VIII. MORPHOGENETIC POLARIZATIONS

Applied electric fields can impose their own polarity on developing cells and organisms (Lund, 1947 in **I**; Nuccitelli, 1988). However, and even though there are many examples of morphogenetic implications of endogenous ion currents generated by bioelectric fields (see **I-III**), caution should be exercised about "an obligatory connection between morphogenesis and bioelectric fields or transcellular ion currents" (Harold, 1986 in **I**).

A. PLANTS

1. *Embryonic polarity*

The apical-basal polarity of the embryo with the shoot and the root meristems located at opposite poles of the main body axis are controlled by patterning genes and their still unknown products (Jürgens *et al.*, 1991). The identified genes act very early in plant embryogenesis. Among these early events, formation of tissues does not require apical-basal polarity. Thus, pattern formation along the axis of polarity and formation of the radial pattern are two separate processes. As for the apical-basal axis it would be initially partitioned into three apical-central-basal regions (Mayer *et al.*, 1991). This early partitioning of the axis bears some superficial resemblance to similar events in the *Drosophila* segmentation process where the gap genes are involved in the initial regionalization of the anterior-posterior axis (see VIII.B.1.g).

Abnormal polarities have been further studied in higher plants and defects have been noticed in the apical-basal pattern before the heart stage of cruciferous embryogenesis (Meyerowitz, 1991). Nine genes have been found to affect three different aspects of the body organization in the *Arabidopsis* embryo: apical-basal pattern in the axial polarity, radial pattern, and shape (Mayer *et al.* 1991).

2. *Organismic polarities*

a) *Mushrooms*

They grow upwards but "how do they know which way is up?" Unfortunately, no gravity-sensing apparatus has yet been identified in fungi. However, perception and response to gravity have been studied: differential elongation of the stipe growth zone was shown to drive gravitropic reorientation of disoriented fruiting bodies (Moore, 1991a); when such cultures of *Coprinus cinereus* were rotated on a clinostat, they aborted at the primordial stage (Moore, 1991b). Curvature experiments with explanted

fruiting bodies of *Flammulina velutipes* demonstrated a restricted localization of the graviperceptive region in the connective area of pileus and stipe of the fruiting body (Kern et al., 1991). Grafting experiments in a *F. velutipes* have shown that the polarity of translocation in the elongating stipe is reversible (Gruen, 1991).

Cytoskeletal elements may be involved in fungal graviresponse (Monzer *et al.*, 1992) as also suggested in moss protonema (c^4). Mitochondria might also be involved in the perception and transduction of the gravitational stimulus (Block and Briegleb, 1989). Future space missions providing microgravity conditions should further answer pending questions about graviperception.

b) *Colonial algae*

A central role has been ascribed to the basal body apparatus, and particularly its microtubular rootlets, in the control of asymmetric divisions that pattern the *Volvox* embryo (Kirk *et al.*, 1991).

c) *Green plants*

According to Sachs' recent review (1991a), there is a positive feedback relation between cell polarization and the transport of auxin: "polarity determines oriented auxin transport while transport itself induces both new and continued polarization". However, polarity could not be specified only by differences in the concentrations of "morphogens" involved in the early establishment of their "prepatterns" (Wolpert, 1971 (**I**), 1989 (**III**), Meinhardt, 1982 (**I**). In his recent book, Sachs (1991b) has proposed an hypothesis of "epigenetic selection" in which decisions on cell fate depend on dynamic prepatterns.

c^4 *Polar auxin transport and tropic curvatures*

Polarly transported indole acetic acid (IAA) is assumed to play an important role in vascular differentiation (see Aloni, 1988) and initiation of lateral roots (see **I**). Elongation growth is correlated with free IAA in etiolated lupin hypocotyls (Sánchez-Bravo *et al.*, 1991). It should now be further confirmed the existence of an IAA gradient between the transporting and the growth competent cells. Polar transport of auxin has also been involved in other growth processes such as phototropic curvature (Firn and Digby, 1980; Baskin *et al.*, 1986).

Gravity is one of the most important formative factors for plants and, among its tropistic effects, there are the modifications of symmetry from radial to dorsiventral or vice and versa (Sinnott, 1960 in **I**). As suggested by space-flight experiments, the orientation of root growth is directed by gravity while that of shoot is guided by both gravity and light. As gravitropic responses to these space-flights, there are cytological abnormalities indicating a disturbance in the mitotic process (Halstead and Dutcher, 1987). These effects of microgravity on mitotic index and root orientation could not be

simulated by clinorotation (Legué *et al.*, 1992). In gravitropically responding moss protonema, microtubules accumulate at the lower flanks of the tip cells (Schwuchow *et al.*, in Herth *et al.*, 1990).

c⁵ *Flowering shoots*

Homeotic changes have been further investigated in floral organs (Coen and Meyerowitz, 1991). The expression pattern of the *Arabidopsis* genes is in part established by regulatory interaction between these genes (Drews *et al.*, 1991). In their attempt to integrate molecular data in genetically grounded models of development, Veit *et al.* (1991) have strategically considered floral development in maize. Among its known mutants, pistil-like structures develop anthers in ear of silky.

B. ANIMALS

In his approach to the old problem of tissue polarity, Waddington (1941 in 1962, see **I**) had advocated the stretching role of attachment bodies and desmosomes at the ends of cells lined up in the anterior-posterior direction of gastrulating amphibian embryos. This interesting suggestion still remains unfortunately speculative. A related problem is the anteriorization of the cylindrical nervous tube which is a crucial event of developing embryos.

In these addenda **IV**, the three possible biopolar axiations will be presented in their developmental sequence (1-3) rather than in their more difficult to define sequential, mono- to triaxial, hierarchized patterns (**I** to **III**). Of the three geometric axes of a vertebrate body, i.e. anterior-posterior, dorsal-ventral and left-right, the formation of this last, displayed in heart and liver development, is still the least understood (Oppenheimer, 1974 in Yost, 1992 and other ref. in **III-IV VIII.3**).

Three pattern-forming sets of genes are necessary and sufficient for the specification of 1) the anterio-posterior (A/P) axis: the anterior system (A), responsible for the segmented region of head and thorax, the posterior system (P) which determines the segmented abdomen, and the terminal system (T) which determines the nonsegmented acron and telson; 2) the dorso-ventral (D/V) axis is determined independently of that of the A/P axis (Nüsslein-Volhard *et al.*, 1987 in **I**; St Johnston and Nüsslein-Volhard, 1992; Lawrence, 1992); 3) the bilateral, left-right or handedness axis. Lipshitz (1991) has further reviewed axis specification in the *Drosophila* embryo and has considered that "three genetic hierarchies control cell-fate specification in largely distinct regions of the antero-posterior axis of the embryo, whereas a single hierarchy specificies dorso-ventral cell-fates". A link between dorso-ventral and anterio-posterior patterning has also been suggested by studies of certain genes (*capu* and *spir*) in *Drosophila* (Manseau and Schüpbach, 1989).

Growth factor and homeobox genes have been implicated in the establishment of positional identity in the embryonic body axes (Melton, 1991 in **III**). Specific effects of

morphogens such as retinoic acid (RA and see 1 . k) on anterior-posterior and dorso-ventral positional identity have been demonstrated in regenerating limbs of vertebrates (Stocum, 1991). Changes in the localization of homeobox proteins can be induced by RA in the anterior-posterior axis of *Xenopus laevis* embryos (López and Carrasco, 1992). Concerning the diffusible signalling molecule RA, there are opinions that it is "a poor candidate for a morphogen" (Williams and Hogan, 1991, see also Slack in III) even though there is some evidence to suggest that RA may direct several different developmental processes (1 . k).

1. ANTERIOR-POSTERIOR (A/P) POLAR AXIATION (MONOAXIAL PATTERNS)

Homeobox genes are an ubiquitous feature of polarity in multicellular organisms and the first gene complexes which control anterior-posterior polarity might have evolved from the level of primitive animals such as rotifers and flatworms. In both invertebrates and vertebrates, homeobox gene clusters on a chromosome are arranged in a precise order and read polarly from left to right; therefore, on the linear DNA molecule "left genes" are expressed in posterior body part and "right genes" closer to the head (D. Duboule and R. Krumlauf in De Robertis *et al.*, 1990, see **III**).

In a recent EMBO Workshop, aimed to unravel the mechanism of action of the homeodomain at the level of its interaction with DNA, it has been highlighted that in the conformational foldings of the α-helices of homeodomain proteins, specific interactions might be mediated by H_2O, "given the strong angle or orientation dependence of H bonds" (Riddihough, 1992).

Members of conserved *Antennapedia*-class homeobox gene clusters (HOM-C) are thought to give specific body regions their identities (Gehring, 1987 see **I**; Kenyon and Wang, 1991). HOM-C genes especially *mab-5* can not only direct region-specific patterns of cell division and differentiation but can also act with migrating cells to program region specific migratory behaviour (Salser and Kenyon, 1992).

a) *Mycetozoa (slime molds)*

The *Dictyostelium* slug is a regulative structure, in which extracellular signals act to direct a polarized cellular differentiation. Positively and negatively acting signals regulate its stalk cell and anterior-like cell differentiation (Ceccarelli *et al.*, 1991). Cell transplantation experiments have shown that "when posterior prestalk cells are transplanted to the prespore zone, respecification of sorting preference is suspended until the cells return to the prestalk zone" and "anterior-prestalk cells acquire posterior-prestalk sorting preferences" (Buehl and MacWilliams, 1991).

c) *Hydrozoa*

Head and foot factors have been involved in the establishment and maintenance of polarity in hydra. The foot regeneration deficiency of a strain of *Hydra oligactis* was shown to be due to a drastically reduced foot activator (Hoffmeister, 1991).

d) *Worms*

The axis of *Caenorhabditis elegans* embryos results of the specific rotational movement of the pair of centrosomes and nucleus (Hyman and White, 1987). Perturbation experiments of the centrosome movement by a laser support this positioning model possibly involving dynamic microtubules (Hyman, 1989). It has recently been found that the order of homeobox genes along the chromosomes of the nematode *C. elegans* corresponds to the order of expression domains along the anterior-posterior axis of the animal (Bürglin *et al.*, 1991).

g) *Insects*

Determination of polarity along the longitudinal axis is carried out by the maternal genes (Nüsslein-Volhard *et al.*, 1987 in **I**; Reid, 1990) while the polarized distribution of their gene products leads to the regional expression of the zygotic gap genes (Ingham, 1988 in **I**). In the anterior system, *bcd* interacts with *hb* while in the posterior morphogen system, *nos* does not seem to interact directly with the posterior gap gene *knirps* (Pankratz *et al.*, 1992).

Maternal regulatory systems function to position a transcription factor asymmetrically thereby contributing primarily to define the longitudinal, anterior-posterior axis. Graded activity of the posterior morphogen determinant *nos* controls the transcripts of the *hb* maternal gene and hence abdominal pattern (Wharton and Struhl, 1991). *Nos* gene has been isolated and molecularly characterized and its RNA shown to be functionally equivalent to the morphogenetic activity found in the posterior pole of the *Drosophila* embryo (Wang and Lehmann, 1991). It has also been suggested (Brönner and Jäckle, 1991) that it is by repression of central gap genes that the terminal gap genes *huckebein* (*hkb*) and *tailless* (*tll*) possibly functioning in the posterior pole region of the *Drosophila* embryo prevent its metamerization.

An interesting case is presented by the early *Drosophila* embryo which is a syncytium that permits the intermixing of regulatory factors by diffusion. The choice in cell fate might involve a slight asymmetry in the concentrations or activities of regulatory factors inherited by daughter cells and setting up on-off patterns in gene expression such as that of segmentation even *skipped* (*eve*) of the strip 2 element (Stanojevic *et al.*, 1991).

k) *Birds*

Retinoic acid has the same effect as the polarizing region of the limb bud when placed at its anterior margin: mirror-image duplicated limbs with additional digits

results (Tickle *et al.*, 1982, 1985). Some authors have cautioned that the role of retinoic acid as an endogenous morphogen is still speculative (see Brockes, 1990, 1991 and **III**). For a review about homeobox genes as possible targets for regulation by retinoic acid and their possible encoding of positional values in chick limb buds, see Tickle (1991).

Morphogens are diffusible substances that can form a concentration gradient across an embryonic field (Wolpert, 1969 in **I**). The controversy about them is continuing (Newman - Slack in *Nature* 354: 26, 1991) ... "We have" (see Slack in **I**) or "may not have" (Slack in **III**) them. However, we now have some candidate morphogens, the retinoids and some candidate primary response elements, the Hox-4 genes (Izpisùa-Belmonte *et al.*, 1991 in **III**; Stocum, 1991) which probably encode positional information (Tickle *et al.*, 1975).

The concept of the Hox complex is a colinearity between the arrangement of the genes along the chromosomes and the spatial extent of their expression domains: 3'-located homeobox genes with *anterior* expression boundaries, *posteriorly* restricted expression for 5'-positions within a Hox complex (Dollé *et al.*, 1991). For further information about polarized molecular models for limb development, especially how "*Hox* genes and the anterior-posterior axis interact", see Tabin (1991), and Stocum (1991) for problems of polarized regeneration.

2. Dorso-ventral (D/V) axiation (Biaxial patterns)

A single set of genes determining an asymmetric distribution of their products is responsible for that pattern (Nüsslein-Volhard *et al.*, 1987 and Govin and Steward, 1991, cited in Casanova, 1991).

d) *Insects*

d[1] *Egg-embryo patterns*

Laser ablation of the oocyte nucleus has shown its requirement for dorso-ventral patterning of the *Drosophila* embryo (Montell *et al.*, 1991).

As now summarized by Stein and Nüsslein-Volhard (1992), twelve maternal effect genes (the dorsal group and *cactus*) are required for the establishment of the embryonic dorsal-ventral axis in the *Drosophila* embryo so successfully analyzed by the genetic approach (Nüsslein-Volhard and Wieschaus, 1980 in **I**; Nüsslein-Volhard, 1991). The ventral formation of a ligand for the *Toll* receptor defines this type of embryonic polarity within the perivitelline compartment. The polarity along the embryonic dorsal-ventral axis is initiated by an extracellular signal, with the dorsal gene *Toll* (*Tl*) product which plays the role of a receptor (Stein *et al.*, 1991). They propose that "the polarizing activity, normally present at the ventral side of the egg is a ligand for the *Toll* receptor". In both the dorsal (*Tl*) and the terminal (*tor*) systems, an evenly distributed membrane protein has been identified and it has been suggested that a ligand for *tor* is expressed (Stein *et al.*, 1991).

d² *Wing patterns*

Segment polarity genes appear to be involved in intrasegmental patterning as suggested by their mutations which alter discrete regions within each segment of the *Drosophila* larva (Ingham, 1990). For instance, in *wingless* (*wg*) larvae the denticles which cover the naked cuticle are oriented towards the midline of each apparent segment (Rijsewijk *et al.*, 1987; also see Ingham *et al.*, 1988 in **I**), whereas in the *ptc* (patched)-*wg* double mutant this loss of polarity cues with respect of *wg* larvae could be explained by the epistasis of *wg* over *ptc* (Hidalgo, 1991).

Unrestricted expression of the gene *ptc* which encodes a transmembrane protein allows a normal segment polarity (Sampedro and Guerrero, 1991). Positional signalling in the cellularized *Drosophila* embryo requires cell interactions expressing the homeodomain protein *engrailed* and the secreted glycoprotein encoded by *wingless*. The receptor for this signal might be the *patched* protein itself (Ingham *et al.*, 1991 and ref. herein).

The generation of dorso-ventral polarity in the early embryo of *D. melanogaster* relies upon the expression of 12 maternal-effect genes termed the dorsal group (Gay and Keith, 1991). In the set of maternal genes which define the D/V pattern, the "dorsal group" acts only on the polarity of the embryo and the gene *dorsal* encodes a nuclear protein acting as a morphogen (Nüsslein-Volhard and Roth, 1989). The other maternally genes act to set up the gradient of this protein in the correct orientation (Roth *et al.*, 1989). Sequence-specific expression of *twist*, a zygotic gene required for differentiation of mesoderm along the D/V axis, is transactivated by the dorsal gene product (Thisse *et al.*, 1991). All these "smart genes" that through elaborate chemical messengers control polarized differentiation and morphogenesis are being further deciphered (see Beardsley, 1991).

f) *Amphibians*

In early embryogenesis, the fibroblast growth factor (FGF) signalling pathway plays an important control in the formation of the posterior and lateral mesoderm (Amaya *et al.*, 1991).

Results obtained by Sokol *et al.* (1991) show that microinjected synthetic mRNA (Wnt oncogenic type) can induce a new and complete dorsal axis. However, injection of Xwnt-8 mRNA can also rescue the development of a dorsally complete anterior-posterior axis in UV-irradiated ventralized *Xenopus* embryos (Smith and Harland, 1991). Cells were thus led to act as a Nieuwkoop center (the vegetal-inducing component of normal dorsal axis formation).

i) *Mammals*

Microtubules play a role in the asymmetrical, epithelial cell divisions (see VII) and the onset of cell polarity during mouse early embryogenesis (Maro *et al.*, 1991).

3. BILATERAL ASYMMETRY OR L/R HANDEDNESS (TRIAXIAL PATTERNS)

This 3rd component of the axial biopatterns complements the two other polar axiations of most advanced organisms, from the worms to the human beings even though it is already "prototyped" in helical bacteria and many protozoa in which it is superposed on their usual anterior-posterior axis according to a biaxial type of pattern. In vertebrates such as *Xenopus laevis* gastrulae, the left-right asymmetry is regulated by a fibronectin-rich extracellular matrix (Yost, 1992).

The question asked by Wolpert and others of why there are left-right asymmetries in organisms from bacteria to vertebrates has been summarized in the proceedings of a recent Ciba Foundation Symposium edited by Bock and Marsh (1991). The central problem remains of how three-dimensional structure, determined by primary structure, is determined by the asymmetry of basic monomers. "An epistemological gap exists in embryology between understanding structures that arise from cellular self-assembly and understanding those that require higher levels of causation" as commented by Slack (1992).