Chapter 20. Topological constraints during embryogenesis, asymmetric partitioning and AMSs.

The cell-cycle oscillation has been inherited in an unbroken chain from single, free-living eukaryotic cells. This cycle may be paused at distinct checkpoints, although none of which corresponds to the origin of the oscillation in any meaningful way. In general, mitotic spindle assembly is initiated from separate polar centrioles, followed by the formation of an equatorial contractile ring [1]. The spindle microtubules extend in straight, rigid bundles, while F-actin microfilaments tend to be flexible and disordered, unless under tension. Thus, the contractile ring remains orthogonal to the spindle apparatus, as tension is developed between antiparallel actomyosin microfilaments, see above **17**. In consequence, the position of the North-South (N-S) polar centrosomes constrains the East-West (E-W) orientation of the contractile ring. The centrosomes themselves, together with the spindle filaments, are formed from cylindrical assemblies of (chiral) α/β tubulin sub-units. Thus, in principle, cytoskeletal components translocated from the opposite poles may assemble around the equatorial ring with polarised E/W alignments. Furthermore, any cargos actively partitioned along the mitotic spindle may require a switch in microtubule motor activity on crossing the equatorial plane. In consequence, the daughter cells separate as reversed, identical twins (I-twins), N-S/S-N. Such chiral reversals would not affect the size, shape, or general metabolism of dividing yeast cells.

In general, cytoskeletal fibrils are less precisely aligned during interphase, but active cargo transport may play a role in enhancing passive diffusion through the cytoplasmic gel. At this stage, the centrosomes are paired, perinuclear structures, which are surrounded by diffuse pericentriolar material (PCM). Each centrosome consists of double, orthogonally aligned centrioles, consisting of α/β tubulin cylinders which may be capped by γ-tubulin rings. The centrosomes replicate during S-phase, with centriole separation being followed by nucleation of an orthogonal microtubule cylinder. One of the two centrioles remains surrounded with PCM, with associated centrobin [2]. Towards the end of interphase, the two perinuclear centrosomes are pushed to the cell cortex by the growth of microtubule bundles; whence they separate across the periplasmic membrane interface to opposite poles. Thus, surface boundary constraints set the alignment of the spindle plane, and the axial coordinates of daughter cells. Meanwhile, the separating centromeres may transmit reversed Cartesian co-ordinates, as in yeast cells. These topological constraints are critical during the early embryonic divisions of multicellular organisms, which show precisely choreographed cell shape changes and oriented mitotic spindles [3 4 5].

While embryonic division patterns vary widely between organisms, regulation of the cell-cycle is strongly conserved, reviewed in [6]. In the mouse, the first division separates mixed-twin (M-twin) embryonic and extra-embryonic fates. The initial spindle orientation may be biased by the plane of the last meiotic division [7 8]. By contrast, in *C. elegans* the plane of the first division is determined by the position of a sperm-derived centrosome. In both these model systems, differential cell fate is dependent on the asymmetrical transport of proteins and ribonucleic particles (RNPs) along the mitotic spindle, regulated by the Par (partitioning-defective) proteins and Cdc42 [4 5 9]. Lack of any of the Par proteins (Par1-Par6), Cdc42 or PKC-3, disrupts the embryonic axes in *C. elegans*. Notably, Par-3 and Par-6 localise to AJs, where they stabilise spindle assembly, while Par-1 localises to baso-lateral cell boundaries [4]. The separation of Par-1/Par-3 and Par-6 is co-incident with an interface between apico-basal (Ap/Ba) cellular compartments, which maintains the epithelial plane during interphase. Thus, microtubules initiated from the Ap/Ba interface may deliver actin microfilament and motor components to the equatorial ring and set the division plane of

dividing cells. In principle, the three Cartesian embryonic axes could be set during the first embryonic divisions, with differential fates allocated by asymmetric partitioning. In the mouse or worm models, the first determinative cleavages would segregate daughter cells with alternative, M-twin, fates; with the chiral centromere orientations reversed around each of the Cartesian axes (X, Y, Z) (Fig. 28).

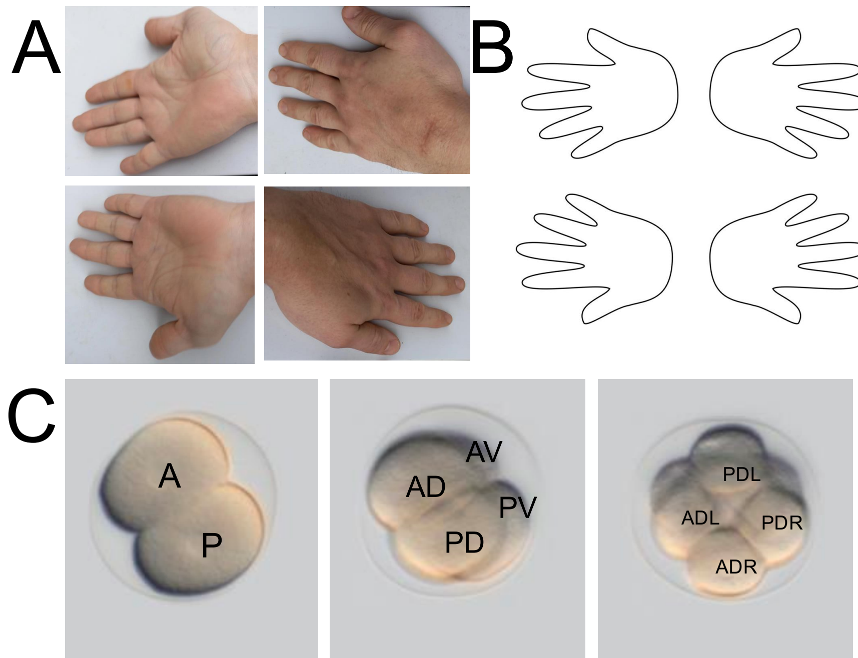

Fig. 28. Reflections, rotations and M-twin fates. Mirror-twin 3D shapes may be aligned along two of their Cartesian axes, but cannot be superimposed without the third axis being inverted **A.** However, the outline of a 2D field may be reflected or rotated around its X, or Y, axis **B.** In principle, embryos with determinative cleavage patterns, might allocate M-twin daughter fates, around each of the Cartesian axes (X, Y, Z), via asymmetric partitioning of fate-determining factors to anterior/posterior, dorsal/ventral and left/right: *eg* two cells A/P, four cells AD/PD, AV/PV; eight cells ADL/ADR, PDL/PDR, AVL/AVR, PVL/PVR, **C**. Such a mechanism must stop at the 8-cell stage, once L/R, A/P, D/V identities are assigned.

The embryonic axial system remains labile, at least by the criterion that early cleavage cells are totipotent when separated. In later stages, however, only two embryonic axes are retained, with the D/V axis defining the L/R division of the body-plan. This reduction of embryonic axes to a long (A/P) axis and a short (D/V, L/R) axis reflects a fundamental topological constraint: namely that 3D reflections of chiral structures are impossible in a universe with less than four spatial dimensions. By contrast, the outline of a 2D epithelial sheet can be reflected around a central AMS, provided that internal cells retain a uniform fate. Such “origami-based” morphogenetic mechanisms would allow the construction of 3D tissues from 2D templates. However, epithelial patterning must be regulated from morphogenetic twin-field boundaries, with 2D patterns transferred to 3D structures via spatio-temporal mechanisms. Following the first determinative divisions, the Par proteins regulate Ap/Ba cell polarity [9 10], although most later embryonic divisions are asynchronous, without coupled checkpoint release.

Despite such strict topological constraints, the early embryonic cleavage patterns are remarkably variable between different organisms. The dextral coiling of snails, for example,

follows the initial embryonic cleavage plane, and is maternally inherited [11]. In both echinoderms and vertebrates, cleavage plane orientations are specified by RhoA activity, with spindle assembly coupled to cortical microfibril alignment via MyoX [12 13]. The synchronised embryonic divisions of echinoderms have been a model system for cell-cycle analysis [3 14]; however, the segregation of "animal" and "vegetal" lineages is delayed until the fourth cleavage division, reviewed in [15]. The adult body forms of echinoderms are radially symmetrical and have altered little since the Cambrian era; without complex hearts, kideys, or CNSs [16]. However, the bilaterally symmetrical larval forms show extremely varied morphologies, even between closely related species [17]. Similarly, the nematodes show remarkably constant body forms, despite extensive metabolic adaptations to varied environmental conditions [18]. By contrast, in segmented organisms the initial L/R axis of bilateral symmetry is sub-divided by additional, orthogonal, AMSs. During gastrulation, cells are allocated parasegmental fates, with segmental infoldings along the long embryonic axis. Each parasegmental twin-field may deploy a serially repeated set of morphogenetic interactions, with incremental modifications along the long (A/P) axis.

In general, morphogenetic twin-field boundaries are not delineated by lineage discontinuities, despite the compartmental boundaries in *Drosophila* imaginal discs. In this system, the adult segmental discontinuities may result from the physical separation of the embryonic histoblast nests, with no lineage discontinuities in the larval epithelial surface [19]. However, the central hypothesis that compartmental fields might be progressively sub-divided is consistent with alternative, M-twin fates being allocated to either side of compartmental boundaries [20 21]. Similarly, lineage restrictions are not generated during vertebrate somatogenesis, as uncommitted cells are recruited to either side of the L/R midline. Notably, vertebrate segmentation is reversed tail-to-head and top-to-bottom (P < A and D > V), with respect to insects. The vertebrates lack D and V thoracic limbs, instead vertebrate limb-buds generate solid tissue behind a zone of polarising activity, like the output of a 3D printer. In this essentially 2D system, proximal joints are formed before distal joints, with a chiral reversal in the distal half of each limb segment. A single femur is followed by (M-twin) tibula and fibula, tarsi, and (five) splayed, segmented digits. Each ossified element carries proximal and distal joint structures, except for the terminal phalanges. Thus, a reiterated set of regulatory interactions may generate fins, wings, arms, or legs: with highly labile morphologies over evolutionary timescales.

Summary:

Cell division is initiated by microtubule growth from centrioles anchored at the cortical membrane interface, driving reversed (chiral) centrioles to opposite poles. These movements set the mitotic spindle axes; together with the equatorial plane, around which the contractile ring will assemble. Active partitioning along the mitotic spindle may generate equal, I-twin, daughter cells with reversed chirality. By contrast, asymmetrical partitioning may allocate differential, M-twin, daughter cell fates. The first embryonic divisions set global Cartesian coordinates, which are maintained during subsequent stages. However, the D/V and L/R axes become conjoined as a single (short) axis; to either side of a midline AMS. These topological constraints derive from the ancestral eukaryotic cell division mechanism, with an obligate orthogonal relationship between microtubule extension and microfilament contraction during cytokinesis. During later development, growth is regulated within morphogenetic twin-fields, with the asynchronous divisions of internal field cells. Strict topological constraints regulate the initial assignment of embryonic axes, differential cell fates and proliferative growth. Both cell-cycle regulation and asymmetric partitioning are highly conserved within

multicellular organisms. However, the symmetry-breaking mechanisms that define the initial embryonic axes are labile.

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