Zeitschrift: Archives des sciences et compte rendu des séances de la Société
Herausgeber: Société de Physique et d'Histoire Naturelle de Genève
Band: 42 (1989)
Heft: 1: Archives des Sciences

Artikel: Polarity : from dipoles to biopolarization
Autor: Turian, Gilbert
Kapitel: VIII: Morphogenetic polarizations
DOI: https://doi.org/10.5169/seals-740080

VIII. MORPHOGENETIC POLARIZATIONS

As a third aspect of development, morphogenesis is related to the multicellular level of organization and involves cellular interactions as opposed to differentiative processes occurring primarily within individual cells. Strictly speaking, morphogenesis therefore refers to the development of the shape and specific form of the organism and its individual parts. As such, it is largely a problem of coordinated movements of cells toward their specific position in the organisms being constructed. On the framework of a pattern of genotypically and phenotypically-controlled polarities, these oriented displacements of cells lead to the organismic morphogenesis in the bewildering array of living forms. All such marvellous formative achievements require the concourse of polarity at all levels of integration.

A. Plants

Polar patterns are very frequent in plant morphogenesis especially in respect to the formation of polar cells (Wardlaw, 1952; Sinnott, 1960). Physical factors, i.e. internal and surface forces are implicated in the patterning of tissues if we consider with D'Arcy Thompson (1942) that division is a mean of restoring equilibrium within an enlarging cell. To explain the polar tissue patterns, the Warren Wilsons (1984) developed an hypothesis that integrates the surface effect and the homeogenetic induction effect in terms of opposed gradients of two morphogens — auxin and sucrose — which have *sources* and *sinks* sufficiently defined to permit a simple simulation of the processes of positional control.

In most cases, the polarity of a cell is established parallely to the axis of cell elongation, and cell division usually occurs in the plane perpendicular to that axis of polarity. The determination of the anchor points of the spindle apparatus is, of course, another important pattern-formation event within a dividing cell. A high activator concentration could initiate the self-assembly of the microtubules and so determine the plane of cell division, even in an initially more or less symmetric cell. Asymmetric cell divisions, which are fairly frequent in plant cells (VII.C), could be explained in this way since the new cell wall may occur closer to the activated site. Of course, a second center of microtubule self-assembly has to be created at the opposite side of the cell and both centers thus generate a *bi*polar field by mechanisms of long-range activation and short-range exclusion (Meinhardt, 1984; Burgess, 1985).

Prat (1948 and 1951), and then Bünning (1953), dealt with the origin of plant patterns in terms of stimulatory or inhibitory fields or gradients around structures

which either produced morphogens or depleted the surroundings of them (Carr, 1984). Prat discussed the origin of histogenetic pattern in terms of physico-chemical gradients (redox, pH, etc.) and considered it as a direct result of the gradient or field. Since, it has been recognized that there is a difference between gradient theories and "positional information", a term coined by Wolpert (1969, 1971, 1981) — although the idea had occurred a century ago to Vöchting and Driesch — to "imply the existence of unknown mechanisms which cause cells in an embryo, an organ or a small animal to respond to the position along a developmental axis or within an aggregate of cells" (Carr, 1984). Wolpert's definition of pattern formation is "the specification of spatial differences", a specification implying that such differences are genetically determined. In plants, however, most patterns are epigenetic in origin and this may have some consequences on the straight application to plants of Wolpert's concept (see below). As first conclusion, the difference between gradient theory and positional information theory is that, "while gradients are primarily held by the latter theory to elicit a cellular or morphological response, it is the position the cell or meristem occupies in the gradient that determines which of a number of courses of differentiation it will take" (Carr, 1984). To assign a "positional value" to each cell in the developmental field would involve the establishment of diffusional gradients of some morphogen scaled so that each cell in the matrix can be considered to reside at a unique intersection. Moreover, each cell should interprete its assigned positional value in terms of an appropriate developmental protocol. "The cell is assumed to possess some form of "reference library" in which it can turn to the appropriate page, specified by its positional value, and read what it must do to complete its part of the overall developmental process" (Lintilhac, 1984).

Nevertheless, as already commented, and following Lintilhac's opinion, the concept of developmental control by means of positional information to explain the events associated with amphibian limb regeneration (VIII.B.2f) cannot be applied directly to plant systems where developmental events occur at regular intervals along the growing axis. Indeed, the extraordinary sensitivity of plants to structural and mechanical stimuli is qualitatively quite different from the positional sensitivity of animal cells, and depends on the mechanical coupling provided by shared rigid cell walls (Lintilhac, 1984). Moreover, "the word "position" is ambiguous since neighbouring cells retain a constant relationship to each other while continually changing their location relative to the apical pole". Lintilhac also differed from Wolpert's view that the genome includes a "set of instructions" for the construction of the adult. Holder attempted, in 1979, to directly apply the positional information theory to plant development. However, Lintilhac considers that Holder's statements can also be misleading "to the extent that they can be taken to mean that an understanding of developmental events will come only by considering them as results of differential gene activity". He has therefore proposed the alternative theory of structural epigenesis, considering plants as architectonic structures and attempting to answer

the question of "how do plant cells sense and respond to mechanical forces?". Plant response to hormonal diffusion gradients (Sachs, 1978) was considered as the most acceptable model of "cell patterning and segmentation patterns in embryogenesis" (Wardlaw, 1952) discussed as a basis for structural epigenesis in ontogeny.

1. *Embryonic polarity*

In thallophytes, bryophytes, pteridophytes, and seed plants, the earliest perceptible embryogenic development is the establishment of polarity. Axial or spindle-like development is found in the embryogeny of all these classes of plants (Bower, 1922). During plant embryogeny the embryo proper becomes polar, establishing the root-shoot axis, and thereby forms two very different types of apical meristems. "How is the root-shoot axis established and what are the developmental parameters involved in organizing the apical meristem?" The only information obtained has come from studies of *de novo* meristem organization in culture (McDaniel, 1984*a*).

At the earliest manifestation of growth in the spore or zygote, there is evidence of polarity and of filamentous or axial development; in all but the very simplest organisms, there is, from the onset, a distinction of apex and base (distal and proximal regions or poles). In short, both the sporeling and the very young embryo afford evidence of orderly or organized development.

In naked, fertilized or unfertilized ova, such as those of *Fucus,* there is apparently no specialized cytoplasmic organization which determines the polarity of the young embryo (see VII.C.3a) and the polar axis may be established in any direction. In another brown alga, *Laminaria,* the extruded but still attached ovum gives rise to an embryo whose axis coincides with that of the oogonium. In that case, as in other developing sporophytes, some of the orderly changes that take place during the ontogenic development may already have been determined by the cytoplasmic organization of the ovum.

Several facts suggest that polarity is not determined by structural features of the archegonium itself. In the encapsulated embryo in bryophytes and vascular plants, the establishment of polarity brings the embryo into a particular position in relation to the gametophyte plant or gametophytic tissue. As this early establishment of a polarized axis has many consequences in the subsequent embryogenic development, it is evident how essential it is to have some knowledge of the factors which may bring it about.

The polarity of the enlarging embryo is determined by inherent factors, or by factors in the environment and the first partition wall is typically laid down at right-angles to the axis of the embryo. In some embryos several successive transverse divisions take place and an elongated filament results: in others, divisions at right-angles to the first wall take place, and in this we see the inception of a tissue mass. This is what occurs in many bryophytes, in *Equisetum* and some other pteridophytes

(Burgess, 1985). In *Anthoceros,* however, the ovoid zygote is divided by a longitudinal wall (Wardlaw, 1955).

The classic species for study of development of higher plant embryo has been the shepherd's purse, *Capsella bursa-pastoris.* One of its most striking characteristics is the polarity of the mature or newly fertilized ovum, in which metabolites are, or quickly become, heterogeneously distributed. Where the ovum is enclosed, the physiological activity of the surrounding tissue is probably important in determining polarity. The basal two thirds of the egg contain a single large vacuole, while the cytoplasm and the nucleus are in the apical (i.e. chalazal) third of the cell. This polarity somewhat parallels that observed in yolky animal eggs, where the nucleus is restricted to the animal hemisphere of the egg. In the polarized zygote, the apical or distal pole becomes the principle locus of protein synthesis, growth and morphogenesis; whereas the basal or proximal pole is characterized by the accumulation of osmotically active substances, its cells become vacuolated and distended.

The zygote may be more or less equally divided, or it may be divided into a small, densely protoplasmic distal cell and a larger basal cell. This first division of the zygote is asymmetric and expresses the polarity which will remain throughout the life of the plant. The two daughter cells of the first division are different in size and developmental potential. The larger basal cell will produce the suspensor, whilst the smaller terminal cell will produce the embryo proper. In short, the initial asymmetric division of the zygote defines the polarity which persists throughout the development of the embryo. Consequently, the mature embryo is a highly polar structure with a differentiated apex, a procambium which will form the vascular system, and a protoderm which will form the epidermic layer. Subsequent development of the plant rests on the activities of the meristematic tissues. The main root-shoot axis of the embryo is located centrally within the embryo, with the shoot tip between the two cotyledons. At maturity, the root tip has a differentiated cap.

2. *Organismic polarities*

a) *Mushrooms*

Polarity of the sporophores (fruit bodies) of higher fungi (Basidiomycetes) has been discovered by Magnus in 1906 when he described the much more vigorous hyphal regeneration of the cortical hyphae on the pileus side than on the foot side of a bisected stipe of *Agaricus bisporus* or of *Coprinus stercorarius.* Similar experiments were also successfully carried out by Weir (1911) in *Coprinus* and Polyporaceae. Polarity is thus evident but it is not fixed as shown by Lohwag (1941) who could graft a segment of the pileus of *Fomes* back to the same pileus in an inverted position. Developmental polarity of the fruit body cap in the genus *Coprinus* has been further studied by Reijnders (1979) who depicted polarity of differentiation where development in some species was described as a rupthymenial (gills differentiation away from the stipe) while others were levhymenial (gills differentiating toward the stipe).

The most highly organized structures of the Basidiomycetes are the fruit bodies, but the vegetative mycelium is also capable of elaborating a range of other organs among which the sclerotia (Watkinson, 1979). Whereas the sclerotium develops a radial symmetry, the fruit body developmental sequence imposes a polarizing influence on the aggregate, and from a very early stage the shape of the developing mushroom is clearly evident (Moore, 1984).

Initiation of fruit body of *Flammulina (Collybia) velutipes* is first indicated by hyphal aggregation in a much-branched region of the dikaryotic mycelium (Williams *et al.*, 1985). Further extension and elongation resulted in a primordium with the typical form of a mushroom in miniature, which showed the basic apico-basal organization of tissues into a stipe, pileus and developing hymenium. Further expansion of the stipes of *C. cinereus* or *A. bisporus* occurs by a uniform incorporation of cell wall material along the length of the growing cells. This intercalary elongation contrasts with the polarized tip growth of mycelial hyphae (Gooday, 1983; Manachère *et al.*, 1983). The development of the intensively investigated fruit body of *Schizophyllum commune* (Aphyllophorales) is not at all like that in Agaricales. The inside of the tiny cup produced is lined with hymenium producing basidiospores. The infoldings of the margin of the fan-shaped fruit-body produce the typical split gills which often seem to radiate out from one point when growth is unilateral. The normal enlargement of the cap is thus multipolar and the mutant *cup* is restricted in hymenial expansion (Wessels *et al.*, 1985).

Physical environmental variables can interact with the polar development of mushrooms as well examplified by the stipitate *Polyporus brumalis* in which the effect of light (Fig. 28 B) and apical transpiration loss are additive (Plunkett, 1961). There are also interacting effects of light and temperature, which have been studied in *C. congregatus* by Manachère *et al.* (1983). In many *Coprinus* species, light can act as an inducer/stimulator and as an inhibitor.

Development of such a highly organized structure as the whole mushroom necessarily implies polarized regulation. Both Borriss (1934) and Gruen (1979, 1982) concluded from their work with *Agaricus* and *Coprinus,* respectively, that the gill lamellae were the origin of controlling factors for stipe elongation and cap expansion. Growth factors produced by the fruit-body cap were shown to influence, perhaps control, growth of the stipe of these mushrooms as experimentally evidenced by total removal of the cap which leads to cessation of stipe growth. When segments of cap were left attached to the stipe, the latter showed a growth curvature with the greatest extension growth being immediately beneath the remaining sector of cap. The gill lamellae could thus be considered as the origin of controlling factors for stipe elongation (Fig. 28 A) and cap expansion (Gooday, 1974).

The cap displays the greatest morphological, and consequently morphogenetic, complexity. By the time it has matured, three morphologically distinct cell types have been produced, for at maturity the hymenium consists of a pavement of highly

inflated paraphyses within which are embedded a great many basidia and a scattering of cystidia.

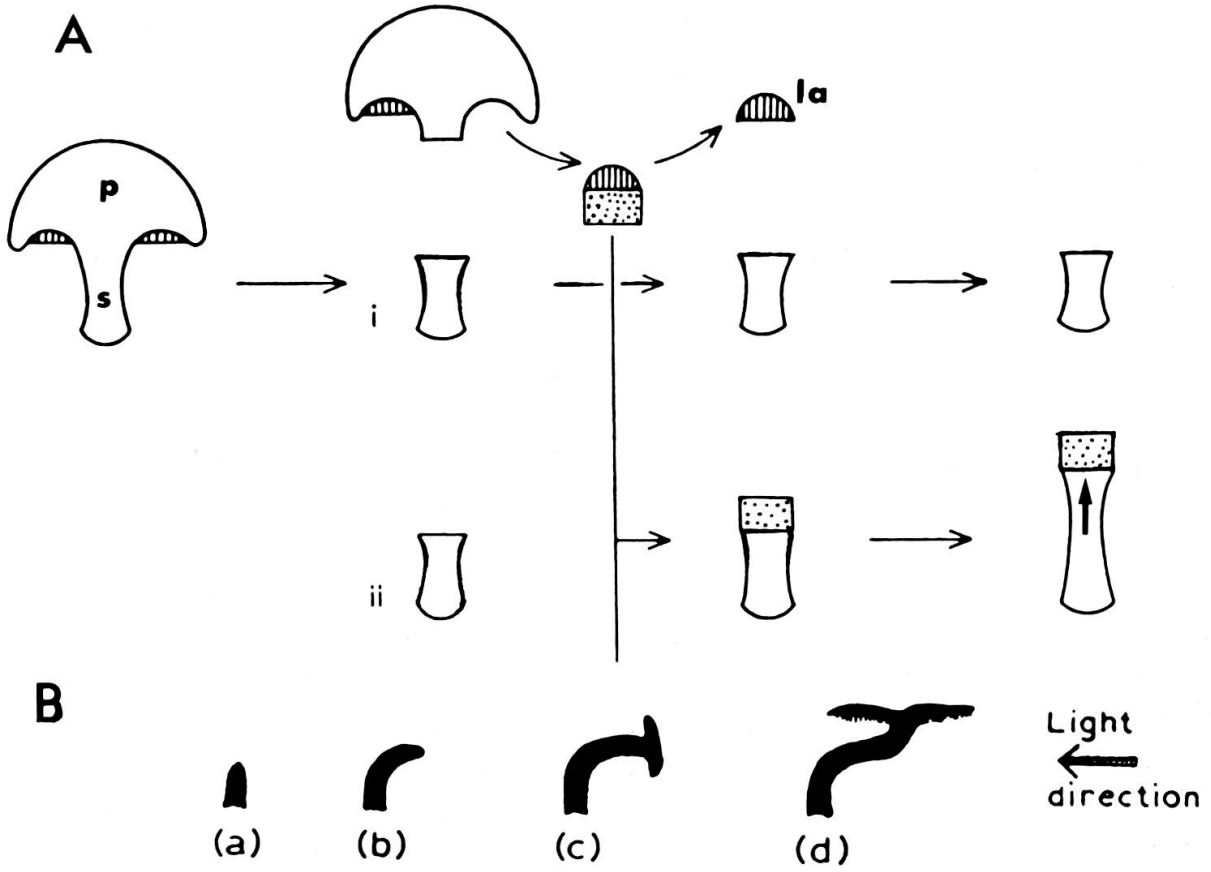

FIG. 28.

Developmental polarity in higher fungi.

(A) Hormonal stimulation of monopolar elongation growth of the basidiocarp of *Agaricus* as demonstrated with lamellae (la) substances transferred into an agar block. i = control; ii = treated elongating (arrow) stipe(s) fragment. From Gruen, 1963, in J. E. Smith and D. R. Berry, 1974, An Introduction to Biochemistry of Fungal Development. Academic Press.

(B) Response of basidiocarp of *Polyporus brumalis* to unilateral illumination: successive curvatures toward the light source (a-c) until the stipe apex is shadded by the expanding pileus when the stipe curves upward (d). After Plunkett, 1961, in Burnett, 1976.

The developmental pattern of a typical mushroom occurs along *two* axes of polarity: from base to apex, and from the inner to the outer surface. The specification of information along these two axes of polarity results in the formation of a palisade layer and thus precipitates the establishment of basic tissue domains. According to Rosin *et al.* (1985) "the concepts of positional information and polarity axes are capable of providing a basic understanding as to how the gill pattern develops, the specification of positional information being determined by the two axes of polarity". These authors then presumed that hyphal cells interprete this positional

information and differentiate into palisades which are the fundamental building blocks of the hymenium and differentiate initially into basidia and cystidia. They conclude that the differentiating agaric gill seems to be an ideal candidate for interpretation along the lines of a diffusion gradient theory as proposed by Meinhardt and Gierer (1974). These authors had illustrated "how two-dimensional patterns very similar to those observed in the agaric hymenium may be generated in response to activators and inhibitors capable of diffusing through the tissues». Rosin *et al.* (1985) further emphasized that "the gill *does* have two developmental axes to which differentiation might be referred, and the hymenial cells do become positionally differentiated in a manner apparently analogous to epidermal cell layers" (see VII.C.5b).

According to Moore (1984), "the development of these structures could provide a classic example of a morphogenetic field, the more so since the gill plate grows in two directions (inwards, towards the stipe, and along the length of the cap as the cap increases in diameter) which are essentially at right angles and which could contribute to a co-ordinating system to which cell differentiation could be referenced". Models of morphogenetic processes based on distribution of morphogens (Meinhardt and Gierer, 1974) are very dependent on adequate communications within the tissue, communications which must extend over many cell diameters. However, the developing basidiomycete hymenium is an array of separate cells in which there is no evidence for any lateral cytoplasmic contact between neighbouring cells. Recently, models involving gaseous or volatile morphogens have been proposed and founded on known metabolic events (Moore, 1981).

Pertinent as conclusions about the polar features of construction of the spore-bearing structures of mushrooms are Burnett's (1976) remarks that "basically, despite the fact that morphogenetic processes appear to transcend the limits of hyphae and apparently affect, in large structures at least, entire hyphal aggregation, their origins lie in the fact of hyphal behaviour. Growth, branching and aggregation are the fundamental processes, together with the control of direction of growth." The key to fungal morphogenesis is thus well at the monopolarly extending hyphal tip (see VI.A.2b).

b) *Colonial algae*

The green algal family of the *Volvocaceae* includes all motile colonial genera in which the cells lie in a disk or a hollow sphere. Vegetative cells are always biflagellate, surrounded by a gelatinous sheath, with a structure like that of *Chlamydomonas*. All colonies are called coenobia which exhibit a definite polarity when swimming through the water, the anterior pole of the colony always being directed forward. There is also a definite morphological anterior-posterior differentiation in size of the carotene-containing eyespots (stigma) at opposite poles of the

coenobium. The single eyespot lies at the anterior pole of a cell. Certain species have not only a progressive diminution in size of eyespots from anterior to posterior cells but eyespots may even be lacking in the lowermost tier of cells. In the most advanced genera of the series progressing from the 8-celled stage (*Gonium*), through the 32-64-celled stage (*Pandorina-Eudorina*) to the more than 1000 cells (*Volvox*), all cells toward the anterior end are vegetative and reproductive cells (gonidia) lie toward the posterior pole (Smith, 1955).

The morphogenetic inversion in *Volvox* is a gene controlled process by which algal embryos turn inside out (Sumper, 1984). By the end of cleavage, the polarity of cells in the hollow spherical embryos is the reverse of the adult orientation: the presumptive flagellar ends of the somatic cells point toward the interior of the cavity and their chloroplast ends are on the exterior surface. The major event of inversion, generation of negative curvature, "requires both microtubules-driven elongation of cells (to produce a classical "flask" shape) and cytochalasin-sensitive active migration of cytoplasmic bridges to the outermost ends of flask cells" (Viamontes *et al.*, 1979).

In the Chlorococcales, cells may be solitary or be united in non-filamentous colonies with the definite number of normally 4 cells in *Scenedesmaceae*. As a result of asymmetrical divisions, the two extreme cells bear each two teeth or spines. These algae are polymorphic and the number of cells in daughter colonies is partially dependent upon the physiological conditions of the parent cells (Trainor *et al.*, 1976).

c) *Green plants*

A vascular plant is an axially bipolar structure, since there are different apices at the opposite shoot and root tips (Sachs, 1981). This division of the plant body into shoot and root is one of the most striking aspects of plant morphogenesis and one which, in higher plants, is normally maintained with great stability throughout their life. The division of the plant body originates in the embryo with the establishment of polarity and the initiation of shoot and root apical meristems, and once established the polarity of the plant body is extremely stable and difficult, if not impossible, to reverse.

Vöchting's (1878) original evidence that the plant axis is bipolar was based on shoot-root inversion of plant axis. His finding (1892) of abnormal development of the vascular system in the inverted plant has recently led Sachs (1981) to question whether this effect of polarity on vascular differentiation is dependent on auxin and its polar transport (see c^4). Furthermore, polarity at a cellular level can also be seen microscopically in the arrangement of specialized cells in vascular strands that connect the shoots with the roots (Sachs, 1986). This observation also raised the other question of whether auxin's transport and the structural expression of polarity are related. Experiments led by Sachs answer it by showing that, when applied locally,

auxin could orient the differentiation of the new tissues and could also replace the effect of organs such as expanding leaves. Additional observations of regenerative wounded plants, further suggested to Sachs (1986) that "auxin movement and tissue polarity are linked by a positive feedback relation: the movement of auxin polarizes the tissue and this polarization orients and enhances auxin movement".

c[1] *Roots*

Moving back from the capped tip along the root axis there are three zones: the meristem zone where cells both divide and elongate; the elongation zone where cells elongate only; the mature zone where elongation ceases. The same zones are also shown by the root cap but with a proximal meristem and distal mature cells. The meristem system would be generated (e.g. in the embryo) and regulated according to such a scheme implicating the asymmetric distribution of diffusible molecules. Such asymmetry is required to account for the frequently observed asymmetric cell divisions (Burgess, 1985).

Roots as whole plants "could contain gradients of morphogens because the cells are literally awash with solutes flowing, usually in a *polarized* direction through and around them". A general proposal is that "in the growing root apex chemical signals direct the course of cellular development and that these signals vary in a quantitative and qualitative manner, according to position" (Barlow, 1984). Such signals exist as gradients, as first proposed in animal systems by Child (1941), and "it is the gradient coupled with the cell's capacity to respond, which is informational" (Barlow, 1984). Various biochemical gradients have been shown to concour to the elongation growth of roots (Cook, 1959; Pilet, 1961; Grison and Pilet, 1978).

Extension along the root is caused by turgor pressure (driving force) within the protoplast and extensibility of the wall enhanced by the acid pH detected in the zone of elongation. Auxin (IAA) is transported for the most part acropetally to this zone (Pilet *et al.*, 1979) where it may promote the extrusion of protons into the cell walls and hence render them more extensible (Rayle and Cleland, 1977). However, the excretion of H ions might not be necessarily correlated with auxin response. The direct target of the hormone action would rather be the wall matrix synthesis, thereby leading to increased epidermal extensibility (Kutschera and Briggs, 1987).

That the root had an electrical polarity was first suggested by Lund and Kenyon's (1927) pre-vibrating probe studies of surface potentials. Schrank (1945) later suggested that root-generated potential differences might affect the distribution of growth regulators. The voltage and/or specific ion gradients that result from ion current generation have then been implicated in several hypotheses of developmental controls (Jaffe, 1986). The electrical polarity of the roots just reported by Miller and Gow (1989) resembles Lund's pioneer data: current consistently enters the meristematic and elongating tissues of intact growing roots; mature non-growing root

regions are responsible for generating the outward limb of the current loop. As in several tip growing eukaryotic systems current again tends to be concentrated at the leak (i.e. the meristic and elongating tissues) rather than the pump (i.e. the non-growing mature root). Also, localized growth occurs at the sites of the leak (Jaffe, 1986). However, "the correlation demonstrated between a pattern of polarising current and root development suggests, but does not prove, a direct causal relationship between the two events " (Miller and Gow, 1989).

c^2 *Vegetative shoots*

The shoot apex is considerably more complex to describe than the root apex. This complexity arises from the wide variety of structures from which the shoot apical meristem may originate. In contrast to the root, the shoot apical meristem is superficial, and branching proceeds from the tip. The most obvious products of the growth of the shoot apical meristem are the leaves and stem of the plant. Angiosperm apical meristems are stable: there are apparently no reports of a shoot meristem transforming itself into a root meristem (Halperin, 1978).

Apical meristems in lower plants exhibit more plasticity than those of angiosperms: at the base of each leaf in *Selaginella* there are a dorsal meristem which normally forms a shoot and a ventral meristem which normally forms a root, a normal rule which can be disturbed by auxin (Wochok and Sussex, 1976; Fig. 5 in McDaniel, 1984*a*).

The phenomenon of the priority of apical over lateral growth is called *apical dominance.* One of its aspects — the correlative inhibition of lateral buds by the apical region of the shoot — is a classical example of hormonally integrated growth in plant, principally brought about by indole-3-acetic acid (IAA). In coleoptiles and roots of *Zea* the polarly transported moiety appears to be pure IAA. Because of the gradual nature of apical dominance, it is reasonable to suppose that it is controlled by gradients in the stem. The correlative signal(s) in this phenomenon and the characterizations of the exact path of transport, rate of movement and primary site of action are presently conjectural (Hillman, 1985).

Vascular differentiation as readily occurring around wounds involved the formation of strands whose polarity is at all possible angles to the original axis of the unwounded tissues (Sachs, 1984). To what extend is the reorientation of vascular differentiation influenced by the original tissue polarity axis? To answer this question, Gersani (1987) compared new vascular differentiation along the original lines of polarity in isolated tissues and at various angles to this polarity. Differentiation of vessels was found along different tissue polarities and the rate of differentiation as a function of time and IAA concentration was the fastest in the original direction of polarity.

Positional controls of shoot organogenesis involve a three-step sequence: (a) abrupt shift in polarity, (b) smoothing, and (c) bulge. The ensuing mechanism of

phyllogenesis and phyllotaxis would imply the early development of one leaf bringing on the polarity discontinuity required for the production of the next leaf. The first signs of leave initiation are periclinal divisions in a small group of cells located in one or more subsurface layers of the shoot apex. This initiation has been viewed as the consequence of preferential enlargement of cells in the direction of future outgrowth from the parental tissue. This hypothesis of polarized cell enlargement as crucial early event in plant organ initiation has been supported by experiments showing that both leaves and roots could be initiated under conditions in which concomitant cell divisions had been inhibited (Foard *et al.*, 1965; Foard, 1971).

The formation of lateral buds in leaf axils proceeds to various extents in different plants. Lateral buds may be easily visible, or they may be very much reduced. They all have in common the fact that, at some stage, their development will be arrested and will not proceed until the growth of the stem results in displacement of the bud well away from the apex, or until the apex is itself removed or physically damaged. Thus, in a typical plant stem there is a gradient not only in the age of leaves with the youngest at the top nearest the growing point, but also in the development of lateral branches. The general form of the vascular system of plant is that of a drainage system; thus a polarity can be readily assigned to all major strains. The leaves of many ferns and most seed plants, however, are supplied by complex vascular networks that can not be understood as an expression of a polar flow (Sachs, 1984). Only their abscission appears to involve a polarized Ca^{2+} regulation (Trewavas *et al.*, 1984).

c^3 *Shoot-root balance and reversal*

The shoot and root meristems may be generated and regulated according to the scheme of long-range activation and short-range exclusion. According to Meinhardt (1984), bipolar fields are generated by these mechanisms of long-range activation and short-range exclusion.

The formation of specific organs can be due to specific substances such as hormones but it may also be affected by concentration gradients of various kinds and "hence by polar (physical) as well as chemical relationship" (White, 1934). However, polar movements of growth-promoting substances such as indole-acetic acid (IAA) were detected, only high concentrations of IAA being transported acropetally (Went and White, 1939). By assuming the existence of such a polarized circulation of growth factors, Gautheret (1944) also derived interesting ideas as to how undifferentiated tissue, growing in culture, becomes organized into an axiate structure with an apical bud, leaves and roots. However, the primary cause of polarity remains still unknown, and in Gautheret's view should be sought, not at the tissue level of development, but at the cellular level.

The suggestion of Czaja (1935) that polarity is due to the movement of auxins and not the cause of it, has not met with general acceptance. The evidence rather indicates that the polar transmission of stimuli is due to the polar transport of auxins. According to Went and Thimann (1937), polarity in auxin transport is probably determined by some inherent property of the living cells: it is therefore difficult to influence it by changing the external environment. The action of growth-regulating substances, or their precursors, is closely bound up with such polarized movement in the plant.

Hormones, like other organic substances, move most rapidly in the phloem and can also be transported through parenchyma and sometimes in the xylem sap. For phloem transport of auxin and other hormones there is no evidence that direction depends on factors other than source and sink locations of the hormones and the sugars with which they are transported. For the specific transport of auxin outside the sieve tubes, the following main types of evidence point to a determined tissue polarity (Goldsmith, 1969, 1977): (a) The direction of transport for any type of tissue is fixed, independently of the presence of growing apices or any other metabolic center. Thus transport towards the roots continues not only in their absence but also when their sink activity is prevented by cold. (b) The polar effects of auxin are clearly expressed in morphogenetic events and their existence at a tissue level can be seen clearly in grafts where one of the two members was inverted. (c) Polarity is maintained even when both the source and the sink are agar blocks, whose location can readily be changed. Finally, (d) polar auxin transport (see VIII.A.2c[4]) continues against an overall concentration gradient, as shown both by measurements (van der Weij, 1934) and differentiation events, though this does not mean that such gradients have no effect on transport.

"A vascular plant can be viewed as a branched axis that grows at its tips" (Sachs, 1984). As first shown by Vöchting (1884) the very same parts of this plant axis are able to develop different apices depending on their location relative to the rest of cutting.

Although axial polarity is normally constant and irreversible (Fig. 29 A, D), in a small number of root and shoot cuttings it may be reversed by simply turning the cutting upside. Reversal seems to be easier to accomplish in seedlings than older plants (Castan, 1940). "Inversion of polarity" was obtained with etiolated pea seedling with epicotyl decapitated, inverted, and placed in water. Roots now grow out from the epicotyl and a shoot from a cotyledonary bud (Fig. 29 B).

Oppositely, the retention of polarity found to be expressed within a cutting of a stem segment of ivy could reflect gradients of hormone concentration (Castan, 1940). According to Burgess (1985) "it would be a reasonable hypothesis that the transport of auxin from the tip of a shoot to its base would give rise to an accumulation of this substance at a cut basal end, and that this would then trigger root formation. Equally, its transport away from a cut apical end would result in the formation of shoots due to a switch in the relative levels of auxin to cytokinin. Taking this view

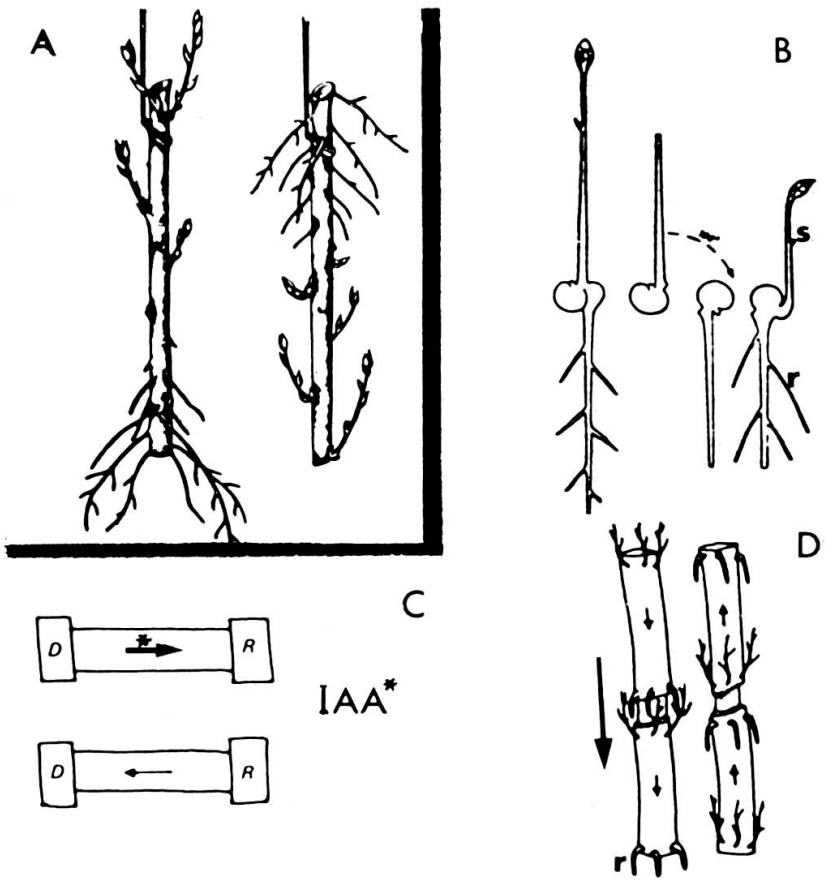

FIG. 29.

Polarity in green plants.

(A) In willow shoots: left, portion of a stem suspended in moist air in its normal root and shoot position; right, a stem similarly grown except in an inverted position. After Pfeffer, 1871, adapted from Sinnott, 1960.

(B) Reversal of polarity in etiolated pea seedling with epicotyl decapitated, inverted, and grown in water; roots (r) now emerge from the epicotyl and shoot(s) from a cotyledonary bud. After Castan, 1940, adapted from Sinnott, 1960.

(C) Polar transport of auxin from a donor agar bloc (D) to a receiver (R). Hormone (IAA*) movement occurs in the same direction as the tissue's polarity (starry arrow). From Sachs, 1984.

(D) Polar regeneration of girdled stem cuttings. The original direction to the root (r) marked by arrows; this polarity can be independent of gravity and the location of cuts and girdles. From Sachs, 1984.

it comes as no surprise to discover that inversion of a cutting in a gravitational field for example does not lead to inversion of the polarity of regeneration''. It can thus be concluded from the ivy experiment that polarity had been remembered and not reversed during the years of growth in the inverted position, despite the fact that presumably during that time the segment of the leg had functioned in a competent manner. This experiment, at first sight highly mysterious in its outcome, in fact confirms the notion of polar gradients of hormones as we shall see below (c^4).

c^4 *Polar auxin transport and tropic curvatures*

Polar transport (Fig. 29 C)

In higher plants, the transport of the auxin is basipetally polar as first proven, in 1932, by van der Weij. The model proposed for this hormonal transport requires that auxin moves from cell to cell by transport across the plasma membrane and the intervening of cell wall rather than primarily through plasmadesmata.

The plant growth substance IAA must be transported basipetally from its sites of synthesis in shoot apices and young leaves to the subapical target tissues in which it exerts its many developmental effects. The current hypothesis describing the mechanism of this polar auxin transport is the "chemiosmotic hypothesis" proposed in 1974 by Rubery and Sheldrake. It includes: H^+ gradient-driven cytoplasmic auxin accumulation by diffusion of lipophilic undissociated IAA molecules and by carrier-mediated cotransport of IAA anions and H^+ ions; transmembrane efflux of IAA anions on a carrier preferentially localized at the basal end of cells in the transport pathway (Rubery, 1980-81). The polar efflux step can be blocked by a group of synthetic compounds examplified by naphtylphthalamic acid (NPA) which bind to a plasma membrane protein receptor. Quite recently, a group of flavonoids (quercetin, apigenin, etc.) have been shown to act as endogenous ligands to the NPA receptor and thereby to perturb polar auxin transport (Jacobs and Rubery, 1988).

In the chemiosmotic hypothesis of this auxin transport, the energy stored in the electrochemical gradient could be applied to polar transport if a carrier protein for auxin anions were preferentially located at the basal ends of cells in transport pathway. The structural basis of cell polarity would thus be an enhanced basal permeability to auxin anions brought about by asymmetrical distribution of carriers. In her review of this model, Goldsmith (1977) termed it the chemiosmotic polar diffusion hypothesis, predicting both quantitative and qualitative features of polar auxin transport in the context of the mathematics and diffusion processes.

In summary, active and polar auxin transport is performed by the cooperation of two specific molecular transmembrane processes at the plasmalemma: an accumulation of IAA into the cytoplasm is driven by a pH gradient and by the electrical membrane potential and an efflux of IAA through polarly distributed exit carriers. In vesicle systems IAA accumulation was also shown to be dependent on pH gradient even though rough estimates of IAA accumulation into vesicles yielded ratios higher that the H^+ gradients (Hertel *et al.*, 1983). However with some modifications, the chemiosmotic theory remains valid, and auxin accumulation but not transport — with efflux limiting — can therefore be considered as electrogenic.

A proton motive force has also been shown to exist between the cytoplasm (pH around neutrality, negative potential) and the cell wall (acidic pH, positive electrical potential). This finding has led its authors (Rayle and Cleland, 1977) to explain auxin action as implicating a stimulation of proton excretion and a subsequent acid-induced

wall loosening. A clear correlation between acidification and growth is however still lacking (see c^1).

Tropic curvatures

Graviperception in roots requires specialized cells, the statocytes endowed with a structural polarity which is a prerequisite for their function. These specialized cells are localized in the root cap and their polar organization is based on a proximal nucleus and distal ER cisternae. Statoliths — mostly containing amyloplasts — serve for the perception of the gravity stimulus.

In higher plants, polar structures in the parietal cytoplasm serve as stimulus transducers. The polarity of the statocytes could not be affected by fast rotating (55 rpm) clinostat — microgravity — treatment and would thus be determined genetically. However, in roots centrifuged (1000 g) in apical direction, stratification of the statocytes was produced but their graviresponse was delayed. This coincidence of restitution of polarity with the delay of graviresponse suggested that a contact between amyloplasts and the distal ER complex is a necessary precondition for graviperception (Sievers and Hensel, 1982). In growing roots tilted at 45° from the vertical direction, the preceding symmetric patterns become asymmetric: the ER complex is differentially compressed by the sedimenting amyloplasts, provoking a potential depolarization (DV_d) in the physically lower and hyperpolarization (DV_h) in the upper statocytes. These changes generate a net asymmetrical current pattern across the root cap, the site of graviperception. Eventually, the cellular asymmetry is transduced to the asymmetry of growth. Parallely, the change from symmetrical to asymmetrical pattern of current flow of positive ions (probably H^+) preceded the graviresponse, suggesting a connection between current flow and the transduction of information from the root cap to the elongation zone. According to Behrens *et al.* (1982), the connection between perception of the gravity signal and its translocation into physiologically effective information appears to be insured by alterations of the membrane potential of statocytes as probable consequence of a local ion gradient generated by the ER and transmitted to the plasmalemma (analogy with photosensor in retina cells).

When exposed to light, primary roots of a few plants respond by a downward curvature or positive gravitropism; this is because light-induced growth inhibitors accumulate in the lower part of their horizontally-oriented roots. Abscisic acid has been strongly implicated as one of the growth inhibitors in the georeaction (Pilet, 1977). It has further been shown that this downward curvature of horizontally-positioned roots may be the consequence of a redistribution of endogenous hormones such as auxin and abscisic acid inducing a differential growth between the upper and lower parts of the roots (Pilet, 1985). Plasmalemmal binding sites could recognize the informational signal of the hormones and control their polar transport from cell

to cell (Kende and Gardner, 1976). Changes occurring in elongating cells from the two sides of gravireacting roots have been investigated using protoplasts prepared from the extension zone of maize roots (Senn and Pilet, 1980). The zeta potential directly depends on the surface charges (ionization of surface groups of proteins, polysaccharides, etc.) of the plasmalemma and should thus reflect its characteristic properties. Increase in the negative potential was found to be higher for plasmalemma from the "higher" located cells than that from the "lower" located cells of gravistimulated roots (Pilet, 1985).

Gravitropic or phototropic curvatures of shoots have also tentatively been explained by the lateral movement of auxin which leaving one side should speed up growth on the other side. However, rather than moving to produce differential growth, as predicted by the so-called Cholodny-Went model, auxin could be differentially released, by an homeostatic reaction, from its conjugated forms on the upper and lower sides of a horizontal shoot (Bandurski, 1980). As working hypothesis for the geotropically-induced IAA asymmetry, Bandurski *et al.* (1986) proposed that the deformation of the cell's bioelectric field provoked by gravity-positioned charged organelles results in "transient potential changes across the plasmodesmata such that these structures open, and or close, resulting in an altered movement of IAA from the vascular stele into the surrounding cortical cells. If plasmodesmatal gating also results in heightened ion gradients from one side of the tissue to the other, a device would also be at hand for amplifying the original weak signal generated by cutting the cell's bioelectric field".

Non hormonal models of differential growth have also been proposed such as that of a proton gradient. This gradient and an influx of K^+ ions leading to osmotic changes have been implicated as a possible cause of gravitropism in sunflower hypocotyls (Mulkey *et al.*, 1981). The zone of H^+ efflux and therefore of auxin-induced increase in extensibility of the longitudinal walls is stimulated on the upper surface, that of positive gravitropic curvature, of horizontally-placed maize roots (Mulkey and Evans, 1981). It is thus possible that the H^+ efflux asymmetry from the elongation zone, associated with the gravitropic reaction, is caused by auxin redistribution. However, the evidence for a correspondance of pH and growth rate changes during gravitropically-induced curvature is not yet convincing (Digby *et al.*, 1982).

Asymmetrical distribution of Ca^{2+} ions has also been implicated in the process as suggested by the reversible loss of gravitropic sensitivity in maize roots after tip application of calcium chelators (Lee *et al.*, 1983; Evans *et al.*, 1986). Further work should therefore be devoted to the calcium (calmodulin)-enhanced movement of auxin to the lower side of the elongating zone to understand the remaining steps of the gravitropic response.

A family of auxin-regulated RNAs from soya-bean has been found to begin to accumulate within 2.5 min after application of auxin. According to a recent report

by MacClure and Guilfoyle (1989), gravistimulation rapidly alters the distribution of these RNAs in responsive organs and the presence of the RNAs is highly correlated with cell extension, suggesting that auxin-regulated gene expression is involved in the response of plants to gravity.

c^5 *Flowering shoots*

The reproductive stages of the life cycles offer striking examples of basic developmental processes among which polarity, pattern formation, and determination. Thus polarity during macrospores and embryonic development is determined at an early stage (Bloch, 1943).

Floral differentiation is a process which includes all events/processes occurring in the meristem or the cells of the meristem which pertain to the production of the flower (inflorescence) as well as its maturation and senescence. Thus, "floral differentiation" is an all-inductive process (McDaniel, 1984*b*). Single algorithms or sets of rules can be used to describe such complex biological patterns which control the inflorescence development — distribution patterns of branches and flowering structures — by either timing or positioning (basi-meso-acrotonic) mechanisms (Lindenmayer, 1984). Flowering constitutes an end-point for the meristem which gives rise to the flower, and the frequent enlargement of the shoot apex during the changes to the reproductive state is paralleled by ultrastructural changes (Nougarède, 1967; Auderset and Greppin, 1977).

The dimorphism of branches presented by certain plants (*Phyllanthes* sp.) results from the plagiotropic orientation of a lateral bud meristem. It was shown to depend on the meristem of the orthotropic axis which formed it and led Nozeran (1984) to draw a suggestive parallel between this process and that of an organizing center ("organizer") in an animal embryo. The informational controls regulating the positioning of floral parts at the apex differ, at least quantitatively, from those which regulate the positioning of leaf primordia (Carr, 1984). Interestingly, "floral induction and fern leaf development appear to be analogous to the example of neural induction in amphibians where induction of competent tissue leads to the expression of a specific development fate" (McDaniel, 1984*b*).

In the conversion from vegetative growth to floral differentiation, the control of the axis of cell elongation appears to be critical. Reorientation of cell elongation axes or polarity shifts have been considered as related to the initiation of lateral plant organs (Green and Poethig, 1982). "In order to understand processes like floral determination one will therefore have to know how polarity shifts are controlled" (McDaniel, 1984*a*).

The conversion of the apical meristem from the vegetative to the reproductive condition is a dramatic example of the switching of a developmental pathway (Bernier

et al., 1981). In photoperiodic plants, this redirection of morphogenesis at the meristem is under environmental control. Exposure of these plants to an appropriate daylength causes formation of a floral stimulus. In the leaves and subsequent translocation of the stimulus to the apical meristem, and upon arrival of the stimulus, the meristem stops making leaves and begins to produce bracts and flower buds (Bernier, 1971). Photoperiodic induction of flowering mediated via phytochrome causes the leaves to transmit a still unknown signal(s) or floral stimulus to the shoot apical meristem(s) which responds by forming a flower.

Recently, floral morphogenesis could be produced in thin layer tissue cultures of *Nicotiana tabacum* (van den Ende *et al.*, 1984). It was shown that the hormone naphthalene acetic acid (NAA) and benzyladenine (BA) in different concentrations affect the formation and distribution of flower buds and callus formation. Polarity occurred in the formation of both callus and flower buds, the degree of which depends upon the hormone concentrations. The suppression of the polarity of flower bud formation by high concentration of NAA was explained by the flooding of a natural NAA gradient in the tissue.

Interestingly, gradients of flowering substances or physiological states were evidenced in cells of leaves which, when placed in an adequate culture environment, produce either vegetative buds or flowers, depending on the location of the explanted cells in relation to the inflorescence (Tran Thanh Van, 1973) suggesting a polar pattern of distribution of the floral inductive factor(s). Translocable flower-inducing substances and flower-inhibitory substances produced from photoinduced leaves presumably interact at the stem apices to determine whether the apex remains vegetative or undergoes evocation and flower formation (Lang, 1980). In the short-day *Sinapis alba* the flowering principle would consist of at least two components, one of which induces an increase in mitotic activity at the stem apex (Bernier *et al.*, 1977). Clearly something moves from photoinduced leaves to stem apices that triggers the onset of flowering (Cleland, 1982). This was already suggested by the circadian changes of membrane potential occurring in the light-stimulated bioelectric response of spinach leaves which can be used as indicators of floral induction (Greppin and Horwitz, 1975).

B. Animals

The idea that axial polarity may be an expression of an underlying cellular polarity is also consistent, to some extent at least, with the fact that the former is often associated with an electrical polarity and can be altered by imposed electric fields (Barth, 1934).

Spatial organization could also be accomplished by graded distribution of substances termed morphogens (Boveri, 1901; Child, 1929, 1941). Wolpert (1969,

1971) developed this idea further into the concept of positional information. He pointed out that the size of an embryonic system is small when determination occurs (order of 1 mm or 100 cells across). Diffusion combined with local production and destruction at opposite ends can form a gradient of this size within a few hours (Crick, 1970).

Morphogens are signal substances emitted from organizing centers controlling position and polarity of cell structures. Such signal molecules are presumed to exist in embryos and to be involved in establishing the spatial pattern of cells during development. The idea is that cells respond to different concentrations by adopting different pathways of differentiation and so produce the characteristic anatomy of the organ concerned (Slack, 1987*a*). Some gradient systems of morphogens are based on diffusion of chemical substances from a more metabolically active "source" at one tissue boundary to a lower point, or "sink ", at the opposite boundary. An active factor from the source diffuses in a polarized manner and is either lost or used up at the sink.

The diffusion of a morphogen between a source and a sink considered in the illustration of Wolpert's ideas, is only one of the numerous candidates potentially responsible for the establishment of positional information and for the subsequent pattern formation. As shown by Crick (1970), the time necessary for a morphogen to diffuse through a field of critical dimensions is compatible with experimental data. The diffusion coefficient appears to be of the order of 0.8×10^{-6} cm^2 s^{-1}, which is about the value of the diffusion coefficient in solution of low-molecular-weight substances such as cyclic AMP. A very instructive illustration of Wolpert's ideas is provided by the so-called "French flag problem" (Nicolis and Prigogine, 1977): consider a one-dimensional field, the position of cells can be defined with respect to one of the extremal points (unipolar system) or with respect to both (bipolar system). In the case of bipolar fields, the state of a cell is determined by two morphogens and is influenced by the state of *both* neighboring cells. Patterns displaying size variance can be generated (Garay, 1977). There exists also a possibility of bifurcations leading to a uniform distribution of the morphogens, as well as of wavelike behaviour (Babloyantz, 1977; Babloyantz and Hiernaux, 1974).

Concentration gradients of chemical morphogens are believed to act across small fields of embryonic cells where different concentrations of morphogen provide "positional information" that determines the future behaviour of the cells. Until recently however, while the logical case for such mechanisms was impeccable, the biochemical evidence was inferential at best. The position has now changed (Slack, 1987*b*) since evidence has been reported strongly implicating gradients of retinoic acid in the specification of antero-posterior digit pattern of the chick wing (Thaller and Eichele, 1987).

To account for pattern regulation (e.g. regeneration) the "polar coordinate model" (PCM) has been proposed (French *et al.*, 1976). It is a formal representation

of a two-dimensional coordinate array of positional values with a circumferential and a radial components. This PCM model has been successful in describing and predicting pattern regulation in imaginal discs, insect legs, and vertebrate limbs. However, remarks have been raised against phase singularities and proposals made for an alternative PCM model without polar coordinate (Lewis, 1981).

1. Monoaxial patterns (Antero-Posterior (A/P) polarities)

Polar patterns are not confined to organisms that have developed in the ordinary way by growth from a reproductive unit such as spore or egg but are found in what are essentially organismic communities.

a) *Mycetozoa (slime molds)*

In the model cellular slime mold *Dictyostelium discoideum,* considered here as a mycetozoan for organizational reasons (protomodel of hydrozoa), the vegetative individuals are tiny myxamoebae which are polarized in their response to changes in chemotactic gradients (V.B.5a). At the end of vegetative growth, some thousands of these become aggregated into a pseudoplasmodium where each retains its individuality. This colonial structure shows a polar organization, for the terminal portion of it can be grafted to the decapitated apex of another pseudoplasmodium, though not to the base. The tip is evidently the dominant region, for if grafted to the side of a pseudoplasmodium it will withdraw from it a group of individuals and start out as a new unit. The sorocarp that ultimately develops has a vertical polar axis and, in some species, lateral axes as well. Polarity in organisms like these appears to be a property not of the individual cells but of the aggregate that they form (Fig. 30 A, B; Raper, 1940; Loomis, 1982).

In the pseudoplasmodium of *Dictyostelium discoideum,* only two cell types are clearly recognizable: the prespore cells, which will eventually form the spores at the top of the fruiting body, and the prestalk cells, which will eventually form slender stalk bearing the mature spores at the apex. Cells at the tip appear to move downwards into this tube and differentiate into stalk cells, i.e. they vacuolate and become surrounded by a rigid cellulose cell wall. When the newly formed stalk makes contact with the substratum, the cells surrounding the stalk are lifted into the air. After the stalk has reached a certain height, these remaining cells become spores. A small group of cells, referred to as the tip, determines the direction of morphogenetic movement and the polarity of the pattern of the two cell types (Schaap, 1986). The tip functions as the classical organizer, a feature which is generally thought to act in embryonic development and tissue regeneration.

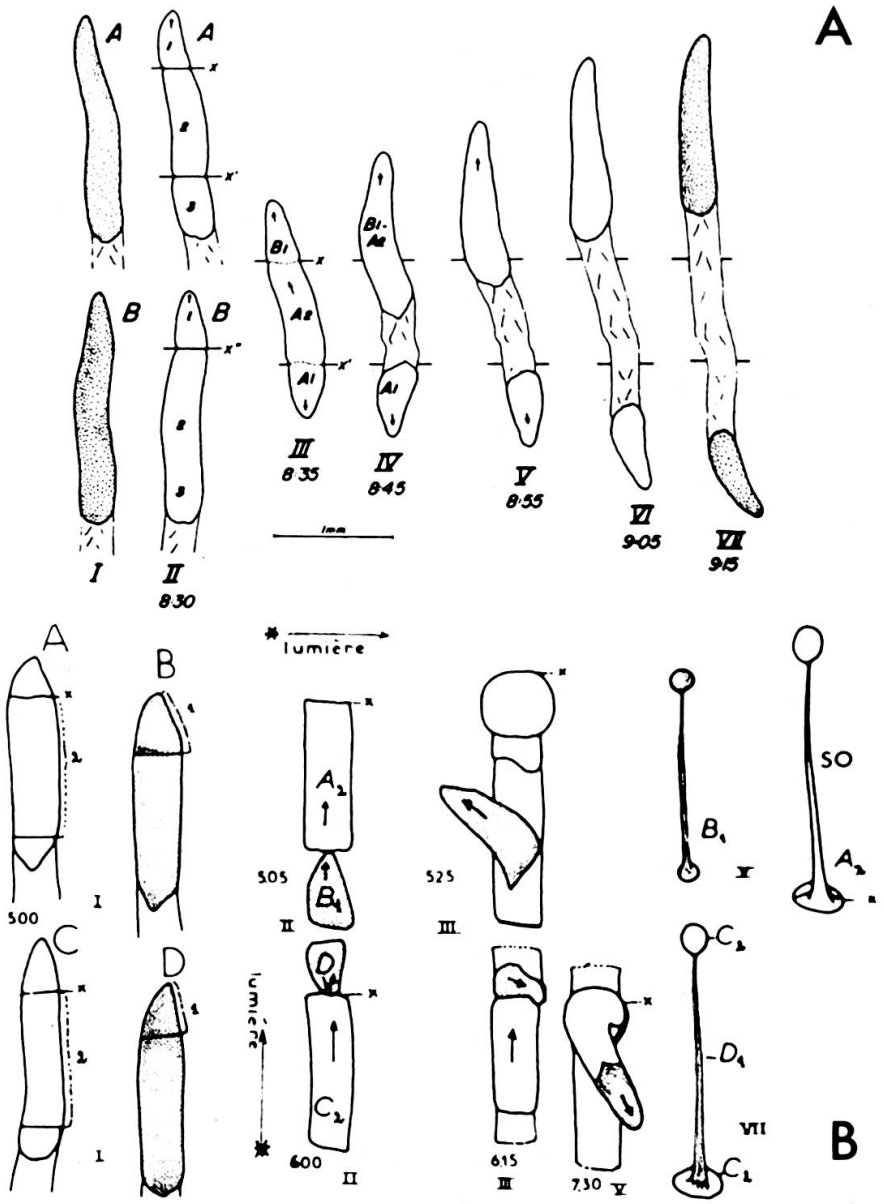

FIG. 30.

Polar axiation in the slime mold *Dictyostelium discoideum*.

(A) Regeneration experiment by Raper (1940) demonstrating polarity after grafting (A) an apical piece (B_1) of a pseudoplasmodium in close contact with the apical end of another, resulting in coalescence of the two pieces (III); (B) by contrast, there is *no* coalescence when the apical piece (A_1) is placed next the basal end (X') either of the same pseudoplasmodium or of another, the terminal piece (A_1) moves off independently (IV-VII). This experiment demonstrated the dominance of the slug tip. From Sinnott, 1960.

(B) Further demonstration of the polar behavior of apical and non apical fractions of pseudoplasmodia (A-D), certain of which (B and D) were stained by the red bacterium *Serratia marcescens* (darkened). When the two grafted pieces have the same polarity (arrows) two stalked fruiting bodies (sorocarps, so) are light (*)-produced while an inversely polarized piece (D) regenerated only one sorocarp. From K. B. Raper, 1940 in J.-P. Larpent "De la Cellule à l'Organisme», Masson & Cie, Paris, 1970.

In *Dictyostelium* the two types of cells can be distinguished biochemically on the basis of their stainability, specific protein content, antigenic properties, biosynthetic capacities, etc. Presumably the differentiation into prespore and prestalk cells reflects the activation of different sets of genes which then provide each cell with the specific templates needed to carry out its own differentiation. The underlying biochemical differentiation manifests itself morphologically, as a specific type of membrane-bound vacuole that is not found in prestalk cells (Karp and Berrill, 1981). The axially polarized slug formed from the aggregated myxamoebae (Fig. 30 A) moves according to a "squeeze-pull" model proposing that prestalk cells are engines and prespore cells are the cargo (Williams *et al.*, 1987).

A number of mechanisms have been proposed for the generation of two cell types in *Dictyostelium* including the choice of cell type being determined by a cell's position within the aggregate (Krefft *et al.*, 1984), or by other, non positional factors. The hypothesis that positional factors determine cell type choice is supported by the observation that a dissected anterior tip will form a new, smaller slug in which prespore cells are formed by conversion of prestalk cells (Raper, 1940). Interconversion can also be seen with purified populations of prestalk and prespore cells (Weijer and Durston, 1985).

Gomer and Firtel (1987) have examined the developmental fate of individual cells in a system that allows *Dictyostelium discoideum* cells to differientiate in the absence of aggregation. Their results show that the propensity of single amoebae to differentiate into either prespore or prestalk cells occurs by a cell-autonomous mechanism dependent on the cell's position in the cell cycle at the initiation of development.

Of primary interest to developmental biologists is the nature of the agents that cause a cell or group of cells to proceed along one path of differentiation as opposed to another (Karp and Berrill, 1981). In some cases, specific chemical substances produce complex responses in competent cells, i.e., cells capable of responding to the stimulus. This is clearly the case for the aggregation response by starved amoebas to cyclic AMP. More recently, another such "on-off switch" has been found to operate at a later stage in slime-mold development. It is evident that low-molecular-weight molecules such as cyclic AMP or ammonia are not substances in which developmental information can be stored. Rather, each serves as a specific trigger to elicit a preprogrammed reaction within the responding cells (Williams, 1988).

One of the consequences of the multicellular state in a pseudoplasmodium, or in any other cell mass, is the potential for intercellular, possibly polarized communication that results from cell contact. The outer edge of all cells forms a complex structure capable of transmitting and receiving a wide variety of stimulatory signals. Cells kept in isolation, and therefore free of cell contact, cannot undergo differentiation into prespore and prestalk cells.

The pattern of initial prespore differentiation suggests the existence of positional cues: in late aggregates, prespore-specific proteins and prespore vacuoles appear — approximately concomitant with tip formation — in the basal/central part of the aggregate and remain absent from the tip region. During subsequent slug formation, prespore-specific gene products remain confined to the posterior part of the slug. The results of such experiments would seem to demonstrate that the induction of prespore differentiation is not necessarily position-dependent (Schaap, 1986). However, it should be noted that, in species other than *D. discoideum,* position-induced (re)differentiation is more conspicuous.

The induction of stalk-cell differentiation is also position-dependent during normal development and, in *D. discoideum,* starts at the ultimate tip and at the base of the fruiting structure. The cellulose stalk tube in fruiting bodies is formed just before stalk-cell differentiation. A differentiation inducing factor (DIF) has been shown to induce such stalk cells (Kay and Jermyn, 1983). The DIF may be related to other morphogens (Watts, 1987) such as the low molecular-mass hydrophobic hexanone derivatives of the retinoic acid type.

b) *Protozoa*

The cellular polarity of a ciliate such as *Paramecium* is shown by a terminal cytostome. At cell division, kineties (rows of basal bodies or kinetosomes) elongate by multiplication of kinetosomes, the cell constricts in its middle and polar formation of a new oral apparatus produces a new daughter cell.

Analyses of cortical patterning of kinetosomes and kineties in ciliates protozoa show that there are two types of developmental processes responsible for the positioning of these organelles. One is the propagation of ciliary rows through localized addition of new ciliary units along the axis of an existing row (Karp and Berrill, 1981). The cortical pattern of ciliary rows seems to be completely independent of the nuclear information. When two differently striped cells of *Tetrahymena* exchange their nuclei, they do not change their cortical pattern. It is perpetuated by the cortex alone.

What controls the polarity of the ciliation wave? Is it the intrinsic polarity of the ciliary row, or is it some other polarity superimposed upon the ciliary row?

There are two systems of polarity in *Tetrahymena* which are both expressed with ciliary rows. This story begins historically with *Paramecium,* in which Beisson and Sonneborn (1965) were able to induce cells to rotate a few of their ciliary rows 180°, following which the paramecia actively maintained and propagated these rows in their inverted state. This demonstration was repeated in *Tetrahymena* over a decade over. Hence there exist two superimposed systems of polarity: "a local one internal to the ciliary rows governing (for example) the direction of nucleation of new basal bodies, and a second large-scale cellular polarity that is independent of the orientation of ciliary rows but nonetheless is able to impose its influence by controlling the time and place of formation of new ciliary structures" (Frankel, 1984).

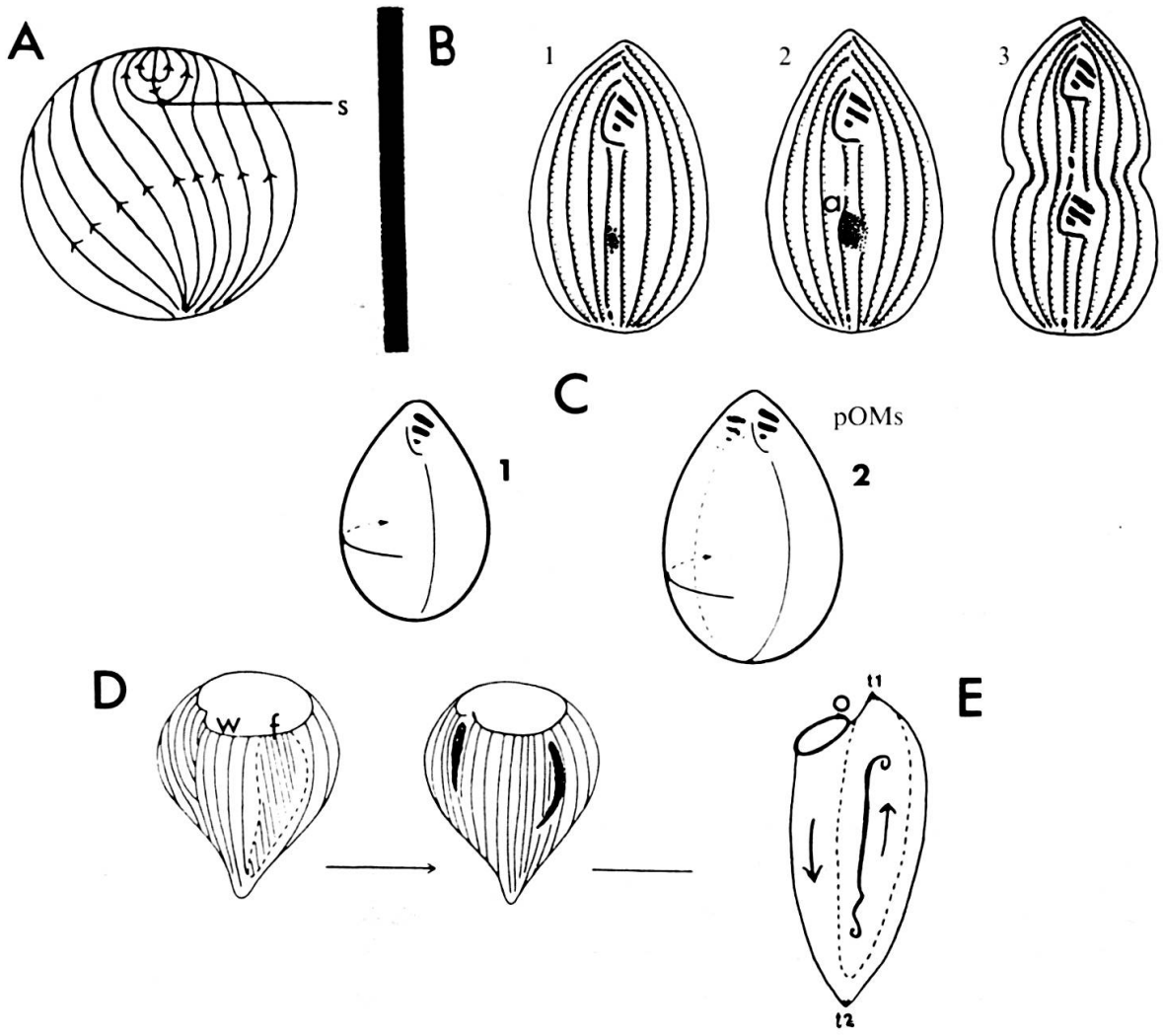

FIG. 31.

Polarity controls in *Protozoa.*

(A) Model of ciliary meridians: the arrows define the direction of morphogenetic field which establishes posterio-polarity saddle(s) point. (B) Cell division stages in *Tetrahymena:* (1) basal bodies initiating a new oral ciliature; (2) anarchic (a) field formed; (3) oral membranelles assembled. From Sleigh, 1973, adapted from Goodwin, 1976.

(C) Schematic lateral view of transparent, nondividing cells of *Tetrahymena* illustrating the geometrical arrangements of oral structures and oral meridians in non-*janus* singlets (1) and homopolar doublets (2) resulting from the fusion side by side of two cells. The resulting two semicells of the parabiotic, homopolar doublet are similarly antero-posteriorly aligned. The demonstrated clonal persistence was the original basis for Sonneborn's contention (1963) that preformed cell structures play an essential role in cell heredity (Fauré-Fremiet, 1948, and Sonneborn, 1963, in Frankel *et al.,* 1984). pOMs = primary oral meridians. Adapted from Frankel *et al.,* 1984.

(D) Double oral regeneration in *Stentor* resulting from grafting an extra fine (f)-stripe zone into the wide (w)-stripe region of a decapitated cell; the new oral primordium is polarly reversed. From Tartar, 1962, adapted from Karp and Berrill, 1981.

(E) Experimental demonstration of the importance of the posterior region of the *Stentor* cell induction of an oral spiral. This spiral is formed at both ends (t1, t2) of the oral primordium (o) following excision of a nonnucleated segment from the region of the contrast-zone and reimplantation following 180° rotation about the antero-posterior axis. The arrows show the intrinsic polarity of the host and of the implant. Adapted from Frankel *et al.,* 1984.

At cell division in *Tetrahymena,* observation of polar disturbance by the anarchic field of proliferating basal bodies led to the conclusion that "the oral apparatus arises at a saddle point in the control field, where polarity vanishes" (Fig. 31 A, B). The anarchic field disturbs the polar organisation of the local ciliary meridians, and then "this oral field becomes organised into the typical ciliature of the mouth" (Goodwin, 1980).

The restriction of the basic pattern to the cell surface, together with the fact that the organism has polarity so that the anterior (or north) pole is different from the posterior (or south) pole, is sufficient to select from among those functions satisfying Laplace's equation (more generally, Poisson's equation) on the sphere a unique one which describes the basic polar organization of the cortical field (Goodwin, 1980).

Cortical structures also propagate in a stable hereditary manner in *Stentor* as demonstrated by the classical, double *Stentor* experiment (Tartar, 1962): a cortical segment from a fine-stripe cell grafted into the wide-stripe region of another decapitated *Stentor* cell results in double oral regeneration, one from the host and one from the graft primordium (Fig. 31 D, E). Each cell division produces two deranged daughter cells. Moreover, decapitation of the double *Stentor* is followed by double regeneration. The genes have therefore not changed, only the cortical structures. Yet cortical structures are also genetically controlled (Loomis, 1986): homozygous cells of the two-faced mutant of *Tetrahymena janus* (*jan/jan*) show two oral apparatuses on opposite sides of the cell in mirror-image orientation (Fig. 31 C). The *jan* allele would modify a reference dorsal border, the dorsal side of the mutant cells being transformed into a reversed ventral one. "Analysis of symmetry-reversals that result from altered gene action and those that result from geometrical anomalies in wild-type cells promise to shed light on the mechanism of cellular pattern determination" (Loomis, 1986).

c) *Hydrozoa*

The fresh-water polyp *Hydra,* a coelenterate of about 5 mm length, of about 100,000 cells and of about 12 types (Saier and Jacobson, 1984), has been a classic model for polarity studies since its discovery more than two centuries ago, when Abraham Trembley of Geneva first succeeded in cutting the small animal in two and observed the regeneration of the amputed parts. With a hair, he could pull the creature inside out, and subsequently observed its recovery to the normal state. The memory of this pioneer of polar regeneration studies (Fig. 32 A) has been honored for the two hundredth anniversary of his death by a special volume of the Archives of Sciences (Lenhoff and Tardent, 1985).

The major changes of form in hydra include the developments of: tentacles during regeneration and budding; new and complete axis during budding; partial secondary axes resulting of induction by grafts (Tardent, 1963; Webster, 1971). Along

its main axis, a hydra may be regarded as a bipolar field, if we define with Wolpert *et al.* (1974) a field as "that set of cells which have their position specified with respect to the same coordinate system or boundary regions". The hydra field thus is bipolar, since the two boundary reference regions appear to be the head and foot ends. As for the variation in positional value along the hydra, it may be represented by a gradient in some cellular property which decreases steadily from the head to the foot end (Wolpert *et al.*, 1974).

The metabolic origin of polarity in hydroids (*Hydra, Tubularia*) has been studied by Child (1941) and has provided him with the conceptual basis for the development of his theory of metabolic gradients, particularly of oxidative metabolism, as the underlying cause of axial order. By the use of inhibitors and dyes, this author could suggest that metabolic activity is the highest at the hypostome while decreasing regularly towards the basal disk of the hydra. Child's ideas have been reviewed by Webster (1971) and as commented by Goodwin (1976) "they remain as a foundation for much thinking about the dynamic nature of polar order in developing systems".

More recent experiments of polarity reversal, with nerve-free hydra, have suggested that hydra polarity is determined by epithelial cells (Marcum *et al.*, 1977). Such suggestion rejoins previous proposals that it might be due to either a metabolic and transport polarity or to a structural asymmetry. Such structural basis for polarity has been ascribed to polarized motions of axially aligned muscle processes (ref. in Marcum *et al.*, 1977, see also Fig. 32 B).

Complete organismal regeneration is possible from a very small fragment even though limited to a minimal size. If a section is cut from the central body part, a new head (hypostome and tentacles) will regenerate distally and a new base proximally, preserving the original polarity of the tissue. Polarity reversal can be produced in an electrical field or by grafting a hypostome proximally and removing it several days later (Goodwin, 1971). During regeneration, multipolar budding forms are often produced and mutants have been found which do not reproduce by budding (Lenhoff *et al.*, 1969). Other mutants, such as regeneration defective strains (Achermann and Sugiyama, 1985) as well as chimeras have been profitably used to explore the gradient underlying pattern formation in *Hydra* (Achermann, 1985).

Two developmental gradients of morphogens play a role in establishing the position of a regenerating head: the head *activation* gradient due to a relatively stable property of the tissue distributed monotonically, with tissue at the upper end of the body column having a greater ability to form a head and the tissue, further down the body column (MacWilliams, 1983) and the head *inhibition* gradient which is also monotonically distributed but very different in character (Fig. 32 C). These differentiation patterns were presumably thought to be controlled by gradients of diffusible molecules or "prepatterns". This concept of diffusible patterning substances was originally proposed by Wolpert (1969), and was later incorporated into reaction-diffusion models by Gierer and Meinhardt (1972) and MacWilliams (1983).

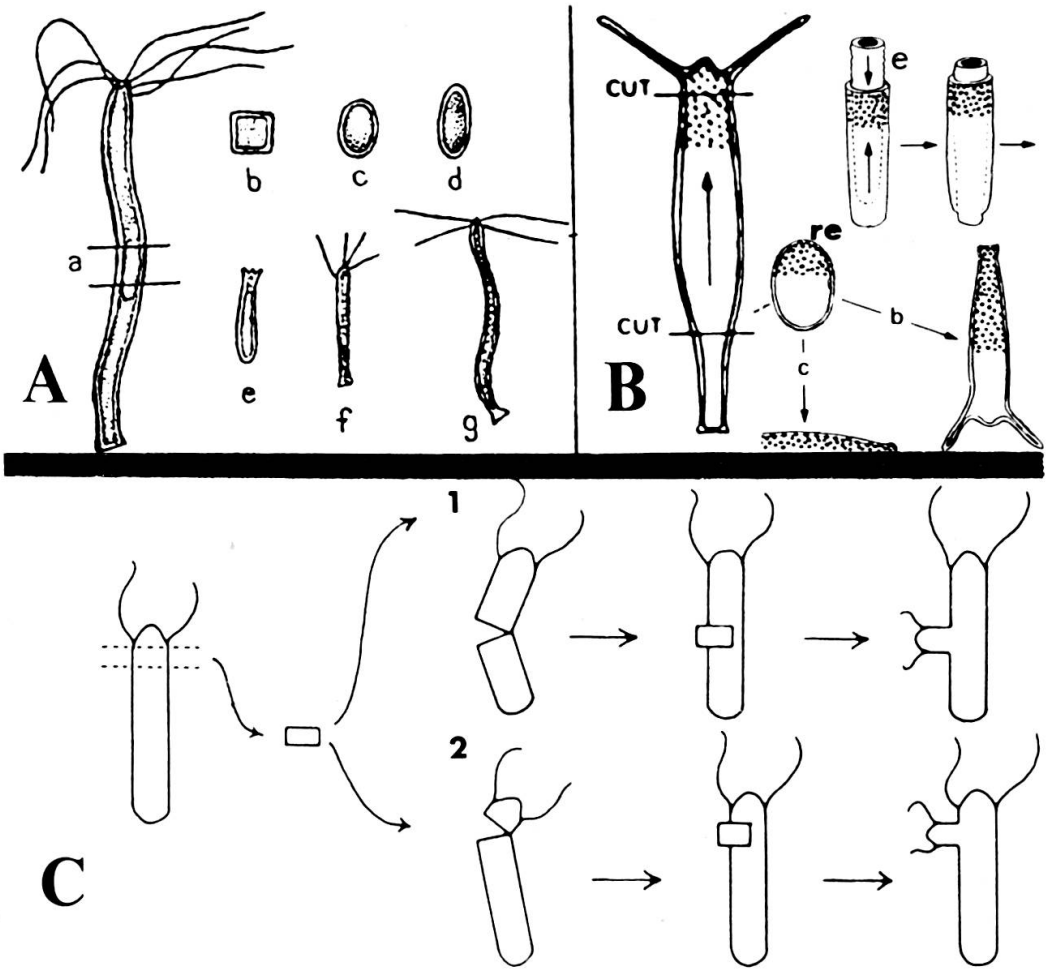

FIG. 32.

Polarity controls in Hydra.

(A) Classical Hydra regeneration experiment by Morgan, 1904: a = fragment cuts; b-g = regeneration steps according to normal apico-basal polarity.

(B) Influences of ecto- and endoderm in determining the axial polarity of *Hydra attenuata* Pall: ectoderm isolated from endoderm (e) and reassembled with opposite polarities (arrows); carbon marks indicate the former distal region of the ectoderm; in regeneration products among the reassembled (re) specimens a few (b) obeyed to the polarity of the endoderm. In (a, not shown) regeneration occurred according to the axial polarity of the ectoderm; in (c) the new axis is perpendicular to the original body axis. Adapted from I. Schmidt and P. Tardent, Wilhelm Roux's Arch. 191: 64-67, 1982.

(C) Transplantation experiments with *Hydra attenuata:* (1) transplant (donor) from head zone tested in the middle of a Hydra receptor (acceptor). The more transplant tested there originates from zones toward the tail zone, the less lateral head formation occurred, thereby demonstrating the existence of an axial head *activator* gradient. (2) Transplant (donor) from head grafted in head region of the acceptor resulting in a minimal (10%) head lateral growth; the closest to the tail is grafted the transplant, the more (ad 85%) is lateral head formation, thereby demonstrating the existence of an axial head *inhibitor* gradient. Adapted from figures by Bode *et al.*, 1987.

The basis of the activation gradient could derive from an intrinsic cell polarity by such mechanisms as directed morphogen transport or electrical conduction. However, patterning in hydra was experimentally showed to be more complex than

a single series of appropriately aligned north and south magnetic poles (Bode and Bode, 1984). In one experiment, rings of the body column were flipped over and then grafted back together again. Thus, each piece retained its original axial position, but had an inverted polarity. If polarity were simply a vector property, head regeneration should then occur at the basal end. Instead, it occurred at the original apical end, as expected if polarity were due to a *scalar* property.

A small peptide has first been isolated as morphogen responsible for the head-forming gradient (Schaller, 1973). It has been further identified as an 11-amino-acid peptide with pyroglutamyl and phenylalanyl residues (Schaller and Bodenmüller, 1981). This "head activator" can be generated by the reaction-diffusion mechanism and the profile of this neuropeptide has been found to "closely parallel the observed pattern of nerve cell commitment and hence the pattern of "free" head activator which was inferred from it" (David and Holstein, 1985).

The phenomenon of head inhibition is commonly assumed to be due to the steady-state production of diffusible "head inhibitor" by the head and its degradation in the tissues through which it diffuses. Recent results provide direct evidence consistent with the involvement of a diffusible hydrophilic and non peptide substance moving through gap junctions in head inhibition in hydra: "antibodies to the major rat liver gap junction protein recognize a gap junction antigen in hydra and are effective in eliminating junctional communication between hydra cells" (Fraser *et al.*, 1987). According to these authors "head inhibition might involve the cooperative influences of both diffusible and nondiffusible components, or an electrically driven passage of ions or small molecules through the gap junctions". These cellular structures are thus deeply involved in the polar patterning process in *Hydra* as extensively discussed by Bode *et al.* (1987).

In marine hydrozoa, the anterior-posterior axis of the planula larva has an integrative function (Teissier, 1931) and is frequently regarded as some sort of gradient running from one pole to the other in the embryo. Freeman (1981) has further shown that, after dissociation of blastula stage embryos into single cells, polarity can be entrained in the reaggregate by grafting a small piece of tissue from any part of an intact blastula to the reaggregate. The grafted cells then organize formation of an axis of symmetry in the embryo. This axis formation has tentatively been explained by chance events occurring during cleavage, translated by a principle of "self-increasing" elongation into an oxidative polar difference (Ostroumova and Belousov, 1971). More recently, this process of emergence of polarity in embryo cell aggregate has been analogized to the acquisition of magnetism by a metal (Freeman, 1981). When polarity is set up, this event can be regarded as "a process whereby a population of polarity dipoles in or near the cell surface become oriented in the same direction". Freeman's experiments have shown polarity to be entrained in reaggregated cells by grafting a small piece of tissue from an intact blastula to a reaggregate, a finding consistent with this dipole model.

As we have briefly described it above (p. 247), the body of work on hydra argues that diffusible morphogens produced primarily at either extremity of the animal play a major role in maintaining polarity. A morphogenetic head activator has also been found in the planula larva of *Hydractinia* (Müller *et al.*, 1977). However, this model of polarity founded on the reaction-diffusion models rather than on the oriented dipoles — disproved in *Hydra* by the scalar model — is probably not applicable to the marine hydrozoan embryo (Freeman, 1981).

2. Biaxial patterns (A/P + Dorso—Ventral (D/V) polarities)

a) *Worms*

The planarians or flatworms provide insight into how the antero-posterior and dorso-ventral axes are kept orthogonal to each other (which is not automatically the case if the patterns are formed by reaction-diffusion mechanisms). A condition for head or foot formation and thus for the re-establishment of an antero-posterior axis is a juxtaposition of dorsal and ventral tissues (Chandebois, 1979). A head or foot can therefore only be formed along the dorso-ventral borderline and never, for instance, within a purely dorsal region. This assures that both axes are orthogonal.

A head, a foot or both regenerate even in very small tissue fragments, indicating that systems which bear an organizing centre at each end are stable over an enormous range of sizes. The head field and the foot field cannot be independent from each other, otherwise they would not appear at opposite sides. The mechanism of mutual activation of two feedback loops suggests an appropriate coupling of a head-forming and a foot-forming system which assures that both structures are present in the system and that they appear at maximum distance from each other (Chandebois, 1979).

As in coelenterates, polarity is an important feature of flatworm regeneration. If a planarian is cut in half perpendicular to the antero-posterior axis, the anterior fragment regenerates a tail at the cut surface and the posterior piece regenerates a head. Regeneration occurs at both wound surfaces, not only by migration of coherent sheets as in coelenterates but also by the proliferation of undifferentiated cells at the cut surface into a blastema that differentiates new head or tail structures (Fig. 33).

Polarity seems to be intrinsic to the piece; changing its position by implanting it at different sites along the longitudinal axis does not alter its rate of regeneration. Anterior fragments regenerate heads more rapidly than posterior pieces, although this property varies from species exhibiting no head regeneration to others exhibiting no gradient where all fragments regenerate heads at the same rate (Grant, 1978).

Polarity expresses itself in many ways: when the wound surface of a posterior fragment is longitudinally cut into two components which are not allowed to fuse, each portion regenerates a complete head. A multiheaded monster may be produced

by making several parallel cuts at the surface. On the other hand, two heads may be fused to form one. The wound surface behaves as a morphogenetic field; that is, when disturbed, it regulates and restores a normal developmental pattern (Grant, 1978).

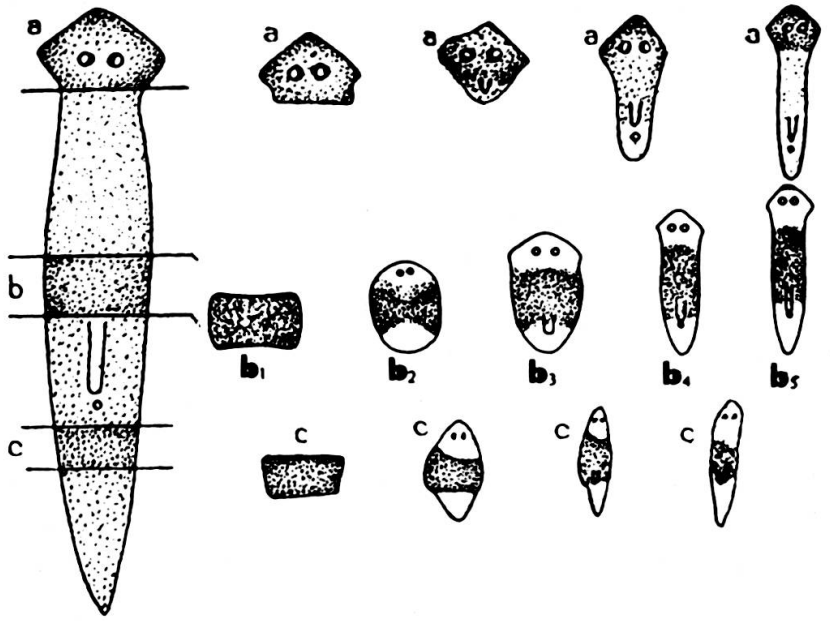

FIG. 33.

Polar regeneration in *Planaria maculata*.

(a) Monopolar regeneration of an excised head. (b_1-b_5) Bipolar regeneration of a transverse section from the middle. (c) Bipolar regeneration of a piece removed from a posterior segment of the body. Regeneration portions unstippled. From T. H. Morgan, Regeneration, 2d ed., New York, Macmillan, 1907.

Bipolar regeneration has been induced in the polychaete worm *Sabella* by means of exposure to colchicine. Colchicine inhibits all regeneration, but when colchicine-treated pieces are returned to normal seawater, a head may regenerate from each end. When such a two-headed piece is bisected, however, the anterior portion regenerates a tail posteriorly, and the posterior portion regenerates a head anteriorly. The original polarity is regained. The interpretation offered is that colchicine reversibly disrupts the normal array of microtubules in the nerve cord, i.e. it causes a temporary depolarization, while the production of neurosecretory granules is not inhibited.

How the epigenetic process responsible for temporal and positional control of cell determination in embryos operates remains a central problem of contemporary developmental biology. The transparent embryos of the round worm or nematode *Caenorhabditis elegans* provide a remarkable experimental system with which to approach such questions (Brenner, 1974; Kenyon, 1988). These embryos develop

rapidly, giving rise to first-stage larvae with only 558 nuclei. Moreover, this worm is convenient for genetic analyses, so that mutations perturbing embryogenesis can easily be isolated and studied for clues to normal developmental mechanisms (Hirsh, 1979).

The fate of each worm cell, including those of the nervous system from the fertilized egg to the adult, has been painstakingly traced by Sulston *et al.* (1983): the zygote undergoes a series of asymmetric divisions, each giving rise to a somatic precursor and a germ line cell. Most of these divisions take place in the direction of the antero-posterior polar axis, but several have dorsal-ventral or left-right components (Laufer *et al.*, 1980).

Boveri (1910) first showed that posterior zygote cytoplasm may act to prevent subsequent chromosome diminution in *Ascaris* embryos. This is consistent with the suggestion that germ plasm material may be important in either the determination of germ-line cells during early embryogenesis or the functions of germline cells during gametogenesis or both. The germ line of the nematode *Ascaris* is established by the inclusion of cytoplasmic components in certain embryonic blastomeres. Since then, nematode embryos have been regarded as classical examples of the "mosaic" type; that is, the fates of blastomeres are fixed at cell division by an unequal partitioning of cell constituents rather than by cell-cell interactions or environmental cues (Gurdon *et al.*, 1985; Emmons, 1987).

Graded signals are known to play a role in structural morphogenesis, in relation with positional information, and the spatial pattern of the inductive signal affects the morphogenetic outcome. In the case of germ cells, the inductive signal emanates from two somatic cells of the gonad. These cells, termed distal tip cells, lie at the distal end of the tubular gonad. If the distal tip cells are displaced from their normal location in the developing gonad, the polarity of the gonad shifts correspondingly so that germ cells nearest the distal tip cell are always mitotic, and germ cells further removed are in progressively later stages of meiosis or gamete formation. Therefore the distal tip cell is responsible for maintaining the normal spatial pattern of cell types in the field of germ cells. This must occur by a diffusible signal, since cells beyond those in contact with the distal tip cells are influenced.

b) *Molluscs*

There are polar lobes as in eggs of annelids. Polar lobes arise by a transient constriction that separates the anuclear vegetal region of the egg cytoplasm more or less completely from the remainder of the egg. The process starts during the maturation divisions, but the constrictions remain rather shallow. During first cleavage the constriction is very deep, resulting in a lobe that is connected by a thin stalk to the egg. This stage is called trefoil stage (Dohmen (1983).

The polar lobe content appears to be of crucial importance for development, as can be demonstrated by removing the lobe at the trefoil stage without injuring

the blastomeres. The egg continues cleaving but subsequent development is severely defective. As for the biochemical nature of the polar lobe determinants, it was found a higher concentration of guanosine triphosphate (GTP) in the polar lobe of *Nassarius* (van Dongen *et al.*, 1976).

The vegetal body of the polar lobe contains the morphogenetic determinants and the localization of the morphogens at the vegetal pole begins during oogenesis. The mechanisms that bring about this localization and provide the strong attachment of the determinants to the egg cortex are unknown but microfilaments might be involved. According to Dohmen (1983), "it remains to be established what significance should be attributed to these regional surface differentiations. They are formed at the vegetal pole of the egg, so they might be instrumental in establishing or maintaining the animal-vegative polarity of the egg without any direct relationship with the morphogenetic determinants present in the polar lobes". However, experimental data have shown that whole egg surface is capable of forming lobelike constriction, and the normal position of the polar lobes at the vegetal pole apparently depends on the correct positioning of the triggering signal. The mitotic apparatus may be instrumental in this process, but its presence is not absolutely required for the appearance of the lobe at the correct time and the correct place (Dohmen, 1983).

In normal development the polar lobes appear in synchrony with the cleavages. The stimulus for furrow formation in cell division is known to be provided by the mitotic apparatus (Rappaport and Rappaport, 1974) and observations suggest that the microtubules of the mitotic apparatus are involved in the initiation of lobe formation. However, the potential to form a polar lobe is apparently restricted to a limited part of the egg. If the vegetal part of an uncleaved egg of *Dentalium* is removed, the egg will not form a polar lobe at first cleavage. Polar lobelike protrusions can be induced anywhere on the egg by exposing fertilized eggs of *Ilyanassa* to isotonic $CaCl_2$ after second maturation division (Conrad and Williams, 1974). Similar effects are observed after iontophoretic injection of calcium, cyclic adenosine monophosphate (AMP) or ionophores (Conrad and Davis, 1980).

c) *Echinoderms*

The embryonic architecture of the sea urchin embryo begins with the initial polarity of the egg endowed with an animal-vegetal polarity (Fig. 34 B) originating before fertilization, far back in oogenesis (Guidice, 1986). In these embryos data relevant to determination along the animal-vegetal axis have been interpreted in a manner consistent with the idea that morphogenesis is regulated by a balance between reciprocal "animalizing" and "vegetalizing" gradients (Angerer and Angerer, 1983). Driesch's success in separating the early blastomeres of sea urchin embryos was a great boost for the argument of "isotropy". It seemed to refute the possibility that *any* predetermination of morphologic outcome can exist, at least up to the four-cell

stage. Driesch's result was a further blow to Weismann's proposal of nuclear differentiation and divergence. It remained for Wilson and Morgan, and for two entire generations to show that there are *not* two perfectly separable and distinct kinds of development, "mosaic" and "regulative" (Jeffery and Raff, 1983). There appears to be architecturally-differentiated domains of the egg cytoplasm, as evidenced by differential organization of the molecular cytoskeleton. They match the domains of those morphogenetic "plasms" so painstakingly traced out by the first investigators of cell lineage.

The location of the early blastomeres, and of the embryonic structures to which they give rise are fixed with respect to the animal-vegetal axis of the unfertilized egg. However, some cell lineages of the sea urchin embryo do indeed develop

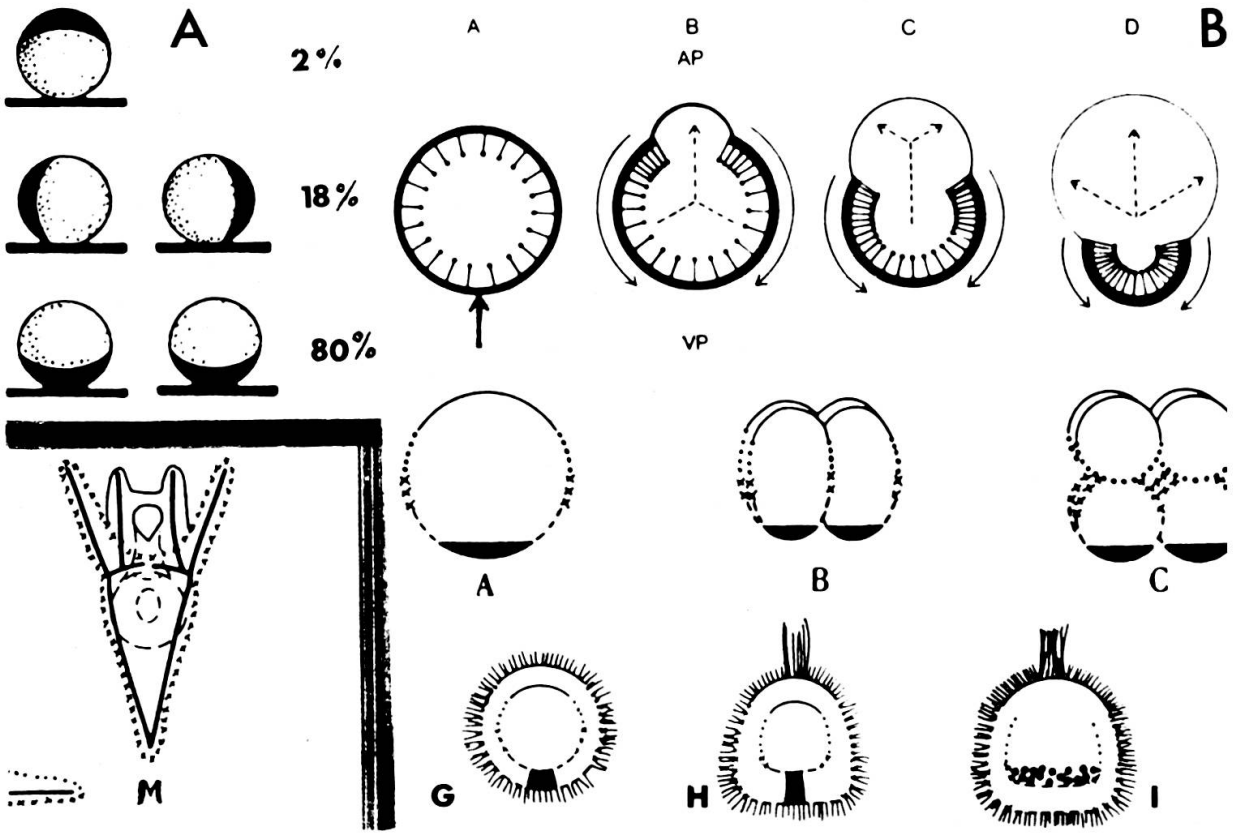

FIG. 34.

Polarization of Ascidian and Echinoderm eggs.

(A) Eggs of the ascidian *Boltenia* showing the direction of their orange myoplasmic crescent predominantly aligned (80%) against a Ca^{2+}-ionophore (A23187) — coated glass rod. Adapted from Jeffery, in Jeffery and Raff, 1983.

(B) Eggs of the sea urchin according to Jeffery's model (1983) in which the local calcium elevation at fertilization causes the plasmalemma to contract toward the point of sperm entry (arrow) in the vegetal hemisphere of the egg; further developmental fate of the various regions (A-I) of the sea urchin egg leads to the pluteus type of embryo (M) as based on Hörstadius, 1939, according to Grant (1978). AP = animal pole; VP = vegetal pole; crescent (black) = micromeres-forming region.

autonomously, but others are evidently specified for their pathway of differentiation by means of intercellular interactions or polarizations (Davidson *et al.*, 1982). In the eight-cell embryo there are four cells containing animal-pole substances and four containing vegetal-pole substances. This third cleavage is a determinate one; if the animal and vegetal halves of the embryo are separated, the animal half will give rise to an abnormal blastula-like larva with overdeveloped cilia, and the vegetal half will give rise to a different type of abnormal larva with an overdeveloped digestive cavity. It is clear, then, that the cytoplasm substances to which a nucleus is exposed must play a prominent role early during embryological development in activating some genes and repressing others. Because the egg cell itself contains different substances in different regions of its cytoplasm — whether these substances are arranged in a pole-to-pole gradient (as they apparently are in sea urchins) or in a less symmetrical pattern (as they are in frog and sea-snail eggs, with their gray crescents and polar lobes) — cells with different cytoplasmic environments for their nuclei are produced early in embryological development.

How the animal and vegetal halves have become so different in their abilities to differentiate in isolation? According to the theory of *double* gradient of Runnström (1975) and Hörstadius (1973), the direction in which a given cell of a sea urchin embryo will differentiate is under the control of two influences acting coordinately throughout the embryo. One of these influences (activities or substances of unknown nature) is at its greatest concentration at the animal pole; the other is at the greatest level at the vegetal pole. Each influence spreads out from its respective pole, decreasing until it has essentially disappeared in the region of the opposite pole. Since each diminishes in strength, each is considered to occur as a gradient, and together they form a double gradient. In this theory, the nature of differentiation of each part of the embryo is thus based on the relative levels of each member of the double gradient.

A correlation exists between the double gradient of oxidative metabolism and the double gradient of differentiation. The causal link between these two phenomena comes from the analysis of the metabolic gradient under conditions that are known to affect the morphogenetic gradients. The two appear to be linked. For example, lithium (LiCl) treatment abolishes the center of metabolic activity at the animal pole without affecting that of the vegetal region. Animalizing agents (thiocyanate SCN^-, trypsin) have the opposite effect and leave the embryo in possession of an intact animal gradient but lacking a counterbalance from the opposite pole. In each case, the ratios of the two influences are greatly disturbed, and the expected abnormal differentiation results (Lallier, 1975).

Developmental changes in the cell surface of the embryo have been examined and some antigens present in the egg found to become localized to the basal lamina or the matrix of the blastocoele (McClay *et al.*, 1983). Although the pattern of deposition is somewhat variable, and each antigen becomes localized to the wall of the

blastocoele, it should be noted that the same cells release the basal lamina material to the basal surface and other antigens to the apical surface of the embryo. This polarized expression of surface- or matrix-related antigens is a dominant theme for most of the antigens observed, and it will be of importance to learn how intracellular trafficking moves proteins in such a polarized fashion.

d) *Insects*

d[1] *Egg-embryo patterns*

The antero-posterior and the dorso-ventral egg axes are readily defined by the shape of the egg, yet the only specialized cytoplasmic organelles known to be localized are the polar granules of the posterior pole. They are included in a distinct clear zone of "pole plasm" and incorporated in the primordial germ cells, the first cells formed in the embryo. Nüsslein-Volhard *et al.* (1987) have recently shown that "the segmented pattern of the *Drosophila* embryo is organized by *two* activities localized at the anterior and posterior egg poles." The two morphogens, positioned at the anterior and posterior egg poles produce signals that spread toward the other pole. Thus this molecular prepattern of two opposing gradients is quite unlike the final fate map (Nüsslein-Volhard *et al.*, 1987).

A/P polarity is controlled anteriorly by the gene product of *bicoid (bcd)* and posteriorly by the gap gene *hunchback (hb)* repressed by the *nanos (nos)* gene. While a morphogenetic gradient underlies the specification of the anterior segment, positioning of the posterior one is determined only by local interaction between the gap genes (Hülskamp *et al.*, 1989; Irish *et al.*, 1989. At least twenty genes have been identified as playing crucial roles in D/V polarity of the early embryo (Anderson, 1987). It has been proposed that interactions among the products of the dorsal group genes are responsible for the specification of a morphogen gradient that ascribes unique positional identities to the different cells along the D/V axis (Anderson *et al.*, 1985). According to this model, the ventral-most regions of the early embryo contain the highest concentration of morphogen, with more dorsal regions containing progressively lower concentrations. Among the eleven maternally active dorsal group genes, *Toll* has been implicated as playing a particularly important role in the differentiation of the D/V pattern (Anderson *et al.*, 1985). However, the cloning and sequencing of *Toll* clearly indicate that it does not control D/V polarity by directly modulating gene expression (Hashimoto *et al.*, 1988). The *dorsal* gene has been recently cloned, and nucleotide sequence analysis reveals extensive homology with the avian oncogene v-*rel* (Steward, 1987; Levine, 1988).

Three kinds of mutations can disrupt the developmental process in *Drosophila*: maternal effect mutations, segmentation mutations and homeotic mutations.

Maternal effect mutations

Certain of these mutations influence the spatial polarity of the embryo and mutants are known which affect either antero-posterior (A/P) polarity or dorso-ventral (D/V) polarity.

A major determinant of A/P pattern is the product of the *bicoid (bcd)* gene (Frohnhöfer and Nüsslein-Volhard, 1986; Nüsslein-Volhard *et al.*, 1987; Driever and Nüsslein-Volhard, 1988). Like many other maternally expressed genes, *bcd* is transcribed in the ovary in specialized cells which form a cluster around the future anterior pole of the developing oocyte and the *bcd* RNA becomes localized at the anterior pole of the oocyte by virtue of its site of entry. Recent results of MacDonald and Struhl (1988) support the proposal that *bcd* transcripts are selectively recognized and trapped as they enter the anterior tip of the oocyte. A discrete *cis*-acting signal is responsible for this anterior localization of *bicoid* mRNA. At the same time that a stable graded distribution of *bcd* protein has formed from the anterior pole, it is assumed that an *osk*-dependent activity, probably, the product of the *nannos (nos)* gene has become inhomogeneously distributed in the posterior part of the embryo (Ingham, 1988; Fig. 35 A, B).

Among the maternal effect genes, at least twenty are known to control dorsal-ventral polarity (Nüsslein-Volhard, 1980). Embryos born from mothers lacking a functional copy of one of these genes die as hollow tubes of dorsal tissue. "In contrast to *bcd,* there is no evidence for a localization of the transcripts of any of these genes in the egg, suggesting that D/V polarity is generated by a mechanism different from that organizing A/P polarity" (Ingham, 1988). These functions have been mainly studied by experiments involving the rescue of mutant embryos by transplantation of wild-type cytoplasm or RNA (Anderson, 1987). The best candidate for a gene encoding such a product is *dorsal (dl).* This maternally active gene directs the progressive localization of *dorsal*$^{+}$ activity to ventral regions of early embryos.

FIG. 35.

Polar axiation in Diptera.

(A) Sequence of initial events of bipolarization in the eggs of *Drosophila:* (1) *bicoid (bcd)* coded-proteins localized at the anterior pole; *nanos (nos)* gene products localized at the posterior pole. (2) graded distribution of the *bcd* protein. *Hunchback (hb)* gene activated while cleavage nuclei have reached the posterior periphery of the developing embryo. Adapted from Ingham, 1988.

(B) Polarity deviation of *Drosophila* embryos mutationally-provoked: wt = wild type; *bcd* = *bicoid,* lacking head and thorax; *os* = *oskar,* lacking abdomen; *tor* = *torso,* lacking head and end of abdomen. Adapted from G. North, Nature, 332: 785, 1988.

(C) Embryological polarity (anterior and posterior indicator proteins) in the eggs of the midge fly *Smittia* disturbed by centrifugation (CE) resulted in double cephalon, by UV-irradiation resulting in double abdomen. In the middle, control eggs. Adapted from Kalthoff, 1984.

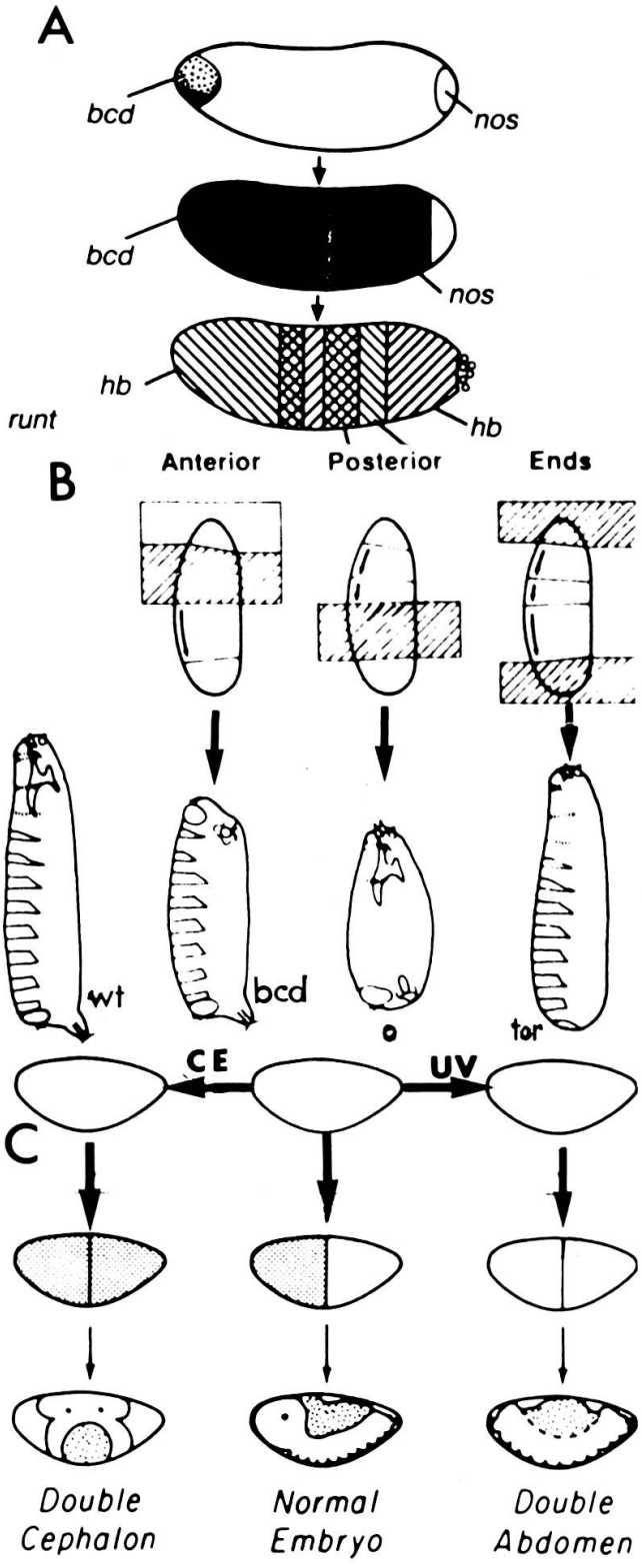
A
bcd
nos
bcd
nos
hb
runt
hb
B
Anterior
Posterior
Ends
wt
bcd
o
tor
CE
UV
C
Double Cephalon
Normal Embryo
Double Abdomen

FIG. 35.

Another of this D/V genes, *snake,* could be rescued by microinjection of a fragment of cloned DNA. Its encoded information is expressed in a serine protease, suggesting a role for this enzyme in the proteolytic cleavage of other spatially localized products (DeLotto and Spierer, 1986). It thus seems that the anterior body pattern of *D. melanogaster* is specified in large part by the protein product of *bcd* and *snake* genes which function as graded morphogens with their peak of expression at the anterior pole of the embryo (Driever and Nüsslein-Volhard, 1988). As a result of the preceding gene activities, when the mature egg is laid, it is both morphologically and molecularly polarized (Ingham, 1988).

The fundamental role of these gene products in pattern specification is illustrated by their mutant phenotypes. Thus, in the diencephalic mutation of *Drosophila,* nurse cells are found at both poles of the egg instead of only near the anterior pole as in a normal follicle. Bipolar follicles give rise to embryos having two sets of anterior structures (two-headed monsters) but altogether lacking posterior structures.

As commented by Gehring, in 1985, "observations of such maternal-effect mutants suggest that the egg cytoplasm contains substances that define the spatial coordinates of the future embryo. After fertilization, when the nuclei migrate to the egg cortex, they encounter these substances and become committed to particular fates according to their position in the cortical cytoplasm. Little is currently known, however, about the substances coded for by the maternal genes that endow the egg cytoplasm with its spatial polarity". There is only some recent evidence that maternal messenger RNA (mRNA) stored in the egg has a role in specifying the dorso-ventral polarity (Nüsslein-Volhard *et al.,* 1987).

Segmentation mutations

Embryological and genetic experiments provide compelling evidence that the segment pattern of the insect body is specified by a dynamic system of spatial cues generated at the blastoderm stage of embryogenesis. Each of the genes providing these spatial cues is defined by characteristic deletions of segments in embryos in which the gene is mutated. Genetic analysis has allowed *three* classes of zygotic segmentation genes, which relate to three different levels of spatial organization during segmentation, to be distinguished: gap genes act on the entire egg as a developmental unit, pair-rule genes act on double segmental units and segment polarity genes at the single segment level.

In the early *Drosophila* embryo, the transcripts of several pair-rule genes accumulate in a series of rapidly evolving and partially overlapping stripes (parasegments) along the antero-posterior body axis (Hafen *et al.,* 1984). During the late cellular blastoderm stage of *Drosophila* embryogenesis, the segmentation genes *engrailed (en)* and *wingless (wg)* become expressed in two series of 14 stripes which will subsequently coincide with the anterior and posterior limits of each parasegment

(Ingham *et al.*, 1988). Homoebox-containing pair-rule genes such as *fushi tarazu* (Japanese for "not enough segments") and *eve* which encode homeodomain proteins have been found to act as regulators of the segmentation genes: positively for *engrailed* controlling the pattern of stripes, negatively for *wingless* (Ingham *et al.*, 1988).

Asymmetrically distributed *dorsal*$^+$ products may play a key role in establishing diverse patterns of zygotic gene expression, and the differentiation of D/V pattern elements (Levine, 1988). For example, one of the crucial steps in the differentiation of the segmentation pattern along the A/P axis is the localization of maternally-encoded *bicoid (bcd)* transcripts to the anterior pole of the developing oocyte (Frohnhöfer and Nüsslein-Volhard, 1986; Frigerio *et al.*, 1986), which appears to be facilitated by an inherent A/P polarity within the *Drosophila* egg chamber.

Homeotic mutations

These dramatic derangements of *Drosophila* development could not be better described than by their most skillful hunter, Walter Gehring (1976), who has been fascinated by such mutations since 1965 when he described the mutant "Nasobemia" that had legs on its head in place of antennae. Previously found mutations in the homeotic gene *aristapaedia* also lead to antennal stuctures being replaced by leg ones (Pasteels, 1951).

The genes first isolated from homeotic mutants of *Drosophila* were defined as the homeobox (Gehring, 1984, 1987). They have DNA sequences in common which code for a protein that, by its functional segment or homeo domain, binds to DNA and, thereby, regulates all the genes needed to control insect morphogenesis.

The developmental pathways for many of the body segments (third thoracic and abdominals) are specified by a cluster of homeotic genes, the so-called *bithorax* (BX) complex (Bender *et al.*, 1983). Both left and right halves of this complex have been explored by chromosomal walks. Discrete genetic units are sequentially activated in each abdominal parasegment. Intricate patterns of BX expression result from the complex *cis*-regulation and the *trans*-interactions between homeotic genes that they mediate (Peifer *et al.*, 1987).

Another cluster of homeotic genes found in the *Drosophila* genome was referred to as the *antennapedia* complex and there are other mutations within the bithorax complex (Lewis' studies, see Gehring, 1985) including *bithoraxoid,* which make the first abdominal segment into a third thoracic segment. Moreover, positional information for the developing limbs is provided by a proximal-distal pattern-forming system controlled by Distal-less genes encoding a homeodomain protein (Cohen *et al.*, 1989).

d^2 *Wing patterns*

A good model system to investigate the generation of polarity pattern is the wing of *Drosophila.* It consists of a dorsal and a ventral sheet of epithelial cells (containing

± 26,000 cells per surface) that produce parallel hairs (Garcia-Bellido and Merriam, 1971). The bristles (four-cell sensory organs) and hairs (cellular extensions that later become sclerotinized) that decorate the wing cuticle have been used as an indicator of fundamental cell polarity (Nüsslein-Volhard and Wieschaus, 1980; Wieschaus and Riggleman, 1987).

The *frizzled (fz)* locus is required for the development of a parallel array of bristles and hairs on the adult cuticle of *Drosophila melanogaster* (Gubb and Garcia-Bellido, 1982). Weak *fz* alleles cause a swirling of hairs in some regions of the wing while strong alleles result in a nearly random orientation of hairs in much of its central region. In their efforts to understand how a group of cells, such as the epidermis of *Drosophila,* coordinate their cytoskeletal outgrowths to produce a parallel array, Vinson and Adler (1987) suggest that "*fz* has two mutably separate functions in establishing hair polarity on the wing. One function involves the transmittance and/or generation of a polarity signal along the proximal-distal axis of the wing. The second function involves the cellular interpretation of a polarity signal." The tissue polarity *fz* locus would encode a protein with seven putative transmembrane domains (Vinson *et al.,* 1989).

e) *Ascidian-Tunicates*

After fertilization, the ooplasm is rearranged by a spectacular episode of cytoplasmic movements known as ooplasmic segregation (Whittaker, 1979). This process begins with the myoplasm and ectoplasm along the vegetal cortex to the future posterior region of the embryo where they are extended into intensely pigmented crescent-shaped patches, the yellow crescent in *Styela* and the orange crescent in *Boltenia.* Ooplasmic segregation is concluded when the ectoplasm streams into the animal hemisphere, accompanied by the male pronucleus, and the endoplasm returns to its original position in the vegetal hemisphere.

Ooplasmic segregation and changes in the distribution of mRNA molecules are among the earliest indications of polarity in the ascidian egg. The colored ooplasmic crescent has been exploited to study this distribution of mRNA with respect to cytoplasmic localization (Whittaker, 1979).

Jaffe (1981) has suggested that a gradient of calcium ions, initiated from the point of sperm entry, polarizes the direction of ooplasmic segregation and mRNA localization in ascidian eggs. The orange crescent in the egg of the tunicate *Boltenia villosa* forms on the high side of a calcium ionophore A 23187 gradient formed with the glass fiber technique (Fig. 34 A). This pigment and mitochondrial segregation normally mark the future dorsal side of the embryo. However, when eggs are activated by the ionophore by placing it in the gradient, 82% of the activated eggs form orange crescents and mitochondrial localization with midpoints 45° or less from the point nearest the ionophore-coated fibers. Moreover, 10% of the eggs positioned

between two coated fibers form two orange crescents, each about half the normal size. This supports the hypothesis that there is *no* predeterminant polarity of cytoplasmic localization in the ascidian egg, and suggests that cytoplasmic Ca^{2+} gradients can strongly influence this axis of polarity (Jeffery, 1983).

Bipolar regeneration also occurs in very short pieces of the abdominal region of the ascidian *Clavelina* (Karp and Berrill, 1981). The anatomy of this ascidian is not important here, except that a nerve cord is not present. In this ascidian, the timing of cuts is as important as the distance between them. If short pieces are isolated by cuts made almost simultaneously, anterior (thoracic) structure forms from both surfaces. If the posterior cut is made an hour, more or less, *after* the first cut, typical anterior and posterior regeneration occurs at the two surfaces respectively; i.e., the original polarity of the tissue persists. The orientation, presumably of macromolecular components, may be more significant than concentration gradients of either ions or molecules in determining and maintaining tissue polarity. The electric DC current of injury may well be responsible for establishing the initial direction of polarization. That is, in short pieces resulting from simultaneous cuts, the polarizing current produces its effect in opposite directions from the two ends.

f) *Amphibians*

At fertilization the egg contains an animal-vegetal polarity that is interpreted morphogenetically during embryogenesis. Thus, the types of cells developing from different regions of the egg can within certain limits be predicted: "the animal cell cap provides ectoderm, neural tissue, and mesodermal cell of various kinds; the equatorial or marginal zone gives rise to a major portion of the mesoderm, as well as to other cell types; and the vegetal region provides endoderm, but also other cell types, including (in anuran amphibians) the germ cell progenitor" (Davidson, 1986). After the egg is activated by sperm penetration, the A/V axis is preserved, but its radial symmetry is superceded by the emergence of bilateral symmetry. In some anuran eggs (e.g. *Xenopus laevis*), the sperm entrance site is visible as a dark spot and conveniently marks the future ventral region (Fig. 36 A), while in several urodele eggs (e.g. *Amblystoma mexicanum*) only a surface pigmentation alteration — the gray crescent — marks the future dorsal area (Malacinski, 1984).

The dorsal-ventral axis is established initially by movements of preformed cytoplasmic consituents following fertilization, and prior to first cleavage. During cleavage the animal-vegetal and dorso-ventral axial specifications are transmitted to the blastomeres that inherit the respective regions of egg cytoplasm, and there is initiated the first of a series of inductive interactions by which the larval body plan is ultimately established (Fig. 36 B). This axis, which is set up during oogenesis, defines the future anterior-posterior polarity of the tadpole; it is thus an example of a developmental outcome predetermined by cytoplasmic localization of informa-

tion in the egg (Dawid and Sargent, 1988). The second major axis of the tadpole is fixed only after fertilization. Sperm entry can occur anywhere in the animal hemisphere and, as described above, the sperm entry point (SEP) normally defines the future ventral side. The way in which this polarity determination comes about

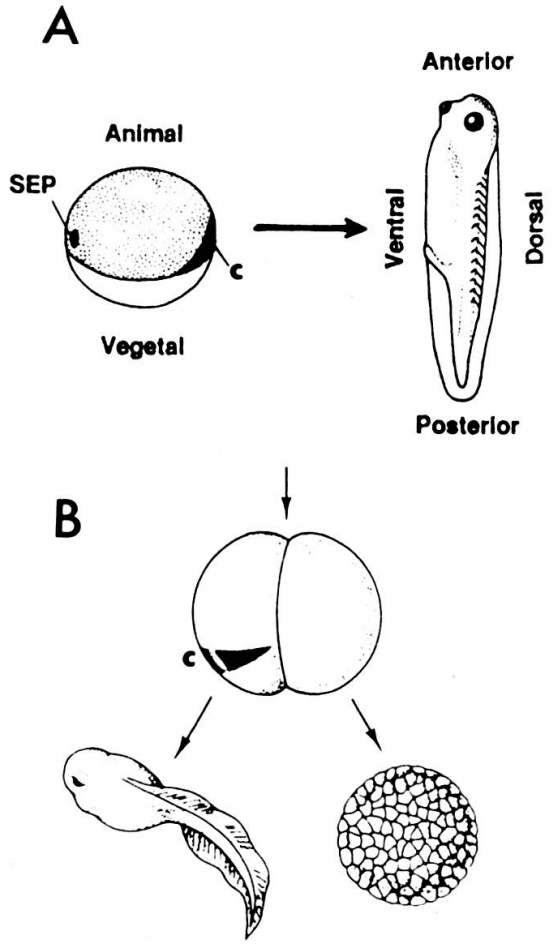

FIG. 36.

Polar axiation in developing Amphibians.

(A) Relation between the polarity of the fertilized egg and that of the tadpole. c = grey crescent; SEP = sperm entry pole. Adapted from Dawid and Sargent, 1988.

(B) Role of the grey crescent (c) in the early development of frog embryo: if the first cleavage (vertical arrow) does not pass through it, only one of the two daughter cells develops into an organized embryo. Adapted from Grant, 1978.

has been explained primarily through the work of Gerhart and colleagues (1983, 1986). A movement of cytoplasm relative to the cortex takes place between fertilization and first cleavage, and it is the direction of this movement that is the most accurate predictor of the future dorsal-ventral polarity of the embryo (Vincent *et al.*, 1986).

In normal development the vegetal pole cytoplasm or "germ plasm" is distributed to all of the first four blastomeres, and each has the capacity to generate a functional germ cell lineage. The primordial germ cells reside in the endoderm during early development (Grant, 1978).

A closer focus on the events occurring at the 8-cell stage reveals a radial intracellular polarization of the blastomere cytology, which is mediated by intracellular contact. At 4th cleavage the peripheral cytoplasmic regions are physically segregated to the outer cells of the 16-cell morula. By the end of the 8-cell stage a major cytoplasmic reorganization has occurred (reviewed in Johnson and Pratt, 1983; Johnson *et al.,* 1984). The peripheral cytoplasm of the polarized 8-cell stage blastomere acts as a determinant for trophectoderm differentiation, while the cells inheriting the internal cytoplasm are destined to become ICM cells, i.e., unless they are placed in circumstances where they are induced to polarize anew. The overall consequence of this polarization is a change in form of the embryo from a loose aggregate of spherical cells to a tightly compacted, radially organized embryo.

Polarization is an inductive process which requires intercellular contact. Furthermore, continued cell contact is needed to maintain the state of polarization initially imposed. When cultured *in vitro,* polar 16-cell blastomeres display trophectoderm-like behaviour, and they wrap around nonpolar cells, while apolar cells cultured together tend to polarize, and to produce aggregates including both external trophectoderm-like cells, and inner, ICM-like cells (Johnson and Pratt, 1983).

Both the cytoskeleton and gravity act in the determination of the dorso-ventral polarity (Ubbels and Brom, 1984). Application of centrifugal forces (Dalcq and Pasteels, 1937) can overrule the strength of the cytoskeletal elements and reverse D/V polarity even shortly before first cleavage. Such experiments strongly argue for a role of gravity in the determination of embryonic polarity.

A secondary dorso-ventral RNA gradient superimposes itself on the primary polarity gradient. As a result of further synthesis and of morphogenetic movements, antero-posterior (cephalo-caudal) and dorso-ventral RNA gradients are present in the neurula and young tadpole. These gradients parallel the well-known "morphogenetic gradients" of experimental embryologists (Brachet, 1967). If, as we have assumed, a dorso-ventral gradient of messenger RNA production exists at the gastrula stage, cephalo-caudal and dorso-ventral gradients in the distribution of polyribosomes, and hence of protein-synthesizing ability, would necessarily be formed in gastrulae and neurulae. The existence of dorso-ventral and animal-vegetal gradients can also be demonstrated in studies on the reduction of vital dyes. Thus dorso-ventral reduction gradients are very apparent in early and late gastrulae and the vegetal pole shows the least activity. The parallelism between the amphibians and the echinoderms (VIII.B.2c) makes it likely that gradients in the distribution of mitochondria also exist in amphibian eggs: a dorso-ventral gradient would

superimpose itself, at gastrulation, on the initial animal-vegetal gradient (Brachet, 1957).

g) *Fishes*

In many shark genera, teeth shapes are polarized, and their cusps are deflected in a lateral-posterior direction parallel to the jaw margin. Developmental studies suggest that polarity and tooth shape are regulated independently as shown by experiments of injury induced polarity reversals (Reif, 1984).

h) *Birds*

On the vitelline surface of birds' eggs, the primitive embryonic streak develops a thickening, the Hensens's node. As the node retreats, the body axis is taking shape anterior to it. Thus, the formation of the anterior end of the body is proceeding well before gastrulation has been completed posteriorly. The polarity of the avian embryo is thus evident prior to gastrulation, during the formation of the area pellucida. The thinning of the area pellucida begins at the presumptive posterior end, where the aggregation of cells to form the hypoblast also begins. This end of the area pellucida retains its organizational capacity at gastrulation as well. However, if the entire area pellucida of an embryo is rotated 180°, the embryo develops with reversed polarity while if a central square — devoid of the posterior margin — is cut out of the area pellucida and rotated, polarity is unaffected. These experiments conducted by Spratt and Hass (1960) provided evidence of the production by the posterior zone of the area pellucida of a morphogenetic factor organizing polar axiation of the avian embryo (Fig. 37 B_1).

The determination of bilateral symmetry of chick blastodiscs, earlier thought to be caused by the rotation of the egg in the mother's uterus, is, in fact, a result of the force of gravity. Axial symmetry of the blastodisc can be changed by altering its spatial position and axis determination shown to be a gradual phenomenon correlated with the morphogenetic process of formation of the area pellucida (Eyal-Giladi and Fabian, 1980).

The polarizing region, a small group of cells at the posterior margin of the avian limb bud, acts as a signalling region to specify the pattern of structures which develop across the antero-posterior axis of the limb. There is indirect evidence that the signal from the polarizing region is a diffusible morphogen (Tickle, 1980). The effects of multiple polarizing region grafts have also shown a positional signalling along the antero-posterior axis of the chick wing (Wolpert and Hornbruch, 1981).

i) *Mammals*

In the mouse, the earliest signs of contact-induced polarization in blastomeres might occur as early as the four-cell stage but throughout this period the polarity that is induced is labile (Johnson and Ziomek, 1981*a, b*).

The induction of polarity in the eight-cell mouse blastomere takes between three and five hours and after this period the axis of polarity is stable. Prior to this, however, a change in the location of the inducing signal will result in a predictable change in the final axis of polarity achieved (Johnson and Ziomek, 1981*a, b*). The axis of polarity can be controlled by the geometry of the contacts (Fig. 37 A): the apical pole always forms at a point opposite the contact, and in the case of multiple contacts, at a point equidistant from them.

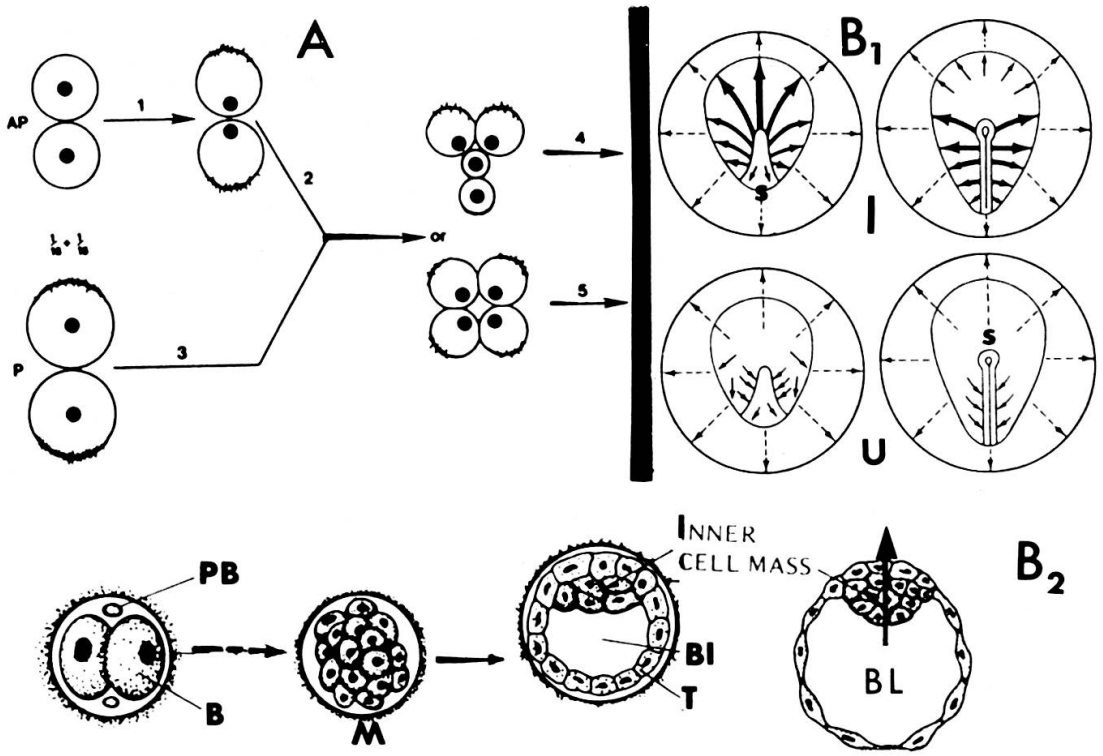

FIG. 37.

Polarity in Birds and Mammals.

(A) Induction of polarity in two apolar cells due to their asymmetry of contacts (1) at early embryogenesis in mouse. A couplet of polar cells then behaves in one of two ways: both cells may divide parallel (3) to the axis of polarity; when they divide normal (2) to the axis of polarity each of them generates one apolar and one polar cell. Adapted from Johnson and Pratt, in Jeffery and Raff, 1983.

(B_1) Polarized movements of embryonic layers in the expanding blastoderm of chick. The thickened arrows from the anterior portion of the primitive streak(s) mark the ingressing cells of presumptive endoderm and mesoderm. Lower (l) and upper (u) surfaces. From Spratt and Hass, 1960, in Grant, 1978.

(B_2) Early human embryogenesis: from the first holoblastic cleavage, through the relatively apolar morula (M) arises a blastocyst (Bl) anterio-posteriorly polarized (arrow). B = blastomere; PB = polar body; ICM = inner cell mass; T = trophoblast. After Loomis, 1986.

During the life of each eight-cell blastomere, the surface of the cell is reorganized fundamentally to yield a polar phenotype with an outward apical pole of microvillous membrane and an inner basolateral membrane largely devoid of microvilli (Johnson and Pratt, 1983). This polarized surface phenotype assumed by each eight-cell blastomere is stable and persists after isolation of single cells and during the division of eight-cell blastomeres to the 16-cell stage either in isolation or in situ (Johnson and Ziomek, 1981*b*; Reeve, 1981).

In conclusion, contacts with competent inducing cells appear to account for polarization of rodent embryos while induction of polarity does not require the formation of intercellular junctional complexes (ionic media, antisera, and drugs that block junction formation do not prevent polarization, Johnson *et al.*, 1984). However, remain the questions rised by Johnson and Pratt (1983) "Since developmental localization within the mouse embryo first becomes stabilized at the eight-cell stage, does this mean that the cell interactions leading to polarization occur under embryonic rather than maternal control? This question cannot yet be answered unambiguously. However, it does seem likely that transcriptional activity is not required during the four-cell and eight-cell stages."

In man, it is only at the postimplantation period of a blastocyst to the uterine wall that the embryonic A/P axis is established (Fig. 37 B_2). Microvilli in the blastomeres surface contain bundles of contractile microfilaments which may function in bringing together the surfaces of adjoining embryo cells. Certain microvilli are localized on the apical surface of these blastomeres, giving each cell a distinct polarity. It is the apposition of the adjoining surfaces which leads to the formation of desmosomes, gap junctions, and tight junctions between the cells of the embryo, which are believed to have important structural and developmental roles (Karp and Berrill, 1981).

3) Triaxial patterns (A/P + D/V + Left-Right (L/R) polarities)

a) *Helical bacteria*

Many types of bacteria are helical. Of the 17 identified species of the Aquaspirilla, twelve are right-handed and three are left (two are straight). It is claimed that within a species all individuals have the same hand.

A temperature-induced switch of hand in the highly organized multicellular structure of *Bacillus subtilis* "macrofibres" has also been observed and studied in some detail (Favre *et al.*, 1985). But are either of these inversions (reflections) genetic in origin?

b) *Green plants*

Cell division of the apical cell of root on the right-handed side of the water fern, *Azolla,* is clockwise (right-handed); similarly, in root of the left-hand side of the plant, division of the apical cell proceeds anticlockwise (Gunning *et al.,* 1978).

c) *Protozoa*

Mirror-imaging by reversal of right-left asymmetry has been observed in many Protozoa. Mirror-image patterns can arise without microsurgically generated polar reversals. The clearest cases have been observed in *Blepharisma* (related to *Stentor*) and in *Tetrahymena.* There are two such situations, one involving a usual pattern juxtaposition in wild-type cell, the other the action of a mutant gene. In the *janus* symmetry-reversal mutant, the ciliary rows of such cells are always normally oriented and never inverted, indicating that there has been an *in situ* reversal of right-left asymmetry without any preceding folding or rotation of cell parts (Frankel, 1984).

A considerable body of circumstantial evidence supports the idea that water temperature has determined the hand of some Foraminifera tests. In *Globigerina* spp., left-handedness is associated with low and right-handedness with higher temperatures (Galloway, 1987).

d) *Worms*

The crown of the sabellid worm is bilaterally distinct, the right and left halves developing independently of each other (see Karp and Berrill, 1981).

e) *Molluscs*

A classical example, discovered half century ago, is that in the mollusc shells the chirality of the spiral cleavage is controlled by maternal genes expressed during oogenesis. Indeed, dextral cleavage depends on a product of the dominant gene (Freeman, 1983). The freshwater snail, *Lymnaea peregra,* has either right-handed (dextral) or left-handed (sinistral) twists to its shells. The patterns of cleavage in *Lymnaea* embryos is also spiral, and even at the first division a handedness can be seen. They cleave in either a dextral or sinistral manner. By the third cleavage the asymmetry is easily observed. The decision as to right- or left-hand cleavage that ultimately results in snails with differently twisted shells is determined by a single gene, *D.* Mutations in this gene result in left-handed cleavage that leads to left-handed snails. The product of the *D* gene must be present in the egg before fertilization for dextral cleavage can occur (Freeman and Lundelius, 1982).

At both the animal and vegetal poles of the red or green colored eggs of the elephant tusk molluscs, *Dentalium,* there are unpigmented regions of cytoplasm relatively free of yolk. The first few cleavages of *Dentalium* are markedly asymmetric. Following fertilization, the bulk of the yolk-free cytoplasm at the vegetal pole is blebbed out into a lobe (Loomis, 1986). Animal-vegetal polarity in the plasma membrane of the molluscan egg has been studied by quantitative freeze-fracture (Speksnijder *et al.,* 1985). The yolk-free material in the polar lobe is essential for differentiation of the mesoderm rudiment in the larva. If the polar lobe is nipped off at the time of either the first or second cleavage, the larva that develops lacks the mesoderm rudiment.

Steric constraints apparently lie behind molecular processes and if they permit one hand, almost without exception, they forbid the other. Could steric considerations similarly explain the preference of right- over left-handed conchospirals? Perhaps surprisingly, the answer may well be yes — at least in part — surprisingly because the steric considerations in this instance are behavioural (Galloway, 1987).

How could evolution discriminate against a rare left-handed form in a predominantly right-handed population and *vice versa*? Sturtevant (1923) may have made the significant observation here. Commenting on rare left-handedness in the European pond snail *Lymnaea peregra,* he pointed out that the left- and right-handed forms might not be able to mate with each other, not having a compatible screw sense! Handedness in snails is a simple mendelian characteristics (although with the interesting feature that it is an example of maternal inheritance — the hand of coiling is determined not by the individual snail's own genes but by those of its mother). Presumably an effect of the left- and right-handed forms not mating with each other would be to create separate breeding populations.

In *Lymnaea,* a trait inherited maternally should be seen in all the offspring from the same hatching. Thus, any broods of snails should be exclusively left- or right-handed. In practice, some broods possess a few snails of the opposing hand, and in sinistral broods the mutation rate to dextrality is quite high. Freeman and Lundelius (1982) have proposed a genetic model for this phenomenon. The power of their model — or alternatives to it — to explain predominant right-handedness needs exploring.

What is now known from studying *Lymnaea* is that the dextral gene is expressed during oogenesis and its product — whatever it is — controls the symmetry of the pattern of very early cleavage. An injection of cytoplasm from dextral eggs reverses the early cleavage pattern of sinistral eggs, but one from sinistral eggs does not influence dextral ones. To resolve this apparent paradox, Galloway (1987) suggested that the normal form of *L. peregra* is actually left-handed, switchable to right-handed by the protein product of the dextral gene. Freeman and Lundelius (1982) speculate that what they have found in *Lymnaea* would be found exactly reversed in a left-handed species like *L. suturalis.* Thus the path from gene locus to cleavage pattern is likely to be far from straight-forward.

f) *Insects*

In insects, the transverse aspect of embryonic pattern formation becomes apparent in the subdivision of the germ "anlage" blastema into the left and right half, with the various structures or organ rudiments arranged from medial to lateral in mirror image symmetry on either side of the midline. Data indicate that bilateralization and transverse patterning can occur fairly late in development, and are systemic properties of the embryonic blastema rather than of the egg cell, despite the bilaterally symmetrical shape of the latter and its shell (Sander, 1984).

g) *Amphibians*

The characteristic left-right asymmetry of the definitive embryo results from slight differences in mesoderm-inducing capacity between the lateral portions of the yolk mass (Nieuwkoop, 1977). Artificial inversion of eggs has also been known for some time to result in the production of double embryos with two blastopores (Pasteels, 1938), and it has recently been shown that the same can occur even at the 2-cell stage if embryos are centrifuged at 30-50 g towards the ventral side (Gerhart *et al.*, 1981).

h) *Mammals*

It is of common knowledge that embryogeny normally directs some way the liver to the right half of the body while the heart is positioned on the left, in an equitable crosslink with the right brain hemisphere, the seat of affectivity and intuition!