EVOLUTION

The skeleton's key

Hox genes control antero-posterior patterning in vertebrates, but previous studies have failed to produce a definitive model of their influence on skeletal development. Research by Wellik and Capecchi now confirms their role as global regulators of the patterning of the vertebrate skeleton, and identifies specific functions for *Hox10* and *Hox11* in the development of the lower spine and hindlimbs.

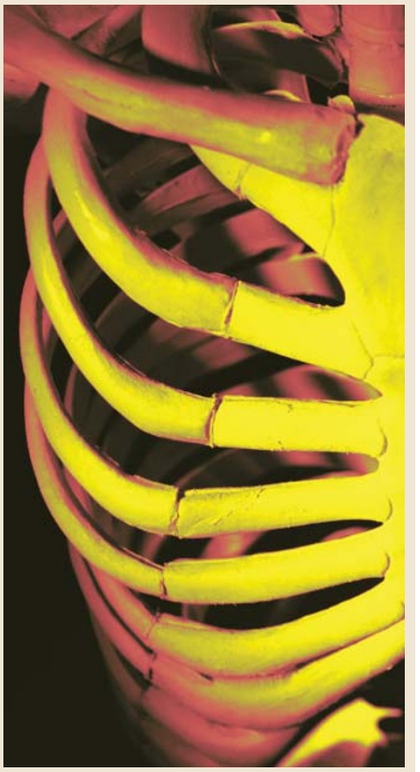
Mice with mutations in single members or subsets of the paralogous *Hox* groups have only minor skeletal defects. Furthermore, combinations of mutations in any five of the six *Hox10* or *Hox11* alleles produce similar abnormalities, which indicates that individual alleles have roughly equivalent effects. By contrast, mutants in which all of these alleles have been inactivated have gross phenotypic defects.

By mutating all members of these functionally redundant groups, Wellik and Capecchi were able to determine their precise roles in patterning the axial skeleton: triple mutants of *Hox10* lack lumbar vertebrae and develop ribs on all posterior vertebrae, whereas *Hox11* triple mutants fail to develop sacral vertebrae.

In light of these findings, the authors propose a mechanism for how changes in *Hox* gene expression might have modified rib formation in the lumbo-sacral region during vertebrate evolution. Their data support the hypothesis that the ground state for this group is the presence of ribs on all vertebrae. Rostral or caudal shifts in the expression of *Hox10* and *Hox11* could have altered the number and position of thoracic and sacral vertebrae, resulting in the range of skeletal patterns seen across vertebrate species.

By highlighting the enormous functional overlap within paralogous *Hox* groups, Wellik and Capecchi have greatly improved our understanding of the role of these master genes in skeletal patterning. These results might also have potential implications for future studies of skeletal birth defects in humans.

Victoria Kitchener



References and links

ORIGINAL RESEARCH PAPER Wellik, D. M. & Capecchi, M. R. *Hox10* and *Hox11* genes are required to globally pattern the mammalian skeleton. *Science* **301**, 363–367 (2003)

TECHNOLOGY

Shining a new light on genetic variation

The detection and quantification of uncommon sequence variants within a population of DNA molecules is central to many areas of biomedical research. Now, Vogelstein and colleagues describe a method that can reliably and sensitively genotype and quantify millions of individual DNA molecules using standard laboratory equipment. The process, which the authors have named BEAMing after four of its main components (beads, emulsion, amplification and magnetics), has six basic steps.

First, biotinylated oligonucleotides are coupled to streptavidin-coated superparamagnetic beads (any unbound oligonucleotides are removed by washing). Second, water-in-oil microemulsions are prepared for PCR. The water phase contains all of the reagents that are needed for PCR plus the oligonucleotide-bound beads and template DNA. Third, PCR cycling of the microemulsions is carried out. Each microemulsion contains an average of less than one bead and less than one template, but if the template and bead are present in the same microemulsion then amplification occurs. Fourth, the microemulsions are 'broken' by the addition of non-ionic detergent and the beads are captured with a magnet. Fifth, fluorescein-conjugated or biotin-conjugated oligonucleotides, which can detect sequence variations among the templates, are hybridized to the templates, and, again, the beads are magnetically captured. The beads are then incubated with fluorescently labelled antibodies that label the bound hybridization probes — after laser excitation the beads that contain a PCR product appear as red or green. Sixth, flow cytometry, using standard two-colour analysis, is used to count the two different populations of DNA molecules.

As well as being sensitive, reliable and accessible, BEAMing has various other benefits: by analysing more beads its sensitivity can be increased; the fraction of variant sequences can be quantified; variant sequences can be purified after flow cytometry and analysed further; and it also has the potential to be automated.

Although the authors chose to focus on the application of BEAMing to sequence variation, they say that "it could also be used to quantify epigenetic alterations such as methylation". The list of potential applications goes on. And so it seems that this technique really does promise to shine a new light on genetic variation.

Natalie Wilson

References and links

ORIGINAL RESEARCH PAPER Dressman, D. *et al.* Transforming single DNA molecules into fluroescent magnetic particles for detection and enumeration of genetic variations. *Proc. Natl Acad. Sci. USA* **100**, 8817–8822 (2003)

WEB SITE

Bert Vogelstein's laboratory: http://www.coloncancer.org