widely separated regions on the surface of the molecule, the two subunits are well related by a non-crystallographic twofold axis (in a direction perpendicular to Fig. 1). Their isologous interaction involves, in part, the loops 70–80 which form hydrophobic pockets around the residues Met 14 from the adjacent subunits. These pockets lie on the edges of the interface (Fig. 1), and the region between them, which is closer to the twofold axis, is more polar in character and includes a number of water molecules and ions that are apparently well ordered in the crystal. The residues principally concerned in the inter-subunit contact are indicated in Table 1.

The active sites

An important incentive for this study was the prospect of being able to observe directly a true enzyme–substrate complex with this enzyme[9]. Accordingly we have also examined crystals of the complex with dihydroxyacetone phosphate (DHAP) which, in 4 M phosphate, are not isomorphous with the crystals of the native enzyme[4]. The results of this study[13] indicate that DHAP is bound to the enzyme at each subunit in the pocket shown in Fig. 3 and containing, among other residues of interest, the Glu 165 side chain that has been labelled by active-site-directed inhibitors[27–29]. This possible active site lies at the carboxyl end of the β-barrel and is formed by residues (see also Table 1) from β-strands a, e, f, g, h, and helices D_1, E_1, H_1, together with a few, which include residues 72–75 from the adjacent subunit, in less regular conformations. In the electron-density map of the native enzyme this pocket includes indications of ordered water molecules and an ion, presumably sulphate, which may be bound near the position occupied by the phosphate group of a substrate (Figs 1 and 3).

Crystals of the enzyme–DHAP complex isomorphous with the native are obtained in 3 M phosphate[13] and studies now in progress should reveal more clearly the position, conformation and orientation of the DHAP molecule bound to the enzyme and the extent to which the conformation of the enzyme changes when substrate is bound. The above details are presented since they suggest chemical experiments of the kind that will be needed to establish the nature and role of the functional groups participating in enzyme action. Thus, it may be relatively easy to modify Lys 13 by semi-synthetic methods[30].

Implications

At this stage in the analysis the most striking result is that triose phosphate isomerase resembles other glycolytic enzymes in being composed largely of alternating segments of α- and β-structure that are often folded on each other in similar ways. Because of the cyclic nature of its $(\beta\alpha)_8$-structure, eight different examples of the $\beta\alpha\beta\alpha\beta$-fold, first observed in subtilisin, flavodoxin and lactate dehydrogenase[31], may be chosen in TIM (aAbBc, bBcCd . . . hHaAb in an obvious notation taken from Fig. 2) and each is generally similar to those found in the dehydrogenases and elsewhere[32]. Two are shown in Fig. 4. Furthermore it may be noted that the active sites of all the molecules with extensive parallel β structures that have been analysed in detail so far, are at the carboxyl ends of the pleated sheets, as we observe here in TIM. These findings must be taken into account in the discussion of the evolutionary significance of such structures now in progress, and they have redirected our attention to the suggestion by Rao and Rossmann[31] that similar super-secondary structures may be found in many protein molecules with widely different amino acid sequences as the result of convergent evolution.

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Control of compartment development by the *engrailed* gene in *Drosophila*

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Our results demonstrate that the normal function of the engrailed (en) *gene is required for the maintenance of the straight boundary between cells of the anterior and posterior compartments in the wing of* Drosophila. *We suggest that the activity of the* engrailed *gene is restricted to the posterior compartment where it 'labels' cells so that they do not mix with anterior cells during growth.*

It has recently been discovered that organs in insects are subdivided into precisely defined regions called compartments[1], each being made by all the surviving descendants of a small group of founder cells[1,2]. This founder group generates a polyclone[3] of cells; that is, all their descendants exclusively form the compartment under consideration and no other cells contribute to it. Cells of the founder group are related to each other by position but not by ancestry. Although the whole polyclone always constructs the entire

Chromosomes	II	III	II	III
Parents	*lt stw en*/+; *M(3) i*^{55}/+	×	*cn en*/*SM5*; *mwh jv*/*mwh jv*	

F_1 (1) *cn en*/+; *M(3) i*^{55}/*mwh jv*

(2) *cn en*/*lt stw en*; *M(3) i*^{55}/*mwh jv*

(3) *cn en*/*lt stw en*; +/*mwh jv*

Fig. 1 Cross used in experiment 1. Three classes of offspring shown were analysed for clones homozygous for *mwh* and *jv* (see text).

compartment, the contribution of each of its members varies from individual to individual. During growth a polyclone can become subdivided into two daughter polyclones; before the subdivision a cell can give rise to progeny in both daughter polyclones, whereas afterwards a cell's progeny falls entirely into only one of the two daughter polyclones[1].

At least some compartments seem to be in the control of a small number of genes ('selector' genes[4]). When one of these genes is mutated, an entire compartment may develop the pattern of cell types appropriate for another: for example, in *Drosophila* mutant for the *bithorax* gene the anterior part of the haltere segment is transformed into anterior wing[5]. The border of this transformation seems to coincide with the antero–posterior compartment boundary. This, and other observations, have led to the hypothesis that the compartments are units for the genetic control of development. The idea is that the combination of active selector genes within a polyclone determines the compartment it will construct. These ideas are discussed at length elsewhere[3,4].

The *Drosophila* wing disk is subdivided into two major polyclones by the first larval stage when the wing disk consists of some 10–50 cells[6–8]. One polyclone forms the anterior part of the wing and most of the dorsal thorax, the other makes the posterior part of the wing and the remaining parts of the dorsal thorax. The boundary separating anterior and posterior compartments in the wing blade runs between veins III and IV and is remarkably straight over most of its length.

The *engrailed* gene is an obvious candidate as a selector gene involved in this compartition of the wing[4], because in *en*/*en* flies the posterior region of the wing blade develops vein patterns and bristles normally only found in the anterior region[9]. The mutation has no effect on the anterior region of the wing itself. The transformation of the posterior region is incomplete so that the posterior wing seems to be a mosaic of anterior and posterior patterns.

We suggest that the proper development and maintenance of a border between two compartments may depend on the confronting cell types being different, possibly as a response to the activity in one cell type, but not in the other, of a single selector gene. Where the cell types in neighbouring compartments are the same or similar we expect the border to be non-existent or abnormal.

One test of this conjecture is to look at the line separating anterior from posterior compartments in *engrailed* (*en*/*en*) flies (experiment 1) where the cells on either side of it, instead of being different, as in wild-type flies, are now similar. We have found that we cannot define an antero–posterior demarcation line in *en*/*en* wings although it is perfectly normal in *en*/+ wings. Experiment 2 was designed to determine whether cells making the boundary depend locally on the action of the *en*$^+$ allele. We therefore marked clones of cells mutant for *engrailed* (*en*/*en*) in an otherwise wild type wing (*en*/+). We find that, whereas *engrailed* clones in the anterior part of the wing have no effect on the pattern and define the normal border, *engrailed* clones in the posterior part frequently cross the border and may extend a long way into anterior territory. A full account of this work is pending (P. A. L. and G.M., unpublished).

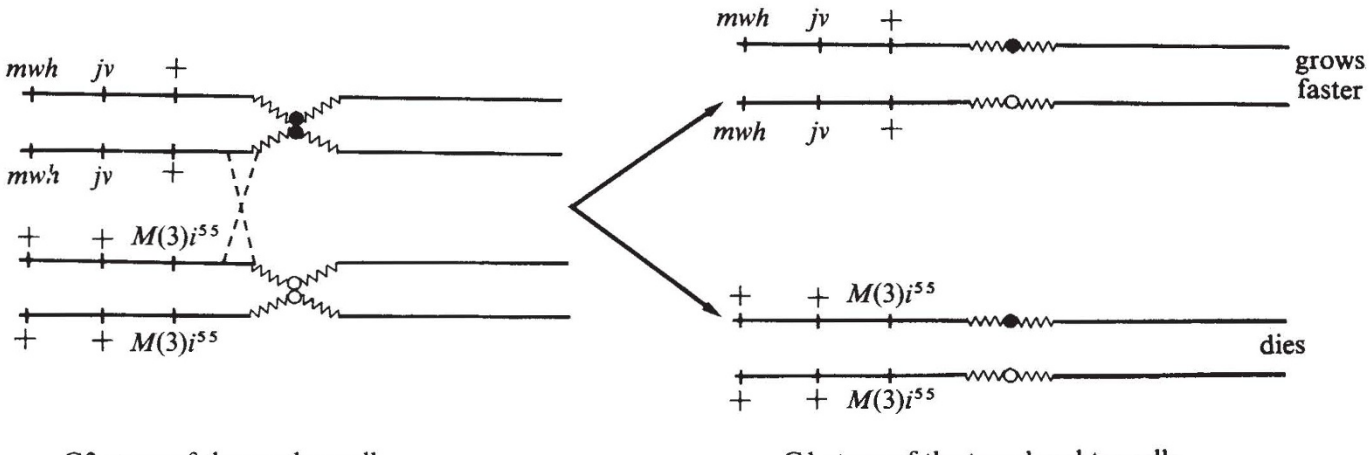
For experiments 1 and 2 we have used the *Minute* technique[10]: as a consequence of a single event induced by X rays, a cell may both become homozygous for a marker gene (which enables us to identify all its progeny) and gain the ability to grow much faster than all other cells in the disk. The clones these marked cells generate consequently give rise to as much as 90% of the compartment but are still unable to cross the compartment border. They therefore define the border for the investigator because their boundaries run along it for hundreds of cells.

The crosses for experiments 1 and 2 were set up as shown in Figs 1 and 3 and the results of somatic crossing over described in Figs 2 and 4. For the description of mutants used see Lindsley and Grell[11]. F_1 larvae were irradiated with 1,000 rad (220 kV at 15 mA, 1 mm aluminium filter, distance 5 cm, rate 500 rad min^{-1}; rad$=2.58\times10^{-4}$ Ci kg^{-1}) at either 36 ± 12 or 60 ± 12 h after egg laying. Normally the insects were cultured at 25 °C, but in some experiments the bottles were transferred to 30 °C after the egg-laying period. The wings were mounted flat on slides for screening.

Experiment 1—marked clones in *engrailed* flies

We studied (Figs 1 and 2) one class of control flies which were *Minute* and wild type at the *engrailed* locus (*en*/+; *M(3)i*^{55}/*mwh jv*). After irradiation of these flies many *mwh*

Fig. 2 Third chromosomes of flies (classes 1 and 2, Fig. 1) to show how mitotic recombination produces daughter cells with different genotypes from the parental cell. Only crossing overs proximal to the *Minute* locus produce homozygous *M*$^+$ clones which grow faster than the parental cell types.



Chromosomes	II	III	II	III
Parents	$M(2)c^{33a}/+$;	$+/+$ ×	*pwn en/SM5; mwh jv/mwh jv*	
F_1	*pwn en/*$M(2)c^{33a}$*; mwh jv/+*			

Fig. 3 Cross used in experiment 2. F_1 offspring shown were analysed for clones homozygous for *pwn*.

clones (*mwh* cells secrete several wing hairs, mwh^+ cells only one) will also be homozygous for $M(3)i^+$ and will therefore grow excessively. As expected from earlier work[1] very large *mwh* clones were found in these flies; many of them filled either the anterior compartment (Fig. 5*a*) or the posterior one (Fig. 5*b*). Out of the 128 clones which filled at least one-sixth of the compartment 69 ran along the antero–posterior boundary for at least half its length; none crossed it. Many of these clones extended on to both the dorsal and ventral wing surfaces.

We also screened two classes of *engrailed* flies. (i) The viability of flies of the genotype *en/en;*$M(3)i^{55}$*/mwh jv* is very poor (less than 1% that of *en/+;*$M(3)i^{55}$*/mwh jv*) and few flies have been scored. There is also a consistent effect on the shape and size of the wing (also noted by R. B. Whittle, personal communication), the posterior compartment being much larger than normal. Of seven *mwh* clones none enabled us to define the antero–posterior boundary and three of them crossed the line where the boundary is in *en/+* flies (Fig. 5*c*). (ii) Because we cannot take advantage of the M^+ effect in flies of genotype *en/en; mwh jv/+*, the clones were rather small and the boundaries much more difficult to define. Nevertheless 53 *mwh* clones were found, nine of which clearly crossed the line where the antero–posterior boundary is in *en/+* flies, and several covered mutually overlapping territories.

These results show there is no straight line separating anterior and posterior compartments in *en/en* wings.

Experiment 2—marked *engrailed* clones in *en/+* flies

For these experiments (Figs 3 and 4) we used the new cell marker mutant, *pawn* (*pwn*, 2–58)[12]. *pwn/pwn* cells secrete truncated bristles and fine wing hairs with a basal spur. We also chose a *Minute* ($M(2)c^{33}$) which is situated on the distal part of the right arm of chromosome II; flies heterozygous for this *Minute* emerge about 2 d later than controls[13]. The irradiated flies were screened for *pawn* clones and many large ones were found. These fell into two distinct classes: (i) those clearly in the anterior region which frequently defined the boundary and had no effects on the bristles or veins; (ii) those in the posterior region, some of which reached the boundary, and if they did, frequently crossed into territory normally occupied by anterior cells. These clones, even if small, had effects on the venation, producing patches of veins and bristles characteristic of the *engrailed* phenotype. We conclude that the anterior clones can only have grown from cells within the anterior polyclone, whereas the posterior clones must have originated within the posterior polyclone. Anterior clones frequently filled the anterior compartment; they had no effect on the pattern of venation and defined the same antero–posterior demarcation line found in *en/+* flies (Fig. 5*d*). Of 34 clones, 15 ran along the boundary for at least half of its length, none crossed it. This result shows that the *engrailed* mutation has no effect on any of the anterior cells, including those at the antero–posterior boundary.

Posterior *pawn* clones ($n=24$) always showed some aspects of the *engrailed* phenotype, and frequently crossed into anterior territory (11 out of 24). No clone defined the boundary and some extended a long way into the anterior part of the wing—even as far as the second vein (Fig. 5*e*). This result suggests that *en/en* cells in the posterior compartment express two kinds of anterior characteristics: they produce veins and bristles similar to those found in the anterior compartment; and they can become more miscible with anterior cells during growth, so that they can invade territory normally occupied by anterior cells.

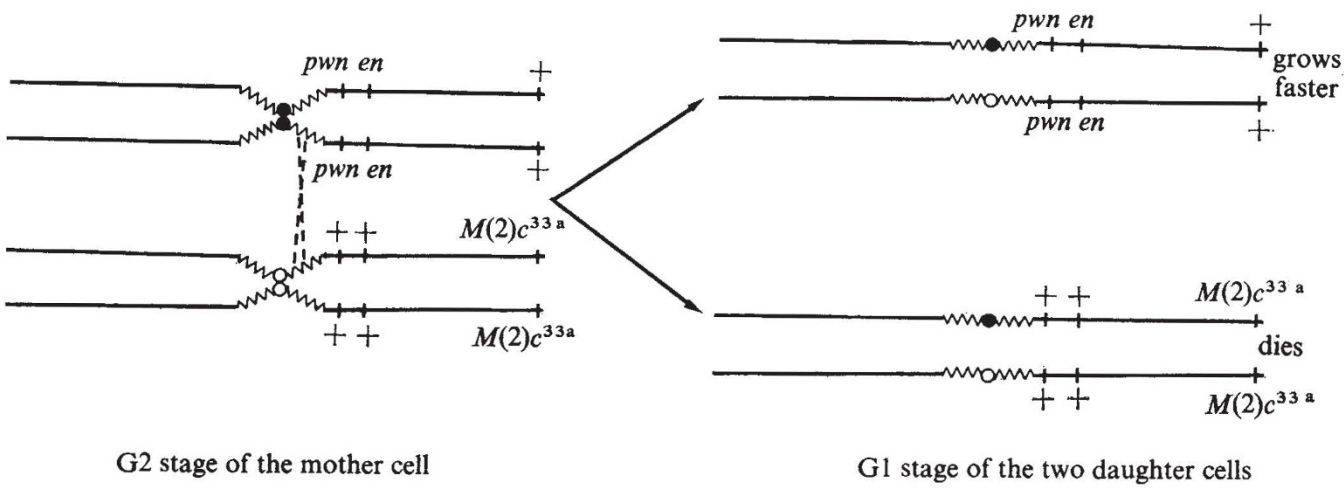
Both these characteristics are temperature sensitive because when experiment 1 was conducted at 30 °C (when the *engrailed* phenotype is very weak, the vein and bristle pattern being almost wild type) the clones respect the border almost perfectly.

In both experiments 1 and 2 clones homozygous for *engrailed* respected the dorso–ventral compartment boundary just as in *en/+* files.

Discussion

A working hypothesis for the genetic control of compartment development can be outlined as follows. At the time of subdivision of a set of cells into two separate subsets (polyclones[3]) a selector gene[4] is activated in one polyclone and remains inactive in the other. After some growth a further subdivision involving another selector gene can occur in the two polyclones and produce two new compartments in each. Thus each compartment is genetically specified by the unique combination of active selector genes,

Fig. 4 Second chromosome to show the result of somatic crossing over between *pwn* and the centromere. All *pwn* clones are also homozygous for *en* and $M(2)c^+$



possibly depending on a binary code[14]. The product of each selector gene is continuously required in every cell so that the elimination of the wild type allele by somatic recombination results in a clone of cells which make a pattern appropriate to another (sister) compartment. The gene product of the selector gene is also involved, not necessarily directly, in producing some label on the cells so that during growth they do not intermingle with cells belonging to a neighbouring sister compartment, but instead form a straight and precisely positioned frontier with them. Elimination of the selector gene from cells in one compartment would remove the label so that the cells become miscible with cells of the sister compartment which always lack that label. Elimination of the selector gene in the sister compartment will be totally without effect.

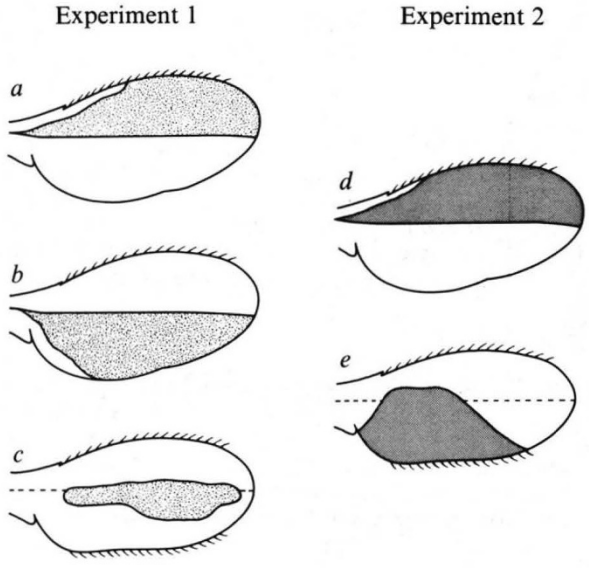


Fig. 5 Examples of clones produced in experiments 1 and 2 (diagrammatic). *a*, Anterior *mwh* clone in *en*/+ wing; *b*, posterior *mwh* clone in *en*/+ wing; *c*, *mwh* clone in *en*/*en* wing; *d*, anterior *pwn en* clone in *en*+ wing; *e*, posterior *pwn en* clone in *en*/+ wing. *mwh* Territory, light shading; *pwn en* territory, darker shading; dotted line shows position of compartment border.

Our experiments with *engrailed* provide substantial support for this hypothesis; they show that the en^+ allele is critical for the normal development of the posterior compartment and for the segregation of its cells from the anterior ones.

Our results—the incomplete transformation of posterior into anterior pattern, the temperature sensitivity—also suggests that *engrailed* is a leaky mutation. This conclusion explains our observations that posterior *engrailed* cells can mix during growth, both with anterior en^+ cells (they cross the compartment border) and with posterior en^+ cells (they fail to sort out from them). We would predict, however, that clones of cells in the posterior compartment, which completely lacked the en^+ gene, would be perfect anterior cells and would mix with cells of the anterior compartment and sort out from those of the posterior. Nevertheless, our results show that when the cells of the posterior compartment are mutant for *engrailed* they cannot define the normal antero–posterior boundary either in an *engrailed* background (experiment 1) or in wild type background (experiment 2).

Experiment 2 also defines the realm of action of the *engrailed* gene: removal of the en^+ allele from all the anterior cells is without effect, whereas its removal from the posterior cells produces changes in phenotype, and may affect the compartment boundary. This strongly supports our hypothesis that the gene is active in all the cells of the posterior polyclone and inactive in the anterior polyclone. The dependence of the posterior compartment on the product of the *engrailed* gene is underlined by the effects of temperature: when the *engrailed* phenotype is nearly suppressed at 30 °C, the mutant posterior cells can now define an almost normal antero–posterior boundary. It is only the antero–posterior compartment boundary that is dependent on *engrailed*; the boundaries separating dorsal from ventral compartments, which are the next to appear on the wing[1], develop normally in *engrailed* flies.

The difference between wild type anterior and posterior cells has been studied *in vivo*. The two cell types (one genetically marked) sorted out and did not form mosaic patterns after being dissociated, mixed and reaggregated[15]. Similar experiments with *engrailed* wing disks demonstrated that at least some of the posterior cells show the same or similar affinities as the anterior cells[9]. Although we know little of the mechanisms subdividing one set of cells into two polyclones, it is possible that these specific cell affinities may function in keeping cells of the two nascent polyclones segregated. It therefore seems likely that the *engrailed* gene is involved from the earliest stages of anterior and posterior compartment formation.

Our experiments suggest that the en^+ allele labels the posterior cells. Removal of this allele is followed by a partial loss of the label and this makes the posterior cells miscible with anterior cells. The label is continuously dependent on the activity of the en^+ allele until late in development[9], the activity of the gene being responsible for the boundary separating cells of the anterior and posterior compartments. The straightness of this compartment border could be caused by two cell populations maximising intercellular contact within each population while minimising the area of contact between them. A similar suggestion has been proposed to explain the straight line separating segmental compartments in *Oncopeltus*[16,17].

The *engrailed* gene is not only crucial to wing development but seems to be involved in a homologous way with the antero–posterior separation in other disks—possibly in the first pair of legs[18] and the halteres and perhaps in all thoracic segments[4].

The ability of cells to sort out from other cell types, and specifically aggregate with cells of the same type, has been studied in many other systems[19,20]. There is the possibility that these properties may be serially acquired during development. Our view, arising from our experiments, is that their role *in vivo* may not be to sponsor aggregation, but rather to stop differently determined sets of cells from mixing.

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