Molecular biology

No introns in insect globin genes

from Athel Cornish-Bowden

ANTOINE and Niessing report in this issue of *Nature* (p. 795) that the genes coding for globins in insects entirely lack the introns hitherto regarded as a stable feature of the globin gene family. In vertebrates, all of the known genes for myoglobin and haemoglobin are organized in the same way, with the coding regions interrupted by introns at positions that correspond exactly from gene to gene. Not only does this imply conservation of intron arrangement throughout the 600–800 Myr of vertebrate evolution, but the discovery (Jensen, E. O. *et al. Nature* **291**, 677; 1981) that the leghaemoglobin genes of leguminous plants contain three introns, two of them corresponding with the two seen in vertebrates, implies a much longer period of conservation.

Despite the embarrassing existence of plant leghaemoglobin, the globins have until now been regarded as characteristic of vertebrates. Nonetheless, the twelve different haemoglobins of the midge *Chironomus thummi thummi* are clearly true globins. Their amino acid sequences agree with that of the β-chain of vertebrate haemoglobin at about 16 per cent of sites, a degree of similarity that would arise by chance with a probability less than 0.01 per cent, and one that is not much less than the 19 per cent identity between human myoglobin and lamprey globin. A possible way to account for the lack of introns in insect haemoglobin genes is by arguing that they are not true genes coding for proteins in the normal living state but are 'processed pseudogenes' that are not expressed *in vivo*. Antoine and Niessing present experimental results, however, that would make this possibility highly unlikely.

The stability hitherto apparent in the organization of globin genes in vertebrates is not observed in other gene families. For example, human α_1-antitrypsin and chicken ovalbumin are two proteins whose amino acid sequences show a clear relationship, but whose genes have completely different exon–intron arrangements (Leicht, M. *et al. Nature* **297**, 655; 1982), which requires one to postulate either that introns have been inserted independently into the two lineages or that both have suffered deletion of introns from a common ancestral gene that contained at least ten. Current work by Nakanishi and co-workers (Tanaka, T., Ohkubo, H. & Nakanishi, S. *J. biol. Chem.* **259**, 8063; 1984) on the gene for angiotensinogen, another protein from the same family, provides additional information: although rat angiotensinogen resembles α_1-antitrypsin considerably less than α_1-antitrypsin and ovalbumin resemble one another, its gene contains four introns located at positions that agree closely with the arrangement in the α_1-antitrypsin gene. This result indicates that intron arrangement is not maintained unchanged forever, and that modifications to the intron arrangement do not occur in step with the evolution of the corresponding proteins.

The lack of introns in the genes for insect haemoglobins is not difficult to accept unless one is committed to the hypothesis of stability of globin genes. Evidence from other gene families certainly allows one to postulate that an ancestral gene may have contained three (or more) introns, of which three remain in the plant genes, but only two in those of vertebrates and none in those of insects. Although, at first sight, it might seem surprising that vertebrates apparently correspond better to plants than to insects, the number of deletions postulated is extremely small in statistical terms, and if three deletions are placed at random on the evolutionary tree relating plants, vertebrates and insects there is only about a 65 per cent likelihood that at least two of them will fall on the part of the tree connecting the plants to the point of separation between vertebrates and insects (assuming, for simplicity, that the vertebrate and insect lines separated about half as long ago as the plant and animal lines).

It may still be worthwhile to consider whether the leghaemoglobin gene might have been acquired by the leguminous plants much more recently than the evolutionary separation of the plant and animal kingdoms. The light-emitting bacterium *Photobacter leiognathi* carries a gene for a copper–zinc superoxide dismutase that is clearly fish-like, though different from the enzyme of its ponyfish host (Martin, J. P. & Fridovich, I. *J. biol. Chem.* **256**, 6080; 1981); thus, transfer of genetic information from a eukaryote to a prokaryote appears to have occurred during the period of symbiotic association. In view of this precedent, it can hardly be regarded as impossible for the leguminous plants to have acquired their globin gene from an animal original. □

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Developmental biology

On to the vertebrates

from Julian Lewis

THE complexity of an embryo is the result of diversification and rearrangement: the tissues become subdivided into parts with different characters, and those parts move and become interwoven to form increasingly intricate assemblies. The body segments of a vertebrate provide a good example. As the neural tube is forming along the axis of the body, the long slab of mesoderm on either side of it begins to split up into a series of little blocks, the somites. Later, each somite in its turn is partitioned to give the dermis of the trunk, the cartilage and bone of the vertebrae and ribs, and the skeletal muscle cells. While these muscle and connective tissue cells are differentiating, other cells, with other origins, move in amongst them. Spinal nerves grow out from the neural tube and the spinal ganglia, while invading endothelial cells create a network of blood vessels. The nerves and vessels, in their own periodic arrangement, mirror the segmental pattern of the somite-derived tissues. Any such structure created by assembly of components from different sources begs the question, how is the assembly coordinated? Does the somite segmentation dictate the neural pattern, or vice versa, or does some common factor cause the tissues to segment independently in parallel? Experiments on chick embryos, described by Keynes and Stern on page 786 of this issue of *Nature*, give an answer to this question, confirming the conclusions of Detwiler from his work on amphibians long ago (*J. exp. Zool.* **67**, 395; 1934). The new findings at the same time throw light on another question that is deeper, subtler and more far-reaching in its implications: how is the physical pattern of segmentation in the mesoderm related to the pattern of intrinsic characters of mesodermal cells?

The methods used are simple: a whole-mount stain for early embryonic nerve fibres, and the traditional embryologist's technique of surgical rearrangement of the developing tissues. The first observation is that the nerve fibres always emerge from the neural tube at the level of the anterior (or cranial) half of each somite, and proceed to grow out through this half of the somite only. If a portion of the neural tube is excised, before any axons have emerged, and is replaced with its anteroposterior (cranio-caudal) axis reversed, the pattern of nerve outgrowth is the same: the axons still pass out through the anterior halves of the somites. Thus, the sites of nerve outgrowth are not determined by the neural tube, but by its surroundings. If, however, a portion of the somitic mesoderm is excised and replaced in reverse orientation at a similarly early stage, the nerve fibres emerge through the parts of the somites that are now posterior, but were anterior with respect to the original orientation of the mesoderm. Evidently the somites