Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

FLIES SHORT OF BREATH

Functional interpretations of the insect tracheal system – a branched series of tubes supplying oxygen from the environment to every metabolizing tissue in the insect's body – are plagued by two related myths. The first is that gases move through the tracheal system entirely by diffusion. This *idée fixe* persists despite many studies showing that, across vast taxonomic distances, insects actively ventilate the tracheal system in astonishing and subtle ways. The second myth is that the tracheal system's direct connection between environment and tissue renders unnecessary other respiratory complications, like circulatory systems and respiratory proteins. Of course, oxygen-carrying proteins (called hemocyanins) are known from insects. But their taxonomic distribution, in basal insects, reinforces the idea that advanced insects, equipped with highly evolved tracheal systems, don't need additional help. Another class of respiratory proteins (hemoglobins) is found in a few groups of insects adapted to parasitic or aquatic habitats that expose them to hypoxia. But these were usually explained away as strange solutions to alternative lifestyles.

What a shock therefore when reports started surfacing a few years ago of a *Drosophila* hemoglobin. An initial report, by Thorsten Burmester and Thomas Hankeln, identified a gene called *dmeglob*1 (for *Drosophila melanogaster* globin). A follow up report from the same group also showed that the protein product was not exported into the hemolymph. It remained intracellular, had oxygen-binding kinetics much like other known hemoglobins, and occurred at high levels in the tracheal system and fat body.

Next the team turned to an evolutionary analysis of hemoglobin gene sequences from eight additional *Drosophila* species separated by up to 65 million years. Publishing their findings in *FEBS Journal*, Burmester and his colleagues describe how that found a remarkable degree of conservation across the *hemoglobin* genes, despite the passage of time.

Comparative data such as these can provide enormous insight into functional questions by identifying whether genes, or regions within them, are under strong selective constraint. The comparative data did not disappoint: the *glob*1 gene appears to be highly conserved across the fly species. Moreover, *glob*1's coding regions contained many more synonymous base substitutions (which do not change the encoded amino acid) than non-synonymous substitutions (which change the encoded amino acid). High ratios of synonymous to non-synonymous substitutions provide good molecular evidence that the gene has been under strong selection in the past. As a bonus, the team identified two additional globin genes in *Drosophila*, *glob*2 and *glob*3. Although their sequences differed substantially from the sequence of *glob*1, the amino acid sites required for heme- and oxygen-binding were conserved. The new genes' functions are unknown. However, expression levels of glob2 protein were much lower than glob1, leading them to exclude a respiratory role for it. They suggest tentatively that it may be related to nitric oxide metabolism.

What is so fascinating about the *Drosophila* story is the possibility it raises. If *Drosophila* can and does make physiological use of hemoglobins, then perhaps insects in general can and do so. As usual, the simple story – in this case, of oxygen supply by simple diffusion through branched tubes – looks increasingly naïve.

10.1242/jeb.02331

Burmester, T., Storf, J., Hasenjager, A., Klawitter, S. and Hankeln, T. (2006). The hemoglobin genes of *Drosophila*. *FEBS Journal* **273**, 468-480.

Art Woods
University of Montana
art.woods@mso.umt.edu

ALZHEIMER'S DISEASE

SOLUBLE OLIGOMER TRIGGERS MEMORY LOSS

It's probable that you won't be acquainted with every detail of this article once you have read it, but you will at least remember that it was about the causes of Alzheimer's disease. Unfortunately, a person suffering with Alzheimer's will most likely have forgotten that he has even read the article soon after finishing. Indeed, losing memory is one prominent symptom of Alzheimer's disease. The brains of afflicted individuals exhibit characteristic and invariant alterations, which are believed to account ultimately for the loss of neurons and synapses. These include the formation of intracellular neurofibrillary *tau* tangles and extracellular plaques of neurotoxic amyloid-β peptide, where aberrant cleavage products of the amyloid-β precursor protein assemble to form insoluble plaques. Although the molecular details of the pathology of Alzheimer's are not completely understood, transgenic mouse models have enabled neurobiologists to dissect some of the underlying pathogenic processes.

One such transgenic mouse model that has successfully reproduced amyloid plaque pathology in an age-dependent manner is called Tg2576. These mice express a human variant of the amyloid-β precursor protein (APPswe), which is associated with the early-onset of Alzheimer's disease. As a result of APPswe expression in the brain, Tg2576 mice develop amyloid plaques as well as damaged neurons and there is good evidence that the occurrence of amyloid-β peptide is responsible for the observed age-related memory loss. However, it was not clear which form of the amyloid-β peptides triggers memory loss; no forms of the peptide had been found that corresponded with the onset of memory decline. Previous studies had proposed that soluble assemblies of the amyloid-β peptide might affect memory, but the existence of such a soluble amyloid-β oligomer was uncertain until a US team of neurobiologists led by Karen Ashe provided evidence in a recently published *Nature* article that the memory-loss trigger may be a soluble dodecamer of the amyloid-β peptide.

The key to their discovery was a new extraction method to quantify and compare amyloid-β species from different subcellular compartments in the brains of Tg2576 mice. The scientists identified a 56-kDa dodecameric form of the amyloid-β protein (Aβ*56) in the extracellular soluble fraction of forebrain extracts that appeared at the same time as six-month old TG2576 mice began exhibiting memory loss. As there was no evidence of a correlation between memory loss and intracellular or membrane-associated amyloid-β species, the finding suggested that Aβ*56 may be responsible for memory loss. However, if Aβ*56 really disrupts memory, it should also cause memory loss when applied externally to the animals' brains. To test this, the team purified Aβ*56 from the brains of impaired Tg2576 mice and injected it into the lateral ventricles of young rats. Subsequent tests on the rat's behaviour in a water maze revealed that Aβ*56 transiently disrupts memory but does not impair the animals ability to learn.

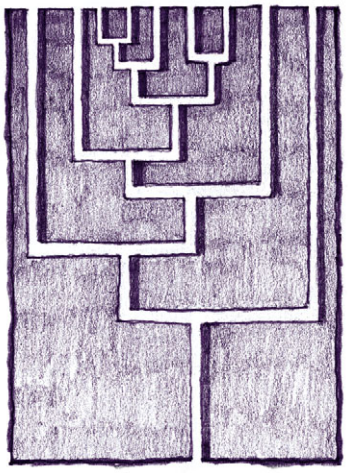
Analysing the pathogenesis of Alzheimer's disease is like solving a complex puzzle. It is clear that altered proteolytic processing of amyloid-β precursor protein resulting in an insoluble molecule is an important piece in this puzzle. Karen Ashe and coworkers' have added another piece to this complex puzzle by clearly showing the transient effects of the soluble Aβ*56 oligomer on memory loss. Although it is not clear precisely how Aβ*56 disrupts memory, the identification of a specific oligomer involved in memory loss may facilitate the development of new diagnostics and therapeutics to combat Alzheimer's disease.

10.1242/jeb.02332

Lesné, S., Koh, M. T., Kotilinek, L., Kayed, R., Glabe, C. G., Yang, A., Gallagher, M. and Ashe, K. H. (2005). A specific amyloid-β protein assembly in the brain impairs memory. *Nature* **440**, 352-357.

Hans Merzendorfer
University of Osnabrueck
merzendorfer@biologie.uni-osnabrueck.de

PROTEOMICS



SNAKE VENOM(E)'S POST-TRANSLATIONAL MODIFICATIONS

Snake venom is something of an all around remedy for heart disease depending, of course, on the dosage. The reason is the venom's potential to activate components of the coagulation cascade without producing blood clots, thereby deactivating a victim's ability to coagulate its blood when necessary. Thus it comes as no surprise that biologists have focused on the characterization of snake venom in great detail. Geoff Birrell and his colleagues from the University of Queensland in Brisbane have succeeded in extending our understanding of snake venom by investigating the venom's proteome or 'venome' in *Molecular and Cellular Proteomics*, by characterising some of the components of this 'healing' mixture.

The team used venom extracted from over 40 Eastern brown snakes (*Pseudonaja textiles*) and analysed the venom's composition with two-dimensional gel electrophoresis. They found that the venom was comprised of about 200 proteins. Analysing the relative positions of the protein spots on the gel, the team noticed several horizontal chains of protein spots that suggested that some of these proteins are undergoing post-translational modification.

Next, the authors applied mass spectrometry to 49 trypsin-digested venom proteins in order to identify them, and found many of the usual venom suspects. Several prothrombin activator complex proteins were present (Factor Va-like protein and Xa-like heavy chain peptides), which activate the coagulation cascade but fail to initiate blood clotting because they cause the disappearance of fibrinogen, the precursor required for clot formation.

They also identified textilotoxin, a neurotoxin that inhibits the release of acetylcholine at the presynaptic membrane, thus, essentially paralyzing the prey. The four subunits of textilotoxin were identified as members of the phospholipase A_2 family, which have also evolved in the Eastern brown snake to prevent blood coagulation and inhibit muscle function in its victims.

Knowing that some of the venom components had been post-translationally modified, the authors tested for two major types of post-translational modification, phosphorylation and glycosylation. Using modification-specific stains the team found that several venom proteins were modified through glycosylation. They further characterized the modifications with proteins that detect glycosylation and found that sialic acids and *N*-linked sugars were incorporated into some of the venom components. Further characterizing the sugars, the team showed the presence of *N*-acetyl-galactosamine and *N*-acetyl-glucosamine as well as sialic acid attached to some venom proteins.

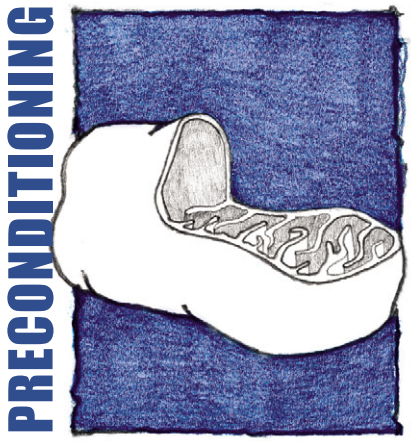
However, the scientists explain that the function of the carbohydrate protein-modifications is unclear. They point out that none of the human homologs that are involved in blood clotting are glycosylated and thus question the role of these sugars in the pseudo-activation of the clotting cascade. Instead, they speculate that these sugars may stabilize the clotting factors in the venom solution. The team also point out that glycosylation may help keep the proteins in solution, as glycoproteins have a tremendously high affinity for water.

As we learn more and more from proteomic studies of biological tissues and materials, the diversity of protein post-translational modification is going to challenge us considerably and leave us with the impression that the genomic diversity that currently overwhelms us could turn out to be relatively simple by comparison.

10.1242/jeb.02333

Birrell, G. W., Earl, S., Masci, P. P., de Jersey, J., Wallis, T. P., Gorman, J. J. and Lavin, M. F. (2006). Molecular diversity in venom from the Australian Brown Snake, *Pseudonaja textilis*. *Mol. Cell. Proteomics.* **5**, 379-389.

Lars Tomanek
California Polytechnic State University
ltomanek@calpoly.edu



MITOCHONDRIA AT THE HEART OF CARDIAC PRECONDITIONING

The study of mammalian ischemia tolerance has focused for many years on preconditioning, in which a brief, sub-lethal period without blood flow or oxygen protects tissues against a later, longer ischemic period that would otherwise prove fatal. Preconditioning studies focus primarily on the heart, and to a lesser extent on the brain and other tissues. A number of compounds have been shown to be related to this protection, including adenosine, ATP-dependent potassium channels, reactive oxygen species (ROS), and a variety of upregulated molecular pathways (heat shock proteins, mitogen activated protein kinases) that induce the preconditioned phenotype. The goal, of course, is to exploit this natural protection in clinical applications, such as myocardial infarctions or stroke, and thus the elucidation of underlying mechanisms is critical to future treatment options.

Much recent research has demonstrated the critical importance of mitochondrial ATP-dependent potassium channels (K_{ATP}) in preconditioning. These channels, present in many cell types, remain closed as long as cellular energy is adequate, but open in response to falling ATP levels. Open K_{ATP} channels, also present in the plasma membrane, thus allow a temporary cellular hyperpolarization, and play a critical protective role in neurons by suppressing the release of excitatory neurotransmitters. In preconditioning, mitochondrial K_{ATP} channel protection is thought to be related to the generation of ROS. However, mitochondria also play a critical role in cell death when oxygen levels are inadequate, as the loss of mitochondrial membrane potential triggers the cascade of caspase proteins that induce apoptosis.

Because of these mitochondrial roles in cell death and cell survival, Sven Vetter and colleagues, associated with Achim Vogt's group in Heidelberg, have developed a novel model of ischemic preconditioning utilizing isolated mitochondria from the rat heart to examine two key questions: Do isolated mitochondria themselves show the preconditioning phenomenon? And does that protection involve the K_{ATP} channels? Preconditioning the mitochondria with a 4-minute anoxic exposure, the team then exposed the mitochondria to complete anoxia for 14 minutes under argon before reoxygenation. The team then monitored the release of mitochondrial enzymes into the medium, which provided a measure of structural damage, while oxygen consumption was monitored continuously to determine mitochondrial function. To test if mitochondrial K_{ATP} channels were involved in the preconditioning response, the experiments were repeated using the specific K_{ATP} channel opener diazoxide or a K_{ATP} blocker to determine if the drugs would either mimic or abrogate the effect.

Following the anoxic/reoxygenation exposure, the mitochondria were no longer able to carry out respiration; however, this loss of function was prevented by anoxic preconditioning or exposure to diazoxide. The team also found that preconditioning was prevented by administration of the K_{ATP} blocker, indicating that mitochondrial preconditioning is directly related to the opening of K_{ATP} channels. Neither ATP-hydrolysis nor mitochondrial enzyme loss differed with anoxic preconditioning or the experimental treatment, indicating that anoxic preconditioning represents a functional adaptation rather than a preservation of mitochondrial structural integrity.

The authors' findings thus support *in vivo* work suggesting that mitochondria are at the heart of natural cardioprotection achieved through preconditioning, and suggest that their model may help resolve the many remaining questions surrounding preconditioning, including the actual mechanisms by which K_{ATP} channels help protect tissues.

10.1242/jeb.02334

Vetter, S. Y., Elsasser, A., Tutdibi, O., Lang, S., Schoels, W., Pott, A., Ackermann, C., Reinhard, C., Wieland, F., Katus, H. A., Kubler, W. and Vogt, A. M. (2006). Brief antecedent anoxia preserves mitochondrial function after sustained undersupply: A subcellular correlate to ischemic preconditioning? *Mol. Cell. Biochem.* E-pub ahead of print: DOI: 10.1007/s11010-005-9071-9.

Sarah L. Milton
Florida Atlantic University
smilton@fau.ed