Chapter 27. Post-translational regulation of cytoskeletal remodelling, rosette cell clusters and mitotic domains during embryogenesis.

Mechanical stress may trigger cytoplasmic remodelling with rapid, post-translational responses within the cortical periplasm. The knockdown of phosphatase and kinase activities can induce embryonic defects, including suppression of the segmentation cascade and denticle formation, see [1]. In particular, knockdown of the Bsk, Cdk4, or Slipper Kinases can suppress A and D regional fates, giving short, rotated embryos and incomplete dorsal closure [2 3 1]. These patterning defects are consistent with mis-regulated microtubule assembly and reduced cargo trafficking, along both the A/P and D/V (L/R) axes, see Chapter **17**. A similar maternal-effect "slipper" phenotype is observed in embryos from *par-1* mutant oocytes [4].

The polar/equatorial movements of cortical periplasm during the acellular blastoderm, may disperse maternally inherited cytoplasmic proteins but should not disturb the nuclear Bcd gradient. By contrast, the maternal Hb gradient may be maintained, and refined, by early zygotic transcription of *hb*. Notably, the equatorial mid-line coincides (at least roughly) with domain of the transcriptional suppressor Kr, and the boundary between the domains of action of the separated halves of the Hox gene complex (Ant-C + Bx-C) in *Drosophila*. Compact, early zygotic functions include *zen*, *zen2* and *ftz* (within the Ant-C) and *eve, en*, *dsh* and *wntD* (at separate genetic loci). The arrival of syncytial nuclei at the embryonic cortex in cycle 11 induces surface cortical blebs, which provide anchor sites for mitotic spindle assembly. Thus, spindle axes align with the embryonic surface topography. However, the curvature of the egg surface is too steep to allow uniformly spaced cortical blebs with hexagonal boundaries, and the distribution of nuclei across the cortical surface is somewhat irregular, see Fig. 5 of [5].

The topography of the egg surface also constrains membrane down-growth and the alignment of cellular interfaces during the mid-blastoderm transition (14th cycle), with irregular spindle orientations in the surface plane. *Zen* and *zen2* are first transcribed during the 11th and 12th cycles, along the D midline and around the polar caps. Zen function specifies dorsal ridge, amnioserosal and optic lobe fates. During cycle14, the first three mitotic domains ($\delta_{14}1$-$\delta_{14}3$) are triggered anterior to the cephalic fold, while $\delta_{14}4$ is slightly delayed at the posterior limit of the ventral furrow [5]. Meanwhile, *ftz* transcription in the syncytial blastoderm is restricted to a broad central band, with the Ftz protein being absent from the poles. By contrast, the Eve protein is distributed between the cortical (and yolk) nuclei from the 11th cycle; until it is cleared from the A and P polar caps during the 13th and 14th cycles . These polar clearances coincide with the loss of yolk granules, consistent with active microtubule trafficking. Meanwhile, the mutually antagonistic activities of Eve and Ftz may reflect their competitive occupation of the same TATA binding sites in different cell populations (see Chapter **26**). By contrast, in the absence of early zygotic transcription of *en*, clearance of the polar yolk granules is defective, extension of the cephalic fold is premature and pole-cell distribution is irregular [7]. After cellularisation, Eve may be suppressed by Kni at the A pole, leaving a wide central band of *ftz* transcription (Caroll and Scott 1986). During normal development, Eve protein then localises to nuclei in alternate pair-rule stripes as transcription of *ftz* is increased. Seven, alternating Eve/Ftz bands form, from A to P [8 9]. Meanwhile, the steroid family receptor Tail-less (Tll) binds hormone response elements (HREs) at both polar caps, and suppresses activity of the Pol11 transcription complex [10]. During the mid-blastoderm transition, *WntD* is transcribed in broad bands on either side of the ventral furrow, and a single row of cells along the lateral mid-line [11 12]. Meanwhile, Eve is required to activate *en* transcription via *slp*, in odd-numbered parasegments; while *ftz* and *odd* are required for *eve* function, in even-numbered parasegments [6 13 14 15]. Thus, the cross-regulatory interactions between these genetic functions sharpens their boundaries and restricts

their domains of expression.

Transcription of *wntD* is initiated in a few nuclei at the A and P poles during the 11th syncytial cycle, which may block activity of the maternally-inherited Dl [12 16]. By the end of the 14th cycle, the first indication of the ventral furrow is apparent along about 70% of the ventral mid-line with Ap surface constriction, while 15 diffuse rings of En protein encircle the D/V (L/R) embryonic axis. The radial (Ap/Ba) embryonic axis is maintained during germ band extension as the terminal abdominal segments advance along the D mid-line. The first mitotic domains ($\delta_{14}1$) are initiated as a dorsal pair, near the A pole; followed by anterio-ventral ($\delta_{14}2$) and central ($\delta_{14}3$) domains. Paired ($\delta_{14}4$) domains form to either side of the P ventral furrow, which by this stage has been pushed onto the D embryonic surface. During this process, amnioserosal cells migrate inwards and separate the cephalic fold from the advancing, terminal abdominal segments. The amnioserosal cells continue to express *zen*, but do not assemble a mitotic spindle and become increasingly polyploid. The next three mitotic domains follow: A ($\delta_{14}5$), P ($\delta_{14}6$) and ($\delta_{14}7$), within the cephalic furrow. As the $\delta_{14}5$ and $\delta_{14}6$ domains reach telophase, the more anterior ($\delta_{14}8$ and $\delta_{14}9$) domains are in metaphase. Some of the $\delta_{14}8$ spindle planes (and all of those in $\delta_{14}9$) are rotated around the radial (Ap/Ba) embryonic axis, as the internal neuronal lineages delaminate from the embryonic surface. The cytoskeletal contractions that drive invagination around the ventral midline take place prior to mitotic spindle assembly, which is delayed in the $\delta_{14}10$ domain, until after the internalisation of the neuronal and mesodermal lineages. Similarly, contraction-extension movements take place during interphase (in the absence of spindle assembly), with the ventral $\delta_{14}14$ domain being delayed with respect to $\delta_{14}11$- $\delta_{14}13$, around the dorsal, dorso-lateral and terminal regions. As the presumptive neuroectodermal and mesodermal cells invaginate, the L and R sides of the ventral furrow are brought together, and one or two rows of cells enter mitosis in $\delta_{14}14$. Notably, the mitotic spindles of $\delta_{14}14$ are aligned preferentially along the A/P axis, to either side of the ventral furrow. During gastrulation, mitotic spindles align with segmental boundaries ($\delta_{14}25$), coincident with the bands of *en* transcription, and orthogonal to the V midline. By contrast, the glial stem-cell lineages delaminate from the Ba surface of ventral cells, before their internal migration [17]. By implication, mitotic spindle rotation may reset local axial coordinates as the surface topography of the cellular blastoderm becomes increasingly convoluted during gastrulation.

The Ap surface contractions along the ventral furrow are followed by pulsatile actomyosin contractions around the D/V (L/R) embryonic flanks [18 19]; see above **24**. Tubulin accumulates preferentially along the lateral interfaces of the ventral midline cells, before they fold inwards along the ventral furrow [5 20 21]. By contrast, the cephalic fold is initiated at the dorsal midline, with radial (Ap/Ba) shortening and Ba nuclear displacement, before Ap surface contraction. Adjacent cells, to either side of the cephalic fold, rotate inwards; so that their apical surfaces become opposed. Meanwhile, the lateral boundaries of cells in the epithelial plane are aligned with the D/V (L/R) embryonic axis. Short columns of cells contract before resolving as rosette clusters along the long (A/P) embryonic axis [22 23]. After cellularisation, cytoplasmic flux is canalised with respect to the ventral furrow, with coordinated actomyosin contractions and kinesin-driven separation of microtubule filaments. The rosette clusters coalesce as irregular polygonal cells, with straight cytoplasmic interfaces that must be under tension. The relationship between rosette clusters and Foe's mitotic domains remains uncertain, although both are constrained by the topographical shape of the embryonic surface. Perhaps the actomyosin-driven cell shape changes displace a raft of rigid collagen rods across the Ap epithelial surface. By contrast, the separation of twin centromeres across the Ap membrane interface is driven by microtubule extension and the Klp61F motor

assembly (see Chapter **30)**. However, the trajectory of the separating centrioles may be constrained by extracellular matrix anchoring, via Arm and E-Cad. On this model, spindle-alignment of would adjust to the shape of their neighbouring cells, whether free-cycling or dividing in synchrony. In either case, cytoplasmic remodelling is driven by a coordinated activities of microtubule and actin microfilament motors, as in single yeast cells. These remodelling mechanisms must be keyed to the cell-cycle, although most embryonic divisions are asynchronous after the mid-blastoderm transition. However, the polar mitotic waves of the syncytial blastoderm divisions, may be triggered by cell-cycle release from the M checkpoint, at virtual polar foci, and transmitted around the embryonic cortex.

Summary:

As migrating syncytial nuclei reach the embryonic surface, they induce superficial cortical blebs to which mitotic spindles become anchored and aligned with the surface plane. Following cellularisation, rosette clusters and discrete mitotic domains are formed, with cytoplasmic remodelling anchored through the extracellular matrix. Spindle alignments are coupled in cells flanking the ventral mid-line, in addition to parasegmental AMSs and the discrete mitotic domains; surrounded by uncoupled, free-cycling cells. As convergent-extension movements push the terminal abdominal segments around the posterior pole, the embryo is divided into a mosaic of morphogenetic twin-fields, with infolded boundaries. During gastrulation, the spindle alignments of synchronously dividing cells may be rotated through 90º, around one, or more, of the Cartesian epithelial axes.

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