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# Cell Lineages and Genes

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*Phil. Trans. R. Soc. Lond. B* 1985 **312**, 101-128
doi: 10.1098/rstb.1985.0181

*Phil. Trans. R. Soc. Lond.* B **312**, 101–128 (1985) [ 101 ]
*Printed in Great Britain*

# Cell lineages and genes

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Fixed lineages are the extreme manifestation of the interrelations between cell division pattern, spatial organization and cell differentiation occurring in embryonic development. These relationships are discussed in the light of recent new detailed descriptions of lineages, experimental perturbations and genetic analysis. Lineages could be phylogenetically old devices under the control of specific genes that are required to organize the morphological space. Such genes would be different from those involved in the specification of lineage alternatives. Specification genes operate independently from each other and are expressed in a combinatorial way in different cells and lineages. Since their activity is reversible during development the possibility exists for changing these combinatorial associations during development and evolution. The classical notion of a hierarchical cascade segregation of histotypes along lineages is discussed.

## Introduction

Embryonic development is classically decomposed into three operations: cell proliferation, morphogenesis and cell differentiation. These operations can be visualized at the cellular level in the three aspects by which cell lineages can be described: the pattern of cell division, the allocation of descendant cells to particular positions, and the specification of different cell types. These three aspects of lineages appear coupled and invariant in normal development. Cell differentiation can be the consequence of either the division pattern of the lineage which segregates cytoplasmic determinants, or of local cell interactions. Examples of both types of processes are found in different organisms and at different stages in the same organism. Lineages are classically defined by the inventory of cell differentiation types they generate. Fixed lineages are operationally identified by the inability of the cells of a lineage, after a given division, to change their normal inventory of descendant cell differentiation types; and indeterminate lineages by that ability under the same experimental conditions. In fixed lineages the founder cell seems to be committed to a given end result, and not committed in indeterminate lineages. Development has been considered as resulting from a segregation of commitments from general to more particular cell differentiation types concomitant with a segregation of lineages. The classical theory of germ layers represented the paradigm of this view.

Detailed lineage analyses in several organisms and genetic data in *Drosophila* and *Caenorhabditis* challenge the strict view of lineages segregating histotypes and subsets of cell differentiation types. In fact, lineages can also be constant as to stereotyped division patterns, independently of the cell types they generate. I will discuss classical and recent data that suggest a role for stereotyped cell divisions in the establishment of a spatial distribution of cells, and for lineages in defining topological discontinuities. Such data show that lineages with histotypic mixed descendants are common and that mutations can affect the geometry and the pattern

of cell division independently of the cell types that appear in the lineages. The genetic analysis of cell commitment in lineages is also challenging both the notion of irreversibility at the genetic level and of a hierarchical deployment of cell specifications. Cell specification seems to result from a combinatorial superposition of different genetic operations independently of lineages. I will discuss how the genetic control of lineages and of cell specification could account for morphological constancy and variation during evolution.

## 1. Lineages: clones and polyclones in developmental pathways

In principle, filling the morphological space of an embryo can proceed according to different strategies depending on the prevalence and causal relationships of one of the three aspects of lineages mentioned in the Introduction: (i) embryos could be formed by indeterminate lineages followed by cell interactions that would cluster cells in regions and subsequently determine their differentiation according to their position; (ii) fixed lineages could segregate different cell types which subsequently migrate and are allocated to certain positions by differential cell affinities; (iii) fixed lineages could topographically subdivide embryonic regions and subsequent cell interactions define the particular cell types to appear in different positions. In fact all three strategies operate at different stages and in different developmental processes of many organisms (figure 1). It is, however, my contention that the third strategy is the prevalent one. This is because data of comparative embryology suggest that lineages lead to topographical or regional organization of the embryo independently of their role generating histotypic differentiations. In the former process topographical organization follows from stereotyped pattern of divisions whereas in the latter histotypical differentiation follows local conditions.

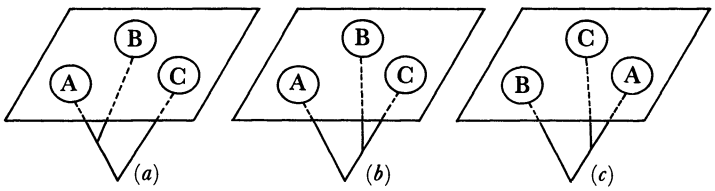


Figure 1. Three formal mechanisms generating specific cell differentiation (A, B and C) in specific positions (circles in squares). Cell specification is topologically invariant in (*a*) and (*b*) although cells derive by different lineages. In (*b*) and (*c*) the same lineage leads to different specification in the same positions.

For this analysis we will have to compare different construction modes as they appear in different animal groups. We should first distinguish several stages and operations in embryogenesis common to these organisms.

(i) Segregation and specification. In pregastrula stages blastomeres segregate presumptive regions of the embryo as dorsal and ventral, animal and vegetative regions, anterior and posterior, etc. To what extent do these blastomeres become specified to either topographical properties or to particular histotypes of the different germ layers?

(ii) Amplification of lineages. Following gastrulation, lineages become assorted to organs and tissues in different sublineages.

(iii) Terminal differentiation. Individual cells show the morphological and biochemical characteristics that permit their identification as belonging to sets of cell differentiation types. To what extent can this result either from the expression of an earlier commitment or from later specifications?


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I will now introduce the terminology to be used in subsequent discussion. Cell division patterns can be distinguished either by the morphological appearance of the resulting cells (equal or unequal in shape and form), or because they segregate differentially along a spatial axis of coordinates, for example, as dividing into a stem cell and a sister cell, or into two equal sister cells. Cell divisions can be defined as symmetrical (or equational) or asymmetrical depending on the inventory of cell types they will generate. Separate lineages can be 'homologous' if the pattern of division segregates topographically and histotypically into the same cell types. The corresponding cells in homologous lineages are called 'analogues'. But analogous cell types can derive from non-homologous lineages (Sulston 1983). Lineages are called 'fixed' when there is a strict correlation between the division pattern and the cell differentiation types it generates. In some cases experimental perturbations (ablation, transplantation) indicate that the invariance of the lineage is due to 'commitment' to the particular pattern of division and inventory of cell differentiation. However lineages could be 'constant' in terms of the stereotyped pattern of division but indeterminate as to the cell differentiation types they produce. 'Pure' lineages are those that give rise to unique inventory of histotypes (mesoderm, germ cells, etc.) or cell types (muscle, sensory neuron, etc.). Mixed lineages differentiate cells belonging to different histotypes. Short of experiment, commitment has been inferred in lineages with a fixed inventory of cell types. These lineages can derive from a single founder cell ('clonal segregation') or from a group of cells not necessarily related by lineage ('polyclonal segregation'). When various cell types originating from different mixed lineages appear in an integrated organic structure the individual cell specification is considered to result from 'topographical assignment' (figure 2).

In the following discussion I will compare the three stages mentioned above in different organisms. More detailed descriptions can be found in other contributions to this symposium or in studies of *Caenorhabditis* and related nematodes (Sulston *et al.* 1983), *Helobdella* and related leeches (Zackson 1982; Weisblat *et al.* 1984), *Chaetopterus* (Polychaeta) and *Ilyanassa* (Gastropoda) (Dawidoff 1928; Anderson 1973), sea urchin (Hörstadius 1973), *Drosophila* (Demerec 1950; Counce 1973), insects (Anderson 1972), *Xenopus* (Keller 1975; Fox 1984) and mice (Gardner 1978).

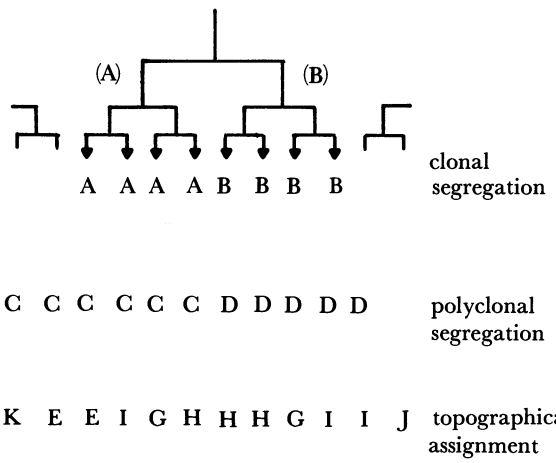


FIGURE 2. The same pattern of division can lead to different cell specification modes. In the mode of clonal segregation the differentiation of A and B cell types can be inferred to result from previous (A) (B) commitment in the lineage. In polyclonal segregation, analogous cell differentiation (C, D) in contiguous cells (compartments) result from cell interactions, independently of lineage origin. Cell types (K–J) appear associated in organs independently of their lineage origin according to local assignments resulting from cell interactions.

### (a) *Segregation and specification*

The concept of fixed cell lineages in early development was put forward by descriptive embryologists around the turn of the century (Whitman 1887; Boveri 1887; Conklin 1905). Fate mapping of blastomeres, using cell shapes, or pigment markers distributed in an asymmetrical way in the egg, suggested regular cell segregation into specific presumptive territories of late embryonic structures. Ablation and transplantation experiments confirmed in some cases that presumptive fate was actual cell 'commitment'. This was the case in certain marine invertebrates (annelids, nematodes, molluscs) as well as in primitive chordata such as ascidians. The same experiments carried out in other embryos (vertebrates, echinoderms) in the same stages showed partial regulative behaviour, interpreted as resulting from lack of commitment.

How does segregation of blastomeres occur and what is the nature of their specification? In *Caenorhabditis* cleavage is holoblastic and highly asymmetrical as to the fate of blastomeres. Lineage is fixed with regards to both pattern of cell division in space and fate; that is, individual blastomeres have a finite number of descendant cells, with definite fates and fixed positions (Sulston *et al.* 1983). Ablation of these blastomeres results in the absence of its cell progeny and none or little compensation is accomplished by progeny of other lineages. The segregation of such lineages is preferentially clonal; that is, lineages of restricted presumptive fate have a single 'founder cell' (figure 3). All segregations from the first cleavage division, follow the rule of dividing asymmetrically into a cell with a restricted lineage (AB) and a stem cell of mixed progeny ($P_1$). The scheme is reiterative in that subsequent cleavage divisions segregate into founder cells and stem cells of the next segregations. Thereafter the fate of such lineages is pure in D (muscle), E (intestine) and $P_4$ (germ line) but mixed in the other lineages: AB gives rise preferentially to hypodermis and neurons (classical 'ectoderm' derivatives in most organisms) and to muscle cells (classical 'mesodermal' derivatives). These appear in particular places in late sublineages. This is similar to what occurs in the MS lineage, giving preferentially mesodermal derivatives with exceptional neurons. The exceptional sublineages are specialized cells: muscles in the AB lineage and neurons in the MS lineage that appear late in particular sublineages of an otherwise pure histotype.

The cleavage divisions of the nematode segregate equal cells with different fates; that is, they are asymmetrical divisions. However, they also allocate cells to different regions of the embryo, symmetrically between left and right, but asymmetrically with respect to the anterior–posterior axis and along the dorsoventral plane. The lack of compensation following cell ablation does not throw any light as to the nature of cell commitment. It could reflect both histotypic and also topological assignments in the segregating cells. Gastrulation occurs by shifting the reciprocal positions of the blastomeres (with no clear relation as to the presumptive fate in terms of germ layers). This restricted, as opposed to global, gastrulation, suggests cell-to-cell specific interactions, a possible indication of the actual differentiation of cells performing that process. After such cellular reorganization the amplification period of the lineages sets in (see below).

Similarly to the situation in the nematode, the early development of the leech *Helobdella* (and others) follows a series of stereotyped cell divisions that segregate different cell lineages. In the four-cell stage three blastomeres (A, B and C) give rise mainly to the endoderm and part of the head and one blastomere (D) gives rise to the trunk segments containing derivatives of the ectoderm and mesoderm. However, asymmetrical divisions simultaneously separate a set of

A, B, C and D macromeres from the corresponding a, b, c and d micromeres. These micromeres remain at the animal pole and their descendant cells produce the head body wall and the supraoesophagic ganglion. These asymmetrical divisions separate, therefore, two presumptive regions of the animal: head and trunk, before subsequent divisions restrict the specification to histotypes. A subsequent symmetrical division in these blastomeres sets the left–right symmetry (figure 4). Blastomeres D divide symmetrically to produce a M stem cell (teloblast) from which preferentially mesodermal derivatives will be produced and a mother cell which will give rise to the NOPQ teloblasts of mainly ectodermal progeny. The latter are stem cells which will bud off 100–400 primary blast cells, designated as n o p q. These will extend by epiboly over the endoderm (gastrulation) forming the individual bandlets of the two paired germinal bands that will give rise to the segments of the trunk. We will see below that the lineages of these teloblasts are mixed.

In many holoblastic organisms cleavage segregates cells of different size in either the first, second or third division which gives rise to anisotropic blastulas along the animal–vegetative axis or the dorsoventral axis or both, as in annelids, molluscs, echinoderms and ascidians. In cases where certain blastomeres can be recognized by their form or size (polar lobe in annelids and molluscs) or pigment (ascidians) their fate has been followed by direct observation and in some cases ascertained by ablation. In these cases fate has been shown to be rather strict. Thus, *Chaetopterus* (Polychaeta) and *Ilyanassa* (Gastropoda) have a D blastomere that in subsequent divisions will give rise to the somatoblasts 2d and 4d. They are stems cells from which the gut (2d) and the caeloblastic mesoderm (4d) of the segmented portion of the trochophora larva will be formed (Dawidoff 1928; Anderson 1973). The ablation of these blastomeres has extreme consequences in the development of the larva because not only are the prospective structures lacking but morphogenesis is prevented also (Wilson 1929; Clement 1962). As in the leech, the geometry of early cleavage divisions suggests that they may segregate cells corresponding to prospective regions of the larva. It is, however, not known whether these segregations correspond to fixed or mixed lineages.

As discussed by Anderson (1973), it is remarkable how constant are the cleavage patterns of different groups of spiralia with trochophora-like larvae, from annelids to molluscs. These patterns differ, however, in the particular blastomeres (cytoplasmic regions of the egg) which will give rise to certain histotypic lineages, such as muscles and the germ line.

Organisms with early regulative development can also show pregastrular specifications. The vegetative micromeres of the sea urchin morula give rise to the mesenchyme and skeleton of the pluteus both in normal development and upon explantation (Hörstadius 1973). This suggests an early commitment to certain specific differentiations. Monoclonal antibodies against cell surface antigens (McClay & Wessel 1984) and *in situ* hybridization to specific mRNAs (Angerer & Davidson 1984) have detected pregastrular localizations related to presumptive regions and cell types (muscles) of the larva. The ascidian *Cynthia*, a primitive chordate, also shows early commitment in blastomeres at the eight-cell stage. Moreover, the blastomere B4.1 precursor of the muscle lineage, expresses acetylcholinesterase autonomously upon explantation or mitotic arrest (Whittaker 1980). It seems to be general that the presumptive fate of these early blastomeres extends over large areas of the blastula at the beginning and only becomes restricted to a particular group of cells (polyclone), or to a clone, later.

Early segregation of lineages with different fate is also apparent in vertebrates. Injection of

label into single blastula cells of *Xenopus* (128–512 cell stages) in different presumptive neurogenic regions results in preferential labelling of discrete regions of the later embryo (Jacobson 1983). In the frog and higher vertebrates there is extensive dispersal and mingling of the descendent cells. Label appears not only in nervous (central nervous system) derivatives but in other tissues as well. The regions labelled become restricted in size and extent with increasing age of the blastula. The cells of the 512-cell stage have clones restricted to four regions, defining topological subdivisions of the central nervous system. Preferential boundaries separate dorsal from ventral parts of the central nervous system (in the retina and along the sulcus limitans) and in the isthmus regions separating mesencephalon from rhombencephalon and spinal cord. The observation of unrestricted cell invasion in other regions but restriction along these boundaries, despite extensive cell mingling, is suggestive of a commitment occurring soon after the 512 cell stage. If there are similar restrictions in other histotypes such observations could be indicative of topological segregations preceding the specification to different histotypes. In *Xenopus* also antibody labelling against α-actin has been found localized already in certain regions of the blastula, related to the lineage that express this gene in the somatic muscle in the larva (Mohun *et al.* 1984).

Polyclonal segregations take place in the early stages of development of the placental mouse (Gardner 1978). Allophenic mosaics uncover differential participation of blastula cells to either the trophoblast or the inner cell mass that will give rise to the embryo. Subsequent subdivisions polyclonally segregate polar from mural trophoblast and primitive ectoderm from primitive endoderm. These segregations are associated to the generation of cells of different histological types and molecular patterns of expression.

*Drosophila*, like most insects, has centrolecithic eggs and cleavage is only nuclear, giving rise to a syncytium. The spindles of the first zygotic divisions are randomly oriented in the egg and there is some nuclear intermingling during the colonization of the yolk. The nuclei migrate to the periplasm of the egg where, after enclosure by membranes, they form a blastoderm of 5000 cells. At blastoderm the global position of the cells can be correlated with their fate. The fate maps of Poulson (1950) (based on direct observations) agree with those resulting from gynandromorph analysis (see Janning 1978) and from ablation experiments (Underwood *et al.* 1980). This map shows along the anterior posterior axis the presumptive regions of a cephalic complex, a repeated pattern of thoracic and abdominal segments and two endodermic caps, one anterior and another posterior (figure 5). Hypodermal presumptive cells occupy a dorsal bandlet along the dorsoventral axis with presumptive neuroblasts lying directly underneath and mesodermal cells ventrally. Two major invaginations occur at about the same time in the blastoderm: a cephalic furrow that separates cephalic from gnathocephalic presumptive regions and a ventral furrow that allows the invagination of endoderm, mesoderm and neurogenic regions (gastrulation).

The combined results of cell ablation by ultraviolet irradiation, of cell transplantation, of clonal analysis in genetic mosaics and of phenocopy experiments suggest an early commitment of the blastoderm cells to topological as well as to histotypical qualities. Whereas blastoderm nuclei show totipotency, transplanted blastoderm cells to ectopic locations develop, integrated or in isolation, according to the fate of the region of origin. The differentiated cuticular structures of these clones correspond to parts of the inventory of the derivatives of segments (Illmensee 1978; Simcox & Sang 1982). Analysis of mitotic recombination clones initiated in the blastoderm stage show restrictions to segment and anterior and posterior subsegments


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(compartments) (Wieschaus & Gehring 1976). Preblastoderm mosaics show later preferential restriction lines along the borders of such compartments. Similar restrictions seem to separate, at blastoderm or shortly thereafter, mesoderm from ectoderm and nervous from epidermal derivatives (see Lawrence (1981) for discussion). More recently the use of molecular probes also show in *Drosophila* topographical restriction of gene activity in blastoderm and during gastrulation (see below).

It seems a general feature in all the organisms considered that individual cells of a lineage tree, or groups of cells related by position, acquire a differential fate or even show a specific molecular trait already in the cleavage divisions, before gastrulation. Presumably the determinants of these commitments or differentiation are cytoplasmic. They become assigned to individual cells by stereotyped cleavages, with or without the concourse of the zygotic genome and with or without cell interactions. In fact, cortical differentiation can occur in pseudoblastulas of *Chaetopterus* without cell division (Lillie 1906) and this is the normal condition in insects. It is generally believed that cleavage divisions merely partition the egg depending on differential cytoplasmic composition and cytoskeleton structures; they are instrumental in segregating oocyte determinants involved in cell determination. However, patterns of cleavage can be modified within the same taxon. Thus, major differences occur between the holoblastic cleavage of crustacea, myriapoda and the centrolecithic cleavage of insecta or between the superficial cleavage of certain reptiles and aves compared with the otherwise holoblastic cleavage of most vertebrates, possibly correlated with the amount of yolk content of the egg. However, it is by no means clear why stereotyped cleavage divisions remain otherwise constant in taxonomic groups (for example, in spiralia). Lineage segregation in specific sequence could be necessary to generate constant topographical organizations leading to specific gene product interactions. This invariance will be discussed when we consider maternal effect mutants.

A second question deriving from the observation of strict lineages relates to the nature of the specification of the committed cells. The specificity of fate is defined by the particular observable differentiation at the end of the lineages. The identification of precursors of muscle, ectodermal cilia or particular neurons is taken as indication of cells being committed to more general properties: mesoderm, endoderm and ectoderm. In particular, the appearance of tissue specific genes products, for example, muscle proteins in cells of the blastula could be considered as marking a set of tissue specific genes of mesoderm and muscle, and therefore of early differentiation of major histotypes. However, it may only reflect the actual expression of certain genes temporarily involved in the later processes of cell movement in cell migration and gastrulation. In any case it remains to be seen how far gastrulation is a consequence rather than a requisite of histotypic cell differentiation.

The regular patterns of divisions seem to partition the egg with respect to topological properties. Such is the case for the cells that are going to be invaginated in gastrulation in *Caenorhabditis* and sea urchin; which will become cephalic (as opposed to trunk) in the leech and possibly in *Xenopus*; and are associated to trophic membranes in the mouse or subdivided into segments in the blastoderm in *Drosophila*. These topographical segregations cut across histotypes, and possibly are independent of histotypic differentiation. But even the topographical segregation of histotypes has a certain phylogenetic constancy, at least within groups. Thus in annelids, as in the leech, and in *Drosophila* the dorsoventral disposition of germinal bands shows the same relative position of ectoderm, neurogenic tissue and mesoderm.


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### (*b*) *Amplification and terminal differentiation*

Around the time of gastrulation or shortly thereafter cells become clonally or polyclonally restricted to specific lineages. In some cases these lineages are restricted to topological rather than to histotypical qualities. We will see now how these fixed lineages are frequently mixed in fate, that is, they include cell types corresponding to different major histotypes or germ layers. Their segregation occurs mainly towards the end of the lineages.

In *Caenorhabditis*, postgastrular cell proliferation occurs following equational (fate-symmetrical) divisions at the beginning and fate asymmetrical divisions towards the end of the

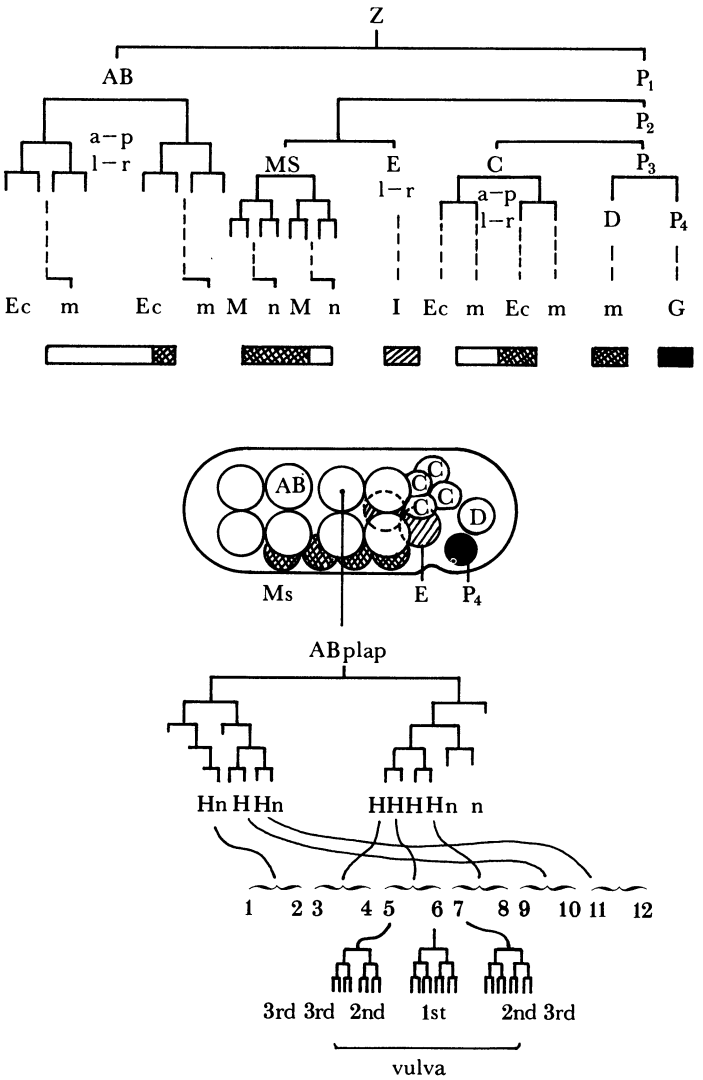


FIGURE 3. *Caenorhabditis elegans*. From top to bottom: lineage segregation and cell specification in cleavage divisions, topological disposition of cells at gastrulation, amplification of the AB plap blastomere and cell differentiation of hypodermis and vulva. (Description of the different stages in text.) Z, zygote; AB, MS, E, C, D and Pc, founder cells of lineages that contribute to Ec, ectoderm derivatives; M, mesoderm; I, intestine; G, germ cells; m, muscles; n, neurons. Divisions segregating topologically, a, anterior, p, posterior daughter cells and l, left, r, right daughter cells. During amplification divisions analogous lineages form H (hypodermis) and n (neural) cells in groups. 1st, 2nd and 3rd, primary, secondary and tertiary states of differentiation relative to the pattern of structures that constitute the vulva.

lineage. Asymmetrical divisions, giving rise to sister cells of different fate, proliferation pattern, cell death, and terminal differentiation, start when the embryo contains 200–400 cells and ends in the 671 cell stage embryos. Cell proliferation continues in only certain sublineages during the juvenile stages, following specific division patterns. There are relatively few cell migrations. Thus morphogenesis of particular organ structures occurs by the grouping of cells that become neighbours by cell proliferation rather than by migration. The embryo is fundamentally left–right symmetrical following an early lineage segregation. There are no morphological segments along the anterior–posterior axis, that is, periodically repeating groups of cells of all the germ layers. There are, however, along this axis, reiterative sublineages, with similar sets of terminal differentiation cells in all the lineages. Homologous lineages, with the same branching pattern, can lead to patterns of homologous cells in fate and differentiation but also to non-homologous cells. Cells of the same fate (analogues) can also derive from different sublineages. In general homologues by lineage are analogues in fate, that is, lineage and fate are tightly related. Homologous sublineages are periodically repeated in the central part of the organism. Sublineages may give rise to pure morphological units like sensory cells, but frequently they are of mixed descent, including epidermal nervous and mesodermal cell types in different proportions, until the end of the lineage (figure 3). There are morphological repeating groups of cells in the lateral hypodermis, although they arise by piecemeal recruitment rather than by cell lineage reiterations. We will see a similar situation in other organisms with partial or real segmentation. In all cases morphological units are formed by neighbouring cells of different lineages and different cell types, possibly arising by topographical assignment.

In the nematode with such strict cell lineages, there are exceptional cases of pattern regulation. Thus, in a particular organ (formed by homologous or non-homologous cells) following ablation of one or several cells, the remaining ones can occupy their position and function (vulva of the ventral hypodermis of the hermaphrodite) or induce compensatory proliferation of neighbouring cells (sensory ray cells of the lateral hypodermis). The change of fate must be dictated by properties of the group of cells. These groups are called 'equivalence groups' because of the capability to be replaced by other cells of the group (Sulston & White 1980). Their operational similarity with compartments will be discussed later.

In the leech, during gastrulation, the teloblasts (M and N, O/P and Q) are the founder cells for the segmented trunk of the animal. Their clonal derivatives have been followed by injecting them with non-diffusible fluorescent cytoplasmic tracers. The teloblasts divide as stem cells producing bandlets of blast cells (m, n, o, p and q) which form the paired germinal bands that expand over the endoderm (figure 4). During the cephalocaudal extension of the germinal bands the blast cells of the bandlets are sampled, singly or in pairs, to become the founders of the segments. The blast cells of the n o p and q bandlets are of mixed descent consisting of epidermal and nervous cells and the m blast cell gives rise mainly to mesodermal derivatives after proliferation in a mediolateral extension. Each blast cell produces, by stereotyped differential divisions, subsets of cells such as specific neurons, epidermal cells and muscle fibres. These division patterns are reiterated in all the segments. Although the m blast cell lineage produces mainly mesodermal derivatives (connective tissue, nephridia and muscle) certain neuromeres are also derived from it. The n, o, p and q blast cells produce specific subsets of epidermal (mainly from O, P and Q teloblasts) and nervous cells (glia, motor- and interneurons, mainly from N and O teloblasts). These subsets of cells in a segment of a given lineage constitute

'kinship groups' (Kramer & Weisblat 1985). The teloblasts therefore give rise to a mixed progeny as to cell types topographically invariant.

Ablation experiments (killing a teloblast or a blast cell by intracellular injection of a toxic enzyme or irradiation) have been carried out to ascertain the degree of cell autonomy of these lineages. In most cases ablation of a teloblast leads to the disappearance of its normally derived kinship group. However, in some cases derivatives of non-ablated teloblasts are also affected. Such experiments show that the O and P teloblasts are interchangeable with respect to their fate. Interestingly, the ablation of either O/P teloblast leads to the surviving teloblast taking the P fate. The irreversible commitment of either o or p fate takes place on the first or second division of the blast cell. This behaviour of the O/P teloblast pair corresponds to that of the equivalence groups of the nematode. Compensation is also found in the absence of nervous cells of an ablated teloblast by the blast cell progeny of other bandlets (Shankland & Stent 1985).

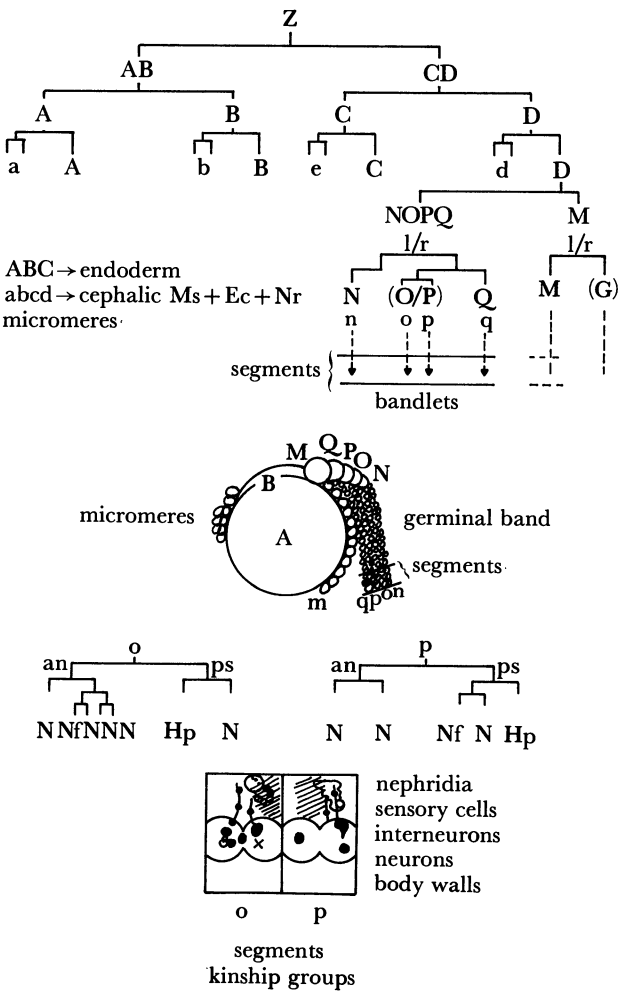


FIGURE 4. *Helobdella*. From top to bottom: early lineage segregations, topological disposition of blastomeres at gastrulation, amplification of blast cells and terminal cell differentiation inventory of cells of the O/P lineages in two segments. Macromeres A, B and C give rise to endoderm, micromeres a, b and c produce cephalic structures of MS (mesodermal), Ec (ectodermal) and Nr (neural histotypes). M, N, O, P, Q founder cells of m, n, o, p, q blast cells. In terminal cell differentiation: N (neuronal cell types) Hp (hypodermis) and Nf (nephridia): appear in the same segment in different pattern, depending on the lineage of the o and the p blast cells. an, Anterior; ps, posterior.

In *Drosophila* cell proliferation continues following gastrulation, mainly in two germ layers, the endoderm (making the midgut) and the neurogenic region, and less so in ectodermal and mesodermal derivatives. Segments are soon morphologically distinguishable by the appearance of intersegmental furrows in the hypodermis and neuromeric and mesodermal clusters. Proliferation ceases at different times depending on the tissue, and visible cell differentiation starts in some cells at about 7 h of development. At hatching the *Drosophila* embryo contains about 20000 cells, the result of two cell divisions, on average, of the blastoderm cells. In *Drosophila* the germ band giving rise to the embryo occupies the whole surface of the egg, and segmentation occurs almost simultaneously along the anterior–posterior axis. In primitive insects (for example, Orthoptera) the germ band occupies only a small fraction of the egg. In addition, segmentation starts in the presumptive region of the last cephalic and first thoracic segments, the so called 'differentiation centre' of Seidel (Counce 1973), and spreads to other segments later. In phylogenetically older insects and in Myriapods, Onychophores and flatworms, segments appear by budding (anamery) out of the gnathocephalic region, between this and the anal exit. These comparative data are important as a frame of reference to understand the genetic control and specification of segments.

Proliferative growth during the larval period of *Drosophila*, as a holometabolous insect, only affects the imaginal discs of the hypodermal lineage, parts of the nervous system, gut and certain mesodermal derivatives (Bodenstein 1950). Most adult structures arise from sublineages which are set apart in embryogenesis. Cell lineage studies have been carried out mainly in the progenitors of these adult structures, and preferentially in imaginal discs (but see Lawrence 1982). These studies are based on genetic mosaics, and more recently on cell transplantation, and antigen–antibody labellings. Classical data on genetic mosaics (gynandromorphs), resulting from chromosome loss in the first zygotic divisions, indicate that imaginal disc growth is indeterminate, that is, even close neighbouring cuticular structures could derive from the progeny of the two first zygotic nuclei (Sturtevant 1929). However, genetic mosaics initiated later, during the proliferation period by mitotic recombination, uncovered clonal restrictions separating morphological regions. The analysis of early and late clones and of mosaics of cells growing at different rates permitted a delimitation of the boundaries of clonal restrictions (García-Bellido *et al.* 1973). The regions delimited were called 'compartments'. Since the clonal origin of cells included in a compartment can be traced back to clones segregated in the first zygotic division, compartments are polyclonal in origin. Moreover, since cell death by injury, mutation or differential growth rate can be regulated within compartments, they are the formal analogues to the 'equivalent groups' of the nematode and the leech. Compartmentalization in *Drosophila* is progressive, subdividing populations of cells topologically into regions, segments, anterior and posterior compartments, and in appendages in dorsal and ventral compartments. Polyclonal compartments segregate dichotomously, like sister cells in clonal lineages. In the ectodermal derivatives these segregations delimit topographical subdivisions rather than different histological cell types (see García-Bellido & Ripoll 1978).

We do not know whether the neighbouring anlagen of ectoderm and mesoderm, hypodermis and nervous tissue, arise by dichotomous, compartmental segregations (see Hartenstein & Campos-Ortega (1984) for discussion on the formation of the neurogenic region and Lawrence (1982) on the lineage of muscles). They arise, however, independently of the segregation of segments. Thus, segments and compartments seem to be developmental units of a topographical diversity, internally subdivided regarding histotypic specifications. During imaginal disc growth

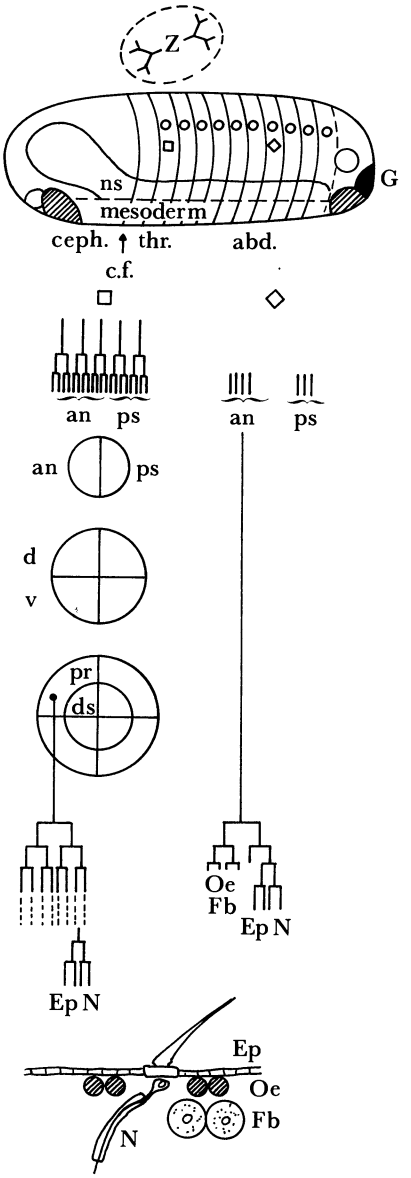


FIGURE 5. *Drosophila*. From top to bottom: patterns of divisions of the zygotic (Z) nucleus, blastoderm map, amplification of cells in the labelled regions □ and ◇ within the anterior (an) and posterior (ps) compartments and terminal differentiation of ectodermal derivatives in the imaginal discs. ceph., Cephalic segments; thr. thoracic; abd., abdominal; G, germ cells; ns, central nervous tissue; c.f., cephalic furrow; barred: endoderm. Compartmentalization steps during amplification in the wing imaginal disc between d (dorsal), v (ventral), pr (proximal), ds (distal) polyclones. Lineages in terminal differentiation of Ep (epidermal cells), N (sensory neurons), Oe (oenocytes) and Fb (fat body) cells.

cell proliferation is exponential but indeterminate, although the shape of clones is region-specific. The pattern distribution of cuticular elements is also region-specific. Those are not related by lineage but are none the less compartment-specific, constituting a fixed inventory of elements or a 'kinship group'. Hypodermal elements correspond to different histotypes. During proliferation of the imaginal disc cells, of ectodermal origin, cell types of a broad histotypic diversity can appear. Clonal analysis has shown that the same mother cell can differentiate


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cuticular (epidermal) as well as nervous derivatives (sensory chaetae and sensilla), and also oenocytes and fat cells, the latter derivatives of mesoblasts in other lineages (Ferrus & Kankel 1981). Thus, as in the nematode and the leech, we find clonal segregations related to topology that are independent of the segregations that give rise to cell types.

The results of a variety of other experiments indicate early commitments at the cellular level. We have already considered the results of ectopic cell transplantation of blastoderm cells (Illmensee 1978). Cells of dissociated *Drosophila* embryos at early stages can differentiate *in vitro* into all the cell types of the final embryo and even of the imaginal tissues (Dübendorfer & Eichenberger-Glinz 1980). Isolated cells of early embryos, grown on a feeding layer of irradiated imaginal disc cells, show after proliferation specific commitments for particular imaginal discs and anterior or posterior compartments (García-Bellido & Nöthiger 1976).

Early imaginal disc or compartment specification, however, can change under experimental conditions leading to extra cell proliferation as it occurs in regeneration and transdetermination. In regeneration of imaginal discs, cells, which are by all criteria determined to their compartmental fate, can give rise to progeny of other compartments of the same disc, although compartmental restrictions set in soon after (Szabad *et al.* 1979; Abbott *et al.* 1981). During transdetermination, proliferation of the regenerating blastema can give rise to cells with fates that are specific to other imaginal discs, to the same segment or to a different segment. We do not know, however, whether transdetermination can occur between cells of different histotypes other than the ectodermal cells of imaginal discs (Hadorn 1978). Thus, commitment, at least within certain lineages, can change during proliferation under experimental conditions. Similar experiments in other organisms also indicate transdifferentiation between cells of different histotypes. We will discuss the genetic implications later.

Cell migration is a morphogenetic operation that allocates cells to a different position than that in which they arose by cell division. It occurs rarely in *Caenorhabditis* and the leech. In *Drosophila* major migration occurs in the germ cells (Poulson 1950) but it is an important operation in the final patterning of chaetes (García-Bellido & Merriam 1971*b*; Lawrence *et al.* 1979). In these cases cell specification precedes migration and location. Cell migration is more common in vertebrates, early and late in development, as seen in allophenic embryos (Mintz 1965). It is specially relevant in the neural crest derivatives (Le Douarin 1980) and in the nervous system. In neurogenic regions consisting of several layers of cells, as in the cerebellum of mice, both horizontal cell migration and vertical cell body movements lead to complex mosaicism. We do not know the reasons for this massive migration. It is, however, possible, at least in the case of vertical migration, that cell specification precedes migration, again as a device to establish complex neuronal connections (Mullen 1978).

### (*c*) *Developmental considerations*

We have seen that division patterns before gastrulation or in the proliferation period are highly regular, following asymmetrical divisions first, amplification through symmetrical division later, and again asymmetrical division at the end of the lineages. Division patterns are very stereotyped in stem cell, parental and grandparental reiteration modes of segregation. These cell division programmes appear frequently reiterated. Such stereotyped divisions suggest a cell internal programme. What does this programme specify? Could it be that there are lineages relegated to generating topographical discontinuities which function earlier and independently of those generating hierarchically different histotypes and cell types? It is interesting that lineages




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can be different for cephalic or preoral organs and for trunk or segmental regions in the annelids, molluscs, insects and possibly chordata. They contain derivatives of all the germ layers. On the other hand, an early segregation of pure histotypes is exceptional. It holds mainly for endoderm and germ line whereas other lineages are usually of mixed histotypic fate. This is the case in AB, MS and C lineages in *Caenorhabditis*, m, n, o, p and q blast cells in the leech and imaginal discs in *Drosophila*. The segregation of these sublineages can occur late in the proliferation period, and therefore such a commitment for, alternatively, 'mesoderm', 'ectoderm' and 'nervous tissue' either does not exist, or does not set in until late, or is reversible.

Segregation of lineages can be clonal or polyclonal. Organs or morphological units such as the vulva in the nematode, the nephridia and neurons in the segments of the leech, the compartments and imaginal discs in *Drosophila*, are not clonal in origin. Rather they come about by the interaction of cells of the same or different lineages in a particular position. These morphological units show cell interactions of regulatory nature: 'equivalence groups' or 'compartments'. They correspond to supracellular entities in which their cells interact, share a common general fate and are possibly genetically interdependent. Clonal or polyclonal lineage segregations are extreme cases of the same operation: the one involved in specification of discontinuous developmental pathways. The role of genes and the nature of their functions in the generation of topological diversity and specification will be discussed in the next section.

## 2. Genetic control of lineages

The observed invariance of lineages and sublineages, the complex stereotyped patterns of division, the specific lineage differences found between species and sexes and the cell autonomy of response to experimental perturbation, all are suggestive of the existence of a cell intrinsic control in their generation. This inference begs the question of the role of genes controlling these processes. I now would like to consider two precise questions amenable to genetic analysis: (i) how do genes affect lineages and (ii) which are the genetic bases of commitment and specification? Unfortunately, relevant genetic data to these questions are only available in two organisms: *Caenorhabditis elegans* and *Drosophila melanogaster*. And these data are still limited to permit general conclusions.

Many morphogenetic mutations have been isolated in both organisms. We will deal here with those called 'pattern mutants', which affect systemic properties, that is changing the distribution of elements, otherwise normally differentiated, in space. The aim of genetic analysis is to identify the role of the normal gene. There are problems in reaching this goal. A given locus may mutate to different allelic states, for example, amorphic (usually lethal) and hypomorphic (viable but with a weak and partial phenotype). The analysis of lethal and null mutations in clones of homozygous cells has allowed us, in some instances, to infer the 'specificity' of the affected function, that is, the realm of action of the normal gene. This analysis has revealed in *Drosophila* that most morphogenetic mutations are cell-autonomous and few are tissue- or lineage-specific. Mutants with altered early stages of development may have their phenotypes modified by perdurance of maternal gene products released in the oocyte. To ascertain the role of these genes requires the study of embryos derived from mutant oocytes, an analysis only carried out in mutants of a small fraction of loci.


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### (a) *Topological segregation and specification of lineages*

In many organisms the bulk of zygotic gene transcription starts in the blastula or early gastrula stages (see Davidson 1976). Thus, it is possible that the stereotyped pattern of divisions at cleavage and the spatial distribution of cytoplasmic determinants in the egg are under the control of oocyte genes. Mutations affecting these processes will therefore have preferentially maternal effects. The classical example is handedness of coiling in certain gastropods (Sturtevant 1923). Mutational screens have been undertaken to ascertain the abundance of maternal effect genes and their role in early development in both *C. elegans* (Hirsh *et al.* 1977) and in *D. melanogaster* (Mohler 1977; García-Bellido *et al.* 1983). These studies clearly show both that the complexity of maternal information is very great and important for both early stages of development and for cell differentiation. Maternal mutants have as a rule also zygotic effects. We have therefore no estimate of the proportion of those functions that are exclusive to early development.

Mutations expressed in the zygote affecting lineages during the amplification period in *C. elegans* have been reported in many recent papers (Chalfie *et al.* 1981; reviewed by Sternberg & Horowitz 1984). Most mutations change sublineages leading to the suppression, prolongation or substitution of one set of elements by another within and between sublineages. They may change the polarity of location of sister cells, reiterate parental or grandparental types, and uncover new sublineages by rescuing cell death in a particular branch. They may be specific to a particular sublineage, or modify several homologous and non-homologous lineages, affecting analogous and non-analogous cells. Occasionally the same mutation can cause several of these perturbations in the same lineage. Thus, mutations in the locus *unc*-86 affect neural precursor cells of several lineages, changing the fate of three segregating cell types A, B, X (X, cell-programmed death) into AAA...cells by reiteration, and extraproliferation of a stem cell for A. The limitations of an incomplete genetic analysis of the corresponding loci, prevents us from inferring the generality or specificity, that is, the 'function', of these genes. Whether these genes determine (or their mutations merely perturb) these decisions is not yet known. However, the perturbation caused by these mutations indicate that alternative states are intrinsic to the genome and entire discontinuous lineage programmes can be repressed, rescued or substituted by mutation. These programmes may correspond to patterns of division without affecting cell differentiation, affecting cell differentiation alone or both.

Homoeotic mutants substitute a sublineage, division pattern and fate, by another, normally found in another part of the organism. Thus, a mutant in the gene *lin*-22 changes the ectoblast sublineages V1–V4, which normally generate hypodermal nuclei, into a V5 lineage which normally gives a sensory cell. Several mutations in the *lin*-17 gene change the mixed fate of gonadal precursor cells into a pure fate, that is, different sister cells acquire the same fate. For example, several loci affect the lineages of the vulva of hermaphrodites (figure 3). The vulva is formed by sublineages of three hypodermal precursor cells P5.p, P6.p and P7.p that produce cells of different morphological fates (P6.p produces a primary lineage; P5.p and P7.p a secondary lineage). They are flanked by precursor cells (P3.p, P4.p and P8.p) of nuclei of a hypodermal syncytium (tertiary lineage). Ablation experiments indicate that P3.p–P8.p are potentially equivalent, that is they form an 'equivalence group'. In the absence of an inductive signal from an 'anchor cell', in the overlying gonad, all the cells produce hypodermal nuclei (no vulva), considered therefore to be the ground state (tertiary). The anchor cell inductive




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signal determines the primary state and their neighbours adopt, according to their position, the fate of secondary and tertiary lineages depending on their distance to the anchor cell. The ablation of precursor cells within the group leads to a lineage substitution. Mutations in five different loci cause all the precursor cells of the equivalence group to enter vulva sublineages (P5.p, P6.p, P7.p), even in the absence of the anchor cell, causing multivulva phenotypes (*muv*). These mutations perhaps cause vulval sublineages to appear constitutively. Contrarily, mutations in the *lin-11* and in five other loci prevent the expression of the primary and secondary lineages causing a vulvaless phenotype (*vul*). Especially interesting is the locus *lin-12* (Greenwald *et al.* 1983) which has several both dominant (*lin-12(d)*) and recessive (*lin-12(O)*) alleles. They have pleiotropic effects in several sublineages including those generating the vulva. In all of them their phenotypic effects are opposite. In the vulva sublineages the *lin-12(O)* mutants show tertiary sublineages in P5–P7 derivatives and *lin-12(d)* mutants show secondary sublineages in all P3–P8 derivatives. In other words, *lin-12(O)* mutants prevent secondary sublineages and *lin-12(d)* mutants cause all the cells of the equivalence group to enter the secondary sublineage pathway. Interestingly, *lin-12(d)* dominance is due to overproduction of a function that can be reverted by mutation into *lin-12(O)*, the recessive lack of function condition. This locus behaves then, genetically, as those known in *Drosophila* to be involved in the control of alternative pathways (see below).

Similar genetic behaviour has been found in mutations that cause the precocious or delayed appearance of sublineages within lineages. They are called heterochronic mutations because lineage substitutions occur along the time-axis rather than in space. These mutants affect the differentiation of elements of ectoblast lineages which are larval-instar-specific. *C. elegans* passes through four juvenile stages (L1–4) before reaching sexual maturity. These mutations affect specific sublineages (for example, sensory elements, cuticular specializations) rather than the whole pattern of a given stage. Precocious mutants (*lin-14* and *lin-28*) show, one or two stages in advance, structures of later moults or of the adult form. Retarded mutants (*lin-4*, *lin-29*) show a repetition of lineages and structures of early stages in subsequent ones. Among these loci *lin-14* is especially interesting because recessive alleles are precocious in phenotype and dominant ones are retarded in phenotype, suggesting again a control function by this locus. Moreover, double mutants and other interactions between *lin-4* and *lin-14* are compatible with the notion that the former encodes for a negative regulator of the latter.

Mutants that transform sexually dimorphic characters are known in both organisms. In fact, some of the loci studied in *Drosophila* have their counterpart and possibly the same role in the sex differentiation system in *Caenorhabditis*. Thus the *her-1* gene seems to be involved in assessing of the X-chromosome: autosome ratio. There are dominant and recessive mutations in this locus and its homologue the *Sxl* locus of *Drosophila*. The function of these loci is expressed (via several other genes) in the activity of the *tra-1* locus of *C. elegans* and the *dsx* locus of *D. melanogaster*. In both loci there are dominant and recessive alleles that change the alternative sexual pathways one into another. These pathways lead to morphological characters made visible in sexually dimorphic traits at the cellular level; specific neurons and vulval cell lineages between hermaphrodites and males in *C. elegans* and segmental anlage, organs and cuticular patterns in *D. melanogaster* (see Hodgkin (1985); Belote *et al.* (1985) for discussion and references). Other genes are hierarchically downstream from *tra* and *dsx* determining the particular lineages that give rise to the visible morphology. Such genes include *lin-12*, *egl-1* and others in *C. elegans*, whose mutants change lineages typical of the male into those of the hermaphrodite or vice versa. Their

counterparts in *Drosophila* would be the genes coding for female yolk proteins and male sex peptides and others responsible for morphological traits. In both organisms sex differentiation mutants substitute common or rescue silent lineages of the opposite sex.

Developmental pathways for segmental and compartmental specification are polyclonal in *Drosophila*; that is, developmental decisions are taken by cells in groups rather than by single cells, as is the rule in *Caenorhabditis*. However, genetic analysis has revealed a similar logic in both organisms in the specification of these pathways.

As seen above several developmental decisions seem to take place in the cells of the blastoderm, or around the time of gastrulation. At this stage and along the anterior–posterior axis topological commitments as to segments, and to anterior or posterior compartments within segments, seem to occur. Simultaneously, or shortly thereafter, epidermal, neural and mesodermal subdivisions may set in along the dorsoventral axis.

Nüsslein-Volhard & Wieschaus (1980) searched for and found lethal mutations that affect segmentation, in about 20 loci. The mutations change segmental patterns in the embryonic cuticle in a locus-specific way, leading to periodic changes in polarity within segmental patterns (polarity mutants) and to deletions in homologous regions of alternating segments ('pair rule' mutants). This results in shortened larvae with half the number of segmental cuticular patterns but normal-sized cuticular elements. Some of the corresponding genes may be involved in providing periodic references for the spatial subdivision of the blastoderm. Moreover, they seem to affect exclusively early operations of segmentation. They show cell autonomous phenotypes in gynandromorph embryos but some have no phenotype in mitotic recombination clones in the imaginal discs (P. Lawrence and G. Struhl personal communication). *In situ* hybridization to RNA with probes of one of these loci (*fushi-tarazu*) shows a generalized pattern of expression over the blastoderm first, but becomes more and more restricted to certain bands later, these regions that appear deleted in the mutant embryos (Hafen *et al.* 1984).

Other mutations, in a dozen loci, change the pattern of differentiation along the dorsoventral axis in a polarized fashion, for example, expanding (or reducing) the extent of the neurogenic region at the cost of hypodermis (Nüsslein-Volhard *et al.* 1980). Genetic analysis has shown that dominant and recessive alleles of the same locus can have opposite effects in the phenotype. Thus *Toll* dominant alleles cause ventralization, whereas its recessive revertants cause dorsalization. Local injection of wildtype cytoplasm or poly $(A)^+$ RNA into *Toll*-revertant embryos, rescue their phenotype in a polarized manner (Anderson & Nüsslein-Volhard 1984). Thus presumably the pattern of expression of the wildtype *Toll* allele is dorsoventrally graded. Interestingly the effect of most or all of these segmental and dorsoventral mutations is maternal as well as zygotic.

The mutants described indicate that segmentation and dorsoventral polarity generate a coordinated spatial frame of reference, which is operationally different from specification of fate. The specification of segments and compartments is affected by homoeotic mutations. These mutations show systemic transformations between segments, with allelic variations in expressivity and specificity. The realm of action of the corresponding genes could be ascertained from the phenotypes of clones of cells homozygous for their null mutations or from homozygous lethal embryos. Genetic analysis has shown that some of them have both maternal and zygotic effects, whereas others have exclusively zygotic ones (Struhl 1981; Lawrence *et al.* 1983).

Among the genes with exclusively zygotic effects are those of the Bithorax complex (BX-C) (Lewis 1978) and the *Antennapedia* complex (ANT-C) (Wakimoto & Kaufman 1981). The genes

of the BX-C are needed for the correct specification of second thoracic and more caudal segments, and those of the ANT-C for cephalic and thoracic segmental specification. In the genes of these two complexes there exist dominant mutations corresponding to excess of function and recessive ones corresponding to insufficiency or lack of function. Their phenotypes show opposite directions of segmental transformation. Clonal analysis has shown that these transformations are cell-autonomous and affect the proliferation parameters of the imaginal disc cells (Morata & García-Bellido 1976). Phenocopy induction experiments indicate that the activation of the BX-C genes occurs in the blastoderm and determines the clonal behaviour of the proliferating cells (Capdevila & García-Bellido 1978, 1981). Generation of preblastoderm mosaics has also shown that the bithorax genes are cell autonomously required for the proper specification of the segmental polyclones (Miñana & García-Bellido 1982). Mutations in the BX-C affect not only the fate of epidermis (larval and imaginal cuticle) but also the central nervous system (Jimenez & Campos-Ortega 1981; Ghysen *et al.* 1985) and muscles (Lawrence & Johnston 1984) in the same segments. More recent molecular analyses confirm the genetic inferences that assigned segment- and compartment-specificity to the genes of these complexes. From the stage of blastoderm onwards, RNA transcripts of the wildtype genes are found in the cells of the segments affected by their mutations and in their epidermal, nervous and mesodermal derivatives (Akam 1983; Levine *et al.* 1983). Interestingly the spatial expression of individual genes of the complexes (Ultrabithorax and Antennapedia) differs not only between various segments but also between anterior and posterior compartments.

Genetic data indicate that the differential activation of the genes of both ANT-C and BX-C in different segments is affected by other genes. Mutant alleles of these loci show phenotypic interactions with mutations or with variable number of doses of ANT-C and BX-C. One such gene, *Polycomb* (*Pc*) behaves genetically as a *trans*-regulator by repression of BX-C genes (Lewis 1978; Duncan & Lewis 1982). Its phenotype in embryos is a generalized transformation of cephalic and abdominal segments to the pattern of the preanal segment: the expected transformation caused by the derepression of all the BX-C genes. Another gene, *Regulator of bithorax* (*Rg-bx*) behaves as if its product is an inducer or antirepressor (Capdevila & García-Bellido 1981; Ingham & Whittle 1980). The hypothesis has been put forward that the graded distribution along the anterior–posterior axis of the egg of the unbound Pc repressor determines the sequential derepression of the thoracic and abdominal genes of the BX-C (Lewis 1978; Capdevila & García-Bellido 1981) and possibly the ANT-C genes as well. Molecular analyses agree with the proposed role of *Polycomb* controlling the pattern of transcription of the *bithorax* genes shortly after the blastoderm stage (Beachy *et al.* 1985). However, other genes are also known with mutant phenotypes and genetic behaviour similar to those of *Pc* and *Rg-bx*. Moreover all those genes seem to affect, as do *Pc* and *Rg-bx*, both the early activation as well as the maintenance of activity of the BX-C genes during cell proliferation of the imaginal discs (J. Botas and A. García-Bellido, unpublished results). All these mutations cause cell-autonomous transformations to posterior segments in clones and have maternal effects. Thus, there is no conclusive evidence that activation and maintenance correspond to two different genetic operations.

Mutations in the gene *engrailed* (*en*) cause a transformation of posterior into the anterior compartments within the same segment. This transformation is observable in imaginal disc derivatives and in the cuticular pattern of the larva (Kornberg 1981). Recent molecular data have confirmed the compartmental specificity of the *en* RNA transcripts in early embryos

(Kornberg *et al.* 1985; Fjose *et al.* 1985). These transcripts are found in posterior compartments in the hypodermis but not in mesodermal cell types, as expected from mutant phenotype and developmental data. The mutant transformation is autonomous in proliferating cells. *en* transforms the pattern but leaves invariably a subjacent mirror image symmetry along the A/P compartment boundary of each segment; it is therefore a specification gene. Double mutant combinations of *en* and mutants of the BX-C and ANT-C show double transformations in segments and compartments, that is, both mutant phenotypes appear combined in posterior compartments, indicating that they perform independent specifications (García-Bellido & Santamaria 1972).

Contrary to the interactive relationships found between BX-C genes and *Pc*-like genes, the pair rule mutants show additive relationships (Nüsslein-Volhard & Wieschaus 1980), that is, the mutant phenotypes of each one appear superimposed. This finding precludes the pair rule gene products being directly involved in the activation of BX-C genes. They could, however, interact with the regulators of these, *Pc* and *Rg-bx*. It is possible that the specification of segments occurs by several steps of subdivision and refinement of topological properties along the anterior–posterior axis. In these processes the product of these genes could modulate the activity of each other until the activity pattern of the BX-C, ANT-C and *en* becomes defined between compartment boundaries. As seen above, the uniform distribution of the gene products of *fushi-tarazu* becomes restricted to segmentally periodic bands in the early gastrula. And similar changing distribution patterns were described for the histotypic antigens and mRNAs in the blastula and gastrula stages of sea urchin and *Xenopus*. In this gradual restriction of gene activity patterns to discrete cellular territories, inhibition and reinforcement of gene activity involving interactions between neighbouring cells, must take place. This is probably the mechanism underlying compartment formation and specification.

The ANT-C and BX-C genes seem to code for anatomical (segmental), rather than for histotypical characteristics. The segmental specifications for ectoderm and mesoderm and epidermis and nervous tissues, which reiteratively appear in all embryonic segments, independently of other specifications could take place by similar spatial restriction mechanisms and be controlled by other switch type genes. Lehmann *et al.* (1983) have discussed candidates possibly involved in the specification of the neurogenic pathway.

The analysis of homoeotic genes of *Drosophila* has revealed a logic similar to that governing the specification of lineages of *Caenorhabditis*. Genes, sometimes belonging to gene complexes, seem to specify developmental alternatives in clonal or polyclonal lineages. The alternatives they specify are anatomical rather than histotypical characteristics, such as segments, compartments or sublineages. Segregations specifying cell histotypes appear in both organisms independently of spatial segregations, as histotypical specifications. I have designated such specification genes 'selector' genes (García-Bellido 1975). They may specify topographic or histotypical pathways. Their differential activity along the rostrocaudal axis of the egg is possibly determined by other genes that I have designated 'activators'. The role of the activator genes would be to produce or transform external cues (maternal products, hormones, growth factors) into molecular signals specifically perceivable by the selector genes. We should include in this scheme the genetic system involved in the specification of the sexual pathway of differentiation dependent on the X chromosome:autosome ratio. Genes such as *Pc* and *lin-12* in homoeotic transformations, *lin-4* in heterochronic transformations, and *her-1* and *Sxl* active in sex differentiation, could be considered as activator genes. The corresponding selector genes would be the *BX-C*, *ANT-C*


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and *en*, some of the *vul* loci, the heterochronic locus *lin-14* and the sex differentiation *tra-1* and *dsx*. The different genetic systems can be activated independently and simultaneously, rather than sequentially, in the same cell, and remain active during subsequent development. The phenotypic effect is a summation of their functions defining the behaviour of the cell and its final differentiation (figure 6).

### (*b*) *Maintenance and implementation*

From the early stage of clonal segregation, or 'commitment', to the actual overt differentiation of the descendant cells several rounds of cell division usually intervene. The proliferating cells during this period are considered to be determined. Genetic data indicate that in *Drosophila* this determination is actual genetic differentiation and is reversible.

How is the information of selector genes implemented in developmental terms? The proliferation patterns of the imaginal disc cells is characteristic for the segment, anterior–posterior compartment and dorsal and ventral appendages from the blastoderm stage. The number of founder cells, clonal patterns and cell affinities are disc- and compartment-specific. In homoeotic mutants the segment of compartment specificity of these parameters are correspondingly changed (García-Bellido & Santamaria 1972; García-Bellido & Lewis 1976; Morata & García-Bellido 1976). Thus, the genetic specification resulting from the activity of selector genes is implemented early in embryogenesis in gene-specific cell behaviour properties and continues during the subsequent period of cell proliferation. In other words, in these cases determination is accompanied by 'proliferative differentiation' (García-Bellido & Ripoll 1978). The genes responsible for this proliferative or overt differentiation are probably subsets of genes under the specific control of the selector genes. I have designated them 'realizator' genes (García-Bellido 1975). Their existence is now supported by molecular identification of the products of the BX-C, ANT-C and *en* genes. A common domain of the protein products of these genes, the 'homeodomain', contain a basic aminoacid sequence, conserved in many taxa- with potentially high binding affinity to DNA (Laughton & Scott 1984). This homeo domain of the selector gene products could function in recognizing the realizator genes and controlling their activity.

We do not as yet know how many realizator genes are in the *Drosophila* genome nor their specific functions. They could be involved in the formation of appendages, cell proliferation patterns and other generic cell properties; their local modulation being responsible for the phenotypes that we observe in the final form. In view of the many mutants known in *Drosophila* that affect these parameters (see García-Bellido & Ripoll 1978) their interactive relationships with selector genes should be studied. This class of genes may include some of the mutants that affect lineage parameters in *Caenorhabditis*, mentioned above. Obvious candidates for realizator genes have been found in the sexual differentiation pathway, for example, the genes coding for yolk proteins in *Drosophila* females (Belote *et al.* 1985).

How is the state of determination maintained during cell proliferation? Mitotic recombination clones of cells homozygous for mutant selector alleles have shown cell-autonomous mutant phenotypes, for example, *Ubx* clones in metathorax show transformation to pro-mesothorax. This finding indicates (i) that the normal function of these genes is required in successive cell divisions to maintain the determined state and (ii) that the implementation state is reversible depending on the on:off state of the selector genes. In these experiments only the selector genotype has changed, and therefore the conclusion applies only to the genes hierarchically


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downstream, the realizator genes. Cell-autonomy of the transformation in clones of mutant regulator genes, *Pc*, and others indicates that it also applies to the off:on condition of selector genes (Struhl 1981; Capdevila & García-Bellido 1981; J. Botas and A. García-Bellido, unpublished results). A similar inference can be drawn from the effects of temperature shifts in *ts* alleles of homoeotic genes (Vogt 1946). Mitotic recombination (Wieschaus & Nöthiger 1982) and temperature shift experiments (Belote *et al.* 1985) with sex determination mutations also show a cell-autonomous reversibility of the differentiation state during the proliferation period. Thus, the maintenance of the activated–repressed state of selector genes is based on an open system of genetic *trans*-interactions.

The question of genetic reversibility of commitment is especially relevant for the understanding of lineages with mixed descent, as described in the leech and the nematode. In these organisms mesodermal derivatives may derive from a mostly ectodermal, or even nervous lineage and vice versa. We have found the same situation in imaginal discs derivatives of *Drosophila*. Thus, oenocytes and fat body cells, on the one hand, and sensory elements (chaetae and sensilla) on the other can differentiate within the same clones of epidermal cells, previously specified as ectoderm at the blastoderm stage. Clones of mixed descent can be explained, in genetic terms, by the existence of subsets of genes that can be independently and reversibly controlled with no memory of the earlier commitment of their ancestors.

Sensory elements appear in all segmental derivatives in segment-specific patterns, both in the larval and in the adult cuticle. They can be considered as members of a kinship group. They have a specific projection to the neuromers of the central nervous system. Mutants in the *achaete-scute* complex (AS-C) affect the differentiation of these elements in the same way in all segments (García-Bellido 1979). Recessive lack of function mutations remove sensory elements in specific places, but the deletion of AS-C causes the lack of these elements over the whole body (García-Bellido & Santamaria 1978) and extensive histolysis in the embryonic central nervous system (Jimenez & Campos-Ortega 1979). Dominant excess of function mutations in the AS-C cause the appearance of extra sensory elements. Other loci are known that determine the activity of the complex by negative regulation. Lack of function mutations in these loci cause the appearance of extra sensory elements, as do the *cis* derepression mutations in the AS-C complex (Moscoso del Prado & García-Bellido 1984*a*). Thus, similar to the homoeotic genes, these of the AS-C may be involved in the segregation of a neurogenic pathway as a sublineage of the ectoderm. But in contrast to these, and more in keeping with the situation in *Caenorhabditis*, these genes affect clonal rather than polyclonal decisions, and act in several homologous lineages. Cell-to-cell interaction, in the activation of the genes of the AS-C, seems to be involved, singling out epidermal cells to enter the neurogenic pathway in species specific patterns (García-Bellido 1981*b*; Moscoso del Prado & García-Bellido 1984*b*).

The preceding discussion uncovers common features in the genetic control of developmental pathways in *Caenorhabditis* and *Drosophila*. Segregation of topographic properties and histotypes that initiate lineages seem to depend on maternal determinants partitioned during cleavage and differentially registered by exclusively zygotic genes: the selector genes. At this stage the major decisions of development take place. These decisions are maintained thereafter by cell heredity in clones. The implementation of these decisions occurs during subsequent proliferation, possibly via specific sets of realizator genes under the control of selector genes. Later during proliferation new decisions are again taken within lineages: they correspond to the final overt differentiation of particular neurons, muscle cells, epidermal specializations, etc. This requires

the activity of new sets of selector genes. Genetic analysis has uncovered three main features relevant for evolutionary considerations: (i) selector genes act independently of each other, activated by specific activator genes and controlling the activity of (partly) different subsets of realizator genes; (ii) lineages are specified by combinations of simultaneously active different selector genes, defining summatorial cell phenotypes; and (iii) the activity of the selector genes is reversible during development: it can be advanced or retarded in a lineage, transferred from one lineage to another, and combined with that of other selector genes in various ways in different cells.

### (*c*) *Evolutionary considerations*

Considered from the resulting cell-type differentiation, differential patterns of cell division appear as the mechanism that segregates lineages leading to that cell-specific differentiation. Hence lineage trees of segregation are the classical paradigm of progressive histotypic specification or commitment as development proceeds. According to the classical view, development results from the deployment of a programme leading hierarchically from general to particular properties via segregation of differential cell types. This is the idea underlying the notion of germ layers of comparative embryologists. It has its ideological counterpart in the concept of progressive phyletic evolution from primitive to the derived forms. According to this classical view development and evolution progress mainly by addition of novel genetic functions which modulate primitive cell types and spatial organizations.

Considerations of detailed lineage descriptions, and of their experimental or genetic perturbations prompts us to reconsider these classical notions. I will comment briefly on some relevant questions. To what extent are lineages genetic devices for distributing cells in specific topographic arrangements, as distinct from their role in histotypic differentiation? Mutants in *Caenorhabditis* show modified division patterns, changes in polarity of segregation, cessation of divisions or expression of hidden lineages. Lineages can then be considered as stereotyped modes of organizing the morphological space, independently of their inventory of terminal differentiations. It is interesting in this context that in the leech different lineages give rise to the head and to the metameric segments in the trunk. This is the rule also in other annelids (polychaeta) and their phylogenetic derivatives, in which segmentation occurs by budding from the gnathocephalic region. Possibly even *Drosophila* retains this developmental scheme. It may not be just a coincidence that the cephalic furrow and the 'differentiation centre' correspond to the segments which are the phenotypic sink of mutations of the BX-C and ANT-C (see García-Bellido (1985) for further discussion). Similarly, even in frogs, early clonal segregation may separate cephalic from trunk and tail regions, before or independently of, histotypic segregations. Clearly in *Drosophila* lineage segregation for segments is a different operation from that segregating histotypes. Division patterns are different in different tissues. Moreover, clone shapes seem to be similar within the series of ventral appendages and different from those in dorsal ones suggesting serial homologies in cell division patterns.

The notion that topographical and histotypical segregations are different processes evokes the next question: to what become lineages specified? We have seen that pregastrula embryos already show cell or regional singularities, expressed in the localized distribution of antigens or gene products related to particular histotypical fates. However, it is difficult to imagine for which fate such blastomeres are specified, if they have a histotypically mixed lineage of descendants. Genetic data in *Drosophila* have shown unambiguously that cells in the blastoderm


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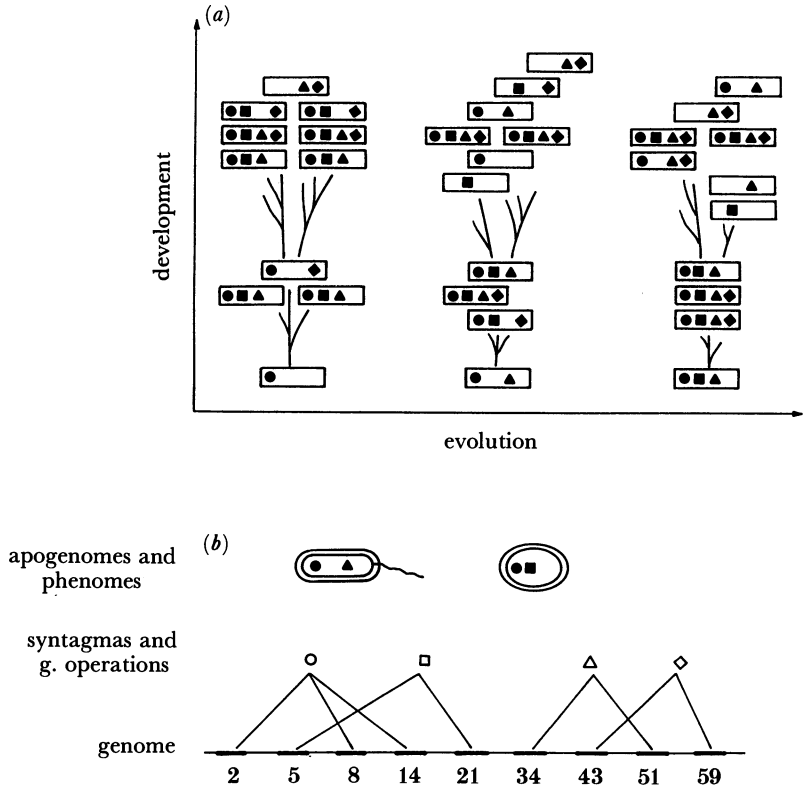


FIGURE 6. Syntagmas and apogenomes during development and evolution. (b) Numbers correspond to individual genes that participate in genetic operations or syntagmas (for example, realizator genes of a selector gene) determining specific molecular (for example, muscle actins), cell differentiation (for example, sensory neurons) and cell behaviour (for example, cell recognition) characteristics. The set of syntagmas operating in a given cell at a given time constitute the cell apogenome expressed in their phenome. (*a*) variations of apogenomes in cells of the same lineages in the development of phylogenetically related organisms. The number and the combination of active syntagmas (full symbols) varies in time and space between related organisms. (Discussion in text.)

become committed to topographical fates such as segment or compartment. The function of the genes responsible for these commitments is partly understood. They seem to control the activity of other genes which determine differential cell proliferation and other cell behaviour properties. Such properties do not directly correspond to histotypes in the classical sense, which appear later in development expressed in terminal differentiation in particular lineages. The assignment of these cells to their fates is again topographical rather than histotypically hierarchical, that is, the cells do not derive from histotypical lineages. The histotypically hierarchical segregation of germ layers to more specific subsets of tissues seems to be the exception rather than the rule.

The existence of histotypically mixed lineages invites another question: is commitment, as inferred from fixed lineages, an indication of irreversible genetic differentiation, that is, does it result from a cascade of sequential gene activations? Cells differentially committed at blastoderm by the differential activity of activator and selector genes, can change their proliferation parameters and differentiation pattern in clones following a change in the genetic constitution or genotype of either of both type of genes. This indicates not only that the commitment is maintained by the continuous activity of these genes, but also that commitment

is reversible, and dictated by the state of these genes during proliferation. This experimental intervention by genetic manipulation, has its parallels in regeneration, transdetermination and transdifferentiation. The totipotency shown by nuclei of differentiated cells in transplantation experiments further support the notion that genetic specification is reversible. If determination is, at the molecular level, genetic differentiation, and this is reversible, then the notion of early commitment to a particular fate loses its heuristic value. In frogs both the lineage restrictions that suggest commitment in advanced blastula cells (Jacobson 1983) and the invoked lack of commitment implied by the response to induction in classical transplantation experiments in gastrula of frogs become compatible. The apparent contradiction is resolved if a possible early commitment is genetically reversible under experimental conditions. Regulative behaviour, following experimental perturbation, is not an indication of a lack of commitment in the same way as the specific fate of a fixed lineage of ancestors is not an indication of irreversible commitment. This conclusion may not apply to certain cases of terminal differentiation, for example, in lymphocytes.

The reversibility of genetic commitment further indicates that although lineages are, by definition, hierarchical, specification does not have to proceed by a hierarchical deployment of decisions, as a cascade process. The additivity of double mutant combinations of selector genes suggests that different specifications are independent from each other and define independently, in the same cell, a combination of realizator gene activities, expressed in composite specific cell behaviour properties (figure 6). The independence of the different specification signals is, at least theoretically, easy to understand in molecular terms. The genes and gene products of the activator, selector and realizator genes are only connected by molecular recognition; they form an integrated unit of specification that I have called a 'syntagma' (García-Bellido 1981*a*, 1985). In it the information flux proceeds downwards from the activator to the realizator genes, that is, realizator genes can not affect the condition of selector genes. Neither can members of a given syntagma affect other syntagmas. In this model a particular cell in a lineage is specified, and behaves, according to the actual combinatorial superposition of operating syntagmas: its specific 'apogenomes' (see García-Bellido (1984, 1985) for further discussion). The independence of syntagmas permits the occurrence of different combinations in different cells, each having a different 'apogenetic code'. According to this notion a species can be described as an algorithm of apogenomes. And related species can be compared by their different apogenomes at work in similar lineage trees. A simile could be the musical notes placed in the five line staff describing different melodies. Evolution would proceed by changing the syntagmic composition of cells in lineages, generating new apogenomic combinations (figure 6).

I envisage the pattern of division of lineages and lineage specifications as resulting from two different operations mediated by different sets of genes. Lineages under the control of specific genes would contribute to the topographical or anatomical discontinuous distribution of cells in space, such as those segregating cephalic and trunk regions in trochophore larvae, micromeres in sea urchin blastula, germ line cells and segments. These construction modes would remain rather invariant within phyla, defining the primitive or archetypic forms. Certainly the complex maternal component involved in the determination of cleavage patterns would tend to maintain them constant. Cell specification would then follow by the activation of particular genes or syntagmas in particular lineages, either by segregation of cytoplasmic determinants, or by cell interactions. The independence and reversibility of the active state of syntagmas would allow


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for their assignment to new lineages or the removal from old ones (figure 6). Obviously, new assignments could change division patterns and lineage trees according to specific requirements: defining the derived caenogenetic forms under the adaptive conditions determined by natural selection. These secondary derivations can appear by different mechanisms. Reiteration of homologous lineages, similar to segments, could be locally modified to generate specialization and heterogeneity. Cell external determinants such as hormones, or inducers, can become internalized, that is, dependent on cell-autonomous molecular interactions and lead to cell autonomous segregations. Later appearing decisions could become more premature in development, leading to heterochronic changes. In this view stereotyped lineages would be more refractory to change than cell type assignments. Changes in space and time of cell specification, by independent syntagmas operating in constant lineages, may account for part of the observable variation and invariance of developmental processes in evolution.

I greatly appreciate the constructive criticism of G. Stent, I. Greenwald, G. Dover, R. Nöthiger and colleagues in the laboratory. The original work presented here was supported by grants from C.A.I.C.Y.T. and F.I.S.

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