

with *Drosophila* (at least in having compound eyes), ought to have differentiation waves:

"The brine shrimp *Artemia* forms dormant, gastrula-stage embryos in cysts (Von Benesch, 1969)... In the late fall, these 0.2 mm cysts float to the surface of the water of certain hypersaline lakes in temperate regions and are deposited on the shoreline.... [After drying] respiration does not begin until water is added (Clegg, 1978; Lavens & Sorgeloos, 1987; [cf. Spooner et al., 1992])....

"When a brine shrimp cyst, with its 4,000-cell embryo is incubated in water, 10-18 hrs later an elongated prenauplius emerges, three times the size of the cyst [but with no increase in the number of cells] (Nakanishi et al., 1962); therefore, development is totally one of cell, tissue and organ differentiation. To account for the increase in size of the animal, and for the complexity of the morphogenesis that follows, there is considerable enlargement of cell diameter and length that accompanies differentiation (Anderson, 1967; Von Benesch, 1969)" (James R. Rosowski, p.c., 1996).

In summary, mitotic waves coincide with differentiation waves in some organisms; in others they are weakly coupled; and in yet others, they are absent, while differentiation waves would appear to persist. Thus it would appear that in terms of evolution, a separation of mitotic waves and differentiation waves has occurred, in such a way that the former are at least now secondary.

9.05 Quantal Mitoses and a Model for Limb Morphogenesis

The initiation of changes in the unfolding of the genetic program (used in the singular, for one species, in this book), what Holtzer (1970) would attribute to a quantal mitosis, I would attribute to a differentiation wave, though if the two are indeed highly correlated, separating cause and effect will take a bit of work. Mammals, for which the neural plate grows while forming (Jacobson & Tam, 1982), in contrast to amphibians (Jacobson & Gordon, 1976a), may particularly show up differentiation waves correlating with mitotic waves. The regional effects of heat shock, for instance, may be due to failure of neural plate differentiation waves to launch or propagate, or

may represent effects on cell proliferation. These alternatives could be distinguished by time-lapse microscopy.

"Synchronized regulation of cell division during gastrulation is essential for the regional proliferation of cells and pattern formation of the early CNS. The neural plate and neuroectoderm cells are a rapidly dividing and differentiating population of cells with a unique and rapid heat-shock response. Heat shock and the heat-shock genes were studied during neural plate development in a whole rat embryo culture system at 9.5-11.5 days. A lethal [heat] shock can cause cell death and severe developmental defects to the forebrain and eye during organogenesis.... The heat shock genes may therefore function as cell cycle regulators in neuroectoderm induction and differentiation" (Walsh et al., 1993). [Heat exposure is implicated in many human neural tube birth defects: Milunsky et al., 1992.]

Duboule (1994b) has implicitly revived the concept of quantal mitosis for vertebrates by suggesting that some sort of 'meta-*cis* mechanism' translates along a colinear sequence of homeobox genes, each step requiring one cell division. Furthermore, the mitoses move on through the tissue from anterior to posterior along the axis, or proximal to distal along a limb, in what I suppose could be called a pulsed mitotic wave. How "slight modifications to the domains of Hox gene expression [which] may have made important contributions to the evolution of vertebrate morphologies" come about is not discussed. These could be but the trajectories of a set of consecutive differentiation waves, each triggering cell divisions in its path. (Such a progression may account for "the close correlation in the progression of acephalism and of aplasia in single embryos [from overripe frog oocytes]": Witschi, 1952.) The 'meta-*cis* mechanism' may indeed exist, and may correspond to my proposal for homeokey molecules (Proposition 270):

"Possible mechanisms underlying meta-*cis* regulation (based on the spatial contiguity of genes; discussed by Horder, 1991) are difficult to conceive, but processes involving spreading of chromatin configurations or DNA replication would fit into this category" (Duboule, 1994b).

Natalie K. Björklund (p.c.) and I would like to take the model of Duboule (1994b) one step further, to show how the epimorphic nature of limb development can be tied to and triggered by differentiation waves. We

model the limb bud as a simple mound of mesenchymal cells in a matrix, overlain by epidermis, which contains a 'zone of polarizing activity' (ZPA: Hinchliffe & Johnson, 1980). We will presume that the ZPA periodically launches a differentiation wave (the 'signal' of Davidson et al., 1991; Francis et al., 1994) that traverses the epidermis of the growing limb bud. This wave in turn triggers a mitotic wave in directly underlying mesenchymal cells, a form of induction, though perhaps by mechanical rather than chemical means:

"The rapid appearance of the transcripts immediately below the apical ridge and the subsequent spread of the regions of expression deeper into distal mesenchyme, resulting in a gradient in abundance of transcripts 24 h after grafting, suggests that the genes are activated in response to a signal emanating from the apical ectoderm" (Davidson et al., 1991).

The mesenchymal cells respond, per the model of Duboule (1994b), with a round of differentiation using the next colinear homeobox master gene. However, the very act of proliferation leaves the more proximal mesenchymal cells at a distance from the epidermis, where they cannot respond to the next epidermal wave. This is consistent with "the rule of posterior prevalence [or distal prevalence]... that a given Hox gene will exert its function essentially in the domain where this gene is the most posterior [distal] of the Hox genes expressed (Duboule, 1991)" (Duboule, 1994b).

Admittedly, the postulated epidermal waves would not cause differentiation of the epidermis itself (though limb bud cilia may form: Jurand, 1965), but rather of the underlying mesenchymal tissue (consistent with expression of homeobox genes only in the latter, at least during limb regeneration: Simon & Tabin, 1993). This is somewhat reminiscent of relationships between neural plate and underlying mesoderm, when we consider the possibility of an effect of the former on the latter, due to the depth of the ectoderm contraction wave being twice the thickness of the ectoderm cells, so that at minimum it should have a mechanical effect on the mesoderm (cf. Figure 7 in Jacobson & Gordon, 1976a, with Figure 10 in Appendix II: Brodland et al., 1994; note that the latter erroneously indicates a 'loose layer of endodermal cells' beneath the ectoderm, rather than a monolayer of

invaginating pharyngeal endoderm, notochord, and lateral mesoderm, as in Figures 10-13 in Appendix V: Gordon, Björklund & Nieuwkoop, 1994). This is the opposite of the normally assumed inductive relationship, which is not excluded. Perhaps we are stretching things a bit, but this would be one way that tissues having the potential for cortical inheritance from the original egg cortex could affect tissues that do not. Perhaps here lies the basis for mirror symmetry of the limbs, which is affected by an overall state of situs inversus (Brown et al., 1989), and thus depends on global properties of the embryo, which may be borne by the cortex.

Exploring consistency of this model with the huge body of literature on limb development and regeneration would require another book. Perhaps, in the meantime, someone will check if ultraslow contraction or expansion waves occur on limb buds or regeneration blastemas (cf. Polezhaev, 1972; Savard, Gates & Brockes, 1988; Oliver et al., 1989; Brown & Brockes, 1991; Izpisúa-Belmonte et al., 1992a; Beauchemin & Savard, 1993; Gardiner, Blumberg & Bryant, 1993; Haack & Gruss, 1993; Spirov, 1993a; Johnson, Riddle & Tabin, 1994; Morgan & Tabin, 1994; Tabin, 1995). If so, differentiation waves could provide an alternative to the dominant positional information model for limbs (Gardiner & Bryant, 1989; cf. the review of alternative models by: Maini & Solursh, 1991). As the model of Duboule (1994b), and our extension of it, is deliberately one dimensional, to correspond with the linearity of DNA, it needs embellishment to account for the full three dimensionality of limb morphogenesis.

9.06 Head and Tail Duplications

Our derivation (Appendix VI: Björklund & Gordon, 1994) of the fate map of urodele embryos (Vogt, 1929a; Nakamura, 1938; Pasteels, 1942; Asashima, 1994b) by interpreting the bottle cells of the dorsal lip of the blastopore as various differentiation waves, is consistent with...

"...the fact that not all bottle cells are the same, a fact that Holtfreter [1943a,b] was aware of [cf. Collazo, Bolker & Keller, 1994]. All the bottle cells of *Xenopus laevis* and the