Compartments and Polyclones in Insect Development

Clones made in early development keep within certain fixed boundaries in the insect epithelium.

F. H. C. Crick and P. A. Lawrence

In this article our aim is to describe recent work on the development of intact epithelia and in particular the important results and ideas of Professor Antonio Garcia-Bellido (*1*) and his group in Madrid which are not yet widely known. We try to explain as clearly as possible what these ideas are and what sort of experiments have been done to support them. Some of the more obvious questions arising from the results and how the new concepts may relate to other ideas such as "gradients" are listed.

Development of *Drosophila*

The development of an adult *Drosophila* is a complex process. The nucleus of the fertilized egg divides a number of times to form a compact mass of about 250 nuclei, near the center of the egg, without cell walls. These nuclei then migrate outward to the inner surface of the egg where for the first time cell membranes are formed. The cells divide several more times to form a single layer of cells, about 4000 in all, lining the inside of the egg. This is called the blastoderm. Behaving as a sheet of cells, the blastoderm undergoes complex folding movements generating a multilayered germ band, which soon becomes visibly segmented. The egg hatches after 24 hours and the animal then goes through three larval stages each separated by a molt. After these larval stages, lasting in all about 96 hours, the animal then pupates and metamorphoses into the adult fly.

This adult is formed mainly from special groups of cells in the larva which themselves take little or no part in larval development or function. These are the histoblasts and the imaginal discs. There are 19 of the latter (nine pairs of discs plus the single genital disc). We shall concentrate mainly on one pair of these, the so-called wing disc. The left wing disc, within the left side of the larva, produces the left wing of the insect and that part of the dorsal left side of the thorax next to the wing.

The wing disc is seen in the first larval stage as a small patch of embryonic epidermal cells (*2*). These cells remain diploid, while the surrounding larval epidermal cells become polyploid (*3*). There are probably only about 15 to 30 cells forming the wing disc at this early stage (*4–6*). During the course of larval growth these disc cells divide in all about 10 or 11 times (on average) to give a total of some 50,000 cells (*5*). Shortly after puparium formation cell division of the disc stops. The disc has now a characteristic size and shape, being somewhat like a flattened and heavily folded balloon (*7*).

At metamorphosis a complicated set of cell movements occurs, and these result in the disc being turned inside out so that it can form the adult structure. The wing itself, for example, is first formed as a bag. The bag is then collapsed to form the adult wing, which thus becomes a single sheet of epithelial cells folded and collapsed to form a double layer of epithelial cells.

Basic Ideas of Clonal Analysis

For the purposes of exposition we now temporarily leave the wing and describe a hypothetical sheet of "white" epithelial cells on the adult fly. We imagine that we have at our disposal a special technique that enables us to mark (say black), at random, a single cell in a developing disc. The mark is such that it does not interfere in any way with the normal development of the animal. Moreover, all the descendants of this marked cell retain the mark and can be recognized in the adult. The method of marking has the advantage that we can choose fairly precisely when, in development, we mark the cell; but it has the disadvantage that we cannot mark a particular cell at that time, but only one chosen at random, and in early stages we usually mark only one cell in any one individual. If we assume that the significant features of the process are effectively the same in all individuals, we can piece together what is happening in development by combining experiments on many different individuals.

What do we find? Naturally, we see a set of black cells in the adult, but how many of them are there, and how are they arranged?

The first observation is what might be

Drs. Crick and Lawrence work in the Cell Biology Division of the Medical Research Council Laboratory of Molecular Biology, Cambridge, CB2 2QH, England.

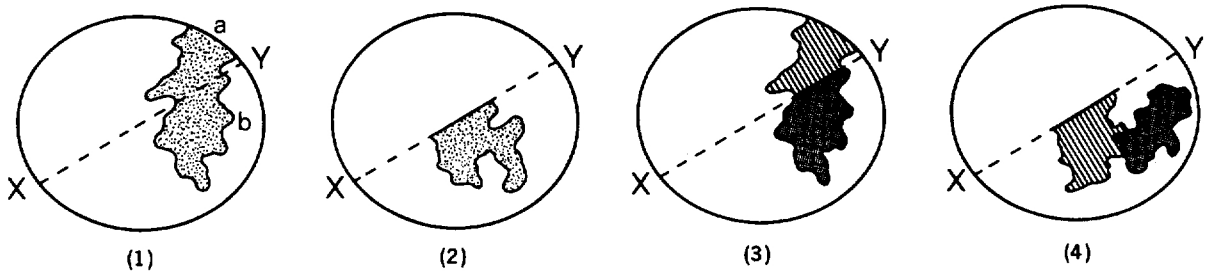

Fig. 1. A clone descending from a cell marked prior to the formation of the compartment border (XY). The clone is smooth at the edge of the structure (a) but rough elsewhere (b). Fig. 2. A clone descending from a cell marked after the formation of the compartment border. Fig. 3. The clones made by the two daughter cells of that cell generating the clone shown in Fig. 1. Fig. 4. The clone made by a cell marked prior to the formation of the compartment border, both of whose daughter cells give rise to clones within one subcompartment.

expected. In general, the earlier a cell is marked in development, the more black cells we find in the adult. A cell marked early leaves more descendants than a cell marked late.

The next obvious question is: What fraction of the total cells are marked? By the total cells we mean the number of cells in that portion of the adult epithelium which has come from the set of cells under consideration in the larva (for example, the 50,000 epithelial cells that come from a single wing disc).

The number of black cells produced by marking at a fixed time is not exactly constant, but the variation is such that we can usefully calculate its average value. If the average number of black cells in the adult is, say, a tenth of the total then making certain reasonable assumptions there were, on average, about ten cells in the larval set at the time they were marked (*8*). As the time of marking gets later and later in development this fraction gets smaller and smaller, and the frequency of marked clones produced increases.

From the arrangement of the black cells we can learn something about their movement during the interval between irradiation and observation. For instance, if there is a pepper and salt mixture, the cells must have been intermingling; while a coherent patch suggests that all the daughter cells have remained in contact during growth. The shape of the patch is also informative. For example, if it is long and thin this may result from the cell divisions being predominantly oriented in one direction. In the case of the wing disc, it is found that the patches are usually both coherent and elongated so that the long axes of the patches are parallel to the long axis of the wing (*4, 5, 9*).

We must next ask: Even though a patch is irregularly shaped, is the shape the same in different individuals? The experimental results show that it is not so. Consider a set of experiments in which the mark was made at more or less the same time in the development of a number of different individuals. Then it is found that the patches produced, when all drawn on the surface of a single idealized adult, do not neatly cover the entire epithelial surface, without either overlapping or leaving spaces (like a jigsaw puzzle). On the contrary, if two patches from separate individuals have ended up in roughly the same place, then it is always found that each partly overlaps the other and are usually of different size. This result shows that the cell lineage in *Drosophila* epithelium is not strictly determined in the same way in all individuals.

After all these preliminaries we can now approach the important result. Let us assume that our hypothetical piece of epithelium is smooth in outline, as shown in Fig. 1. Then perhaps it is not too surprising to learn that a black patch near the borders of this piece of epithelium has itself a rather smooth outline where it follows the boundary of the area but has a rough outline elsewhere. We assume that at the earliest stage of marking (that is, when the disc is first formed) a black patch can be produced anywhere within our area. In particular it may have the size and shape shown in Fig. 1. We now ask: Suppose the mark is made a little later, say, one cell generation later, what will the patch be like? Naturally, it will, on an average, be half the size, and we expect it to have an irregular outline except where it touches the area border. But now in some cases a new and totally surprising restriction appears. When all the results from many different patches are combined, it is found that a rather smooth line (marked XY) can be drawn, dividing our hypothetical area into two distinct parts, such that no black patch, made at this later time, will ever cross this line. Moreover, the outline of a patch touching this line is smooth where it runs along the line but rough elsewhere (Fig. 2). And this in spite of the fact that a patch marked one cell generation earlier can cross this special line.

The surprising nature of this result can be seen by going back and considering the entirely irregular patch illustrated in Fig. 1. We drew this particular patch (marked at the earlier stage) across the special boundary XY. We now ask: What would Fig. 1 look like if instead of just marking that particular cell we had been able to put a different mark on each of its two daughter cells, produced one generation later? We should now find two adjacent patches, each with an irregular outline except where the patches touched. Along the line of contact their outlines would be smooth and fairly straight (Fig. 3). This result is true only if the double patch crosses the special line XY. Otherwise the contact outline of the two daughter patches would be irregular (Fig. 4).

Garcia-Bellido, Ripoll, and Morata (*10*) have called an area bounded by these special demarcation lines a *compartment*. The progeny of a cell marked at about the time of the drawing of boundary lines never fills a compartment completely, but often occupies an appreciable proportion of it. A compartment is thus made by the descendants of a small group of cells. We propose to call the cells in the compartment a *polyclone*. Just as a clone is a group of cells which are all, without exception, the descendants of a single cell, so a polyclone is a group of cells that are descended from a certain (small) group of cells—the founder cells—which were present in the embryo at an earlier time. Moreover, in our terminology they are *all* the (surviving) descendants of that small group. This last point is vital since necessarily all the cells in a compartment are, for example, descendants of the fertilized egg. The distinction is that some of the descendants of the egg make up other parts of the body; that is, they end up in other compartments. The members of a polyclone, however, all fall within one compartment and account for all the cells in that compartment.

This point can be made more sharply. Consider the small group of cells, the founder members of the polyclone, and then consider their immediate ancestors. Then (except in rare cases) this earlier

group will not form a polyclone for the compartment under consideration. That is, we will usually find that some of the descendants of these cells end up outside the compartment we are considering. The cells in the compartment are necessarily all descended from this smaller group, but they are not all the surviving descendants. Therefore, this earlier group are not the founder members of the polyclone for that compartment.

The other side of the idea must also be mentioned: a compartment is never a clone, except perhaps accidentally in rare cases. That is, for most cases, the cells in a compartment cannot be traced back to any single cell, all of whose descendants fall within the compartment. This idea, which implies that for these properties cells are switched not singly but in groups, is important (*11*).

We thus see that the idea of a compartment and the idea of a polyclone are, at the moment, intimately connected. As things stand at present we have no other reliable criterion for the sharply defined region we call a compartment except that a marked clone produced after a certain time in development will never cross over the compartmental boundary and include any part of any other compartment; whereas clones formed earlier may well do so. Reciprocally, we cannot say that a group of cells form a polyclone unless we first define the compartment to which the polyclone refers.

We must now consider the second major fact about certain compartments, namely that as time goes on they become subdivided. Let us call a certain compartment comp 1; at a later time it will be subdivided into two compartments which we may call comp 1A and comp 1B. These two subcompartments are not necessarily equal either in area or in number of cells but together they add up exactly to comp 1.

By definition all these compartments are polyclones. That is, the ancestors of all the cells contained in each compartment can be traced back to a founder group, early in the embryo, all of whose descendants end up in a compartment being considered. It is an experimental fact that one marked clone of cells, started from a single cell at a certain early stage, may stay entirely within comp 1 and yet go across the border between comp 1A and comp 1B. A marked clone made at a slightly later stage, however, will never cross this boundary. This implies that in any particular case the cells that are the founder members of the polyclone for comp 1 form three classes: those whose descendants will fall (i) wholly within comp 1A, (ii) wholly within comp 1B, and (iii) partly into comp 1A and partly into comp 1B.

It is this third class which explains why early clones can cross a subcompartment boundary whereas later ones cannot. However, at a slightly later stage in embryogenesis some further developmental step must take place, since at that time the descendants of the founder members of comp 1 will fall strictly into the first two classes listed above. No cell will then be found with the properties of class iii. Every cell in this enlarged group will be either a founder member for comp 1A or a founder member for comp 1B. In short, whereas before only one polyclone existed, that polyclone can now be considered as the sum of two distinct polyclones.

The work of Garcia-Bellido and his colleagues shows that this process of forming subcompartments within larger compartments can happen several times in succession. The data suggest, but do not prove, that the division takes place each time into just two parts.

The Methods

We shall now illustrate the methods used in clonal analysis by describing in outline the techniques employed by Garcia-Bellido *et al.* (*10*) in their detailed studies of the wing disc of *Drosophila melanogaster*. The wing disc is strictly called the dorsal mesothoracic disc. There are two of them in each larva, one for each side of the adult animal. Each disc produces the entire epithelium for a wing and that part of one side of the thorax near the wing. The dorsal part of the thorax is called the notum and the lateral part the pleura.

The method used to mark a clone is mitotic recombination produced by x-rays delivered at a chosen time in development, usually during the larval stages. The genetic makeup of the animal is designed so that certain mitotic recombinants will be phenotypically different. For example, if the animal is heterozygous for the recessive gene *yellow* ($y/+$) then mitotic recombination may produce two daughter cells. One of these ($+/+$), will be phenotypically wild-type and therefore indistinguishable from unaltered cells, but the other will be homozygous for *yellow* (y/y). All the descendants of this cell will also be (y/y). If such a descendant in the adult is colored at all then it will be yellow rather than the normal darker color.

An ideal genetic marker would be easily scored in all types of cell, have complete expression, and be cell-autonomous. That is, the phenotype would depend only on the genetic makeup of the cell in question and not at all on that of neighboring cells. Unfortunately few such markers are known. Markers often used are: *multiple wing hairs (mwh)* which produces groups of two to five hairs (trichomes) on the wing instead of one per cell as in the wild type; and *forked* (*f*) and *singed* (*sn*), which produce deformed bristles and hairs. To assist recognition, the mutant allele with the most extreme phenotype among those available is usually used; and to minimize mistakes more than one marker is often employed. Double marking also allows the degree of expression and cell autonomy to be checked.

The markers used so far in this work do not allow a marked cell to be recognized when it is first produced in the imaginal disc, or even after a few divisions. The cell phenotypes employed can only be scored by the observer at the adult stage when the cells have differentiated. Moreover, only cells that form (or can be induced to form) hairs or bristles can be scored at all easily, so that if these are lacking or sparse in some particular area it is often difficult to find the exact edges of a marked clone in such regions. Fortunately most of the wing disc derivatives, being covered with hairs, are relatively easy to score.

If the growing disc in the larva is irradiated at the early stages of development, there will be few target cells and most individuals examined will not show any mutant patches. This cannot be overcome by increasing the x-ray dose (which is usually 1000 roentgens) as too big a dose will interfere with development. One simply has to examine a fairly large number of flies. If the x-rays are given later in development, more mutant clones are produced (since there are more target cells); but the average size of each clone will be smaller since a cell altered at a later stage produces few descendants. This small clone size means that it is more difficult to recognize compartment boundaries since most of the clones will be in the middle of a compartment rather than near its edge and even those at the boundary, being small, will not display the boundary so graphically. This is somewhat offset by the subdivisions making the compartments smaller as time goes on but in spite of this it becomes progressively more difficult to recognize compartment boundaries. It would in any case involve much more work if enough patches are to be scored to make an apparent boundary statistically significant.

However, Garcia-Bellido *et al.* devised a method of overcoming this difficulty. There exist a series of dominant *Minute* loci (*12*) which are lethal when homozygous. When heterozygous, the insects grow slowly and the bristles are small. They needed a mutant which (after mitotic recombination) would make the marked clone grow faster than the unaltered cells

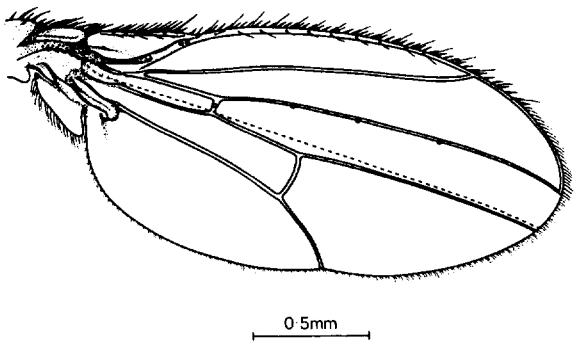

Fig. 5 (left). Drawings of *Drosophila* wing to show the position of the antero-posterior compartment border.

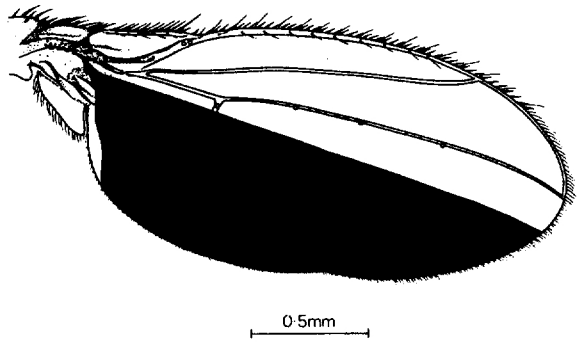

Fig. 6 (right). Outline drawing of *Drosophila* wing to show the area covered by a typical M^+/M^+ clone in a M/M^+ background.

and thus produce a much bigger patch. Morata and Ripoll (*13*) showed that homozygous wild-type cells (M^+/M^+) produced by mitotic recombination divided more rapidly than the slow-growing heterozygous *Minute* (M/M^+) background, which was the effect they needed. In addition, for reasons which are obscure, the frequency of mitotic recombination for (M/M^+) larvae after irradiation is apparently increased (*14*). This is especially useful in the early stages of development when the normal rate is inconveniently low. A second somewhat unexpected result was that in spite of the (M^+/M^+) clones being much larger than normal, the overall size and shape of the wing was not altered (*13*). This implies that there are special mechanisms to regulate size and shape which can cope with differential cell division rates—an important result in its own right. These mechanisms can also regulate for the loss of cells both due to x-rays and the formation of M/M cells.

The Results

Having given an indication of the methods used in this type of clonal analysis we must now mention some of the earlier results. Becker (*15*) was the first to use x-rays to produce clones at particular stages of development, in his study of the *Drosophila* eye. Later Garcia-Bellido (*9*) noted that clones produced after the 1st instar larva never crossed from dorsal to ventral on the wing; and Bryant and Schneiderman (*8*) that they were confined to single leg segments when larvae older than early third-stage larva were irradiated. Bryant (*4*) made the important observation that clones in the wing disc could cross from dorsal to ventral if produced early enough, but not when produced late. Similarly, in *Oncopeltus* (*16–18*) up until the late blastoderm stage, clones may extend to two or more abdominal segments, but after that stage clones are strictly confined to a single segment. These observations all show that within three different discs of *Drosophila* and in the *Oncopeltus* abdomen the "anlagen are represented by separate populations of proliferating cells" (*4*).

The most detailed results so far have been obtained by Garcia-Bellido and his colleagues studying the development of the wing disc. As might have been expected the earliest clones (irradiation of first-stage larvae) are contained exclusively within the fairly large area of the adult cuticle produced by the entire disc. This shows that effective separation of the wing disc from the other discs producing the adult epithelium must have occurred before the first larval stage. However, even at these early times a compartment boundary is apparent within the disc. This was first clearly demonstrated by the Madrid school using the *Minute* technique. The boundary, which separates anterior regions from posterior regions, runs along the middle of the wing between the third and fourth vein. The actual demarcation line is near the fourth vein but is distinct from it (Fig. 5). The line runs along both surfaces of the wing and continues on the body where it divides the notum into two distinct areas. Even a very large clone (Fig. 6) will observe this demarcation line although at this stage it may well cross the wing margins, thus appearing on both dorsal and ventral surfaces and extending onto the notum.

The edges of the clone are somewhat irregular except where they run along the demarcation line. It is not very likely that this line marks the frontier where two initially remote and separate groups of cells have moved together, since both anterior and posterior regions are within the same nascent imaginal disc and thus probably fairly close together (*6*). Since about twice as many clones appear in the anterior compartment as in the posterior one, it is surmised that at this early time there are about twice as many anterior as posterior cells. That is, the antero-posterior division is not exactly into two equal parts but more like a 2 : 1 ratio (*10*).

Some time later, during larval development (the exact time is not quite clear), each of these two compartments is found to be divided into four parts, giving eight compartments in all. The demarcation lines divide dorsal from ventral areas and wing from thorax. The final size of these compartment areas varies somewhat (from 10^4 cells to 10^3 cells or less). The evidence that late clones really observe these demarcation lines is very strong. They are observed by very large clones, which in some cases make up as much as 90 percent of a compartment. Such clones may border a demarcation line for as many as a thousand cells. Nor is the effect solely due to the fact that clones are often elongated in a direction roughly parallel to a demarcation line. The main axis of these clones meets the demarcation line at various angles, sometimes even perpendicularly. Nor on any simple model can the demarcation lines be lines of fusion of quite separate groups of cells if only for the fact that marked clones made at a slightly earlier stage will go straight across these lines.

As development proceeds the recognition of new subcompartments again becomes somewhat more difficult because the effects of differential growth (due to M^+/M^+ cells in a M/M^+ background) have less time to produce larger clones. Garcia-Bellido, Ripoll, and Morata suggest that there may be two further demarcation lines formed about the same time. On the adult fly these separate two areas on the body, one dividing the notum into two parts and the other the pleura. These compartments were all discovered by the use of M/M^+ flies, but similar experiments on non-*Minute* flies (which have, of course, smaller clones), show that the demarcation lines are also observed in this more normal situation. The *Minute* flies thus serve to make the subcompartments more easy to observe: the phenomenon itself is not peculiar to them alone.

Further Problems

Having now described the results on compartments in outline we must ask how widely the idea is applicable and what are its limitations. One limitation is that the evidence obtained so far relates only to epidermal structures. This is mainly because in insects they are so easy to observe and so rich in detail. Internal structures, for example, the exact arrangement of the muscles, cannot be studied satisfactorily without the use of more difficult experimental methods.

However, the properties of internal tissues may be partly imposed by the pattern of the enclosing epithelium (*19*), and they may well also be compartmented.

With regard to compartments in imaginal discs, there are a series of outstanding questions that need answering. Are all subdivisions binary? We have seen how the first division of the wing disc, after the very early antero-posterior divisions, appears to yield four parts rather than two. It is natural to ask if this is really two separate binary steps in quick succession, and this question focuses attention on the exact timing of the subdivisions. Even for an obviously binary step one can ask whether the decision is an abrupt one or is spread over a period. Does it necessarily require cell division? Are compartment boundaries always smooth? The edge between the dorsal and ventral surface of the wing is very well defined, and clones that border it are smooth to the nearest cell (*4, 5*), but is this true for all boundaries?

The problem of how a compartment boundary is formed and how it gets so straight appears to be a difficult one. Factors that may have to be considered are strictly oriented mitoses near the boundary (*17*), straightening effects due to differential cell affinities, and possibly cell death for cells which get themselves into the wrong places, so that the compartment edges are trimmed. It is claimed (*20*) that extensive cell death is unlikely because otherwise clone size near the boundary would be smaller, which is apparently not the case.

Nor is it completely clear where the process of the subdivision of compartments stops. Even the technique for spotting compartment boundaries, using relatively fast-growing marked clones, has its limitations as, at later times, even these clonal patches are rather small. How can we be sure that these are not further subcompartments? Even the definition of a compartment becomes difficult at this point. Although formally, for example, the descendants of a single bristle mother cell [for example, the trichogen, the tormogen, the sense cell, and the neurilemma cell making up a bristle in *Oncopeltus* (*21*)] which are most certainly a clone and which stay together, could perhaps be regarded as a compartment, we feel that this is stretching the term too far. It would seem sensible to restrict the term "compartment" for the moment to fairly large groups of cells and to those groups which form a polyclone rather than a clone.

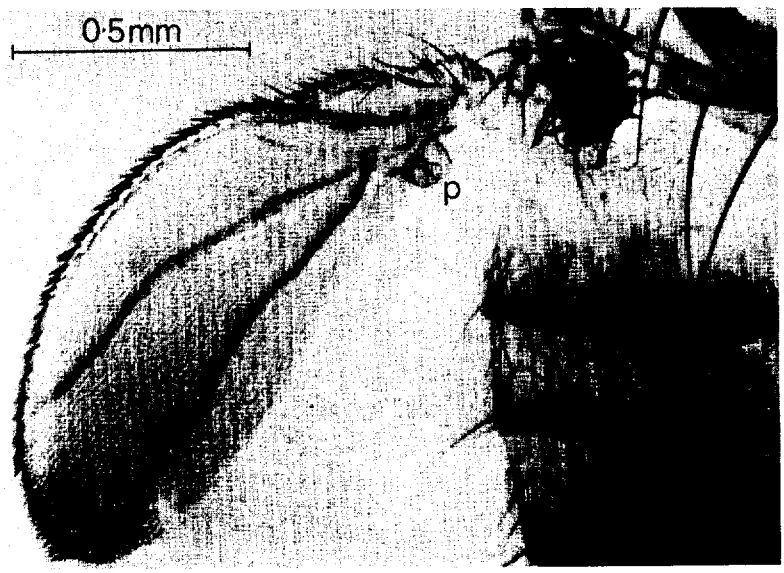

Fig. 7. A metathoracic appendage from a *Drosophila* carrying an extreme allele for *bithorax*. The posterior haltere develops normally (*p*) while the anterior haltere is transformed into an apparently normal and complete anterior wing compartment.

Other Possible Characteristics of Compartments

We have seen that, at the moment, a compartment is defined by its boundaries and these alone, since clones, made after a certain time in development, never cross them. Are there other properties that allow us to identify a compartment?

One such property may be the area affected by a homeotic mutant. There are mutants that shift an imaginal disc, or part of an imaginal disc, into another developmental pathway. For example, *aristapedia* (ss^a) transforms part of the antenna into leg segments (*22*).

It is rather rare for a mutant to turn one whole disc into another whole disc. Possibly such a drastic change would be lethal and thus escape observation. It is more common for a part of one disc to be turned into part of another one. Even in these cases the transformation is not always complete, because of partial and variable expressivity. We can, however, ask the general question: In such cases do the (maximum) boundaries of the transformation coincide with a compartment boundary found by the clonal method?

Morata and Garcia-Bellido (*23*) have shown by clonal analysis that the haltere disc (the metathoracic disc) has within it an antero-posterior boundary; but locating it precisely is difficult because of the absence of suitable landmarks on the haltere. It has been known for many years (*24*) that various mutants in the bithorax system turn various parts of haltere into wing (or vice versa) with different degrees of expressivity. A number of mutants appear to respect the antero-posterior boundary of wing with some precision and probably also of the haltere although here the precision is more difficult to judge. For example, an extreme allele of *bithorax* (bx^3) turns the anterior part of the haltere into anterior wing while leaving the posterior part of the haltere (which is much smaller) unaltered. The boundary of this transformed half-wing is very close or identical to the antero-posterior boundary found by clonal methods in the wild-type wing (Fig. 7). Another mutant in this complex locus (*postbithorax*) also delineates this boundary because its effect is restricted to the posterior part of the haltere.

The gene *engrailed* also delineates the boundary, and in an especially interesting way. In flies mutant for *engrailed* the posterior part of the wing is transformed and resembles a mirror image of the anterior part (*25*). The *Minute* technique has recently been used to show that the realm of action of the *engrailed* gene precisely coincides with the posterior compartment,

there being no effect on the anterior. If large *engrailed* (*en/en*) clones are made in a wide-type wing (*en/+*) they may fill the anterior compartment right up to the antero-posterior boundary but never cross it. They are completely without effect on the pattern. However, all *engrailed* clones in the posterior part express the phenotype (*26*) and, as discussed later, may cross the antero-posterior boundary.

Another possible correlation is between gradient discontinuities and compartment boundaries. These discontinuities can be of at least two kinds. The first has a discontinuity in the value of the gradient but not its slope, as shown in Fig. 8. The other has no discontinuity in the value but a change of slope, in particular a change of sign of the slope to give the mirror-image situation shown in Fig. 9.

The first of these is found between the segments of the insect cuticle in *Rhodnius* and *Oncopeltus*. Lawrence (*16, 18*) has shown in *Oncopeltus* that marked clones do not cross the intersegmental boundary, so here at least we have one clear case where a clonal boundary coincides at least approximately with a gradient discontinuity (*27*). Another possible case is suggested by the mutant *engrailed* mentioned above. Since this produces a rough mirror image across the antero-posterior compartment boundary of the wing, one might be tempted to think that the underlying gradient (or "prepattern") might have the mirror image form shown in Fig. 8 both in the mutant and the wild-type. Otherwise the experimental evidence for this possible correlation is either scanty or absent.

There are several other properties which we can speculate about. Experiments designed to show how mixtures of cells from imaginal discs appear to sort out show clearly that cells from different discs will segregate, suggesting rather strongly that they have different surface properties (*28*). Moreover, such segregation also occurs between marked cells from different parts of the same disc. For example, cells from the anterior part of the wing disc will segregate from those of the posterior part (*29*). This obviously suggests the generalization that each compartment has characteristic cell surface properties, different from every other compartment, which allow cells from any two compartments to segregate. Thus the normal development and maintenance of the antero-posterior boundary in the wing might depend on the confrontation of cells of a different type, that is "anterior" with "posterior" cells. If so, one might expect that boundary to be malformed or nonexistent in *engrailed* flies where the posterior cells are partially transformed into those of the anterior type. Clonal

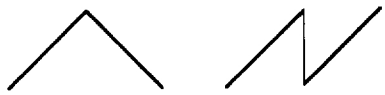

Fig. 8. The probable gradient situation in *Drosophila* wing; the slope, but not the altitude, changes near the antero-posterior compartment border. Fig. 9. The probable gradient situation in two adjacent abdominal segments of Hemiptera. The step probably coincides with the intersegmental compartment boundary.

analysis of *engrailed* flies has recently shown that clones do frequently cross the line where the border normally is (*26*). This never happens in flies wild-type for the *engrailed* locus, a result that strongly supports the idea that the role of the en^+ allele is both to control the development of the posterior pattern and to instruct the cells so that they do not intermingle with cells of the neighboring anterior type.

An additional possibility is that there is a gradient of cell surface properties within each compartment. This is certainly suggested by the observation (*30*) that in the epidermis of *Oncopeltus* a graft takes better if it is from the same level in the segmental gradient, even if from a different segment, than if moved to a different position in the gradient in the same segment. These speculations go far beyond the experimental data now available, but they do suggest that direct methods of characterizing cell surface properties, preferably in situ, would be very valuable. If such a method could be developed it would have the enormous advantage that it might work for the cells of the developing imaginal disc so that one could spot compartments and their boundaries at the moment, or soon after, they are formed.

It is also possible that, even though all the epithelial cells of a disc appear very similar, the compartments within them could differ by a particular enzyme or set of enzymes. For this reason there is a case for testing all the imaginal discs, both in their mature and their developing states, by as many histochemical tests as are available. A beginning has already been made in this approach by Janning (*31*) using a test for aldehyde oxidase.

Another histological feature that may correlate to some extent with compartments is the distribution of nerve axons. Hasenfuss (*32*), studying the epidermis of *Galleria* and *Rhyacophila*, noticed that the nerve axons of the sensillae in the abdominal epidermis were collected into groups each of which went to one segmental ganglion only. He suggested that this was because each group came only from a single epidermal segment. This, however, was true only of the axons since the dendrites were observed to extend over considerable distances and thus could not be confined within one segment. A similar phenomenon has also been observed by Lawrence (*33*) in the abdomen of *Oncopeltus*. In this case, the intersegmental boundaries are clearly delineated by color and cell shape. No axons have been observed to cross these boundaries, although they do cross the midline. (It is known that the midline is formed in the embryo by the fusion of two separate groups of cells.)

One is thus led to the speculation that the fields outlined by well-defined groups of nerve axons may perhaps coincide in certain cases with compartments or subcompartments. This might be because compartmentalization may often occur before the separation of the neuroblasts from the presumptive epidermis, so that any cell surface differences or other labels associated with a compartment may be shared by both the epidermal cells and the neurons.

The hypothetical properties so far discussed would be possessed by all or most of the cells within a given compartment or subcompartment. They could be described as area properties. Another rather different property would be one which characterized boundaries between compartments, that is, an edge property. For example, the cells on one side of the intersegmental boundaries in *Oncopeltus* are markedly elongated in the direction of the boundary (*17*). Do all compartment boundaries have this property? For the antero-posterior wing boundary it seems that the adult cells have no unusual appearance; but nevertheless a detailed scrutiny of several such boundaries might be worthwhile. Another obvious hypothesis is that whereas there may be free diffusion of certain chemicals within compartments it may be greatly restricted across compartment boundaries. This suggests that compartments might not be electrically coupled to each other, but a direct test across the intersegmental boundary in *Rhodnius* (*34*) showed coupling to be normal. Moreover, a careful cytological study by electron microscopy has shown no observable difference in the various types of cell junctions (gap junctions, septate desmosomes, attachment desmosomes) for the corresponding intersegmental boundary in *Oncopeltus* (*35*). One is thus not exactly encouraged to look for these same differences at compartment boundaries in structures from imaginal discs. Nevertheless, it would be surprising if there were not some important cytological difference at compartment boundaries.

Possible Mechanisms for Compartment Formation

We must now consider the nature of the step which partitions the cells that are the ancestors of one compartment in such a way that some of them become the founder cells of one subcompartment while the others become the founders of the other subcompartment. As we have seen, this step is often a partition into two parts (rather than three, four, or more), and it is possible that this is always the case. For the moment we will only consider the case of binary partition.

At present, little can be said about any underlying biochemical mechanism, but we can usefully discuss the problem at the cellular level. Unfortunately, we have rather few facts to go on. In view of the existence of size and shape regulation (as shown by the experiments in which a relatively fast-growing clone within a compartment does not alter its dimensions), it is not obviously a requirement that the partition need be always exactly the same, since any variation, if it is not too big, can probably be corrected by subsequent growth. We consider three possible types of mechanism.

1) *The partition of daughters.* All the cells divide once, one daughter of each division being allocated to one subcompartment and one to the other.

2) *Random allocation.* The cells are allocated at random, with a fixed probability which we shall assume to be about one-half. Because of the number of cells involved, the chance of all the cells being accidentally allocated to one subcompartment is so small that it can be ignored (for example, for 20 cells this chance is 1 in 2^{19} or about 2 in 10^6). Even if all cells but one are allocated to one subcompartment, the single cell allocated to the other could, conceivably, compensate for this numerical handicap by an increased rate of multiplication.

3) *Geographical partition.* The patch of epithelial cells is divided, the dividing line separating the founder cells of one subcompartment from those of the other.

The difficulty with the first two mechanisms is that, in order to get the cells of each subcompartment together in one patch, a certain amount of relative cell movement would have to take place. Since the partitioning into subcompartments takes place several times in succession, one would not expect marked clones to stay in one piece, as they usually do. Thus these two mechanisms seem unlikely, except perhaps for the first of the several partitioning steps.

The mechanisms can be saved to some extent by an additional hypothesis; that any cell which is surrounded by cells of the other type commits suicide. It is difficult to make this model precise, but it would appear to lead to a fair amount of cell death. Moreover, the cells which migrated would still have to move to the correct place in the epithelium relative to other surrounding tissues.

The third proposed mechanism—geographical partition—seems to us to be by far the most likely one, especially as it does not need to be extremely precise. Consider, say, a patch of 20 cells. Let each cell divide once to give 40 cells. Each of these cells will be surrounded in the epithelium by several other cells (the average number is usually a little above five), one of which will be its sister cell. Now draw an arbitrary (but moderately straight) line partitioning the patch into two parts. This line will separate some cells which are sisters. The problem is to estimate the fraction (averaged over many cases) of the original 20 cells which will have daughters separated by the line. It is only these particular cells that can produce a clone of descendants which will go across the boundary between the subcompartments.

Several approximate estimates have been made by Ripley (*36*) using various simplifications. The fraction defined above can be written as equal to $C/N^{1/2}$ when N is the number of cells at the time the line is drawn (40 in the example above) and C is a parameter which is approximately constant. The values of C found were not far from 0.55. Thus for $N = 25$ the fraction is about 11 percent. This calculation shows rather clearly that on this simple mechanism the existence of clones which cross the subcompartment boundary will not be a rare event if they are marked one generation before the compartment is divided.

A more detailed mathematical study of this problem would be worthwhile since it is important to compare the detailed experimental data (what fraction of clones crosses a border, what fraction runs alongside one, and the like, as a function of exact time of irradiation) with what would be expected on the various theoretical models.

Conclusion

We have seen that the work of Garcia-Bellido and his colleagues has clearly brought out the formation of compartments and successive subcompartments in the epithelium produced by the wing disc of *Drosophila* and that there is evidence that a similar process occurs in the production of other regions of the insect epithelium. We have also seen that the phenomena, although clearly demonstrated in outline, need further detailed study, especially quantitative study. The mechanism that produces these subcompartments is obscure although a plausible model can be suggested for the general nature of the process.

It is therefore pertinent to ask what is the novelty of these ideas, viewed from the general perspective of development studies. To do this we must ask what the experiments show does not happen.

We are not talking about the determination of cell type in the usual sense—for example, a muscle cell as opposed to a fibroblast—but about cell position. In this system the determination and differentiation of cell types—for example, bristles as opposed to epithelial cells—probably comes later and may well also be dependent on the compartment to which the cells belong. What we are concerned with is geographical position in the organism and, moreover, not about exact geographical position but whether a cell is somewhere within one well-defined region or another one.

What has been demonstrated in this system is that once a major developmental step of this type has been taken by a cell it is not reversed in the progeny of that cell, at least in normal development. If reversal was possible, a cell which had been determined for the dorsal side of the wing and which found itself on the ventral side could be reprogrammed to be a ventral cell. What clonal analysis has shown is that this never happens. Either such a cell cannot get to the wrong side of the wing, or, if it does so, it must either move back to the right side or be killed. The exact mechanism is obscure. Whatever it is, it is clearly of interest, even though the basic concept, the irreversibility of major developmental steps, is not in itself especially novel.

But it would be both novel and exciting if it turns out that the compartments and subcompartments are used by the organism as units for the control of shape and size; if gradient systems meet at compartment boundaries, if cell surface properties changed abruptly there, if size regulation occurred partly independently within each of these domains, and so forth. It may be that the normal development of each imaginal disc can usefully be divided into a precise succession of major steps each of which produces a set of new compartments. If so, by studying compartment formation, one could both enumerate these steps and determine their times of action. On this picture each compartment would be specified by a unique combination of a small number of controlling genes [selector genes (*1*)] that are active in it. (The steps that follow—for example, the determination of a bristle in a particular position within a compartment—may be of a

somewhat different and more complex character.) For the first time there is the real prospect of understanding the logic behind gene deployment in pattern formation. As we have seen, the speculative ideas about compartments in this section are not supported by hard evidence. The best we have so far is a series of hints. But it is exactly this possibility, that compartments may have a wider significance, which makes the study of them at the present time so important and so interesting.

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