Notes on the genetics of pattern formation in the internal organs of *Drosophila*

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We now have the beginnings of a working hypothesis to explain genetic control of pattern formation in the epidermis of Drosophila. *This hypothesis treats the epidermis as divided up into compartments, each being made by a distinct population of cells with distinct genetic instructions. Many of the compartments have been mapped onto the pattern of the adult fly and some of the genes involved in regulating their constituent cells have been identified The other organs of the insect body – such as the central nervous system, the muscles and the gut – are almost completely uncharted territory Here I discuss how far the principles worked out in the epidermis can also be applied to the internal organs*

The formation of patterns of cells in developing animals is a mysterious process that has foxed generations of embryologists – it is not even clear whether it can be best understood at the level of molecules, genes, cells or organs The insect epidermis is a convenient system in which to study pattern formation and some progress has been made towards a genetic and cellular description – particularly in *Drosophila* We find that the *Drosophila* epidermis is made part by part; each compartment deriving from a small group of founder cells which are under specific instructions that we call a genetic address[1] We imagine that the genetic address is made up of a small number of special 'selector genes'[2] (a different combination in each compartment) which in some inscrutable way work together to determine the portion of anatomy that is constructed[1]. The ways the groups of founder cells are defined and the steps which lead to the deployment of selector genes within them are little understood, but it is clear that these processes are part of the first steps in segmentation

In the epidermis a segment is defined by the cell lineage and consists of two compartments, one anterior (A) and one posterior (P)[1,3] In the *Drosophila* embryo the main portion of the body ectoderm is probably made by 28 compartments arranged in a continuous chain, beginning with a P compartment and ending with an A (see Ref 4) One distinction between all A and all P compartments is that there is a special gene called *engrailed* which is active only in cells belonging to P compartments[3]. We think that this gene is responsible for labelling posterior cells in some way so that they make appropriate patterns and mingle less freely with anterior cells In consequence of this, A and P compartments are maintained as discrete groups of cells that do not get mixed up with each other as they divide and increase[3]. Distinctions between compartments along the axis of the body depend on differential deployment of selector genes such as elements of the bithorax and Antennapedia complexes (see Refs 5 and 6 and see Levine's article).

A picture of the genetic control of pattern formation in the epidermis is beginning to take shape – what about the rest of the fly? Segmentation of the internal organs of *Drosophila* is so little understood that a martian scholar with access to our scientific literature might conclude that the fruitfly is an empty box Here I look at development of the soft parts of *Drosophila* and try to see how they compare with the epidermis. Are the CNS, the mesoderm and the gut divided into chains of A and P compartments? Does the diversification of the soft parts depend on differential activity of selector genes? The answers to these and other related questions depend on putting each of the internal organs through an experimental interrogation in which four questions are asked. (1) Where does the organ arise from in the embryo? (2) Is it divided up into precise domains with independent cell lineages? (3) Is there expression of selector genes in the cells of the organ in question? (4) Do selector genes have a direct role in the cells – that is if the wildtype gene is removed from the cells is there an autonomous effect on the pattern? I will apply these questions to the CNS, the mesodermal derivatives and the gut

The central nervous system

It used to be thought that the neuroblasts, which generate the CNS, arise in the blastoderm stage from a separate strip of cells that lies between the ventrally located mesoderm and the more lateral cells that give rise to the epidermis[7] This has now been shown to be incorrect, presumptive neuroblasts and epidermal cells are intermingled in the entire ventral ectoderm[8] Soon after the blastoderm stage the presumptive epidermis becomes subdivided into compartments[1] Although there is no direct evidence for compartition of the CNS, the way in which presumptive neuroblasts and epidermal cells are jumbled up at blastoderm strongly suggests that groups of cells founding compartments will consist of both cell types. Probably therefore the CNS is divided into P and A compartments

The existence of P compartments in the CNS can also be confirmed genetically by close study of cells mutant for *engrailed* This gene can be used diagnostically because mutations should affect posterior but not anterior cells[3] and might therefore be expected to have direct effects only in specific (posterior) parts of the CNS To test this, genetically marked nuclei which were also carrying a lethal allele of *engrailed* were transplanted into young eggs (The nuclei of the donor were able to make the normal form of an enzyme while the host egg could only make a thermolabile form) When the transplanted nuclei colonized part of the host all their derivatives could be distinguished by first heating the mosaic fly and then staining for the enzyme Most of the fly did not stain but the remainder stained blue and was therefore mutant for *engrailed*-lethal In two of the mosaics that resulted, patches of *engrailed*-lethal tissue colonized the CNS, in some parts of the CNS the pattern appeared normal but in others it was not[9] It seems likely that these abnormalities were due to the *engrailed* mutation affecting the P compartments of the CNS However, there is some evidence against the existence of P compartments there. Kornberg and colleagues[10] report no expression of the *engrailed* gene in the embryonic CNS when they use a labelled probe and *in situ* hybridization – but they point out that their probe may not detect all *engrailed*$^+$ transcripts

What is the role of other selector

genes in the CNS? *In situ* hybridization experiments by Akam[11] and Hafen *et al* [12] have shown that *Ubx*$^+$ (part of the bithorax complex[5,13]) and *Antennapedia*$^+$ (part of the Antennapedia complex[6]) are expressed in specific parts of the CNS, more or less exactly where genetic experiments on the epidermis would predict function[5,13,14]. No one has yet removed wildtype alleles from genetically marked cells of the CNS to test the role of the bithorax complex or Antennapedia complex directly but mutant phenotypes strongly suggest that the CNS needs wild type function of at least the bithorax complex[15,16]

It would seem that the CNS and the epidermis develop according to a very similar program Divided up into P and A compartments, the development of pattern depends on proper deployment of *engrailed*$^+$, the bithorax complex, the Antennapedia complex and, presumably, other selector genes

The somatic and visceral mesoderm

The mesoderm arises from a ventrally located strip of cells which rolls into the embryo during gastrulation and comes to lie inside the ectoderm Soon after gastrulation the mesoderm can be seen to be in two separate parts; a thin inner layer which later wraps around the gut and other organs and is called the visceral or splanchnic mesoderm and a thicker mass of cells which is adjacent to the ectoderm[7] This mass is made up of metameric units, each being separated from the next by a groove[7]. From morphological arguments[4] and *in situ* hybridizations[17] it is probable that these mesodermal units are one segment in length Strangely, they appear to be out of register with the segments of the epidermis and we have therefore called them parasegments[4] In the epidermis a parasegment consists of an anteriorly-located P compartment from one segment and a posteriorly-located A compartment from the next. The mesodermal parasegments are first defined in the embryo as packages of cells that are arranged precisely in register with the parasegments of the ectoderm. But it seems that later on, when the germ band shortens, there is a relative shift between the mesoderm and ectoderm (see centrefold)[4,17] This shift is important when trying to make sense of the effect of mutations on the muscles – as we shall discuss later. First we should look at the cell lineage of the somatic muscles

The cell lineage of the somatic muscles is not so well known as that of the epidermis but in the adult thorax the muscles of each segment are separate from those of the next – that is they are segregated into lineage compartments[18] In the ventral abdomen the muscles are largely made in segmental units although there is some evidence for occasional and later mixing across from one segment to another[19] There is no evidence for A or P compartments in the muscles, and there is no effect of lethal *engrailed* mutants in the muscle cells[18] – even when large masses of muscle in several segments are derived from *engrailed*-lethal cells[9] Moreover, *in situ* hybridizations with probes specific for the *engrailed* gene product show stripes in the ectoderm[10,20], confirming the existence of A and P compartments there, but in the mesoderm expression of *engrailed*$^+$ is only ephemeral[10,20] Apparently the somatic mesoderm is subdivided one less time than the epidermis, meaning that it is divided into parasegments but not into A and P compartments[9]

The genetic determination of muscle pattern presents an intriguing puzzle which is by no means solved *In situ* hybridizations designed to monitor the spatial expression of the *Ubx* element of the bithorax complex, show that there is deployment in the mesoderm[11,17] Initially in at least the blastoderm stage *Ubx*$^+$ is active in the cells which will form parasegments 6–12 but later when the germ band has extended, Akam and Martinez-Arias[17] detect that *Ubx* transcription parasegments 5 and 13, but only in the ectoderm. *Ubx*$^+$ transcription remains confined to parasegments 6–12 in the mesoderm This means that the realm of action of *Ubx*$^+$ and therefore the domains affected by mutants, might be expected to be different in the two germ layers. The regions of the epidermis that are mainly dependent on *Ubx*$^+$ are parasegments 5 and 6, that is the four compartments between the A/P boundary in T2 and the A/P boundary in A1 (see centrefold) *Ubx* mutations therefore transform parasegments 5 and 6 of the epidermis each towards parasegment 4 (see Refs 5, 13 and 21) However, if there is a direct role for *Ubx*$^+$ in the mesoderm, as the transcription pattern suggests, it should be in parasegment 6 but not 5 (see Refs 10, 17) *Ubx* mutations should therefore have no effect in the mesoderm of parasegment 5 (which probably constructs the muscles of T3 (see centrefold), and should transform parasegments 6 towards 5 Consider for example the famous four-winged flies of Lewis[5]

Lewis made flies in which T3 is homeotically transformed by mutants in the *Ubx* domain: these flies have two perfect pairs of wings and two perfect mesothoraces. It has been a mystery as to why dorsal fibrillar muscles are well developed in the normal T2 but absent in the ectopic one[22,23]. However, under the hypothesis spelt out above, the flight muscles of T2 arise from parasegment 4 which is not the responsibility of the bithorax complex The normal muscles of T3 arise from parasegment 5 where *Ubx*$^+$ is not active in the mesoderm[17]. So *Ubx* mutants should not affect the muscles of T2 or T3 which will remain untransformed as observed There is a complication however and that concerns the ventral muscles of the four-winged fly. Here we see two sets of leg-associated muscles which are now very similar – that is the T3 leg muscles now look like T2 leg muscles[23] In thinking about this we should remember that muscle pattern cannot usually be seen out of context, most muscles are recognized by the epidermal contacts they make that help define their size and shape These attachment sites are programmed in the epidermis[24] and in the case of the four-winged fly there is a perfect transformation of the epidermis from T3 to T2 giving an extra set of T2 attachment sites These extra attachment sites are clearly not alone sufficient to result in the development of an extra set of indirect flight muscles in the dorsal part of the transformed T3 segment Here, some genetic change in the muscle nuclei themselves may also be necessary By contrast, in the ventral parts, the change in the attachment sites may perhaps be sufficient to transform T3 leg muscles into T2 ones – without any apparent alteration of the genetic address of the myoblasts. Together these results suggest that the muscles arising from parasegments are possibly differently genetically determined but the development of the muscle pattern involves a two-way exchange of information between muscle and epidermis[24].

How much is muscle pattern dependent on the genotype of the muscle cells themselves and how much on the epidermis to which they attach, or to

innervation? The short and accurate answer is that we do not know. In one experiment we tried to map the embryonic cells which determine the development of a special muscle found in the male abdomen in segment A5 (Ref 25). These embryonic cells are located far ventral to the primordia of the adult epidermis and are probably close to, or identical with, the precursors of the muscle cells themselves This ruled out the adult epidermis (which presumably specifies the attachment sites for the muscle[24]) as the determinant of the muscle itself, but did not prove that the genotype of the muscle cells is alone responsible The conclusive experiment would be to remove genes presumed responsible for the development of muscles from the myoblasts (but not from other cells) and to ask if the muscles are transformed For example, if *Ubx*$^+$ were removed from only the mesodermal cells in parasegment 6, would those cells of A1 now differentiate as if they were parasegment 5 and make thoracic muscles?

Whatever applied to the somatic muscles might be expected to apply to other derivatives of the somatic mesoderm such as the heart and, probably, the fat body[7] The situation in the visceral mesoderm might well be different The visceral mesoderm develops as a layer on the inside of the somatic mesoderm and is distinct from very early on[7] *In situ* hybridization give the impression that genetic specification of the visceral mesoderm is different from the somatic For example, in the embryo, *Ubx*$^+$ expression is limited to a single parasegment of the visceral mesoderm[17], suggesting that segmentation might be less complex in the visceral than the somatic mesoderm Nothing is known of the cell lineage of the visceral mesoderm, so it is not clear whether it is divided into parasegmental or other lineage units

The endoderm

Rather little is known about the cell lineage of the endoderm which constructs only the midgut In the embryo the endoderm consists of two widely separated primordia called the anterior and posterior rudiments and these invaginate and meet to form a simple tube[7] According to Janning[26] the two primordia construct well defined regions at least in the adult but there are no other apparent lineage restrictions. The two rudiments are located outside the chain of 14 parasegments and this supports the view that the endoderm is not divided up into metameric units Also, mutations which disrupt segmentation of ectoderm and mesoderm have no effect on the midgut which, even when the epidermis is grossly deformed, develops well[27] As expected therefore neither *Ubx*$^+$ nor *Antennapedia*$^+$ is transcribed in the midgut[10,12,17] and large sections of the midgut develop normally when they are homozygous for lethal alleles of *engrailed*[9]

Conclusions

The idea that different parts of multicellular animals have evolved at different rates and reached disparate levels of complexity is not new. For example, in Woody Allen's film 'Everything You Wanted to Know About Sex ' this point is skilfully made, the brain being shown as a hitech space center trying to control the medieval genitalia Our present provisional view of the insect body is not so different, the ectoderm, both epidermis and CNS, is most evolved and is divided up into parasegments and subdivided into compartments Each of these compartments has a unique combination of active selector genes (the genetic address[1]) and therefore forms a specific piece of the pattern The somatic mesoderm is more primitive, it is divided up only into parasegments (which each may have different genetic addresses) while the visceral mesoderm is simpler still, at least as far as *Ubx* expression is concerned[17] The endoderm appears to have been inherited relatively unchanged from unsegmented ancestors

Acknowledgements

I would like to thank Michael Akam and Alfonso Martinez-Arias for their advice and criticisms which have contributed greatly to the work reported here

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