

well-established techniques for manipulating electron beams in a vacuum. Two decades later the new field of mesoscopic physics arose, in which pieces of metal or semiconductor were made so small and cold that the conduction electrons moved around in them as coherent waves. Fundamental to mesoscopic systems is the interference between a host of different paths available to electrons passing from one point to another. In a magnetic field the Aharonov–Bohm effect should occur between every pair of possible paths, with important but generally rather messy consequences, because the paths are often very complicated and numerous.

It was predicted⁴ however that, for the particular geometry of a small, thin-walled hollow metallic cylinder in an external magnetic field (Fig. 1b), the Aharonov–Bohm effect should cause the electrical conductance to oscillate with the magnetic flux through the cylinder's bore. When researchers evaporated a normal metal film onto a two-micrometre-thick insulating fibre they found exactly this behaviour⁵. Moreover, since then the Aharonov–Bohm effect has become thoroughly established as a basic principle in the physics of mesoscopic systems (many of which, incidentally, are created by drawing patterns with good old electron beams).

Nearly two decades further on, mesoscopic devices have now become 'nanostructures', whose size scales reach down to the molecular level, and which sometimes even employ individual molecules at their heart. Rapidly becoming the archetype of these is a particular class of wire-like molecules with extraordinary electrical properties — the carbon nanotubes⁶. The simplest nanotubes are long, flexible, hollow cylinders, each like a drinking straw rolled from a single graphite layer, where the atoms are arranged in a hexagonal lattice. Because these tubes are so narrow (around 1.5 nm in diameter), electrons are quantum mechanically restricted to move only parallel to the tube axis. In the past couple of years this has opened up a real-life laboratory for one-dimensional physics^{7,8}, long the preserve of theorists. Another, more common type of nanotube contains many coaxial graphitic cylinders. In these thicker (typically 10-nanometre or more) multiwalled tubes, the electrons can move relatively freely over the outer cylindrical surface — just the thing for observing the Aharonov–Bohm effect.

Bachtold *et al.*¹ attached metal leads to individual multiwalled tubes, and found conductance oscillations consistent with the metallic cylinder theory. Their results dramatically demonstrate the potential for science in such nanostructures, where physics and chemistry merge. The electron-beam paths in the Aharonov–Bohm experiment have effectively been replaced by molecular

orbitals. Bachtold *et al.* were also surprised to find extra oscillations with a smaller period, corresponding to electron paths that revolve several times around the cylinder before interfering. Although there is no specific explanation for these oscillations yet, they could well represent the underlying nature of the orbitals in the nanotube, such as might be caused by a built-in twist in the graphite lattice. This illustrates the likelihood that, thanks to the infinitely rich nature of molecular orbitals, future molecular-scale experiments will lead to new science that goes far beyond the domain of the old-fashioned macroscopic laboratories.

It was not by coincidence that this seven-orders-of-magnitude reduction in the size of physics experiments came over the same period that electric valves, arc lamps and cathode ray tubes were replaced by transistors, solid-state lasers and flat-panel dis-

plays. The technology of nanostructures is driven largely by the urge to make ever smaller and faster electronics⁹. However, scientists are both pushing and riding the wave, and physicists will be busy for a long while exploring and exploiting their new nanometre-sized laboratories. □

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Development

Hox proteins reach out round DNA

Matthew P. Scott

It might be misquoted of Hox genes that “in the field of development, never was so much owed by so many tissues to so few genes”. All animals have Hox genes, and the multifarious roles of Hox proteins in building vital organs such as brain, muscle, bone and gut endow them with honorary status among the transcription factors, both in controlling the fates of different cells and in arranging them into working structures. Valuable insights into how Hox proteins work are offered by two new papers, one by Passner *et al.*¹ on page 714 of this issue and the other by Piper *et al.*² in last week's *Cell*, which each describe the structure of a Hox protein on its DNA-binding site in combination with a cofactor protein.

Over a century ago, Bateson³ described homeotic transformations as a change in one body part into the likeness of another, broad-mindedly including misplaced insect appendages and transformations of mammalian vertebrae. The vertebrae pattern switches were both anterior to posterior and vice versa, which are the sorts of changes that are now known to be caused by ectopic or by reduced Hox gene function.

Hox genes encode transcription factors that coordinate the expression of genes necessary for the implementation of different developmental pathways, so they can generate some bizarre phenotypes when mutated: for example, *Ultrabithorax* mutations in the fruitfly *Drosophila* create an extra set of wings, and *Antennapedia* mutants grow legs in place of antennae; in the nematode *Caenorhabditis elegans*, Hox mutations reroute migrating cells; in mouse, they convert one type of vertebra into another or change

the pattern of hindbrain development; and in humans, they cause fusions and duplications in hand and foot digits.

Hox genes are a specific subset of the hundreds of homeobox genes⁴. The homeobox is a 180-base-pair DNA sequence that encodes a 60-amino-acid DNA-binding domain, the homeodomain. Hox genes are distinguished from other homeobox genes both by their more closely related homeodomain sequences and by their clustered positions on chromosomes. Four clusters of mammalian Hox genes, 39 genes in all, are thought to be derived from one ancestral cluster — in flies, the single ancestral cluster evidently split into two parts. Within each fly or mammalian cluster, Hox genes are conveniently organized according to the body region they affect: at one end of the cluster, genes are found that are involved in anterior (head) development; at the other end, the genes work for more posterior structures. In between, genes are arranged roughly in the order of their influence along the body, proceeding from head to tail. Each mammalian complex is designated as A–D, and each gene within the complex by a paralogue number, 1–13: paralogue 1 genes act on the most anterior regions of the body axis, and paralogue 13 genes on the most posterior ones. Genes that have the same paralogue number but are located in different mammalian clusters are highly related and sometimes partially redundant in function.

The *Ultrabithorax* (*Ubx*) protein studied by Passner *et al.*¹ is produced and functional in the posterior thoracic and anterior abdominal segments of *Drosophila*⁵. *Ubx* mutations lead to posterior-to-central tho-

rax transformations, giving rise to Lewis's famous four-winged fly⁵, and to abdomen-to-thorax transformations. The human HoxB-1 protein, the subject of the study by Piper *et al.*², is required for normal development of rhombomere 4 of the hindbrain⁶, where *hoxb-1* is expressed. *hoxb-1* is most related to the *Drosophila* 'head' gene *labial*, and is therefore in a different class from *Ubx*, which is most closely related to Hox6–8.

Hox protein products of both *Ubx* and *hoxb-1* interact functionally with another group of homeodomain proteins called Extradenticle (Exd) in flies and Pbx in mammals. These proteins enhance the specificity and affinity of DNA binding by Hox proteins⁷. The *pbx1* gene was first found at chromosome breakpoints associated with human leukaemia. Mutations in *exd* cause diverse homeotic transformations, and yet *exd* does not transcriptionally regulate most Hox genes. Instead, Exd cooperates with Hox proteins to bind to DNA, so loss of Exd function leads to a compound phenotype incorporating multiple Hox phenotypes. Similarly, Pbx1 has been found to cooperate with Hox proteins in, for example, *hoxb-1* autoregulation⁸. Thus, Exd/Pbx1 proteins act as cofactors that increase the DNA-sequence specificity of Hox proteins.

The two new crystal structures that have been solved for the Ubx–Exd–DNA and HoxB-1–Pbx1–DNA complexes^{1,2} both display the three α -helices that are characteristic of homeodomain structures. The DNA contacts for Exd and Pbx1 differ from those of most homeodomains, however, and may be weaker or less discriminating. The cooperative binding between the Hox and Exd/Pbx1 proteins is due to a hexapeptide outside and amino-terminal to the homeodomain. The crystal structures reveal how the individual components cooperate in binding to one another: the two homeodomains bind to opposite sides of the double helix of DNA. The Hox protein hexapeptide, which is on the end of a long linker arm, reaches around to Exd or Pbx1 on the other side, inserting itself into a special pocket in Exd or Pbx1 which is created partly by a tripeptide loop peculiar to the Exd/Pbx1 group of homeodomains. Because both homeodomains make sequence-specific contacts with bases (sometimes the same bases) and with backbone atoms, the specificity and binding affinity of the whole complex are enhanced.

Remarkably, a region of Pbx1 that is outside the homeodomain on the carboxy-terminal side, and which is essential for the cooperative binding of Pbx1 and HoxB-1, does not touch either HoxB-1 or the DNA. Instead, it forms a fourth helix that packs against the third helix, which is the most important determinant of sequence specificity. This fourth helix is presumed to stabilize or modify the helix-3 configuration in

order to impart greater DNA-binding strength and to hold helix-3 in an optimal contact position for insertion of the hexapeptide. In addition, the DNA is bent by about 10° by both Pbx1 and HoxB-1, which may influence the strength of binding.

What are the implications of the features revealed in these structures? First, Hox proteins of different paralogue groups associate in quite a similar way with Exd/Pbx proteins. Second, the basis for sequence specificity is now clearer than when Hox proteins alone were tested on DNA, but additional genuine target-gene *cis*-regulatory sequences need to be identified before we can find out how the combinatorial sequence recognition is actually employed. Third, the different actions of various paralogue groups could be accomplished by association with proteins other than Exd/Pbx. Some paralogue groups have a hexapeptide sequence of Y/F-P-W-M-K/R (single-letter amino-acid code), whereas others contain a tryptophan (W) residue in a different context⁴. Hexapeptides of different paralogue groups have distinct sequences, paralogue groups have characteristic linker lengths between hexapeptide and homeodomain, and some paralogue groups have characteristic residues carboxy-terminal to the homeodomain⁹. Each type of hexapeptide could insert into a different cofactor if more proteins like Exd/Pbx exist. One candidate is the Meis/Hth protein, a cofactor for Exd/Pbx^{10–12}. Alternatively, the linker length between the hexapeptide and the homeodomain could affect which target-gene sequence can be bound by the combination of a Hox protein and Exd or Pbx. A stretched

or distorted linker could alter the insertion orientation of the hexapeptide. Fourth, the sequence conservation found among some Hox paralogues carboxy-terminal to the homeodomain may indicate that such regions have an indirect role in DNA binding, as in the case of Pbx1.

Satisfaction comes from seeing universals emerge, such as structural similarities between homeodomains and bacterial helix–turn–helix proteins and yeast mating-type homeodomain proteins — but at the same time, the new Hox–Exd/Pbx structures are rich in novelty. Bateson would be pleased. □

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Bioenergetics

One price to run, swim or fly?

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It has long been accepted that it is metabolically cheaper for animals to fly than to run, and cheaper still for them to swim¹. Measurements of oxygen consumption have shown, in comparisons between animals of equal mass, that the energy used by a mammal to run one kilometre is enough to enable a bird to fly about two kilometres, or a fish to swim up to ten kilometres. But in a recent paper in *Philosophical Transactions of the Royal Society*, Williams² repeats the analysis for mammals alone and reaches the surprising conclusion that their three types of locomotion are almost equally priced. Thus, a 100-kg seal needs as much energy to swim a kilometre as a 100-kg pony would need to run a kilometre, and a 1-kg fruitbat needs only a little less energy to fly a kilometre than a mongoose of the same mass needs to run it².

The energy cost of animal locomotion is expressed as the cost of transport (energy used)/(body mass \times distance travelled). It

may be defined either to include all the metabolic energy used on the journey (total cost of transport) or only the extra energy, excluding that which would have been used if the animal had been resting (net cost).

Williams has compared total costs of transport for marine mammals with different degrees of aquatic specialization, ranging from muskrats and sea otters, which have bodies and paws like those of their fully terrestrial relatives, to sea lions with streamlined bodies and flippers, and even whales, which have evolved tail flukes for efficient swimming. She finds that swimming is very expensive for semi-aquatic mammals: a 20-kg sea otter swimming at the surface uses five times as much energy per kilometre as a sea lion of the same mass swimming under water. For this reason, semi-aquatic mammals are limited to low speeds. Sea otters are slower than sea lions, and dolphins are five times as fast, over 50 metres, as world-record-holding human