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Book of abstracts

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Book of abstracts

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ABSTRACTS

A prospect for a novel drug for short-term thrombosis prevention based on the nose-horned viper venom component

Igor Križaj[1], Mojca Dobaja Borak[2,3], Adrijana Leonardi[1], Kity Požek[1,3], Katarina Reberšek[4], Helena Podgornik[4,5], Aljaž Pirnat[6], Alenka Trampuš Bakija[6], Simona Kranjc Brezar[2,7], Tomaž Trobec[8], Monika Žužek[8], Milka Vrecl[8], Robert Frangež[8], Miran Brvar[2,9]

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Contemporary antiplatelet drugs exert their antithrombotic effects by reducing platelet count and inhibiting platelet function, often resulting in excessive bleeding—a major side effect that complicates procedures in interventional cardiology and angiology. Profound and transient thrombocytopenia of functional platelets without bleeding was observed in patients envenomed by *Vipera a. ammodytes* (Vaa). This condition was rapidly reversed by Fab fragments of antibodies raised against the whole venom, leaving platelets fully functional. It was proposed that snake venom C-type lectin-like proteins (snaclecs) were responsible for this effect. To test this hypothesis, we purified snaclecs from crude venom, biochemically characterized them and studied their interaction with platelets. Six Vaa-snaclecs were isolated from the venom using a combination of five consecutive liquid chromatography steps and structurally analyzed. Platelet count, agglutination and aggregation assays, along with standard blood coagulation tests, identified Vaa-snaclec-3/2 as the most potent antiplatelet molecule. This snaclec is a covalent heterodimer composed of Vaa-snaclec-3 (α-subunit) and Vaa-snaclec-2 (β-subunit). Flow cytometry revealed that Vaa-snaclec-3/2 induces thrombocytopenia by binding to the platelet receptor GPIb thus triggering platelet agglutination. Importantly, this effect was reversible leaving platelets functionally competent. We further evaluated the antithrombotic efficacy of Vaa-snaclec-3/2 in a murine model of ferric chloride-induced carotid artery thrombosis. This substance induced profound thrombocytopenia in a dose-dependent manner, with a median effective dose of 4.7 μg/kg. Although it prolonged tail bleeding time, bleeding remained within the physiological range, and no spontaneous hemorrhage was observed. Histological analysis also showed no signs of acute bleeding. Vaa-snaclec-3/2 efficiently protected mice from carotid artery occlusion. The lowest dose that induced severe thrombocytopenia and completely inhibited ferric chloride-induced thrombus formation was 20 μg/kg. Our findings highlight the potential of Vaa-snaclec-3/2 as a promising agent for short-term thrombosis prevention in interventional cardiology and angiology.

Secreted phospholipases A2 - their physiological role as antagonists of nicotinic acetylcholine receptor and therapeutic potential

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels, which are found overexpressed in various cancers. Agonist binding to nAChRs is associated with tumor-promoting effects such as increased proliferation, resistance to apoptosis, and angiogenesis. In contrast, antagonists of nAChRs counteract these effects, showing therapeutic potential. Among nAChR antagonists are snake venom secreted phospholipases A2 (sPLA2s). They were shown to suppress ACh-elicited ion currents, independently of their enzymatic activity. To explore whether mammalian sPLA2s also act in this way, we prepared recombinant sPLA2s and their enzymatically inactive mutants: snake venom ammodytoxin A (AtxA) and AtxA(D49S), human group V and X sPLA2s (GV and GX) along with GV(H48Q) and GX(H48Q), and rat group IIA sPLA2 (GIIA) and GIIA(D49S). Electrophysiological studies were performed on neuronal α7 and adult muscle-type nAChRs expressed in Xenopus laevis oocytes. AtxA, GV, GX and GIIA inhibited ion conductance of both nAChR subtypes, while some of the enzymatically inactive sPLA2 mutants showed reduced or no activity on nAChRs. GV was the exception among the tested sPLA2s for displaying the nAChR subtype selectivity – it preferentially inhibited α7 over the muscle-type nAChR. Interestingly, the active site mutation of GV (i.e. H48Q) ceased the inhibition of the muscle-type nAChR but not the inhibition of α7 nAChR. Our results suggest that the inhibitory effect of sPLA2s on nAChRs can also be independent of PLA2 activity and that the active site mutations may induce conformational changes that affect receptor binding. Supporting the latter, circular dichroism spectroscopy of GV and GV(H48Q) revealed alterations in α-helical structure. Our findings expose the potential physiological role of GV in the modulation of α7 nAChR activity, as well as the therapeutic potential of its GV(H48Q) mutant. Therefore, we are currently evaluating the effects of the GV molecules on proliferation and cell death of lung cancer and non-tumorigenic lung epithelial cells.

Mitochondrial Targeting by Secreted Phospholipases A2: From Venom Neurotoxicity to Neurodegenerative Diseases

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Snake venoms are a well-known reservoir of pharmacologically active secreted phospholipases A2 (sPLA2s), which exert diverse biological effects through their enzymatic activity and by acting as ligands for membrane and soluble receptors. These receptors mediate processes such as cellular transport, signalling, and modulation of sPLA2 activity, making sPLA2–receptor interactions an important area of pharmacological interest. Using the presynaptically neurotoxic sPLA2 ammodytoxin (Atx), a major component of *Vipera ammodytes ammodytes* venom, we have elucidated key molecular events underlying presynaptic neurotoxicity. Through the identification and characterization of several Atx-binding proteins, and the investigation of its enzymatic and non-enzymatic actions, we have significantly advanced the understanding of Atx trafficking and action within nerve cells. Among its intracellular targets are mitochondria, which are severely affected, structurally and functionally, by Atx. In line with this, a key finding was the identification of a mitochondrial Atx-binding protein, subunit II of cytochrome c oxidase (CCOX-II), an essential component of the respiratory chain. Notably, both enzymatically active and inactive forms of Atx impair mitochondrial respiration by inhibiting CCOX physiological activity.
Building on these findings, we investigated whether the endogenous sPLA2 group IIA (GIIA) affects mitochondria in a similar way. We confirmed that both the recombinant rat GIIA (rGIIA) and its enzymatically inactive mutant, rGIIA(D49S), interact with CCOX-II as well. Despite lower CCOX-II-binding affinity than Atx, both rGIIA forms impaired mitochondrial function by strongly inhibiting CCOX activity. This suggests a conserved mechanism of impairment of mitochondrial respiration by sPLA2 molecules and revealed GIIA as a potential physiological regulator of this process. Our ongoing research is focused on the identification of additional protein targets of GIIA in mitochondria to further clarify mechanisms by which it influences mitochondrial function under physiological or pathological conditions, for example in neurodegenerative diseases.

Rational Design of Anticoagulant Peptides Inspired by a Snake Venom Serine Protease Homologue

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Venous thromboembolism (VTE) is one of the leading causes of death and disability in the developed world. It is characterised by clot formation in the venous system, which can lead to serious complications such as deep vein thrombosis and pulmonary embolism. Current anticoagulant therapies effectively prevent VTE, but their non-selective mechanisms carry a significant risk of bleeding, emphasising the urgent need for safer alternatives. Animal venoms offer a rich source of potent and selective bioactive molecules that have been shaped by evolutionary pressure. In the venom of *Vipera ammodytes ammodytes* (*Vaa*), we have identified a serine protease homologue, VaaSPH-1, which exerts a specific anticoagulant effect by targeting the intrinsic coagulation pathway. It is a 34 kDa, enzymatically inactive protein that competitively binds to the A2 domain of activated factor VIIIa (FVIIIa), blocks its interaction with activated factor IX (FIXa), and prevents the assembly of the intrinsic tenase complex—a crucial step in the intrinsic coagulation cascade. As a result, the cofactor activity of FVIIIa is inhibited, leading to reduced catalytic efficiency of FIXa and suppression of clot formation. Despite its promising anticoagulant properties, the large molecular size and immunogenicity of VaaSPH-1 limit its therapeutic potential. With the ambition to develop an inventive anticoagulant drug, our research focuses on the formulation of fragments of VaaSPH-1 that retain the anticoagulant activity of the intact protein, and on the optimization of this activity using structural modelling and site-directed mutagenesis. We evaluate the activity of the designed peptides using various coagulation assays and surface plasmon resonance analysis, aiming to identify a key pharmacophore for the rational design of small molecule intrinsic tenase inhibitors – next-generation anticoagulants.

Evolution of actinoporins

Gregor Anderluh

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Actinoporins are pore-forming proteins derived from sea anemones. They are characterized by a relatively simple structural fold consisting of a β-sandwich and an α-helix, both of which are crucial for pore formation. The molecular mechanism by which actinoporins form pores is well understood, and recent structural studies of membrane pores in several actinoporins and their homologues have provided detailed insights into membrane architecture and protein–lipid interactions.

Actinoporin-like proteins occur in a variety of organisms and are found in protein families such as fungal lectins, aegerolysins, thermostable direct hemolysin, and Nep1-like proteins. Although the molecular process of pore formation is well described, comparatively little is known about the biological roles of actinoporins and their homologues across different species.

Recently, numerous homologues have been identified in molluscs, particularly in marine bivalves and snails. In this presentation, I will outline the current understanding of actinoporin function and explore their potential biological roles, with an emphasis on their significance as key components of animal venoms.

Stoichiometric Variability and Lipid-Dependent Architecture of Actinoporin Pores

Gašper Šolinc, Marija Srnko, Ana Crnković, Marjetka Podobnik, Gregor Anderluh

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Pore-forming toxins (PFTs) are proteins that create pores in lipid membranes typically formed by protomers that assemble in circular arrays, often incorporating lipids into their structures, not just as substrates but as integral components. Actinoporins are, a group of α-PFTs from cnidarians that form pores in membranes containing sphingomyelin. Until recently the only known pore structure was octameric although different stoichiometries were proposed. We explored the structure of actinoporin Fav from the mountainous star coral (*Orbicella faveolata*), using cryo-electron microscopy (cryo-EM). Our findings reveal that Fav forms funnel-shaped pores associated with numerous lipids some of which play an important role in the structure of the pore. With the variation of lipid composition used in pore preparation and the length of the N-terminal region of Fav, we were able to prepare and determine the structure of pores of varying stoichiometries namely, heptamers, octamers, and nonamers. High-resolution cryo-EM maps reveal a similar pattern of lipid arrangement and protomer fold between the three observed stoichiometries. Our study reveals that actinoporin pores are structurally versatile and stabilized by specific lipid interactions, underscoring the dynamic interplay between protein and membrane in pore formation.

Actinoporin-like proteins in cone snails – what is their biological role?

Matija Ruparčič, Gregor Anderluh

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Cone snails are a large group of marine gastropods that produce a complex mixture of toxic compounds to hunt prey and defend themselves against predators [1]. The main components of the venom are small, bioactive peptides known as conotoxins that target membrane receptors, while a smaller portion of the venom consists of larger proteins that are thought to aid in conotoxin maturation and the envenomation process [2]. Interestingly, conoporins have been found to be highly expressed in the venom gland of several cone snail species, suggesting an important role in the envenomation process. In spite of this, the exact biological role of conoporins remains unknown, as only one conoporin has been experimentally characterized [3]. With respect to the mechanisms and functions of other pore-forming toxins, we propose four hypotheses for the function of conoporins: (i) permeabilization of epithelial barriers, (ii) facilitation of membrane translocation of conotoxins through pores, (iii) antimicrobial activity, and (iv) involvement in digestion.
We performed a bioinformatic search for annotated conoporin sequences, which yielded 95 unique sequences belonging to 27 species. Compared to actinoporins, conoporins feature extensions at the N- and C-termini, while the predicted structure of the β-sandwich core appears to be conserved, similar to other actinoporin-like proteins from molluscs [3,4]. Interestingly, phylogenetic analysis revealed that conoporins are clustered into at least three distinct clades. Experimentally, we successfully expressed and purified four conoporins: ConM3 and ConM6 from the piscivorous *Conus magus*, as well as ConEb1 and ConEb3 from the vermivorous *Conus ebraeus*. Preliminary results show that ConM3 causes hemolysis when incubated with bovine red blood cells, whereas this effect was not observed with ConM6 and ConEb1.

Structure-Guided Mutagenesis of the Fav Actinoporin-like Protein to Improve Pore Stability

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Pore-forming proteins (PFPs) are a widespread and evolutionarily conserved group of membrane-associated proteins that are essential in various biological processes. Actinoporins, a subset of α-pore-forming toxins, are known for their high specificity toward sphingomyelin-rich membranes, where they assemble into oligomeric structures to create functional transmembrane pores. These proteins are primarily found in marine organisms such as sea anemones, where they serve protective and predatory roles. Recent research on the actinoporin-like protein Fav, derived from the coral *Orbicella faveolata*, highlights its interaction with lipids, especially cholesterol, which enhances pore stability.

Due to their ability to form highly selective membrane pores, biological nanopores have emerged as powerful tools for molecular sensing applications. Nanopore technologies are transforming the field of single-molecule analysis by enabling precise detection of biomolecules such as nucleic acids, proteins, and small metabolites. However, to expand their practical use, it is crucial to identify and develop nanopores with improved stability, selectivity, and gating properties. Investigating the structural and functional characteristics of actinoporin-based nanopores, such as Fav, may open new possibilities for enhancing biosensing technologies and molecular diagnostics.

To enhance the structural stability of the Fav nanopore, specific residues in its protomers were substituted with cysteines to promote disulfide bond formation. Analysis of the data from Molecular dynamics simulations, provided insights into the structural impact of these mutations. Analysis of Cα distances from the pore's center of mass revealed a narrowing of the pore at the mutation sites, suggesting a more constrained conformation. Additionally, RMSF and RMSD analyses demonstrated reduced structural flexibility in the mutated variant compared to Fav, indicating enhanced rigidity and stability. These findings suggest that cysteine mutations effectively reinforce the structural integrity of the nanopore, potentially improving its suitability for biotechnological applications.

Actinoporins in nanopore biosensing

Ana Crnković, Gregor Spruk, Gašper Šolinc, Gregor Anderluh

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Actinoporins, members of a family of cnidarian toxins, form homooligomeric pores on sphingomyelin-containing membranes. This property of forming mainly non-selective water channels on lipid bilayers has been exploited for various biotechnological purposes. Here I will present the application of actinoporins in nanopore biosensing, where a single actinoporin pore allows the passage of molecules under an applied electric field. Since the passage of each molecule through the pore is accompanied by a characteristic current signature, this method should enable the detailed detection, quantification and study of a variety of molecular analytes. However, the size and charge of certain molecular analytes - analytes that translocate too quickly or are too large to translocate under standard conditions - require new approaches to classical nanopore biosensing. The facile preparation of soluble actinoporin monomers and their remarkable conformational plasticity allow us to easily develop new actinoporin nanopores tailored for the detection of analytes of interest.

From biological actions of pore forming sponge toxins to political printmaking

Roderick Scott

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In Aberdeen we began our part of the collaboration working with cocktails of polyalkylpyridinium salts (poly-APS) sponge toxins using electrophysiology and Ca2+ imaging methods. We found that the sponge toxins evoked large conductance (>145pS) pores which were permeable to Ca2+. Further experiments showed that poration could be transient and that intracellular Ca2+ could be mobilized by poly-APS. A critical breakthrough in this field was made when synthesis of defined different sized poly-APS was achieved. The large reversible pores in the membranes suggested that poly-APS could be used for delivery of macromolecules into cells. This was achieved using human Tau protein and cDNA for enhanced green fluorescent protein and human TNFR2. But not siRNA against β-actin or the anticancer drug Doxorubicin. As poration and transfection reagents poly-APS showed a negative temperature coefficient (12, 21 & 37°C). We speculated that poly-APS in nature as well as forming a chemical defence may also play a role in natural transfection, giving rise to new species of microorganisms.

A laboratory and an artist's studio are very similar, so it was no hardship in 2014 to change occupations. My printmaking and sculptures are mainly inspired by urban life and political events. I conduct experiments in printmaking using linocut, drypoint, monoprinting, letterpress and collagraph methods. I also construct 3D installations and make prints using rubbish. In 2018 I co-founded Experimental use of Space, a group that creates pop-up exhibitions in places not usually used for art (https://www.instagram.com/experimentaluospace/).

Cnidarians, their toxins, and interactions with researchers

Dušan Šuput

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I became familiar with cnidarian toxins when, as an electrophysiologist, I visited Prof. Lebez and his PhD students Peter Maček and Bojan Sedmak. A few years later, another interesting and curious PhD student joined his team: Tom Turk. He liked to study the actions of cnidarian toxins, comment on everything, and was willing to participate in any experiment or endeavor, no matter how odd. I will mention only a few memorable moments and happenings:

- In Singapore, some 40 years ago, we attended the IST meeting and entered the conference room just at the time when a respectable foreign researcher explained his view of the action of EqTx. With new data, I gave a new, different view of EqTx II action. This has also been readily accepted, as Mr. Turk was there with me. Of course, Tom Turk, not the »famous« prof. Vito Turk. I am not sure that anybody realized his real identity by the end of the conference.
- At the time of the financial crisis in Yugoslavia, the Adriatic was also infested with Pelagia noctiluca. With Bojan Sedmak we decided to produce and sell a substance that would fix the nematocysts and prevent the escalation of the effects of contact with this jellyfish. Tom was diving to find Pelagia, so that we could test the substances on volunteers = us. Since then, Pelagia disappeared from the Adriatic Sea for several decades... maybe he scared them off by diving.
- We met at several meetings of EST/IST. On one occasion, at the border between Israel and Egypt, Tom suggested going snorkeling at about midnight. Shouting and other loud noises brought us back to the shore. Luckily, the personnel at the marine biological station were warned of a possible terrorist attack – they realized that these must be two crazy Slovenians, and not terrorists, so we survived...

Tom will certainly find many ways to enjoy life, and I am sure that he will never be fed up with watching birds and marine animals.

From Toxins to Marine Carbon Cycles: A Family Journey

Timotej Turk Dermastia

Marine Biology Station Piran, National Institute of Biology, Piran, Slovenia

Growing up in a family of two scientists deeply engaged in the study of biological systems, it might seem inevitable that I would follow a similar path. While my sister took a different direction, I was drawn—perhaps through a mix of genetics, upbringing, and shared fascination—into biology and eventually marine science. My father played a major role, introducing me early to the beauty of the marine world. Like him, I was initially captivated by fish, whales, and the dramatic dynamics of visible ocean life. But as often happens in science, interests evolve and careers follow the opportunities available. My father's focus shifted toward biochemistry and specifically marine invertebrate toxins, and mine diverged in a different, yet unexpectedly complementary direction. After starting with whales in my master's research, I transitioned during my PhD to studying harmful algae—microscopic phytoplankton capable of producing potent biotoxins that contaminate shellfish and pose serious environmental and public health risks. These microscopic organisms, form the base of marine food webs and are critical to ocean ecosystems. This intersection between our fields led to a collaborative effort with my father: a Slovenian-language book on harmful algal toxins, aimed at informing medical professionals, veterinarians, and aquaculture stakeholders. Since my PhD, my research has expanded to ecosystem-scale processes, focusing on the role of viruses in phytoplankton ecology and their influence on global carbon cycling. Fieldwork has taken me on research cruises across the Pacific and Antarctica—profound experiences that deepened my appreciation for collaborative science. In many ways, my career reflects the evolution of my father's early marine aspirations, shaped by his enduring passion for the sea and scientific discovery. While our paths differ, I continue to be inspired by his work and hope, one day, to match the depth of his accomplishments.

Verticillium-specific effector SSP4.2 is a pore-forming protein with unknown function

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The sordariomycete *Verticillium nonalfalfae* secretes VnaSSP4.2 into the xylem sap of susceptible hops and is required for full wilt disease development [1]. Bioinformatic analysis shows that VnaSSP4.2 is a *Verticillium*-specific, 14 kDa, basic, lysine-rich cytoplasmic effector with a predominantly beta-secondary structure and no known protein domains [2]. VnaSSP4.2 has no specific protein target within host plant cells and is not recognized by the plant immune system. In this study, we use novel approaches to better understand its biological role.

Live cell imaging of hop protoplasts showed that the VnaSSP4.2-eGFP fusion protein targets the plasma membrane and interacts with the negatively charged lipids cardiolipin, sulfatides and phosphoinositides (PIs), as revealed by protein-lipid overlay assays. The binding was confirmed with large unilamellar vesicles (LUV) composed of dipalmitoyl phosphatidylcholine and various PIs at a ratio of 9:1 (mol/mol) in both flotation assay and surface plasmon resonance. Finally, we found that VnaSSP4.2 permeabilized calcein-loaded LUVs, with the highest percentage of calcein release occurring from LUVs supplemented with 5 mol % PI4P. Considering that PI4P mainly accumulates in the plasma membrane of plants and performs essential cellular functions related to structural organization, intracellular trafficking and signalling [3], we hypothesize that VnaSSP4.2 is a novel fungal PI effector that may modulate host plant immune signalling.

EryA-mCherry as a biomarker for surface-exposed cardiolipin in apoptotic mammalian cells

Kristina Sepčić

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Erylysin A (EryA), an aegerolysin protein produced by the edible king oyster mushroom (*Pleurotus eryngii*) interacts strongly with an invertebrate-specific membrane sphingolipid ceramide phosphoethanolamine. Recently, a fluorescently fused variant of EryA was shown to bind to artificial and bacterial lipid membranes containing cardiolipin (CL). This tetra-acylated glycerophospholipid, present in bacteria and in inner mitochondrial membranes of eukaryotic cells, was shown to be externalized to the plasma membrane surface during the process of apoptosis. In this work, we evaluated the interaction of EryA-mCherry with CL-containing artificial lipid vesicles and with mammalian cells undergoing apoptosis, and compared its binding affinity and specificity to that of the well-established apoptosis marker, annexin V-FITC. Our results show that, in contrast to annexin V-FITC which binds different negatively charged glycerophospholipids, EryA-mCherry specifically recognizes and binds CL in artificial membrane systems. Experiments using mammalian cells showed the ability of EryA-mCherry to selectively label the surface of apoptotic cells, exhibiting the same labelling pattern as anti-CL antibodies. Our data suggest that EryA-mCherry might be used as a marker of early apoptosis, as well as a marker of CL in biological and artificial lipid membranes.

Translating aegerolysin research into sustainable crop protection

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Aegerolysins, lipid-binding proteins from *Pleurotus* species, interact specifically with ceramide phosphoethanolamine, forming cytolytic pores when paired with pleurotolysin B. These proteins exhibit potent activity against agricultural pests, notably the Western corn rootworm and Colorado potato beetle, by compromising insect gut membranes.

Building upon foundational studies of the aegerolysin/PlyB complex's activity and specificity, we have translated this knowledge into practical applications for crop protection. Our GMO potato field trials in Europe demonstrated substantial pest resistance in the modified plants, confirming the *in planta* efficacy of these proteins. In addition to potato, we have developed transgenic corn lines expressing the aegerolysin/PlyB complex, achieving robust expression and effective pest control.

Currently, we are extending this technology to other economically important crops, including soybean, to broaden its impact and application scope. These advancements are especially significant for agricultural markets such as the United States and Brazil, where corn and soybean production is vital and heavily threatened by these pests. Europe, meanwhile, offers an emerging opportunity for deploying these genetically modified crops within frameworks prioritizing sustainable agriculture and integrated pest management strategies.

Our work highlights the critical role of protein-based biopesticides in meeting global food security challenges while reducing reliance on chemical pesticides. By leveraging natural protein toxins and modern biotechnology, we aim to provide environmentally responsible pest management solutions that align with the principles of sustainability, biodiversity preservation, and agricultural resilience.

Huge diversity of the aerolysin superfamily in basal metazoans

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The aerolysin superfamily of β-pore-forming toxins is present both in prokaryotes and eukaryotes. The members of this superfamily, which are mostly toxins, have important roles for the survival of organisms, being used in attack, defense, and also digestion *via* pore formation followed by cytolysis of targeted cells. Proteins of the aerolysin superfamily contain a pore-forming aerolysin domain and a receptor-binding domain (RBD). In contrast to the highly conserved pore-forming domains, RBDs are highly variable, and their structural variations lead to differences in target recognition and, consequently, in the way of action. Although the aerolysin superfamily has been the subject of many studies, knowledge about its diversity, origin, and evolution in basal metazoan lineages is limited; thus, I aimed to address this long-standing knowledge gap. I traced the origin and expansion of the aerolysin superfamily with a phylogenomic analysis, using sequence data from numerous basal metazoan proteomes, transcriptomes, and genomes. I identified the full complement of the aerolysin superfamily in all basal metazoan lineages (cnidarians, ctenophores and sponges). Unexpectedly, I discovered a large diversity and many novel domain architectures of aerolysin superfamily basal metazoans. No less than 10 novel domain architectures for the aerolysin superfamily can be found in basal metazoans. I found that different evolutionary forces operated on the aerolysin superfamily in basal metazoans, including domain accretion, gene duplication, horizontal gene transfer and gene loss. This study challenges the current understanding of the origin and evolution of the aerolysin superfamily in metazoans and provides valuable insights into their early diversification. The findings of this comprehensive study provide guidelines for future structural and functional studies of the aerolysin superfamily.

Nemerteans Are Not Just a "Can of Worms"

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Nearly half of the animal phyla are worms: nematodes, annelids, flatworms, sipunculids, nemerteans (nemertines), etc. Most marine biologists are unaware of nemerteans because they are usually inconspicuous! We think of most worms as primitive animals but this isn't true with nemerteans; like us, they possess a one way digestive system, separate sexes, red blood cells and a myogenic heart. Bacq (1936, 1937) reported the presence of a substance, "amphiporine," in the Atlantic hoplonemertean (*Amphiporus lactifloreus*) that contracts frog muscles and elicits repetitive action potentials in crab nerves. In 1971 we reported the isolation of this potent muscle contractant from a Pacific hoplonemertean. However, anabaseine has no effect on nerve action potentials; it acts upon several nicotinic receptors (nAChRs). The anabaseine derivative GTS-21 selectively activates α7nAChRs, which are implicated as therapeutic targets for treating cognitive problems, stroke and brain inflammation. Phase 2 clinical tests of GTS-21 for cognitive improvement produced mixed results, possibly because there is an additional "dominant negative" α7 monomer gene product expressed in ~70% of humans that lacks the ACh binding site. However, human macrophages expressing α7 receptors composed of monomers from both genes seem to mediate anti-inflammatory effects of GTS-21. Many (>15) related pyridines were found in another *Amphiporus* species, so hoplonemertines should be an excellent source of new toxins. Heteronemerteans contain peptide neurotoxins and cytolysins. The neurotoxins are 3,500 (α-nemertide) and 6,000 (β-nemertide) dalton basic peptides that elicit spontaneous action potentials in crustacean neurons. The 10,000 dalton cytolysins form pores in lipid bilayers and have interesting predicted folded structures. Our colleague Dr. Turk and collaborators have purified, sequenced and cloned an interesting cytolysin from a large Antarctic species, *Parborlasia corrugatus*. In the future we hope others will take up where we stop, as all nemerteans excepting parasitic species are likely to be toxic!