Chapter 17. Cytokinesis, the cleavage plane and female germ-line assembly.

During cytokinesis, a diffusion barrier around the contractile ring separates yeast cells into discrete membrane compartments [1] [2]. Trafficking of the cytoplasmic membrane, and associated cortical material, away from the contractile ring is regulated by the Sterile20 (Ste20) Kinase [3] [4]. As the ring contracts, cytoplasm is displaced towards the centromeric poles, and an equatorial furrow is formed. In multicellular organisms, a large family of Ste20 Kinases regulates cell shape, together with associated Ras GTPases (*eg*: Rho and Cdc42), GTPase activating proteins (GAPs) and guanine exchange factors (GEFs) [5]. In particular, the Rho GTPases are membrane-targeted, via prenylation of their C-terminal CAAX motifs [5], while Rho activating factors are transported along the mitotic spindle [6]. In consequence, Rho activating factors delivered along spindle microtubules drive the assembly of an orthogonal ring of actomyosin microfilaments [7]. Similarly, the cleavage planes in the first embryonic divisions of echinoderms and amphibians are coincident with rings of activated RhoA. Meanwhile, reduced E-cad localisation to AJs drives increased actomyosin flux [8].

Similarly, a membrane diffusion barrier is maintained in epithelial cells during interphase, at least roughly coincident with AJ localisation along the Ap/Ba boundary. This diffusion barrier may be dependent on active trafficking of membrane, and associated cytoplasmic components, along microtubules that are preferentially aligned along the Ap/Ba axis. Actin microfibril assembly continues to be regulated by RhoA during interphase, with RhoGEF2 controlling the assembly of MyoII motor complexes [9] [10]. All these processes require the transmission of contractile force between the cortical actin cytoskeleton and the extracellular matrix, separated by the hydrophobic membrane interface. In consequence, junctional complexes tend to become foci for post-translational protein modifications and cytoskeletal remodelling. The lipid bilayer is labile, with little inherent mechanical strength, while the cytoplasmic gel resists passive deformation. These properties are evident during Ap surface contraction along the ventral furrow, which is initiated before Ba cell surfaces are sealed-off, without the extrusion of basal cytoplasm [11].

In general, the position of the Ap/Ba membrane boundary of epithelial cells is regulated by the Par-1/Cdc42/CDK-A complex [12], acting in conjunction with Par-3, Par-6 and multiple Ste20-related kinases. Rotation of this annular interface may displace the axial system prior to the mitotic spindle assembly. Such rotations may require thickening of the cortical cytoskeletal layer, with increased rigidity and trans-membrane anchoring to the extracellular matrix. Whatever the details, the yeast Sterile20 (Ste20) Kinase family is greatly expanded in multicellular organisms. In particular, the germinal centre kinases (GCKs) and p21-activated kinases (PAKs) have multiple, overlapping functions in the regulation of epithelial growth, neuronal extension and motile cell migration [3]. For example, the *Drosophila*, Ste20 kinase, *misshapen*, regulates dorsal closure, neuronal growth cone motility, nuclear migration and wound-healing [13] [14] [15] [16] [17]. Similarly, *mushroom bodies tiny*, affects learning and memory, dorsal closure and cell adhesion [18] [19] [20] [21]. Meanwhile *hippo* regulates epithelial growth, cell proliferation and apoptosis, R cell fate, morphogenetic field boundaries, dendritic neuronal growth and stem cell maintenance [22] [23].

The GCK kinases retain particular functions during female germline assembly in *Drosophila*, with cellular migration being guided by heterotypic adhesion molecules [24] [25]. Briefly, the ovaries consist of 12-14 parallel chains of ovarioles within a composite germarium [26](King 1970). Both somatic and germline stem cells derive from a terminal ovariole filament. The germline cells give rise to fused, 16-cell cystoblasts, while the somatic cells provide the follicle cell lineage [27]. During ovariole assembly the germline cystoblasts are passed posteriorly between their surrounding escort cells, before being surrounded by a

sheath of follicle cells [28]. Germline morphogenesis is regulated via Wg, Hh and Dpp; with Wnt4 acting through Focal adhesion kinase (FAK) [29 30 31]. In this system, the PAK1 kinase co-ordinates cell shape changes, through Rho1 and the actin cytoskeleton [31]. In *pak1* mutants, radial duplications of the germarium are formed [32]. Defects in germ cell migration and convergent extension are also associated with PCP mutations during female germline assembly in mice [33 34 35]. None of the *Drosophila* PCP mutants have been reported be female sterile, although dsh^{1} mutants show reduced sheath cell motility and fertility [29]. However, the $pk^{MI07065}$ splice-trap insertion, is associated with a GOF female sterility and a complete block in egg laying. In the transgenic $pk^{MI07065}$ strain, the N-termini of the three Pk isoforms are coupled to GFP, while transcription of the common exons is blocked. The $pk^{MI07065}$ sterile phenotype shows gaps between the sheath cells, and weakly dorsalised oocytes. This sterility is rescued by *E-cad* (*shg*) overexpression in $pk^{MI07065}$; $E\text{-}cad^{Ubi::mTFP1}$ flies, unpublished observations.

Summary:
Cytoskeletal remodelling is dependent on surface boundary effects at the cortical membrane interface during cytokinesis. Rho activating factors are delivered along the mitotic spindle as an actomyosin ring assembles around the equatorial plane. The displacement of cortical cytoplasm away from the contractile ring is regulated by Ste kinases, with a membrane diffusion barrier separating the two halves of the dividing cell. Thus, the contractile sliding of labile microfilaments may form a cortical furrow and regulate the equatorial division plane, with cytoplasmic flux constrained by viscous drag. During interphase, a membrane diffusion barrier divides epithelial cells into Ap and Ba compartments and may maintain the axial cellular plane. The GCKs regulate contact-dependent interactions via junctional complexes, with AJs localised near the Ap/Ba interface. In turn, AJs act as foci for spindle assembly, and post-translational protein modifications. Assembly of the female germline is largely driven by post-translational modification of cytoskeletal components at the cortical actin/membrane interface. Co-ordinated cell shape changes generate cylindrical epithelial tubes, with heterotypic affinities between intercalating cells.

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