References

1. Clapham, D.E. (2003). TRP channels as cellular sensors. Nature *426*, 517–527.
2. Damann, N., Voets, T., and Nilius, B. (2008). TRPs in our senses. Curr. Biol. *18*, R880–R889.
3. Venkatachalam, K., and Montell, C. (2007). TRP channels. Annu. Rev. Biochem. *76*, 387–417.
4. Nilius, B., Owsianik, G., Voets, T., and Peters, J.A. (2007). Transient receptor potential cation channels in disease. Physiol. Rev. *87*, 165–217.
5. Dietrich, A., KalwaFuchs, B., Grimminger, F., Weissmann, N., and Gudermann, T. (2007). In vivo TRPC functions in the cardiopulmonary vasculature. Cell Calcium *42*, 233–244.
6. Schlingmann, K.P., Waldegger, S., Konrad, M., Chubanov, V., and Gudermann, T. (2007). TRPM6 and TRPM7 – gatekeepers of human magnesium metabolism. Biochim. Biophys. Acta *1772*, 813–821.
7. Clapham, D.E., Julius, D., Montell, C., and Schultz, G. (2005). International Union of Pharmacology. XLIX. Nomenclature and structure-function relationships of transient receptor potential channels. Pharmacol. Rev. *57*, 427–450.
8. Katz, B., and Minke, B. (2009). Drosophila photoreceptors and signaling mechanisms. Front. Cell. Neurosci. *3*, 2.
9. Yau, K.W., and Hardie, R.C. (2009). Phototransduction motifs and variations. Cell *139*, 246–264.
10. Gaudet, R. (2008). TRP channels entering the structural era. J. Physiol. *586*, 3565–3575.
11. Abramowitz, J., and Birnbaumer, L. (2009). Physiology and pathophysiology of canonical transient receptor potential channels. FASEB J. *23*, 297–328.
12. Huang, J., Liu, C.-H., Hughes, S.A., Postma, M., Schwiening, C.J., and Hardie, R.C. (2010). Activation of TRP channels by protons and phosphoinositide depletion in *Drosophila* photoreceptors. Curr. Biol. *20*, 189–197.
13. Yuan, J.P., Kim, M.S., Zeng, W., Shin, D.M., Huang, G., Worley, P.F., and Muallem, S. (2009). TRPC channels as STIM1-regulated SOCs. Channels *3*, 221–225.
14. DeHaven, W.I., Jones, B.F., Petranka, J.G., Smyth, J.T., Tomita, T., Bird, G.S., and Putney, J.W., Jr. (2009). TRPC channels function independently of STIM1 and Orai1. J. Physiol. *587*, 2275–2298.
15. Hofmann, T., Obukhov, A.G., Schaefer, M., Harteneck, C., Gudermann, T., and Schultz, G. (1999). Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. Nature *397*, 259–263.
16. Chyb, S., Raghu, P., and Hardie, R.C. (1999). Polyunsaturated fatty acids activate the Drosophila light-sensitive channels TRP and TRPL. Nature *397*, 255–259.
17. Raghu, P., and Hardie, R.C. (2009). Regulation of Drosophila TRPC channels by lipid messengers. Cell Calcium *45*, 566–573.
18. Nilius, B., Owsianik, G., and Voets, T. (2008). Transient receptor potential channels meet phosphoinositides. EMBO J. *27*, 2809–2816.

Ludwig-Maximilians-Universitaet Muenchen, Walther-Straub-Institut of Pharmacology and Toxicology, Goethestr. 33, 80336 Munich, Germany.
*E-mail: Thomas.Gudermann@lrz.uni-muenchen.de

DOI: 10.1016/j.cub.2010.01.013

Convergent Evolution: Pick Your Poison Carefully

Genomic and biochemical analyses reveal that two independently evolved serine protease venoms in mammals and lizards have converged on nearly identical protein structures. Likewise, in two groups of frog, an identical toxin, caerulein, has arisen repeatedly from unique genes in those lineages.

Edmund D. Brodie, III

Are the outcomes of evolution foreseeable destinations on the journey of adaptation? Or are Darwin's "endless forms most beautiful" just haphazard stops along roads to nowhere in particular? Adaptation is driven by an engine of natural selection continually preserving the forms with higher fitness, but ultimately that engine is fueled with random mutations. The engine cannot push evolution in a direction that does not arise by chance, and so the dogmatic view of evolution is one of unpredictability. Two recent papers in *Current Biology* about the genetic and biochemical basis of venoms and toxins challenge this dogmatic preeminence of chance in determining evolutionary outcomes [1,2].

Is this perspective of ruling contingency fair? Evolutionary convergence stands as one of the most cited lines of evidence for the power of natural selection. Countless cases of taxa that have evolved similar features suggest that there is some predictability to adaptation, at least in a general sense. *Anolis* lizards of the Caribbean, for instance, have repeatedly radiated from common ancestors to fill similar ecological niches with associated behavior and body plans [3]. Different light sensitive opsin proteins have arisen in bacteria and metazoans to fulfill comparable functions with different chemical structures [4]. Plants of the cactus and euphorb lineages have arrived at confusingly similar shapes, defenses, and stem based photosynthesis to inhabit xeric habitats [5]. The list goes on and on.

Most examples of convergence, however, describe similarities of general function and performance. From the outside, convergent adaptations appear alike, but in the few cases where their mechanistic bases have been revealed, we typically see that evolution has built them in different ways. Bat wings and bird wings both evolved from tetrapod forelimbs, but bats support their wing membrane across the expanded digits ('fingers'), whereas birds have reduced digits and a flight surface extending off the arm and hand bones. The devil may not be in the details, but contingency certainly seems to be.

New molecular and genomic evidence confronts this view across wide phylogenetic divides. In a recent paper in *Current Biology* [1] comparing insectivorous mammals and lizards (Figure 1), we learn that these disparate vertebrate lineages have arrived at nearly identical venom proteins modified from serine proteases. Natural selection appears to have driven the proteins to the same fundamental structures as determined by the biochemical properties that determine enzyme activity. In a second paper in this issue of *Current Biology* [2], phylogenetic analyses of genomic data reveal that an apparently identical defensive compound, caerulein, found in two distantly related frog species has evolved twice from two independent genetic starting points.

Despite their wide distribution across almost all groups of animals, venoms are only known from a few mammals, including shrews and the insectivore solendon. Named for a genus of North American shrews (*Blarina*), blarinatoxin (BLTX) is a serine protease that is clearly related to kallikrein-1 in structure. BLTX is venomous to the prey of shrews because it has high catalytic activity compared to other serine proteases. This elevated catalysis consequently floods the circulatory system with bradykinin, resulting in paralysis and death. Tracking this increased catalysis as the source of toxicity, Aminetzach

and colleagues [1] were able to predict and test structural changes to kallikrein that would be likely to modify a digestive enzyme into one with toxic effects.

Using molecular dynamic simulations, the team determined two probable characteristics of BLTX that were likely to increase enzyme activity and that differed from more harmless kallikrein relatives. Flexibility of the protein would allow rapid conformational changes and increase binding to the active site of the enzyme. As predicted, BLTX differed from other shrew salivary proteins and human kallikrein in the length and polarity of the loops in the active site generating a more flexible active surface. Secondly, a contrast in charge between the active site and surrounding surface is expected to increase enzyme activity. BLTX was found to have a uniquely positively charged surface surrounding a negatively charged active site. Thus, the BLTX protein matches neatly the predicted three-dimensional structure of a toxic serine protease. These lines of evidence alone suggest that the specific modifications of a protein to acquire toxicity are somewhat predictable.

What is most amazing about the structural evolution of BLTX, however, is that it is mirrored almost perfectly by another venom in a lineage of predators separated by at least 300 million years of evolution. The new world helodermatid lizards (gila monsters and beaded lizards) also possess a venom, GTX, derived from kallikrein-related enzymes. Like BLTX, its toxicity obtains from increased catalytic activity and a consequent deluge of bradykinin in the circulatory system of the prey. When Aminetzach and colleagues [1] examined the structure of GTX, they found remarkably identical modifications from lizard serine proteases. Just like BLTX, GTX had an insertion of 21 base pairs lengthening the critical loop 1 of the protein and providing increased flexibility. Other substitutions generate a hydrophobic and positively charged loop 1 that results in a charge contrast in the area of the negatively charged active site comparable to that found in BLTX. Notably, no similar changes were found in any of the 24 other kallikrein-like proteins that were studied.

On the other side of the ecological road, innumerable prey species have evolved toxins to defend against predators. Caerulein was originally identified from the skin of an Australian frog (*Litoria caerulea*) [6], and is a relatively nasty toxin that disrupts the digestive system by binding to receptors of gastrin and cholecystokinin (cck), leading to gastrointestinal distresses, drop in blood pressure and inhibition of feeding. Caerulein has since been identified from a number of frog species, and was logically assumed to result from a single evolutionary origin of the toxin early in the divergence of frogs.

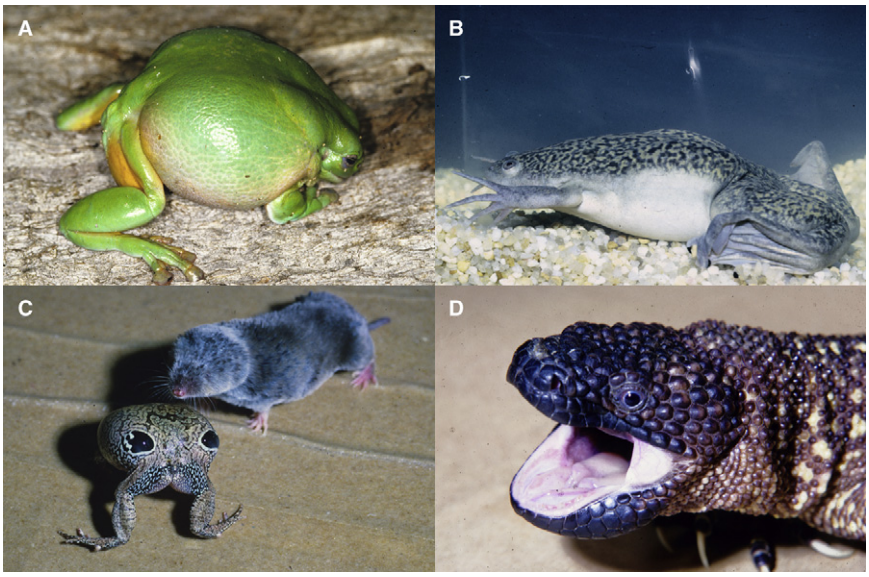

Figure 1. Evolutionary convergence of toxic compounds.

The frogs *Litoria caerulea* (A), in defensive posture, and *Xenopus laevis* (B) produce structurally identical caerulein toxin that has evolved independently from different genes. The serine protease venoms of shrews (*Blarina* sp.) (C) and beaded lizards (*Heloderma horridum*) (D) have converged on a common protein structure that results in toxic levels of enzyme activity. (Photos: E.D. Brodie, Jr.)

A deeper genomic analysis performed by Roelants and colleagues [2] reveals that caerulein has not only independently evolved within frogs, but also has evolved from unique and independent genetic precursors. Caerulein in *Litoria* derived from changes to the *gastrin* gene, while caerulein in the African clawed frog, *Xenopus laevis*, arose from a recent duplication (and subsequent modification) of the *cck* gene in that lineage. Both *gastrin* and *cck* genes are found throughout vertebrates, usually on different chromosomes, arising from a duplication event deep in vertebrate phylogeny approximately 500 million years ago. Patterns of synteny between *cck*/*gastrin* and other gene pairs support the deep duplication event.

Caerulein was a relative latecomer to the evolutionary path of these lineages. The signature of strong directional selection is still evident in *cck*-derived caerulein, but not in the gastrin-derived group. Expression of *cck*/*gastrin* in the skin evolved only recently and only in those taxa that evolved caerulein. Moreover, even the ecological contexts of selection for caerulein toxicity in these groups are different: *Xenopus* (*cck*-derived) has a fully aquatic lifestyle, while *Litoria* (*gastrin*-derived) has a terrestrial habit. The phylogenetic data thus clearly point to a compound that, while apparently identical in protein structure, emerged through two distinct evolutionary roads that began from different genetic starting points.

Taken together, these studies suggest a remarkable predictability of the biochemical details of evolution. Venoms and toxins may be somewhat unique in this sense because their molecular form so directly determines their function [7]. Although evolutionary convergence now is widely recognized at the molecular level, most examples involve common changes to the same gene in different species, such as substitutions that confer resistance to natural or anthropogenic toxins [8,9]. The extraordinary insight provided by these new results is that evolution sometimes starts from different genes

and still arrives at the same place on the adaptive road.

References

1. Aminetzach, Y.T., Srouji, J.R., Kong, C.Y., and Hoekstra, H.E. (2009). Convergent evolution of novel protein function in shrew and lizard venom. Curr. Biol. *19*, 1925–1931.
2. Roelants, K., Fry, B.G., Norman, J.A., Clynen, E., Schoofs, L., and Bossuyt, F. (2010). Identical skin toxins by convergent molecular adaptation in frogs. Curr. Biol. *20*, 125–130.
3. Losos, J.B. (2009). Lizards in an Evolutionary Tree (Berkeley, CA: University of California Press).
4. Larusso, N.D., Ruttenberg, B.E., Singh, A.K., and Oakley, T.H. (2008). Type II opsins: Evolutionary origin by internal domain duplication. J. Mol. Evol. *66*, 417–423.
5. Bennici, A. (2003). The convergent evolution in plants. Riv. Biol. *96*, 485–489.
6. Bowie, J.H., and Tyler, M.J. (2006). Bioactive peptides from Australian amphibians: caerulein and other neuropeptides. In The Handbook of Peptides, A.J. Kastin, ed. (Amsterdam: Academic Press), pp. 283–289.
7. Fry, B.G., Roelants, K., Champagne, D.E., Scheib, H., Tyndall, J.D.A., King, G.F., Nevalainen, T.J., Norman, J.A., Lewis, R.J., Norton, R.S., *et al.* (2009). The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. Annu. Rev. Genomics Hum. Genet. *10*, 483–511.
8. Feldman, C.R., Brodie, E.D., Jr., Brodie, E.D., III, and Pfrender, M.E. (2009). The evolutionary origins of beneficial alleles during the repeated adaptation of garter snakes to deadly prey. Proc. Natl. Acad. Sci. USA *106*, 13415–13420.
9. ffrench-Constant, R.H., Daborn, P.J., and Goff, G.L. (2004). The genetics and genomics of insecticide resistance. Trends Genet. *20*, 163–170.

Mountain Lake Biological Station, Department of Biology, University of Virginia, Charlottesville, VA 22904, USA.
E-mail: bbrodie@virginia.edu

DOI: 10.1016/j.cub.2009.12.029

Lipid Kinases: Charging PtdIns(4,5)P_2 Synthesis

Phosphatidylinositol (4,5) bisphosphate is a lipid second messenger that controls diverse cellular processes. Phosphatidylinositolphosphate-5-kinases synthesise this lipid at the plasma membrane, although it is not clear how the localisation of these kinases is controlled. A recent study suggests that the intrinsic surface charge of the plasma membrane may be an important factor.

Nullin Divecha

Different permutations of phosphorylation of the 3, 4 or 5 position of the inositol head group of phosphatidylinositol generates seven different phosphoinositides that form the basis of a ubiquitous membrane-associated signalling system [1]. There are over eighty isoforms of phosphoinositide kinases, phosphatases and phospholipases that modulate the level of phosphoinositides to regulate processes such as membrane trafficking, cell survival, proliferation and migration. Phosphoinositides carry out their functions by regulating the activity of proteins that harbour specific phosphoinositide-interacting modules (such as the PH, FYVE, FERM, and PHOX domains) [2]. Phosphatidylinositol (4,5) bisphosphate (PtdIns(4,5)P_2) is one of the busiest phosphoinositides, being the substrate for receptor-activated phospholipase C (PLC) and phosphatidylinositol-3 kinase, as well as having its own unique signalling functions in plasma membrane trafficking and actin polymerisation [1]. Furthermore, polarised synthesis of PtdIns(4,5)P_2 is important for the maintenance of epithelial cell morphology [3], podosome function, and membrane ruffling [4] and for the first asymmetric cleavage after fertilisation in *Caenorhabditis elegans* [5]. How localised synthesis of PtdIns(4,5)P_2 is achieved is far from clear. A new study into the role of phosphoinositides in phagocytosis by Grinstein and colleagues [6] has now revealed that the plasma membrane localisation of PIP5K is dependent on the electrostatic interactions between PIP5K and the negative surface of the plasma membrane.

Phagocytosis is the major mechanism by which apoptotic bodies and foreign particles such as bacteria are eliminated from the organism [7]. Opsonisation of the bacterial surface promotes phagocytosis, leading to the interaction of the bacteria with specific cell-surface receptors on macrophages. The ensuing pseudopod formation extends the macrophage plasma membrane around the bacterium, eventually leading to the isolation of the bacterium in an intracellular membrane-bounded vacuole (the phagosome). The phagosome matures over time, becoming acidic and rich in hydrolases and anti-microbial agents capable of degrading bacteria. In a series of elegant studies the Grinstein laboratory has previously demonstrated exquisite spatial and temporal changes in phosphoinositides as the phagosome forms and matures (for a review, see [8]). Initially, upon cell surface binding of the particle, there is an increase in PtdIns(4,5)P_2 [9] and then PtdIns(3,4,5)P_3 at the base of the particle, which is maintained as the pseudopods traverse around the particle. The increased PtdIns(4,5)P_2 accumulation probably helps to drive actin polymerisation, which is required for pseudopod extension. At the point of engulfment and sealing, the PtdIns(4,5)P_2 at the base of the phagosome — and only at the base — dramatically decreases, leading to actin depolymerisation and sealing and internalisation of the phagosome (Figure 1).

Interventions that compromise either PtdIns(4,5)P_2 synthesis or its degradation also compromise phagocytosis [10,11]. PIP5K isoforms (α, β and γ) phosphorylate PtdIns4*P* on the 5′ position and are responsible for the synthesis of the majority of cellular PtdIns(4,5)P_2 [1]. During phagocytosis, PIP5Kγ is required for clustering receptors that interact with bacteria, while PIP5Kα regulates actin polymerisation and bacterial internalisation. PIP5Kα localises to the forming phagosome [12] and, together with PIP5Kγ, probably increases PtdIns(4,5)P_2 in the pseudopods. But how is the decrease in PtdIns(4,5)P_2 at the base of the phagosome achieved? Increased PtdIns(3,4,5)P_3 synthesis is critical as it can activate PLC-γ, which hydrolyses PtdIns(4,5)P_2. However, although PIP5Kα is targeted to the phagocytic cup at early stages, it is lost just before phagosome internalisation [11], suggesting that reducing PtdIns(4,5)P_2 synthesis may also be important in