Homoeotic gene transcripts in the neural tissue of insects

Homoeotic mutations, studied primarily in the fruit fly Drosophila, transform one body segment or part into another. The molecular analysis of some of the genes in which such mutations occur has led to the surprising finding that the most abundant, though not exclusive, accumulation of homoeotic gene RNA transcripts is in neural tissue. The transcripts are localized specifically in certain ganglia, corresponding to the body segments which previous genetic and developmental studies had shown to be the sites of gene function. The questions now being asked are how is the positional specificity of transcription achieved and how does the expression of homoeotic genes guide particular tissues into particular developmental pathways?

Homoeotic mutations result in the replacement of a part of an insect with a different part normally found elsewhere. For example, mutations in the proboscipedia (pb) gene, originally described in the fruit fly Drosophila in 1933, result in parts of the proboscis being transformed into legs or antennae. Some alleles are temperature sensitive, resulting in the proboscis to leg transformation if the flies are raised at one temperature, and the alternative proboscis to antenna switch if another rearing temperature is used. Other genes in which mutations induce such changes in developmental programming include Antennapedia (Antp, legs for antennae and vice versa), Ultrabithorax (Ubx, vestigial wings or 'halteres' into wings), Contrabithorax (Cbx, wings into halteres), and Ophthal-moptera (Opt, eyes into wings). There are many others, not only in Drosophila but also in other insects.

The progression of the field from viewing homoeotic genes as a set of curious oddities to understanding them as a coherent, interacting group of loci was largely dependent on the work of E. B. Lewis at the California Institute of Technology. Other important perspectives on the significance of these mutations come from the work of Antonio García-Bellido.

Lewis' work is focused on a cluster of Drosophila homoeotic genes which he named the Bithorax Complex (BX-C), which has recently been reviewed. His critical observations were that multiple homoeotic genes are clustered in a tightly linked group and that genetic tests could be used to demonstrate functional interactions between the genes. From his work came the idea that the pattern of differentiation of a particular body segment, or part of a body segment, depends on the array of homoeotic genes that is active in that segment. Since insects evolved from ancestral forms with one pair of legs for each segment, Lewis reasoned that one evolutionary step would be the appearance of a gene that suppresses leg development in some of the body segments. Thus an abdominal segment with no legs is more advanced evolutionarily than a thoracic segment with legs. Mutations in the gene bithoraxoid (bxo), a component gene of the BX-C, can result in the most anterior abdominal segment developing with a pair of legs. A normal function of the wild-type allele of bxo (bxo+) is apparently to suppress leg development in the first abdominal segment.

Characterization of more of the BX-C genes revealed an intriguing organization of the loci: with one exception, the sequence of the genes along the chromosome corresponds to the sequence of the body segments which they affect. Lewis proposed that the different genes had arisen by multiplication of an ancestral gene, followed by divergence and specialization of the genes to permit and govern unique segmental characteristics.

The BX-C includes loci required for proper differentiation of the posterior thoracic segment and the abdominal segments. The expectation was that other genes would be found to be involved in the control of the differentiation of the other thoracic segments and the highly evolved head segments. T. C. Kaufman and his colleagues at Indiana University recognized a second cluster of genes, subsequently named the Antennapedia Complex (ANT-C), which are important for head and thoracic segment differentiation. The ANT-C includes pb and Antp, mentioned above, as well as several other loci essential for proper differentiation of the head segments.

![Fig. 1. Part of the embryonic nervous system (14h) of Drosophila showing the localization (black grains) of regions of hybridization with a Ubx probe. The 'ladder' formed by the commissures in neuropil is clearly visible. Each neuromere in the thoracic and abdominal region is associated with two lateral commissures separated by cell bodies. The approximate positions of each neuromere is indicated, but the precise boundaries of each segmental derivative cannot be identified. S = sub-esophageal neuromere. P, M, T = pro- (first), meso- (second) and meta- (third) thoracic neuromeres. 1-8 = abdominal neuromeres. (Reproduced with permission from Ref. 21.)](image-url)
for normal development. Complex genetic interactions among the ANT-C loci are reminiscent of genetic interactions among BX-C loci. In both cases the genetic interactions are thought to be indicative of functional relationships. Most importantly, it has been shown that it is the combination of ANT-C and BX-C genes active in each segment that determines the pathway of differentiation. There may, of course, be other as yet unidentified genes that are also involved.

If in normal development each segment's developmental fate is regulated by the array of homeotic genes activated in that tissue, it is to be expected that another class of gene products is involved in controlling when and where the ANT-C and BX-C genes are active. Three genes have been identified in which mutations result in excessive and inappropriate BX-C and ANT-C gene activity. The three genes Polycomb (Pc), Polycomblike (Pcl), and extra sex comb (esc) all behave formally as position-specific repressors of BX-C and ANT-C gene function. Pc, Pcl and esc must be differentially active in different parts of the early embryo, when many pattern-determining 'decisions' are made and the continued activity of the Pc and Pcl (but not esc) regulating genes is required later in development for maintenance of the decisions. If Pc, Pcl and esc are the next step upstream from the BX-C and ANT-C in the regulatory hierarchy, a critical and unanswered question is what lies just downstream of the BX-C and ANT-C genes. How do these genes make segments differ when the structural components of the different segments are largely the same?

Most of the genetic investigation of the BX-C and ANT-C genes has depended on recognition of segment-specific cuticular structures. It was clear from these studies that epidermal cell differentiation was regulated by the homeotic genes. But what of the internal cells, the neural ectoderm, the mesodermal tissues, and the gut? Investigation of the precise locations of homeotic gene expression had to await molecular analysis of the two gene complexes. When molecular techniques were applied, those studying homeotic genes suddenly found themselves to be intensely interested in neural development.

The molecular studies of homeotic genes began with pioneering experiments in D. Hogness' laboratory at Stanford University. Hogness and his colleagues W. Bender and P. Spliter developed methods for isolating long stretches of chromosomal DNA as a series of overlapping cloned recombinant DNA pieces. One of the first applications of these 'chromosome walking' techniques was the isolation of BX-C DNA (Ref. 19). Subsequently, many other Drosophila genes and gene complexes were cloned, including the ANT-C (Refs. 20, 21). The isolation of DNA from the two complexes allowed the molecular mapping of a large number of mutations, primarily insertions of transposons and break-points of chromosome inversions and translocations. The locations of the mutations and mapping of transcripts revealed many new puzzles, mostly too complex and too unresolved to be discussed here, but one startling fact became clear quickly: the apparent transcription units of several of the BX-C and ANT-C genes are extraordinarily large, for example about 73,000 base pairs for the Ultrabithorax (Ubx) unit of the BX-C (Ref. 22) and about 103,000 base pairs for the Antp locus of the ANT-C (Refs. 20, 21). The purpose of these enormous transcription units is not yet known, but in each case differential processing gives rise to multiple RNA products. The genetic complexity of Ubx and Antp is probably in part due to different functions encoded by component parts of each locus.

The availability of molecular probes for Ubx and Antp has led to an exciting series of recent reports on the localization of homeotic gene transcripts in tissues and cells. M. Akam, now at the Department of Genetics at Cambridge University, and a group led by Walter Gehring at the Biozentrum in Basel, each developed improved methods for in situ hybridization of radioactively-labelled DNA probes to RNA in sectioned tissues. Hafen et al. estimate the sensitivity of their hybridizations to be sites of transcription when sectioned larvae were examined. No transcripts were detected in endodermal tissues. Transcripts were found to be distributed within each tissue in a segment-specific manner, so, for example, an Antp probe labels primarily the mesothoracic neuromere of the ventral ganglion of an 18-h-old embryo. The large dorsal hemispheres of the brain do not contain detectable levels of Antp (or Ubx) transcripts.

The striking concentration of homeotic gene transcripts in neural tissue does not come as a complete surprise. It has been shown that at least the Ubx locus functions in neural tissue. Ubx mutations cause partial transformation of neural tissue. Each mutation affects a different segment of the neural axis. Ubx transcripts are found in tissues of the third thoracic and abdominal segments, while Antp transcripts were seen in all three thoracic segments and in the abdominal segments. (The activity of Antp in the abdomen had been revealed by studies of embryos deficient for BX-C genes.)

The distribution of RNA for each gene need not have correlated with the results of developmental and genetic studies. It was conceivable, for example, that the genes could be transcribed in all tissues and that the transcripts would be differentially processed and expressed to give tissue specific effects. The in situ hybridization results show that transcriptional control is in fact important, but do not help us, as yet, with the question of the importance of other levels of control. Another reasonable question might be whether the homoecotic genes could be expressed in one tissue (as RNA) but exert their effects on other tissues, as is the case for many hormones. However, many studies have shown that patches of tissue that are mutant for a homeotic gene will behave autonomously, having the appropriate mutant phenotype even when surrounded by wild type tissue. These results suggest that most homeotic gene act in a cell autonomous fashion, with each cell developing according to its own genotype. (Some, probably minor, exceptions have been noted.) The in situ hybridizations demonstrate that the sites of gene function are also the sites of transcription.

The specific localization of Antp and Ubx transcripts in certain body segments is not accompanied by a comparable tissue specificity. In embryos, the most dramatic accumulation of RNA from both homeotic genes is in the nervous system. However, other ectodermal and mesodermal tissues (e.g. epidermis, tracheal cells, muscles) were also found to be sites of transcription when sectioned larvae were examined. No transcripts were detected in endodermal tissues. Transcripts were found to be distributed within each tissue in a segment-specific manner, so, for example, an Antp probe labels primarily the mesothoracic neuromere of the ventral ganglion of an 18-h-old embryo. The large dorsal hemispheres of the brain do not contain detectable levels of Antp (or Ubx) transcripts.

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The in situ hybridization results have strengthened the idea that mesodermal and neuroectodermal tissues are regulated in their development by homoeotic genes. A recent paper provides additional support for the importance of BX-C genes in the mesoderm24. Homoeotic genes may be important in the organization of neural development, and similar genes may operate in vertebrates. The usefulness of the in situ technique undoubtedly extends well beyond the homoeotic gene applications and may be important, for example, in localizing the sites of production of particular neuropeptides. The application of the in situ technique to homoeosis has once more confirmed that our trust in the geneticists is well placed.

Reading list
1 Bridges, C.B. and Dobzhansky, Th. (1933) Wilhelm Roux Arch. Entwicklungsmech. 172, 575–590
15 Duncan, I. M. (1982) Genetics 102, 49–70
26 Duncan, I. L. (1982) Genetics 100, 820

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British neuroscience unit to close

The Medical Research Council (MRC), which funds a wide range of biomedical research in the UK, decided in principle last month to close its Neurochemical Pharmacology Unit in Cambridge. The unit was set up in the early 1970s under Leslie Iversen, who remained its director for 12 years. During that time the unit established an international reputation for research into the neurochemical basis of neurological disease. It also set up a "brain bank" of neural tissue, unique in the UK, which has been invaluable in the study of Huntington's disease and other disorders.

In 1982 Iversen announced his intention of leaving the unit a year later to join the new neuroscience research laboratory of Merck. Sharp and Dohme. At that time the MRC did not plan to close the unit, and the council advertised for a new director. But to date it has been unable to find a suitably qualified candidate who is willing to accept the post. For this reason it has decided to disband the research team (who will be redeployed at other MRC units) and turn the purpose-built laboratories over to some other use.

The details of this redeployment have still to be worked out in consultation with staff members and other interested parties. Similar research may be undertaken at the new neuroanatomical unit the Medical Research Council plans to open in Oxford.