by the Slovenian Research Agency [Z1-2142 to M.B., J4-2111 to D.Ž.B.] and the UK BBSRC.

REFERENCES

Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K.A., Tomita, M., Wanner, B.L., and Mori, H. (2006) Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol Syst Biol* **2**: 2006 0008.

Bingle, L.E., and Thomas, C.M. (2001) Regulatory circuits for plasmid survival. *Curr Opin Microbiol* **4**: 194-200.

Butala, M., Busby, S.J., and Lee, D.J. (2009) DNA sampling: a method for probing protein binding at specific loci on bacterial chromosomes. *Nucleic Acids Res* **37**: e37.

Butala, M., Klose, D., Hodnik, V., Rems, A., Podlesek, Z., Klare, J.P., *et al.* (2011) Interconversion between bound and free conformations of LexA orchestrates the bacterial SOS response. *Nucleic Acids Res* **39**: 6546-6557.

Cascales, E., Buchanan, S.K., Duche, D., Kleanthous, C., Lloubes, R., Postle, K., Riley, M., Slatin, S., and Cavard, D. (2007) Colicin biology. *Microbiol Mol Biol Rev* **71**: 158-229.

Courcelle, J., Khodursky, A., Peter, B., Brown, P.O., and Hanawalt, P.C. (2001) Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*. *Genetics* **158**: 41-64.

Ebina, Y., Kishi, F., and Nakazawa, A. (1982) Direct participation of *lexA* protein in repression of colicin E1 synthesis. *J Bacteriol* **150**: 1479-1481.

Eraso, J.M., Chidambaram, M., and Weinstock, G.M. (1996) Increased production of colicin E1 in stationary phase. *J Bacteriol* **178**: 1928-1935.

Herschman, H.R., and Helinski, D.R. (1967) Comparative study of the events associated with colicin induction. *J Bacteriol* **94**: 691-699.

Jerman, B., Butala, M., and Zgur-Bertok, D. (2005) Sublethal concentrations of ciprofloxacin induce bacteriocin synthesis in *Escherichia coli*. *Antimicrob Agents Chemother* **49**: 3087-3090.

Kamensek, S., Podlesek, Z., Gillor, O., and Zgur-Bertok, D. (2010) Genes regulated by the Escherichia coli SOS repressor LexA exhibit heterogeneous expression. *BMC Microbiol* **10**: 283.

Kleanthous, C. (2010) Swimming against the tide: progress and challenges in our understanding of colicin translocation. *Nat Rev Microbiol* **8**: 843-848.

Kuhar, I., and Zgur-Bertok, D. (1999) Transcription regulation of the colicin K *cka* gene reveals induction of colicin synthesis by differential responses to environmental signals. *J Bacteriol* **181**: 7373-7380.

Lewis, K. (2000) Programmed death in bacteria. *Microbiol Mol Biol Rev* **64**: 503-514.

Lill, R., and Muhlenhoff, U. (2005) Iron-sulfur-protein biogenesis in eukaryotes. *Trends Biochem Sci* **30**: 133-141.

Little, J.W. (1991) Mechanism of specific LexA cleavage: autodigestion and the role of RecA coprotease. *Biochimie* **73**: 411-421.

Lloubes, R., Baty D., and Lazdunski, C. (1986) The promoters of the genes for colicin production, release and immunity in the ColA plasmid: effects of convergent transcription and LexA protein. *Nucleic Acids Res* **14**: 2621-2636.

Majeed, H., Gillor, O., Kerr, B., and Riley, M.A. (2011) Competitive interactions in *Escherichia coli* populations: the role of bacteriocins. *ISME J* **5**: 71-81.

Mrak, P., Podlesek, Z., Putten, J.P.V., and Zgur-Bertok, D. (2007) Heterogeneity in expression of the *Escherichia coli* colicin K activity gene *cka* is controlled by the SOS system and stochastic factors. *Mol Genet Genomics* **277**: 391-401.

Mulec, J., Podlesek, Z., Mrak, P., Kopitar, A., Ihan, A., and Zgur-Bertok, D. (2003) A *cka-gfp* transcriptional fusion reveals that the colicin K activity gene is induced in only 3 percent of the population. *J Bacteriol* **185**: 654-659.

Nesbit, A.D., Giel, J.L., Rose, J.C., and Kiley, P.J. (2009) Sequence-specific binding to a subset of IscR-regulated promoters does not require IscR Fe-S cluster ligation. *J Mol Biol* **387**: 28-41.

Otsuka, Y., Miki, K., Koga, M., Katayama, N., Morimoto, W., Takahashi Y., and Yonesaki, T. (2010) IscR regulates RNase LS activity by repressing *rnlA* transcription. *Genetics* **185**: 823-830.

Salles, B., Weisemann, J.M., and Weinstock, G.M. (1987) Temporal control of colicin E1 induction. *J Bacteriol* **169**: 5028-5034.

Schwartz, C.J., Giel, J.L., Patschkowski, T., Luther, C., Ruzicka, F.J., Beinert, H., and Kiley, P.J. (2001) IscR, an Fe-S cluster-containing transcription factor, represses expression

of *Escherichia coli* genes encoding Fe-S cluster assembly proteins. *Proc Natl Acad Sci USA* **98**: 14895-14900.

Tokumoto, U., and Takahashi, Y. (2001) Genetic analysis of the isc operon in *Escherichia coli* involved in the biogenesis of cellular iron-sulfur proteins. *J Biochem* **130**: 63-71.

Wade, J.T., Reppas, N.B., Church, G.M., and Struhl, K. (2005) Genomic analysis of LexA binding reveals the permissive nature of the *Escherichia coli* genome and identifies unconventional target sites. *Genes Dev* **19**: 2619-2630.

Wu, Y., and Outten, F.W. (2009) IscR controls iron-dependent biofilm formation in *Escherichia coli* by regulating type I fimbria expression. *J Bacteriol* **191**: 1248-1257.

Yang, T.Y., Sung, Y.M., Lei, G.S., Romeo, T., and Chak, K.F. (2010) Posttranscriptional repression of the cel gene of the ColE7 operon by the RNA-binding protein CsrA of *Escherichia coli*. *Nucleic Acids Res* **38**: 3936-3951.

FIGURE LEGENDS

Fig. 1. Delayed induction of the *cka* gene promoter after trigerring the SOS response. Measured β-galactosidase activities (full lines) of JCB387 carrying pRW50*cka*, with a *cka-lacZ* transcriptional fusion, and of strain ENZ1257 harboring a *sulA-lacZ* fusion, as indicated. Each value represents the mean ± SEM of at least three independent measurements, the arrow indicates the time of addition of nalidixic acid (NAL) where relevant and the dashed lines represent optical density measured at 600 nm.

Fig. 2. LexA and IscR regulate induction of the *cka* gene promoter. (A) Coomassie stained protein profile of flow through (FT), protein standards (M), denatured beads and LexA (lane 1) and eluates from the control (lane 2) or p*cka* affinity chromatography (lane 3). Proteins in three gel slices (denoted by boxes) were trypsin digested and analyzed by mass spectrometry. Proteins in the bands marked A, B and C were identified as DeoR, GlcC, UlaR; Lrp and IscR; H-NS and NsrR, respectively. (B) Expression of the *cka-lacZ* fusion either in wild type BW25113 (wt), or in the Δ*deoR*, Δ*glcC* or Δ*iscR* mutants. Each value represents the mean ± SEM of at least three independent measurements, the arrow indicates the time of addition of nalidixic acid (NAL) and the dashed lines represent optical density measured at 600 nm.

Fig. 3. Role of IscR in regulating *cka* expression. (A) Regulatory elements of the *cka* promoter region. The boxes indicate the predicted palindromic target for IscR binding which corresponds to the consensus (Nesbit *et al.*, 2009). The promoter -10 and -35 elements are in bold type, and the SOS box targets for LexA, the Shine Dalgarno sequence (S.D.) and the translation start site (*cka*) are also indicated. Positions of the site-directed mutations described in the paper are indicated above the sequence. (B) Expression of the *cka* promoter in strain BW25113 (wt) or the Δ*iscR* mutant derivative complemented with holo-IscR (p*iscR*) or apo-IscR (p*iscR*-CTM). Empty parent vector pFWO2 was used as a control plasmid. L-arabinose was added at the time of inoculation and the arrow indicates the time of addition of nalidixic acid (NAL). For panels B and C each value is the average ± SEM of at least triplicate experiments and the optical density measured at 600 nm is shown as dashed lines. (C) Measured β-galactosidase activities in BW25113 (wt) or the Δ*iscR* mutant carrying either the p*cka*, p*cka* p-44G or p*cka* p-28C fragments subcloned into pRW50. The arrow indicates the time of addition of NAL as indicated.

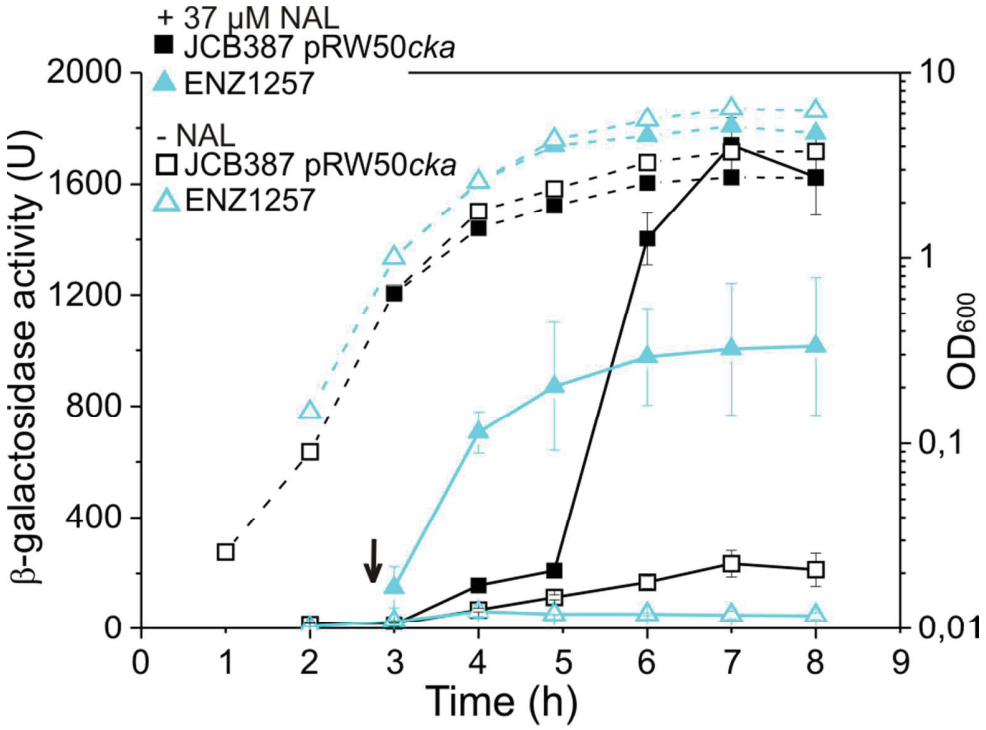
Fig. 4. IscR interacts with the *cka* regulatory region. (A) Biotinylated DNA fragments used in the SPR analysis. The DNA linker by which fragments were attached to the chip surface is indicated in blue font, the palindromic sequence in red and the point mutations in green. (B) SPR sensorgrams of interactions of IscR (62 nM to 1 μM) with chip-immobilized wt DNA fragment. (C) Sensorgram of 1 μM IscR interacting with either wt DNA fragments or derivatives with mutations in the predicted IscR site.

Fig. 5. Regulation of IscR activity. Measured β-galactosidase activities in BW25113 (wt) or Δ*iscR* cells carrying pRW50*cka* with the p-12C mutation in p*cka*, with or without the UP3 substitutions that stop LexA binding (see Fig. 3A). The arrow indicates the time of addition of 0.2 mM H_2O_2, where relevant. Each value is the average ± SEM of at least triplicate experiments and the optical density measured at 600 nm is shown as dashed lines.

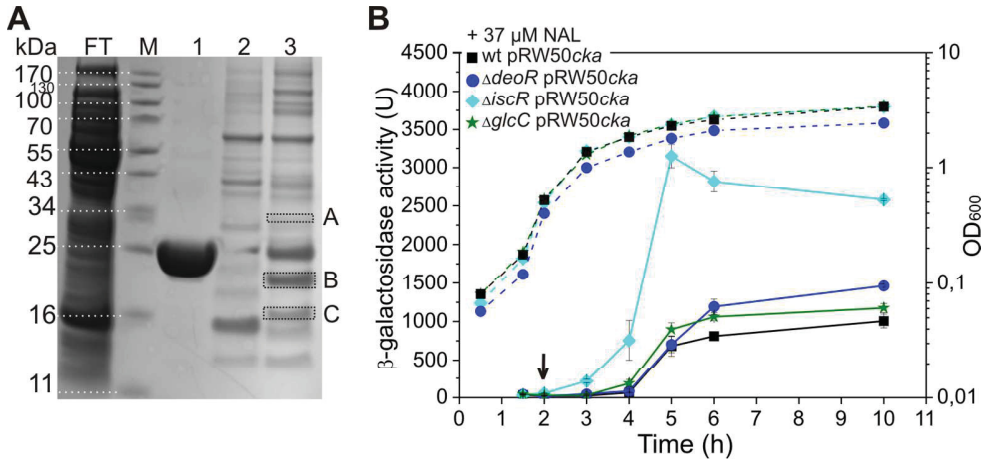
Fig.6. IscR levels decrease as cell growth slows. (A) Western blot analysis of the growth phase-dependent variation in the levels of RecA and FLAG-tagged IscR at various growth phases in MG1655. Samples were taken at time intervals before or after induction of the SOS reponse with nalidixic acid (NAL) at OD_{600} 0.3 (0h), as indicated, or during normal growth. Cells entered the stationary phase of growth after 2.5 h. Purified RecA$(His)_6$ (0.18 μg) loaded in the last lane was used as a control. Quantitation of proteins is presented below the gels as the ratio (%) of the protein density value of the initial sample (0h) relative to the density value obtained from the samples harvested throught the bacterial growth, shown with the standard deviation. (B) Model for the delayed expression of p*cka*. During normal growth, LexA and IscR bind and repress transcription from p*cka*. Upon DNA damage, e.g. caused by antibiotics,

SOS DNA repair commences due to the decrease in intracellular LexA concentrations, but IscR levels are not affected. p*cka* becomes de-repressed after long-lasting DNA damage due to decreased IscR levels as cell growth ceases.

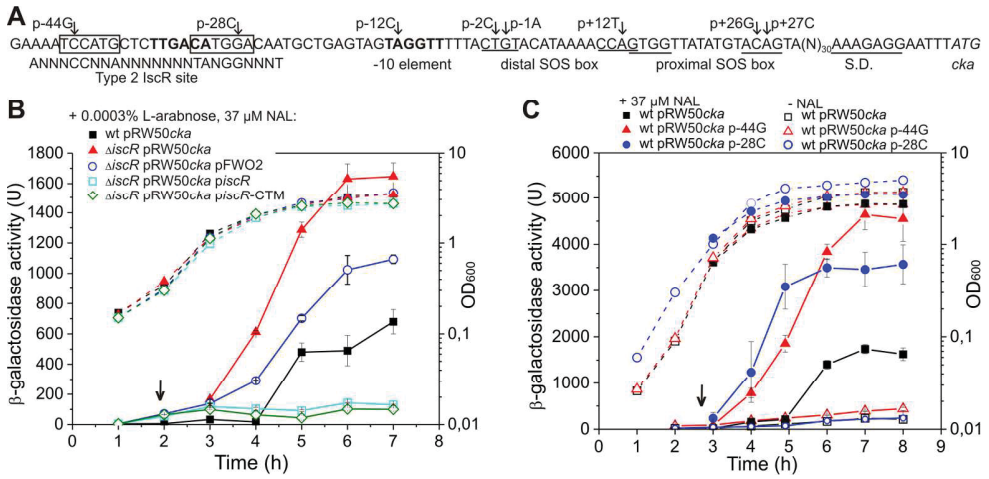
Fig. 7. IscR protein manages temporal induction of different colicins. (A) Growth curve of BW25113 (wt) or Δ*iscR* cells harboring naturally occurring plasmids encoding pore forming colicins either K (pColK), E1 (pColE1), A (pColA) or N (pColN). The arrow indicates the time of addition of nalidixic acid (NAL), each value is the average ± SEM of duplicate experiments. (B) Assays of colicin production in cells harboring colicin-encoding plasmids. Equal amounts of cells were collected at hourly time points from the time of addition of nalidixic acid (0 h) and cell extracts were placed into wells in an LBTc plate overlaid with soft agar harboring an indicator strain. Numbers below the lysis zones indicate the fold increase of colicin production in the Δ*iscR* strain compared to the wild type strain at the same time point, as determined from the dilution of crude colicin extracts (Fig. S2). Experiments were performed in duplicate. (C) SDS-PAGE analysis of total cell extracts of BW25113 or Δ*iscR* cells carrying pColK. The arrow indicates the position of colicin K as determined in comparison to the size of the purified $(His)_6$-tagged colicin K. (D) The sequence alignments highlight regulatory elements in colicin gene promoter regions, annotated as in Fig. 3A, and the predicted IscR sites are marked with dashed boxes.



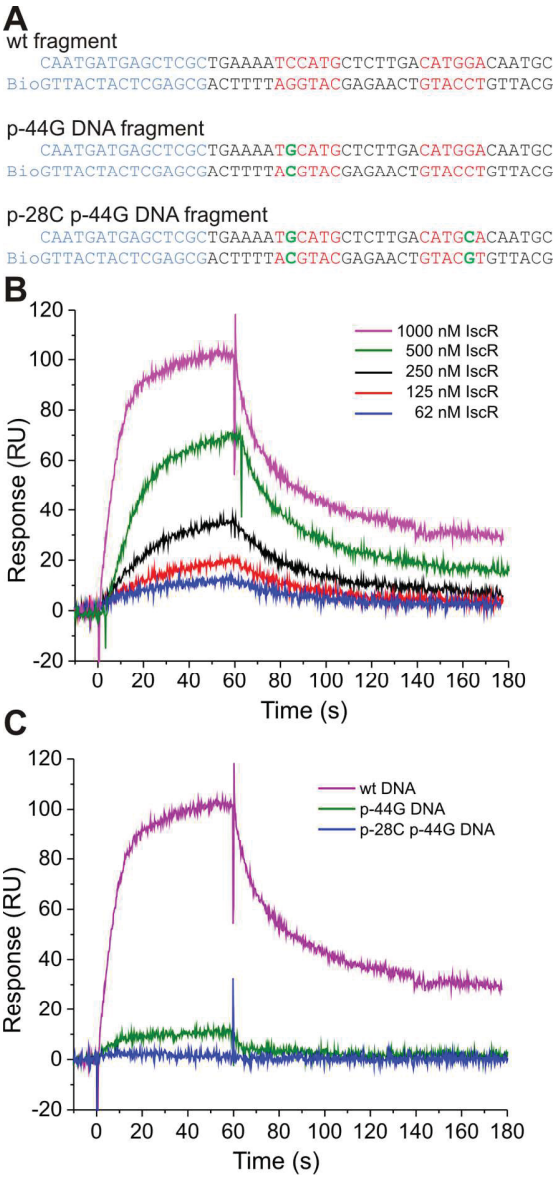
79x59mm (300 x 300 DPI)



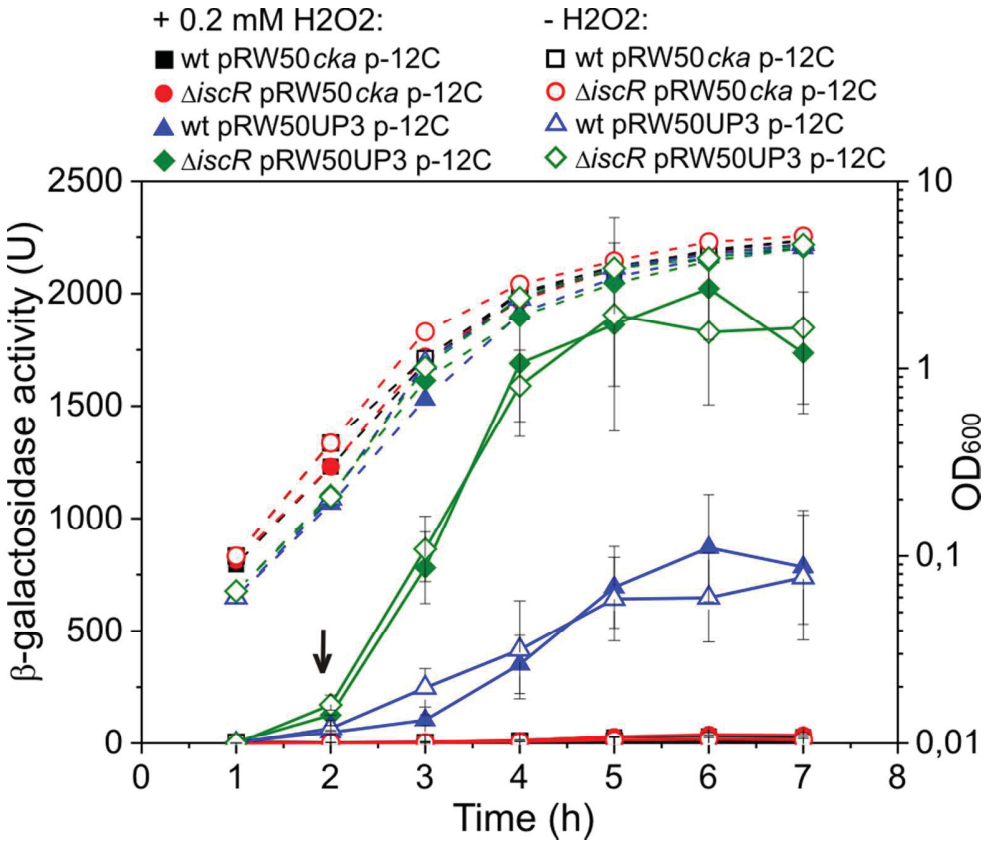
139x65mm (300 x 300 DPI)



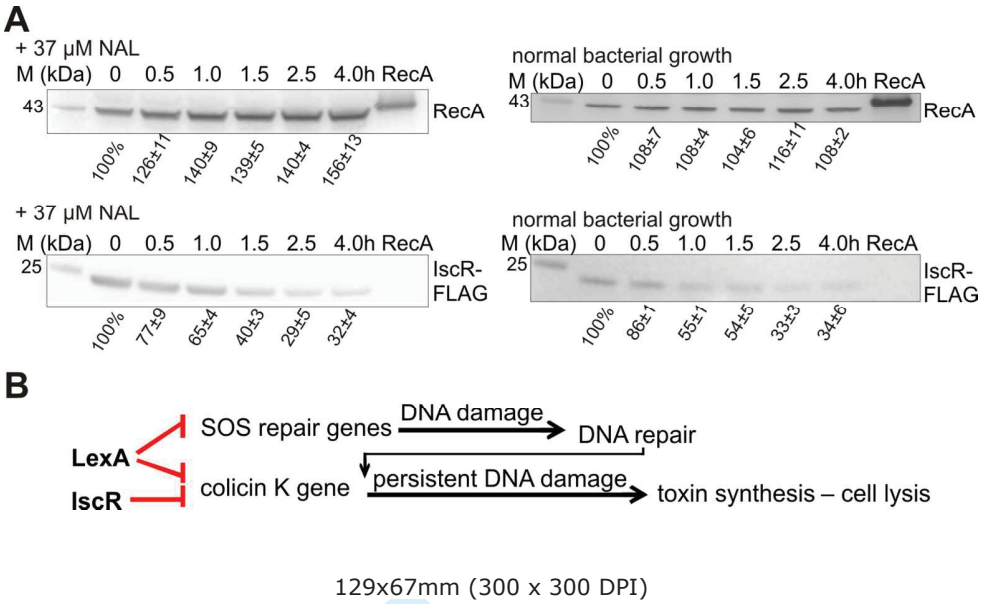
168x81mm (300 x 300 DPI)



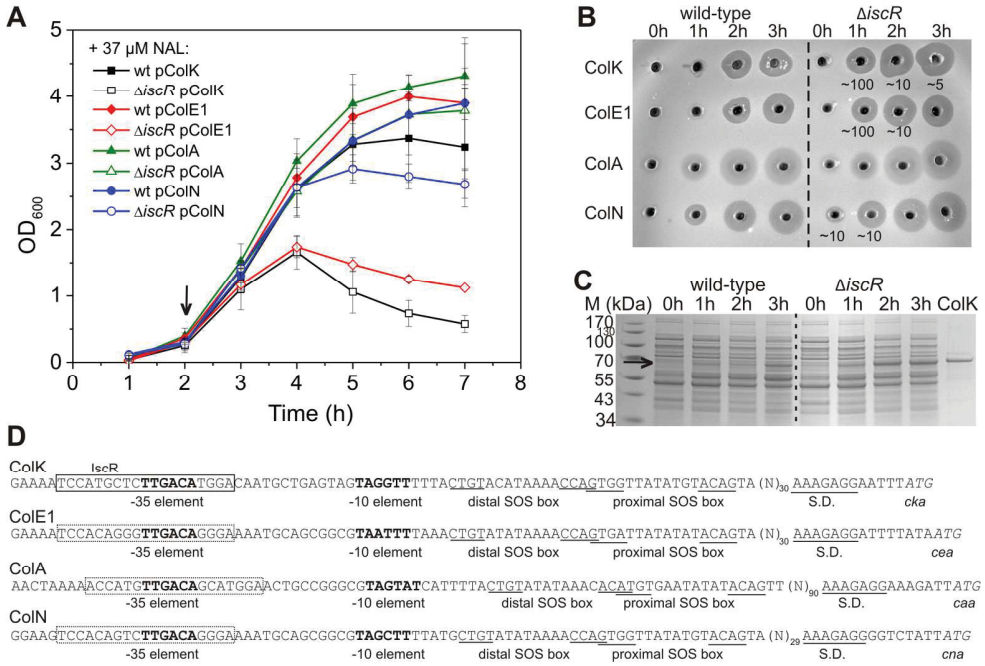
79x169mm (300 x 300 DPI)



79x68mm (300 x 300 DPI)



129x67mm (300 x 300 DPI)



168x115mm (300 x 300 DPI)