IN THE NEWS

***Hox* shock**

The homeotic (*Hox*) genes establish the body plan in early embryonic life, and are arranged along the chromosome in order of their spatial and temporal expression. The *Hox* locus is regarded as the jewel of evolutionary biology, as cross-species conservation of the clustered arrangement and expression patterns of its constituent genes is strong evidence for our humble evolutionary beginnings.

But now, a small marine organism has broken the rules, as Daniel Chourrout and colleagues showed in a study in *Nature*. Unlike all other animals that have bilateral body symmetry, the *Hox* genes of *Oikopleura dioica* are located far apart with no indication of clustering — a finding that, so far, is restricted to *Caenorhabditis elegans*. With regard to the precise position of the *Hox* genes in *O. dioica*, Nipam Patel, from the University of California at Berkeley, USA, speculated that "for all we know they'll be on different chromosomes." (*The Scientist*, 2 September 2004).

So, if *O. dioica* can develop normally despite the separation of its *Hox* genes, what is the driving force behind the conservation of *Hox* clustering?

Similar to *C. elegans*, the chromosomal position of the *Hox* genes still correlates with spatial expression in *O. dioica*, but the coordination of temporal expression is lost. As Patel points out, the rapid embryonic development of these small organisms might allow them to "employ mechanisms that don't require strict anterior and posterior timing." The need for a coordinated timing mechanism would, however, favour the maintenance of *Hox* clusters in higher organisms.

Shannon Amoils

MEMBRANE FUSION

Taking steps to unite

Contrary to their textbook portrayal as static, oval-shaped organelles, mitochondria are highly dynamic, tubular structures that are continually dividing and fusing. Mitochondrial membrane fusion is of particular interest, because it's distinct from other membrane-fusion processes — it requires the fusion of a double membrane and doesn't involve core secretory fusion components such as SNAREs. However, the steps that lead to mitochondrial fusion have remained unclear, because of the difficulty of replicating the process *in vitro*.

Now, though, in *Science*, Nunnari and colleagues have provided new insights. They've developed an *in vitro* assay to measure mitochondrial membrane fusion. They mixed mitochondria that contained either matrix-targeted green fluorescent protein (m-GFP) or matrix-targeted red fluorescent protein (m-DsRed), concentrated the organelles using centrifugation and then resuspended them in the presence of cytosolic extract, ATP, GTP and an energy-regeneration system. Fusion could be assessed by observing the mixing of matrix content, and they showed that fusion was highly dependent on exogenous NTPs and the energy-regeneration system.

Two mitochondrial membrane proteins — Fzo1 and Mgm1 — are required for mitochondrial fusion and are thought to be GTPases. However, whether GTP hydrolysis is required for fusion has been unclear. Nunnari and co-workers therefore used their assay to examine the nucleotide requirements of the fusion reaction, and found that GTP, but not ATP, hydrolysis is needed for fusion. Using ionophores, they also showed that the electrical potential of the inner mitochondrial membrane is required for content mixing.

Next, the authors divided the assay into two experimental stages — stage 1 involved the centrifugation, incubation and resuspension of mitochondria, and stage 2 involved adding exogenous NTPs and the energy-regeneration system. They found that fusion intermediates form during stage 1 and fuse in an energy-dependent manner during stage 2. When they analysed the fusion intermediates using electron microscopy, they found both non-deformed, tightly associated mitochondria and deformed mitochondria, in which the outer membranes had fused while the inner membranes remained separate.

They confirmed these results by developing a fluorescence-based assay for outer-membrane fusion. They mixed mitochondria that had been labelled with m-DsRed and outer-membrane-targeted GFP with mitochondria that had been labelled with matrix-targeted blue fluorescent protein, and observed outer-membrane fusion in the absence of inner-membrane fusion. This fusion was driven by the relatively low amounts of endogenous GTP and was dependent on the inner-membrane proton gradient. The outer- and inner-membrane fusion events are therefore distinct, with the latter requiring higher levels of GTP, as well as the inner-membrane electrical potential.

In the final part of their study, Nunnari and colleagues studied the role of the outer-membrane protein Fzo1 in mitochondrial fusion, and their results indicate that homotypic *trans* interactions of Fzo1 on neighbouring mitochondrial membranes are needed for both outer- and inner-membrane fusion. So, although the outer- and inner-membrane fusion events are distinct and can be uncoupled, interactions of Fzo1 with inner-membrane proteins are important for inner-membrane fusion.

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Meeusen, S. *et al.* Mitochondrial fusion intermediates revealed *in vitro*. *Science* 5 Aug 2004 (doi:10.1126/science.1100612)

FURTHER READING Mozdy, A. D. & Shaw, J. M. A fuzzy mitochondrial apparatus comes into focus. *Nature Rev. Mol. Cell. Biol.* **4**, 468–478 (2003)