

observe. On the other hand, ion current waves are not universal during activation, so we shouldn't necessarily expect two components in differentiation waves:

"Four vertebrate eggs have been investigated during activation with either the extracellular vibrating probe or patch clamp techniques during fertilization: the eggs of the frogs *Xenopus*, *Rana pipiens* and *Discoglossus pictus* (Jaffe, Kado & Muncy, 1985; Kline & Nuccitelli, 1985; Kline, 1986; Nuccitelli et al., 1988), and the egg of the medaka fish, *Oryzias latipes* (Nuccitelli, 1987). In all but *D. pictus*, the activation current was found to enter the site of activation and spread across the egg in a ring-shaped wave. However, the egg of *D. pictus* exhibited no such current wave, despite the presence of a wave of increased intracellular calcium concentration" (Nuccitelli, 1988a).

9.02 Cleavage Waves

Amongst the various waves that travel over the surface of uncleaved amphibian (Kirschner et al., 1980) and other fertilized eggs (Schroeder, 1975; Stricker, 1995), the contraction differentiation waves most resemble the surface contraction waves which occur in amphibian eggs just prior to the midblastula transition (Sirakami, 1958a; Dan, 1960; Agrell, 1964; Hara, 1971, 1977; Sawai & Yoneda, 1974; Sawai, 1979, 1982, 1985, 1987; Kirschner et al., 1981; Kirschner & Gerhart, 1981; Yoneda et al., 1982; Boterenbrood, Narraway & Hara, 1983; Shinagawa, 1985; Boterenbrood & Narraway, 1986, 1990; Ohsumi, 1987; Mabuchi et al., 1988; Dabauvalle et al., 1988; Asada-Kubota & Kubota, 1991; Gillis, 1991):

"...When eggs of one species are fertilized by sperm of another species [cf. Proposition 226], it has been found in both echinoderms and amphibians that the cleavage rate is typical of the species to which the egg belongs (see Raven, 1954, for review). These observations refer to the period of cleavage when all cells are dividing synchronously" (Deuchar, 1975a).

See Nelsen (1953) for drawings of cleavages versus time for a number of species, and Bavister (1995) for the use of time-lapse videomicroscopy of cleavage rate as an predictor of normal development. The time interval between cleavage waves (i.e., their period) has been shown to allow

dimensionless comparisons of developmental rates, heterochronies, etc., between individuals and species (Dettlaff, 1991a). Note that...

"The number of cleavages undergone by the animal and vegetal cells is always the same, except for the point that the vegetal cells cleave later than the animal cells by the time required for the wave to travel between the two poles" (Dan, 1960).

Synchronous division is accomplished in consecutive waves over the surface of the embryo, giving the name 'cleavage waves' (Sirakami, 1958a). Perhaps these are contraction waves, since "treating *Xenopus* embryos with anti-microtubule agents... led to cleavage arrest but not to inhibition of the nuclear cycle" (Manuel-Dominguez & Paiement, 1989). It would be interesting to know if inhibition of actin or associated molecules (Drechsel et al., 1997) prevents a wave of nuclear replication. Synchronization of cell division disappears by the midblastula transition (Boterenbrood, Narraway & Hara, 1983; Masuda & Sato, 1984a; Boterenbrood & Narraway, 1986).

Whether amphibian cleavage waves have a role in differentiation has not been asked, let alone answered. Early cell division synchrony and mitotic waves are widespread phenomena, suspected of being involved in differentiation (Agrell, 1964). (It should be noted that the distinction between synchronous division and mitotic waves is primarily one of temporal resolution in the observation, since synchrony generally would require entrainment and its propagation from one nucleus to the next.)

I would like to consider the hints in the literature that cleavage waves are, indeed, differentiation waves. Research on cleavage waves peaked some time ago, and perhaps, with new tools available, it is time for experimentalists to take another look at them:

"It seems to me that on the basis of currently available evidence, assigning a general functional (= evolved, via natural selection) role for cleavage waves is tricky" (Vidyanand Nanjundiah, p.c., 1996).

A number of phenomena may correlate with cleavage waves:

1. The so-called 'competence prepattern' of activin (Kinoshita, Bessho & Asashima, 1993) suggesting "that the first inducing effect may start between the 8- and the 16-cell stage" of *Xenopus* may be correlated with cleavage waves.
2. Lillie & Cattell (1923) found that the rate of cell division in sea urchin eggs depends on the ionic conductivity of the medium, and earlier Lillie (1916a) found rhythmic changes in resistance to osmotic disruption of these dividing eggs, which he presumed reflected changes in electrical properties. These properties have been investigated for *Xenopus* cleavage waves by Gingell & Palmer (1968).
3. Tompkins & Rodman (1971), reprinted and discussed in Fulton & Klein (1976), found regional and temporal changes in protein composition of *Xenopus* cortex during Stages 1 to 4 [cf. Figure 4; Table 1].
4. The contractions on Stage 5 *Xenopus* embryos released by ionophores interfere with cell division (Osborn, Duncan & Smith, 1979), and therefore probably alter the cleavage waves.

A correlation that may help uncover a relationship between cleavage waves and differentiation waves is that fact that the former are initiated in the same location (top of the animal cap) as the animal cap expansion wave (Figure 8a in Appendix V: Gordon, Björklund & Nieuwkoop, 1994):

"Looking at the propagation of the 'cleavage waves' in fast projection one gets the impression that there are some 'pacemaker cells' in the animal hemisphere, and that signals generated periodically are transmitted in [the] vegetative direction" (Hara, 1977).

Earlier accounts of repetitive 'travelling waves' during 'polar globule' formation, and other 'waves of contraction', are reviewed by Whitman (1888), who notes: "The source of the initiatory impulse would still be an open question", as it is today.

The point from which the waves start may correspond to the point of extrusion of the polar bodies (Balinsky & Fabian, 1981). This deserves a close look. Cf. the modelling of "autonomous oscillations... and the lengthening of cycle times at the mid-blastula transition of intact embryos"

(Novak & Tyson, 1993). The suppression of extrusion of the second polar body during triploid formation by heat shock (Fankhauser & Godwin, 1948) or hydrostatic pressure (Gillespie & Armstrong, 1979; Armstrong & Duhon, 1989) might affect the launching of cleavage waves and the animal cap expansion wave. Since there is a local change in 'susceptibility' (Bellamy, 1919; Gilchrist, 1929a) and upon cortical rotation a streak in the pigment is caused by something at the very point of second polar body extrusion (Chung & Malacinski, 1980), this is a plausible expectation. In fertilized ascidian eggs the activation wave starts at the site of sperm entry (Speksnijder, Sardet & Jaffe, 1990a,b), which may be a related phenomenon. Cf. Speksnijder (1992). Kubota (1967) shows that the activation includes a spreading wave of cortical rigidity in the frog *Rana nigromaculata* that starts from the sperm entry point and travels 25-40 $\mu\text{m}/\text{min}$, though this is correlated with the underlying sperm aster.

The observation that calcium oscillations persist when cleavage is suppressed (Kubota, Yoshimoto & Hiramoto, 1993; cf. Schatten et al., 1982b), if applicable to differentiation waves, would undermine our concept that mechanical events involving the cytoskeleton are the first events of differentiation. On the other hand, this may be the key observation distinguishing the two kinds of waves.

It would be curious to know if the synchronous divisions of various algae (Berrill, 1961; Spratt Jr., 1971a) also involve cleavage waves. If so, they may be the evolutionary precursors of differentiation waves. Some comparative research on contraction and cleavage waves may be well worth undertaking:

"Perhaps the most dramatic example of rhythmic cortical contractions in single cells are displayed by eggs of the goose-neck barnacle, *Pollicipes* (Lewis, Chia & Schroeder, 1973). For a period of hours, trains of constriction pass from animal to vegetal pole in peristaltic waves. These traveling constrictions propagate at a rate of approximately 20 $\mu\text{m}/\text{min}$ A band of microfilaments resembling a contractile ring has been identified beneath these constrictions.... Traveling disturbances of this general type have been reported to occur in

eggs of fish (Yamamoto, 1940), crustacea (Rappaport, 1960a), and insects (Vollmar, 1972)" (Schroeder, 1975).

Similar peristaltic contractions of single newt neural plate cells have been observed in culture by Burnside (1973b) (cf. the periodic motions of microfilament rings in cultured human lymphoblasts: Guyader & Hyver, 1997).

We can expect an ionic component to differentiation waves (Appendix IV: Björklund & Gordon, 1993b) and thus to cleavage waves. This may be a function of the 'inducible' ion channels that occur in amphibian and mammalian oocytes and early embryos:

"The oocyte of the frog *Xenopus laevis* contains interesting voltage-sensitive Na^+ channels that are not normally detected. It is only after a prolonged depolarization to a potential more positive than about +30mV that these channels appear (Baud, Kado & Marcher, 1982; Baud, 1983; Baud & Kado, 1984). This process has been described as the 'induction' of Na^+ channels.... The mechanism by which these Na^+ channels are induced and their function remain unknown. Induction probably does not depend on new protein synthesis (Baud & Kado, 1984). This suggests that channels are present in the membrane of the oocyte before induction. Under physiological conditions, the oocyte membrane would not experience depolarizations strong enough to induce the channels.... A voltage-activated Ca^{2+} channel is present in growing oocytes, eggs, and early cleavage-stage embryos of the mouse, but there is no evidence to suggest... an 'inducible' Na^+ channel in the mouse oocyte or egg (Yoshida, 1985)" (Kline, 1991a).

If cells are dividing, then what happens to a cell caught in the act when a differentiation wave passes? Does it respond to the wave, does it sense a wave is coming and avoid dividing, or does it not participate in the wave? If the latter, does it remain in its previous differentiated state, or somehow acquire the news of the passed wave from its neighbors and then differentiate? If there is a conflict between differentiation and cleavage, perhaps we have an explanation of why the cleavage waves are synchronous. The cells are large, and each represents a substantial fraction of the whole embryo. If the cleavage waves are differentiation waves, and one large cell didn't participate in the current step of differentiation, there

could be major consequences to subsequent development. One way to test this may be to create chimaeric organisms using embryos of different ploidy, for which the speeds of cleavage waves, and perhaps differentiation waves, differ:

"In sea-urchin eggs, the... segmenting eggs may... be either haploid (parthenogenetic), diploid (normal, fertilized eggs) or tetraploid (fertilized monaster-eggs) and the size of the resulting cells differs correspondingly.... The size-differences... result from... different rates of cleavage, the haploid cells dividing most rapidly, and the tetraploid most slowly" (Wilson, 1925).

Cleavage waves in amphibians extend up to the midblastula transition (and probably in sea urchins too, at which point cilia appear: Masuda & Sato, 1984a,b). Their fundamental distinction from differentiation waves may be that cleavage waves correlate with translational control of maternal mRNA, while differentiation waves, we presume, correlate with transcriptional control. However, "the concept of an initial transcriptional activation of exogenous genes [Newport & Kirschner, 1982a,b] at amphibian MBT [midblastula transition]" (Shiokawa et al., 1990a) has been challenged:

"We have recently shown that *Xenopus* embryos synthesize heterogeneous mRNA-like RNA and a small amount of low-molecular-mass RNA during the pre-MBT stage (Nakakura et al., 1987; Shiokawa et al., 1989a)... The level of synthesis measured on a per-cell (hence per-nucleus) basis was not greatly different before and after the MBT stage" (Shiokawa et al., 1990a).

"At any given time in [mosaic] leech embryos, some cells are using maternal regulators and others zygotic regulators" (Bissen, 1995).

"...A sperm-supplied factor... participates directly in development of the early [nematode] embryo" (Browning & Strome, 1996).

The most recent evidence is that paternal mRNA comes into the egg with the sperm, and may be functional and necessary from the beginning of development (Kramer & Krawetz, 1997). Thus it is not out of the question that cleavage waves could be involved in altering specific gene expression.

Either type of wave results in temporal changes in the expression of specific proteins. Differentiation waves, being of localized extent or trajectory over the embryo, also determine spatial changes in gene expression. Both thus appear to be variations on a theme, and we might presume that differentiation waves evolved from cleavage waves. Given the distinction we have made, it appears that mammals, which initiate zygotic mRNA translation as early as the two cell stage (cf. Browder, Erickson & Jeffery, 1991), dispense with cleavage waves altogether (but cf. Section 9.03). The pregastrulation cleavages in nematodes are insensitive to massive chromosome deletions (Storfer-Glazer & Wood, 1994), suggesting a possibly exploitable homology with cleavage waves and the midblastula transition. Furthermore, in nematodes "...it is now clear that some new transcripts appear during early cleavage" (Wood & Edgar, 1994). The coincidence of contraction waves (Proposition 243), mitotic waves (Section 9.04), and segmentation (Section 8.01) in the *Drosophila* syncytial blastoderm, with the period during which a "block to zygotic gene transcription" (Schejter & Wieschaus, 1993b) has no obvious phenotypic effect, suggests that *Drosophila* has a 'midblastula' transition, prior to which differentiation is indeed occurring.

It is possible that the whole question of when an embryo starts differentiation is a mechanical one:

"Since the total volume of the embryo remains basically the same as that of the fertilized egg, the cytoskeletons in the cells will get steadily smaller as division proceeds: fewer microfilaments, shorter tubules. The mechanical properties of the cytoskeleton should not scale in proportion to size. For example, short microtubules should be stiffer, stronger and less springy when they are shorter. Perhaps the cell state splitter does not have all the necessary properties until its size is in a certain range. This would explain why a fertilized egg can go through several divisions before the cells begin to differentiate" (Steve McGrew, p.c., 1997).

This would imply that embryos that start out with smaller cells exhibit differentiation sooner, a correlation that seems at least qualitatively correct.