

## 9.04 Mitotic Waves

Mitotic waves in the *Drosophila* syncytial blastoderm, which involve possibly contractile, transient actin caps (Postner, Miller & Wieschaus, 1992), and the furrow of the eye imaginal disc (Wolff & Ready, 1991a) are strictly correlated with rounds of nuclear division. The length of a *Drosophila* syncytial blastoderm embryo is about 300  $\mu\text{m}$  and the time between each of the four mitotic waves at the cortical surface is 15 min (Browder, Erickson & Jeffery, 1991). Their speed is thus about 20  $\mu\text{m}/\text{min}$ . This may be slow enough to qualify these waves as ultraslow waves (Jaffe, 1995). While Kauffman (1973, 1975) sees these waves as 'associated' with regulatory 'circuits' and multiple gradients of morphogens, it may be easier to look upon them as differentiation waves. For the last waves travelling "from the ends to the middle", the speed is reduced by half to 10  $\mu\text{m}/\text{min}$ , and it may be quite reasonable to consider them as myosin/actin based ultraslow waves.

The *Drosophila* embryo has been found to differentiate by a set of 'wave-like' localized rounds of cell division after cellularization, which are believed to correlate with steps of differentiation:

"In embryos of *Drosophila melanogaster* all the nuclei in the syncytial egg divide with global synchrony during the first 13 mitotic cycles. But with cellularization in the 14th cycle, global mitotic synchrony ceases. Starting about one hour into the 14th interphase, at least 25 'mitotic domains', which are clusters of cells united by locally synchronous mitosis, partition the embryo blastoderm surface into a complex fine-scale pattern. These mitotic domains, which are constant from one embryo to the next, fire in the same temporal sequence in every embryo. Some domains consist of a single cell cluster straddling the ventral or dorsal midline. Most consist of two separate cell clusters that occupy mirror-image positions on the bilaterally symmetric embryo. Others comprise a series of members present not only as bilateral pairs but also as metameric repeats. Thus a domain can consist of either one, two, or many (if metamericly reiterated) clusters of contiguous cells. Within each cluster, mitosis starts in a single cell or in a small number of interior cells then spreads wave-like, in all directions, until it stops at the domain boundary. Each domain occupies a specific position along the anteroposterior axis - as determined by the expression pattern of the engrailed protein, and along the dorsoventral axis - as determined by cell count from the ventral

midline. The primordia of certain larval structures appear to consist solely of the cells of one specific mitotic domain. Moreover, cells in at least some mitotic domains share specific morphogenetic traits, distinct from those of cells in adjacent domains. These traits include cell shape, spindle orientation, and participation by all the cells of a domain in an invagination. The specialized behaviors of the various mitotic domains transform the monolayer cell sheet of the blastoderm into the multilayered gastrula. I conclude that the fine-scale partitioning of the newly cellularized embryo into mitotic domains is an early manifestation of the commitment of cells to specific developmental fates" (Foe, 1989).

These mitotic domains (cf. Foe & Odell, 1989; Edgar & O'Farrell, 1989; Arora & Nüsslein-Volhard, 1992; Maldonado-Codina, Llamazares & Glover, 1993) may thus be fully equivalent to our differentiation waves, including specific launching domains. In particular, it is noteworthy that...

"...the pattern of mitotic domain formation in *Drosophila* is determined by the position of each cell, with no effect of cell lineage" (Minden et al., 1989).

This implies a regulating, rather than mosaic, character to this stage of *Drosophila* development. It will be curious to see if there are any one-to-one homologies between *Drosophila* mitotic domains and vertebrate tissues. While preliminary evidence in zebrafish embryos suggests not (Kane, Warga & Kimmel, 1992), this may be because of observational difficulties, and a presumption that one is looking for waves of cell division, rather than waves of expansion or contraction. (Loss of synchrony appears to be less abrupt in zebrafish: Kane & Kimmel, 1993.)

We have observed some correlation between the yolk endoderm contraction wave (Appendix V: Gordon, Björklund & Nieuwkoop, 1994) and cell division (Natalie K. Björklund, p.c.), suggesting a continuity between cleavage waves and differentiation waves in the axolotl. In this case a few waves of cell division precede and are adjacent to a contraction wave (Miura et al., 1998). Flickinger (1970) reported "...increased cell division in the neural plate - dorsal mesoderm region of early [frog] neurulae, compared to the lateral epidermis-mesoderm regions (Flickinger, Lauth & Stambrook, 1970)", (cf. Suzuki & Miki, 1983), which may reflect a similar correlation for the ectoderm contraction wave. There is "a wave of DNA synthesis

[that] passes along the dorsal ectoderm during gastrulation (Maleyvar & Lowery, 1973)" in *Xenopus* (Maleyvar & Lowery, 1981) that may reflect the presumed ectoderm contraction wave. This wave of cleavage is probably disrupted without stopping the ectoderm contraction wave, by x-rays, suggesting that they are quite separable, at least at this stage:

"[H.A.L.] Trampusch has studied the effects of carefully dosed X-irradiation on the Axolotl embryo at the pregastrula and at the neurula stage. The most remarkable fact concerning the brain is that adequate irradiation at the younger stage results in a microcephaly in the etymological sense of the word, i.e., a considerable reduction in size of the optic vesicle, together with a shortening of the brain itself, but without cyclopia or monorhinia. After irradiation at the neurula stage, the microphthalmia is no longer obtained, but the shortening of the cerebral parts is still present.... It... seems important that the general pattern of the brain has been maintained without monorhiny or cyclopia, but with a considerable reduction of size" (Dalcq, 1946a).

Agrell (1964) reviewed the relationship between invagination (launching and propagation of differentiation waves?) and mitosis across phyla:

"With continued embryonic development the rate of the mitotic activity slows down. The division synchrony disappears, mitotic gradients fade off and gradually the cell divisions are rather evenly distributed within the embryo. Later, renewed mitotic activity can be observed in restricted parts of the embryo. This is for instance the case in the sea urchin embryo at the time of gastrulation. Increased mitotic activity sets in at the vegetal pole, the site of the first invagination (Agrell, 1954; Kinnander & Gustafson, 1960). A vegetal-animal mitotic gradient thus reappears. Generally, the different foldings of the embryo which occur in embryogenesis tend to be accompanied by local mitotic activity. Cases in point are the fly egg (Agrell, 1962), the mammalian brain (Frank, 1925; Källén, 1956b), the bone primordia in the eyeball of the chick embryo (Hale, 1956), and the primitive streak of the chick embryo (Emanuelsson, 1961)" (Agrell, 1964).

Since "Premature progression through mitosis causes the abortion of nascent transcripts in *Drosophila* (Shermoen & O'Farrell, 1991)" (Brentrup & Wolf, 1993), it may be necessary, in rapidly developing embryos, to retain a tight correlation between mitotic waves and differentiation waves. In *Drosophila*, this correlation is so tight that the cell divisions are synchronized:

"There is no cell division in the [eye imaginal disc morphogenetic] furrow, and there is a coordinated and final wave of mitosis following it (Ready, Hanson & Benzer, 1976; Wolff & Ready, 1991a)" (Ma et al., 1993).

"A striking feature of eye development is the coordination of cell cycle progression with the onset of pattern formation. The conversion of an unpatterned epithelium to a repeated pattern of developing groups of cells in the eye disc occurs at a structure called the morphogenetic furrow. All the cells within the furrow are synchronized in the G1 phase of the cell cycle.... Mutations in *roughex* lead to a failure of cells to enter G1 in the morphogenetic furrow and their direct entry into S phase. Although the epithelium is divided into clusters in a normal fashion, cell fate determination in the clusters is abnormal..." (Zipursky, 1996).

The correlation between mitotic waves and differentiation waves may have a basis in nuclear/cytoplasmic transport:

"We speculate that nuclear localization of anillin serves to prevent the protein's interaction with cortical actin. Release of anillin from the nucleus during mitosis could play a role in organizing the cortex for its specialized function in cleavage. Anillin could thus play a role in coordinating the cytoskeletal changes that occur during cell division with respect to the cell cycle.... In the precellularization [*Drosophila*] embryo, anillin never enters nuclei.... The change to the more typical regulation with nuclear uptake in interphase may require zygotic transcription.

"How anillin becomes localized to a subset of the cell's actin filaments in contractile domains is not known. The same question may be asked for myosin, peanut, radixin, and other actin binding proteins that localize to contractile rings" (Field & Alberts, 1995).

The correlation of mitotic waves with differentiation waves is one of cell division with cell differentiation. This correlation may revive the concept of the 'quantal mitosis', i.e., a cell division 'needed' before a step of differentiation (Holtzer, 1970; Dienstman & Holtzer, 1975; Grounds & McGeachie, 1987; Jeffery, 1989a; Weigmann & Lehner, 1995):

"The various kinds of precursor cells in each lineage possess a unique genetic program permitting them to function as obligatory precursor cells to the succeeding members of the series.... The core problem is how replicating cells are genetically reprogrammed to produce *predictable* discontinuities in the synthetic activities between parent and daughter cells....

"Our work on myogenic, chondrogenic, and erythropoietic cells has led to the following proposals:

- (1) The genetic reprogramming which permits cells to yield progeny with different synthetic repertoires is integrated into a sequence of cell cycles in an obligatory fashion.
- (2) Cell cycles which yield daughter cells having the same genetic program as the parent cells have been termed 'proliferative' cell cycles; cycles yielding one or two daughter cells having different genetic programs have been termed 'quantal' cell cycles....
- (3) Basic changes in nuclear activity are initiated by cytoplasmic cues. The stepwise differences in the cytoplasm of cells in an evolving lineage stem from a modest number of derepressions which are coupled in an obligatory fashion to particular quantal cycles.... Genes define, permit, and sustain biosynthetic programs; they do not initiate them" (Holtzer, 1970).

Flickinger (1994) asks the question:

"Although cell division frequently has a role in the process of cell differentiation, not all cell divisions lead to differentiation. What is special about those divisions that lead to differentiation?" (Flickinger, 1994).

The answer may be: those that are part of a mitotic wave are correlated with, or caused by, a differentiation wave; those cell divisions not triggered by a differentiation wave are 'proliferative' cell divisions.

Consider work on the differentiation of wheat seedlings in which all cell division has been stopped. Differentiation appears to continue in this circumstance, suggesting that mitosis and thus mitotic waves are secondary to differentiation, not causes of it:

"Wheat grown from gamma-irradiated (800kr) grain can germinate and produce small seedlings ('gamma-plantlets') without undergoing any DNA synthesis, mitosis, and cell division.... The occurrence of a high degree of differentiation after doses of radiation that produce extensive chromosome breakage indicates that in the absence of mitosis the chromosomes need not remain intact for the cells to continue differentiation" (Foard & Haber, 1961).

There are two objections to these results: 1) it is not clear if any events of determination occurred after gamma-irradiation ("the initial phases of differentiation have occurred during embryogeny and thus before the irradiation": Foard & Haber, 1961), although to be sure "differentiation continued to the tip of the root"; 2) differentiations involving asymmetric cell division, which we have taken as single cell differentiation waves, are suppressed:

"Conspicuously absent from gamma-plantlets, except for the extreme apical portion of the first foliage leaf, are those cell types for whose differentiation unequal cell divisions presumably play an immediate role: guard cells, subsidiary cells, and hair-bearing cells (Esau, 1953)" (Foard & Haber, 1961).

More work in which mitotic waves and differentiation waves are deliberately uncoupled is warranted:

"...The broad acceptance of the quantal-cell-cycle hypothesis has deterred testing for a lack of coupling between [cell] division and differentiation.... The... hypothesis - that mitoses are for achieving population goals and are not used in any direct way to arrive at and stabilize differentiated states - has not been fully tested..." (Beresford, 1990).

Hartenstein & Posakony (1990a), using the *string* mutant of *Drosophila*, in which "cell division is blocked following blastoderm formation", nevertheless obtained some normal differentiation. This suggests that mitotic waves are dispensable even in *Drosophila*.

Perhaps mitotic waves are indeed secondary to differentiation, but asymmetric cell divisions are not, i.e., mitotic waves are triggered by differentiation waves, but asymmetric cell divisions include differentiation waves.

Brine shrimp apparently represent an extreme, natural case of uncoupling of mitosis from differentiation (cf. 'dissociability' in: Needham, 1950). As we shall see in Section 9.21, they are probably regulating, and by homology

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