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REVIEW

SUBJECT COLLECTION: CYTOSKELETON

Cytoskeletal mechanics and dynamics in the *Drosophila* syncytial embryo

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ABSTRACT

Cell and tissue functions rely on the genetic programmes and cascades of biochemical signals. It has become evident during the past decade that the physical properties of soft material that govern the mechanics of cells and tissues play an important role in cellular function and morphology. The biophysical properties of cells and tissues are determined by the cytoskeleton, consisting of dynamic networks of F-actin and microtubules, molecular motors, crosslinkers and other associated proteins, among other factors such as cell–cell interactions. The *Drosophila* syncytial embryo represents a simple pseudo-tissue, with its nuclei orderly embedded in a structured cytoskeletal matrix at the embryonic cortex with no physical separation by cellular membranes. Here, we review the stereotypic dynamics and regulation of the cytoskeleton in *Drosophila* syncytial embryos and how cytoskeletal dynamics underlies biophysical properties and the emergence of collective features. We highlight the specific features and processes of syncytial embryos and discuss the applicability of biophysical approaches.

KEY WORDS: *Drosophila*, Microtubule, Actin, Mechanics, Active matter

Introduction

The cells of a living organism are highly dynamic – they divide, migrate and change shape to fulfil designated functions. In addition, numerous processes, such as positioning of organelles and vesical transport, take place inside a cell. These mechanical aspects play a key role in cell physiology (Iskratsch et al., 2014). A core component defining the shape and movement of cells is the cytoskeleton. It acts as a mechanical integrator that responds to forces inside and around the cell (Fletcher and Mullins, 2010). The cytoskeleton is composed of polymer filaments that form a dynamic network with the help of regulating, crosslinking and translocating polymer-binding proteins (Wickstead and Gull, 2011). This network provides a structural support to the cell, not only to define cell geometry but also to instruct the internal organization of organelles (Frederick and Shaw, 2007). In addition, this network provides tracks for intracellular transport, and a system for generation and transduction of mechanical forces (Pegoraro et al., 2017). Consequently, the composition and structure of the cytoskeleton determine the mechanical properties of the cell. Moreover, the dynamics and organization of cytoskeletal networks are tightly regulated depending on the type, fate and cell cycle stage of the cell (Vining and Mooney, 2017). The organization can change at a timescale of seconds from one developmental stage to the next. A prominent example is the cell cycle-dependent organization of microtubules, changing from interphase asters to the assembly of the mitotic spindle within minutes (Sharp et al., 1999). As a result, the mechanical properties and the shape of transitioning cells undergo changes as well. Thus, detailed knowledge of the cytoskeletal dynamics during these transitions is a close proxy for the biophysical properties of the cells. Metazoans contain three types of cytoskeletal filaments – F-actin, microtubules and intermediate filaments (Wickstead and Gull, 2011). As the mechanical properties of intermediate filaments and their consequences for cell function have been covered elsewhere (Block et al., 2015; Etienne-Manneville, 2018), in this Review, we will discuss the regulatory elements of F-actin and microtubules in *Drosophila* syncytial embryos and their roles in controlling cellular mechanics in living systems.

The *Drosophila* embryo develops as a syncytium in early stage

A syncytial or coenocytic (hereafter referred to as syncytial for simplicity) cell features multiple nuclei within a shared cytosol. Syncytial embryo cells are comparatively large and undergo rapid developmental transitions that affect groups of nuclei (Grbic et al., 1998), rendering them an excellent model system to study the dynamics of cytoskeletal networks, their regulatory machinery and the collective behaviour emerging from their direct interactions. Syncytia are observed in multiple organisms, including the slime mould *Physarum* (Alim et al., 2013), muscle tissue (Manhart et al., 2018), the germline in *Caenorhabditis elegans* (Hubbard, 2007) and the yolk syncytial layer in zebrafish (Carvalho and Heisenberg, 2010). The early embryo of *Drosophila melanogaster* is an extensively studied syncytial system, due its genetic tractability and the amenability of the embryo for live imaging. During the first 2 h, the *Drosophila* embryo undergoes rapid nuclear divisions without cytokinesis. The architecture of the cytoskeleton is tightly linked to the nuclear division cycle (described in Box 1) (Daniels et al., 2012; Foe and Alberts, 1983; Frescas et al., 2006; Schejter and Wieschaus, 1993; Sullivan and Theurkauf, 1995). In addition, sharing the same cytosol enables the physical and biochemical interaction of structures associated with each nucleus, such as the spindle microtubules and the cortical F-actin (Sullivan and Theurkauf, 1995). These interactions and the resulting cytoskeletal networks lead to emerging collective behaviours, such as the formation of a regular nuclear arrangement (Kanesaki et al., 2011), and the flow of organelles and cytoplasm (Chowdhary et al., 2017; Deneke et al., 2019).

Cytoskeletal dynamics during syncytial embryo development

The developmental process of early *Drosophila* embryo is divided into several stages based on the distinct morphology. In this section,

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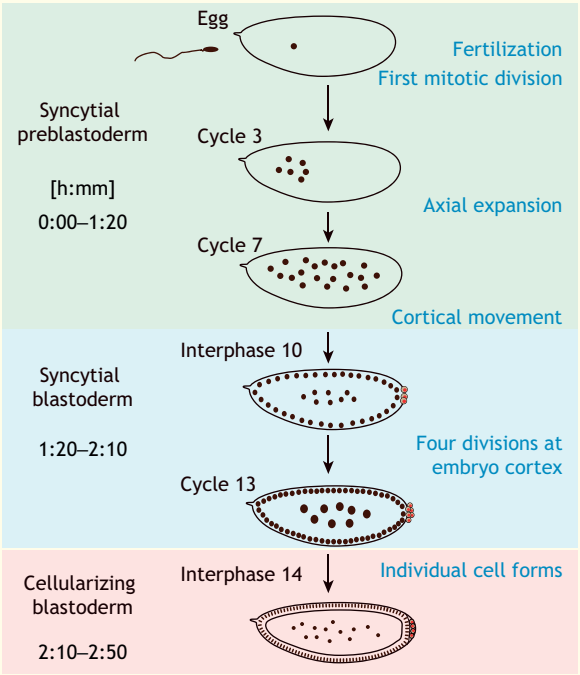
Box 1. Brief overview over *Drosophila* syncytial embryogenesis

The cell biology of early *Drosophila* embryos has been studied for more than four decades, resulting in a detailed understanding of gene regulation, morphogenesis and cellular dynamics (Foe and Alberts, 1983; Schejter and Wieschaus, 1993; Sullivan and Theurkauf, 1995). After fertilization, the embryo undergoes 13 rounds of nuclear division, but cytokinesis only occurs in the final mitotic cycle (see figure). The syncytial nuclei share a common intracellular space of an ellipsoid of ∼500 µm in length and ∼180 µm in width. The first three rounds of nuclear division take place at the anterior third of the embryo and deep inside the cell. These initial divisions are followed by a pronounced spreading of nuclei along the anterior–posterior (A-P) axis during nuclear cycles 4 to 6 in a process called axial expansion. Thereafter, the nuclei and their associated centrosomes and cytoskeleton migrate towards the cell cortex in a cyclic fashion during mitoses 7 to 9. The arrival of the nuclei at the cortex in nuclear interphase 10 is easily observed by the formation of an optically clear zone devoid of yolk granules, leading to the so-called blastoderm. The embryo undergoes another four rounds of nuclear division (cycles 10 to 13), giving rise to ∼6000 nuclei that are arranged at the embryonic cortex as a single two-dimensional layer. After 13 divisions, in interphase of cycle 14, the cortical nuclei are incorporated into cells by full ingression of plasma membrane around them in a process called cellularization.



we will discuss the cytoskeletal dynamics and its regulations in these developmental stages (details in Table 1).

Pronuclei fusion and first three nuclear division cycles

After sperm entry into the egg, the zygote is established by pronuclear apposition (Fig. 1A) deep inside the anterior region in a process dependent on microtubules and the kinesin-like protein at 3A (Klp3A) (Williams et al., 1997). Paternal and maternal chromosomes are spatially separated during their alignment at the metaphase plate, and they only fuse late during mitotic division 1, in a process termed gonomeric division (Callaini and Riparbelli, 1996; Landmann et al., 2009). The subsequent mitotic divisions are normal and occur every 8 to 9 min (Rabinowitz, 1941). Division cycles 1 to 3, occurring in the anterior region of the embryo, produce eight nuclei that are distributed isotropically, presumably by microtubule aster-based transport (Telley et al., 2012) (Fig. 1B), which then migrate slowly to the posterior region (Baker et al., 1993).

Axial expansion

Cycles 4 to 6 are distinct because the increasing population of nuclei spreads predominantly along the anterior–posterior (A-P) axis, a process called axial expansion (Baker et al., 1993; Deneke et al., 2016, 2019; Royou et al., 2002; von Dassow and Schubiger, 1994; Zalokar, 1976). Two distinct mechanisms have been proposed for nuclear distribution along the embryo axis, which each propose a central role for one of the two cytoskeletal elements, F-actin and microtubules, respectively. The actin model is supported by pharmacological data showing that inhibitors of F-actin prevent spreading of nuclei into the posterior half of the embryo (Callaini and Riparbelli, 1996; Hatanaka and Okada, 1991; Zalokar, 1976) (Fig. 1C,D). F-actin and non-muscle myosin II (referred to hereafter as myosin) form a thin cortical layer. Within the largely uniform distribution, an enrichment of F-actin and myosin at the anterior-lateral side is found directly above the nuclei prior to axial expansion (Chodagam et al., 2005; Deneke et al., 2019; Royou et al., 2002) (Fig. 1C). It has been proposed that this cortical ring of contractile actomyosin generates a cytoplasmic flow, which passively spreads the nuclei along the A-P axis in cycles 4 to 6 (Deneke et al., 2019). Support for a prominent role of cortical F-actin and a role of cytoplasmic flow comes from the observation that ectopic activation of Rho signalling leads to the re-distribution of nuclei in a predictable manner (Deneke et al., 2019). The alternative model stresses a crucial role of microtubules and centrosomes. Mild doses of drugs affecting microtubules do not affect distribution along the embryonic axis but rather the lateral spreading (Hatanaka and Okada, 1991) (Fig. 1D). High doses cannot be applied due to the essential mitotic role of microtubules. Despite this, astral microtubules are important for keeping the nuclei at a distance, as asterless spindles lead to a collision of nuclei and defective nuclear migration during telophase (Kao and Megraw, 2009; Vaizel-Ohayon and Schejter, 1999). Importantly, cytoplasmic transplant assays (described in Box 2) have revealed long-range microtubule-based interactions, which separate nuclei in telophase and interphase (Telley et al., 2012). In addition to these two opposing models, an in-between mechanism involving both F-actin and microtubules may be possible. A potential link between F-actin and microtubules is provided by the centrosomal protein 190 (CP190) (Chodagam et al., 2005), which is essential for axial expansion as revealed by loss of axial expansion in CP190 mutant embryos (Chodagam et al., 2005). CP190 is known to activate myosin, and this activation depends on CP190 binding to centrosomes and microtubules (Chodagam et al., 2005).

Cortical movement

Following the distribution along the embryonic axis, the nuclei synchronously migrate toward the embryo cortex in an episodic manner during telophases of cycles 7 to 9 (Foe and Alberts, 1983; von Dassow and Schubiger, 1994) (Fig. 1E). Although the phenomenon of cortical movement is clearly distinct from axial migration, similar mechanisms may be involved. Pharmacological perturbations suggest a critical role for microtubules, but not actin, in this cortical migration of the nuclei (Hatanaka and Okada, 1991; Zalokar, 1976). Centrosomes, together with their associated microtubules, can migrate towards the cortex even in the absence of a nucleus (Raff and Glover, 1989). The astral microtubule-based movement of nuclei may be a major mechanism for cortical

Table 1. Nuclear distribution and involved cytoskeletal elements during preblastoderm development

Nuclear division cycle	Nuclear motion	Involved cytoskeleton	Reference(s)
First cycle	The female pronucleus is often considered mobile, but it is still unresolved whether the male pronucleus is nonmigratory. Gonomeric division.	Microtubules; Klp3A	Callaini and Riparbelli, 1996; Landmann et al., 2009; Loppin et al., 2015; Williams et al., 1997
Cycles 2 to 3	Small centring movement within the embryo space.	Unknown	Baker et al., 1993
Cycles 4 to 6	Axial expansion. Inhibition of microtubules or loss of astral microtubules affects inter-nuclear distances, whereas axial distribution is not affected. Inhibition of F-actin assembly or ectopic cortical contractions prevents spreading of nuclei to the posterior region of the embryo.	F-actin; microtubules	Baker et al., 1993; Deneke et al., 2019; Hatanaka and Okada, 1991; Royou et al., 2002; Telley et al., 2012, 2013; von Dassow and Schubiger, 1994; Zalokar, 1976
Cycles 7 to 10	Cortical migration. Plus-end-directed motors may link and act on neighbouring arrays of overlapping astral microtubules to generate long-range nuclear separation in telophase and interphase and drive outward migration to the cortex. The nuclei divide with native characteristics in the absence of cortical membrane and down-stream mechanisms.	Microtubules	Baker et al., 1993; Deshpande et al., 2020 preprint; Telley et al., 2012; von Dassow and Schubiger, 1994

movement. Long astral microtubule arrays extending toward the centre of the embryo are observed during telophase and early interphase when the nuclei are moving to the cortex (Baker et al., 1993). The long microtubules that originate at the centrosomes and are attached to the neighbouring nuclei form antiparallel arrays, which are necessary for homogenous nuclear positioning at the cortex (Deshpande et al., 2020 preprint). However, in the earlier cycles of cortical movement, the astral microtubules pointing to the cortex are too short for cortical interactions that would pull nuclei to the cortex to occur (Telley et al., 2012). Thus, a long-standing hypothesis is that the force that drives nuclear cortical migration is produced by motor proteins crosslinking and sliding antiparallel microtubules from neighbouring asters (Baker et al., 1993) (Fig. 1E, insets).

Blastoderm nuclear divisions

Once the nuclear cortical migration is completed, the nuclei anchor to the embryo cortex in a microtubule- and centrosome-dependent manner (Fig. 2), which involves the microtubule motor protein dynein together with dynactin (Robinson et al., 1999; Sharp et al., 2000). F-actin polymerization inhibitors, such as latrunculin, or depolymerization agents, such as cytochalasin, induce a so-called 'nuclear fallout' phenotype, which is where the nuclei cannot stay at the cortex and fall inside the deep yolk in the mutant embryo (Postner et al., 1992; Sullivan et al., 1993), implying that F-actin plays a role in linking nuclei to the cortex, either by viscoelastic or molecular anchoring. The cortical nuclei undergo four division cycles with the spindle in parallel to the embryonic cortical plane, resulting in a single-layered pseudo tissue (Kanesaki et al., 2011). Actin accumulates at the cortex upon being induced by the centrosomes and forms dome-like structures called actin caps. These caps are located between the ruffled plasma membrane and the centrosome, and distributed regularly along the surface of the embryo (Foe et al., 2000). Actin caps are highly dynamic and undergo expansion during interphase (Sommi et al., 2011; Sullivan and Theurkauf, 1995). In fact, the actin caps keep expanding radially until they contact a neighbouring cap. Thereafter, membrane protrusions flatten to increase the membrane portion needed for the ongoing invagination (Sullivan and Theurkauf, 1995). This process allows the invagination of incomplete membrane boundaries during mitosis (metaphase furrows), thereby separating adjacent mitotic spindles. By late mitosis, these metaphase furrows regress, the protrusions of plasma membrane reform and F-actin reorganizes back to the individual cap structures above each daughter nucleus, which are associated with a pair of centrosomes (Riggs et al., 2003; Sullivan and Theurkauf, 1995).

The actin cytoskeleton changes in synchrony with the cell cycle and, concomitantly, with a massive microtubule reorganization. In interphase, microtubules emanating from the centrosome form three distinct populations. First, they surround each nucleus, forming a basket-like structure around the nucleus, with a basal opening towards the interior of the embryo (Wessel et al., 2015). Second, astral microtubules from neighbouring nuclei interdigitate and mediate either repulsive or attractive interactions by means of crosslinkers (Lv et al., 2018; Winkler et al., 2015), a mechanism likely involved in maintaining nuclear distances while membranes remain retracted (Deshpande et al., 2020 preprint). Third, microtubules connect the centrosomes and the nucleus to the plasma membrane (Buttrick et al., 2008; Riggs et al., 2003). During mitosis, microtubules still remain assembled in dynamic astral arrays at each spindle pole. Interaction between astral microtubules from neighbouring spindles is thought to be progressively inhibited by the presence of more-prominent plasma membrane invaginations from one mitosis to the next (Holly et al., 2015; Kanesaki et al., 2011). As soon as the furrows retract in anaphase, the physical barrier disappears, and microtubules from neighbouring asters can interact again. In contrast to what is seen at mitosis, at every interphase, an increase in the number of possible microtubules interactions in space and time is expected due to the duplication of nuclear density and the gradual prolongation of interphase (Foe et al., 1993).

Cellularization

During interphase of cycle 14, the embryo transforms from a syncytial into a cellular organization. The plasma membrane invaginates between adjacent nuclei during extended cytokinetic furrow formation until nuclei are fully enclosed. A single-layered epithelial cell sheet is generated, which includes individualized cytoskeletons, cell junctions and the full apical-basal polarization (Mazumdar and Mazumdar, 2002; Schmidt and Grosshans, 2018).

What can we learn from the *Drosophila* syncytial embryo?

The special properties of the *Drosophila* syncytial embryo enable us to investigate cytoskeletal regulatory mechanisms, mechanical interactions between cytoskeletal components and the resulting

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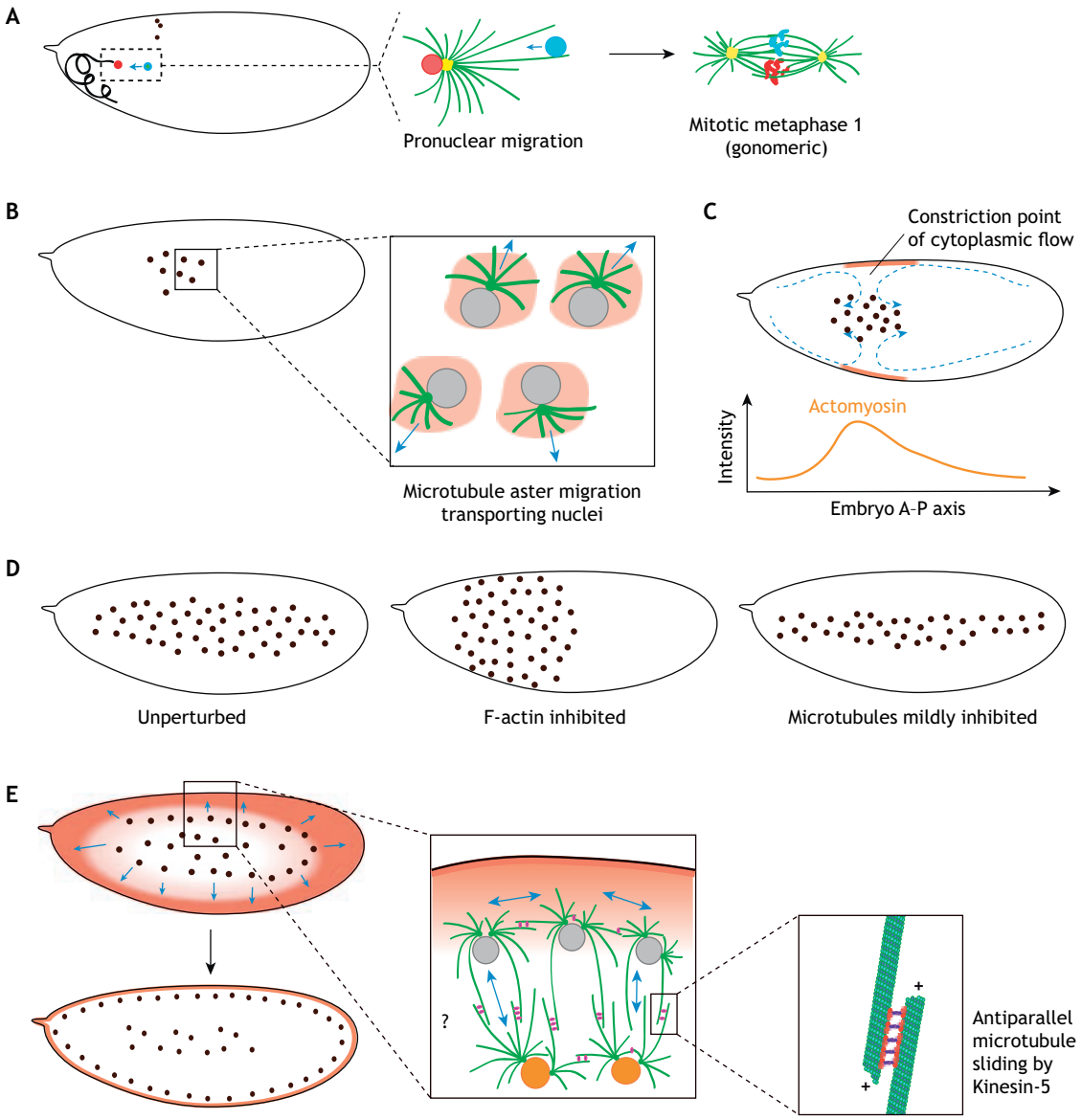

Fig. 1. The involvement of actin and microtubules in preblastoderm development in *Drosophila*. (A) In the *Drosophila* embryo, after entry of the sperm carrying a single centriole at the anterior end, a centrosome is formed that nucleates microtubules into a large aster. The current model proposes a 'catch' mechanism and transport of the female pronucleus along astral microtubules. Once male and female pronuclei are apposed, their chromatin is condensed but kept spatially separated and a gonomeric metaphase spindle forms. The fusion of male and female contribution occurs after chromosome segregation. (B) During the early divisions, the nuclei move and distribute together with their centrosomes and microtubule aster (inset). Nuclei cease their migration if the centrosome is removed or absent, microtubules are shorter or F-actin is perturbed. (C) Axial expansion. Actomyosin gradient drives the cytoplasmic flow, which passively spreads the nuclei along the A-P axis in cycles 4 to 6. (D) After the third mitotic nuclear division, the nuclei start spreading in the anterior and posterior directions. By division 6, they form an ellipsoid cloud (left). Injecting an F-actin inhibitor abolishes the A-P spreading, but nuclei still arrive at the cortex (middle). Perturbing microtubule polymerization causes a mildly stronger A-P spreading. (E) Cortical migration. During divisions 7 to 9, nuclei move towards the embryo cortex in an orchestrated fashion. An as-yet-untested model proposes an astral-microtubule-dependent repulsion that pushes peripheral nuclei away from the deeper yolk nuclei. The repulsion is suggested to be generated by motor-driven sliding of anti-parallel aligned microtubules of neighbouring asters.

emergent features, and the contribution of cytoskeletal elements to the material properties of developing embryo.

Microtubule–actin interaction in the *Drosophila* syncytial embryo

F-actin and microtubules often work together. They are crosslinked by associated proteins such as Sort Stop (Shot) (Lee and Kolodziej, 2002) and Pod1 (Rothenberg et al., 2003). In addition, actin bundles guide microtubule growth by Actin Cross-linking Factor 7 (ACF7, also known as Short stop in flies) (Bernier et al., 2000) and microtubule plus-end-tracking protein CLIP170 (cytoplasmic linker protein of 170 kDa; also known as CLIP-190 in flies) (Henty-Ridilla et al., 2016; Lewkowicz et al., 2008). Furthermore microtubule plus-ends are anchored to the cell cortex by several F-actin binding proteins, such as Moesin (Jankovics et al., 2002; Solinet et al., 2013), Afadin (also known as Canoe) (Carminati et al., 2016) and Anillin (also known as Scraps) (Gregory et al., 2008; Hickson and O'Farrell, 2008), suggesting that the regulation of the F-actin-microtubule interactions are linked by several pathways. The highly dynamic and coordinated behaviour of F-actin and microtubules in the syncytial embryo make it an

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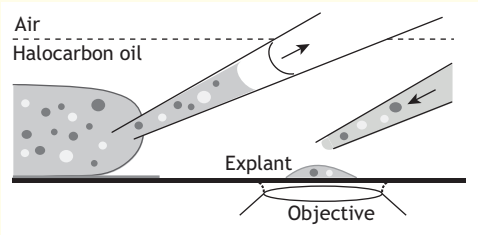
Box 2. Embryo extract assay

One of the drawbacks of the *Drosophila* syncytial embryo is the limited optical transparency that is predominantly caused by the large amount of yolk and lipid spheres, which highly diffract visible light. Consequently, high-resolution live-cell imaging is performed during the blastoderm stage, offering a nuclear and cytoskeletal arrangement that is sufficiently close to the microscope objective. For this reason, we know relatively little about fertilization and the preblastoderm stage embryo, when cellular events and developmental transitions occur deep inside the large embryo. This limitation has been overcome by means of micromanipulation-assisted extraction of the embryo content and generation of small explants (de-Carvalho et al., 2018; Telley et al., 2013) (see figure). This extraction method allows for a considerable reduction in volume, while maintaining the native intracellular processes. Here, a single embryo is punctured with a micropipette and embryo 'explants' are generated by deposition of cytoplasm onto the cover glass. These explants recapitulate intracellular processes that are not dependent on the cell cortex, such as mitotic divisions and distribution (Telley et al., 2012).



interesting model to address the regulatory mechanisms of crosstalk between F-actin and microtubules, which could be generalized to other biological systems.

The cell cortex of the syncytial blastoderm is considered a hub for the mechanical and biochemical interactions between microtubules and F-actin (Fig. 2A–C), linking the coordination of both cytoskeletal networks and their dynamics with the nuclear division cycles. Cyclin-dependent kinase 1 (Cdk1) temporally coordinates nuclear division (Deneke et al., 2016), while centrosomes, acting as microtubule-organizing centres, are considered the spatial coordinators (Blake-Hedges and Megraw, 2019; de-Carvalho et al., 2020 preprint; Schejter, 2005). In interphase, a pair of centrosomes lies beneath each actin cap (Fig. 2D). Upon entry to mitosis, during prophase, this centrosome pair moves apart to the nuclear equator due to dynein and dynactin activity (Robinson et al., 1999; Sharp et al., 2000). Meanwhile, the actin caps expand and their margins merge. The metaphase furrows form at the same time as chromatin condensation and alignment at the mid-plane between both centrosomes (Fig. 2E). The furrow retracts in anaphase and telophase and, at this time point, the already duplicated centrosomes move apically, in close contact with the blastoderm cortex (Rothwell et al., 1999).

The centrosome is likely a molecular hub and the spatial instructor for F-actin organization in the *Drosophila* syncytial embryo (Raff and Glover, 1989). Centrosomes have been described to organize actin into aster-like structures *in vitro* and in cultured cells (Farina et al., 2016). In mutant embryos lacking the pericentriolar material protein Centrosomin (Cnn), spindle assembly and nuclear separation is abnormal, but some embryos can reach the blastoderm stage, while showing irregular nuclear distribution (Megraw et al., 1999; Vaizel-Ohayon and Schejter, 1999). However, actin caps are not formed in these mutants (Vaizel-Ohayon and Schejter, 1999). Conversely, centrosomes dissociated from the nucleus can induce cortical actin structures in the embryo (Kanesaki et al., 2011; Peel et al., 2007; Raff and Glover, 1989; Yasuda et al., 1991). In summary, these observations suggest that the centrosome is necessary and sufficient to induce cortical actin reorganization into cap-like structures at the cell cortex. However, the molecular mechanism is still unclear. The engulfment and cell motility (ELMO; also known as Ced-12 in flies)–Sponge complex is a promising candidate to govern this link (Schejter, 2005). Sponge is a member of the dictator of cytokinesis (DOCK180) family and acts as an unconventional guanine nucleotide exchange factor (GEF) in actin remodelling processes (Postner et al., 1992). The ELMO and DOCK180 family members are evolutionarily conserved from *C. elegans* to human (East et al., 2012; Meller et al., 2005). Binding of ELMO to DOCK180 is essential for GEF activity (Lu et al., 2004; Lu and Ravichandran, 2006). The cortical actin is unstructured in *ELMO* and *sponge* mutant embryos; however, centrosomes are unaffected in these mutants (Postner et al., 1992; Winkler et al., 2015). Based on studies in other species, it is highly likely that the ELMO–Sponge complex is involved in Rac–SCAR–Arp2/3-mediated F-actin polymerization (Katoh and Negishi, 2003). However, how ELMO or Sponge receive the signal from centrosomes is still unclear. Investigation on the regulatory mechanisms of ELMO–Sponge complex in *Drosophila* syncytial embryo might bridge this gap.

The emergence of features at tissue level

An ensemble of elements engaged in interactions may evolve toward a novel arrangement whose emergent properties cannot be directly predicted from the individual elements. Such a self-organization process is widespread and assumed to contribute to fundamental biological functions. For example, mixtures of microtubules and motor proteins with ATP form asters or antiparallel pattern under certain conditions (Sumino et al., 2012). The numerous and complex interactions between individual cells are often an obstacle towards a mechanistic understanding of feature emergence in a tissue. Conversely, because it has extended cytoskeletal networks and lacks membranous inter-nuclear boundaries, the syncytial embryo employs simplified and more direct 'cell–cell' interactions, which reduce the complexity of emergence features.

When cortical migration ends with division cycle 9, the nuclei are embedded close to the cortex in a surprisingly regular array, where 60% of the nuclei have six neighbours, and the remaining 40% are distributed equally between having five and seven neighbours (Kanesaki et al., 2011) (Fig. 2A). This regularity emerges rapidly and robustly following each division cycle, although nuclear divisions profoundly distort regularity by separations of the daughter nuclei during mitosis (de-Carvalho et al., 2020 preprint; Kanesaki et al., 2011; Koke et al., 2014). On a whole-embryo scale, uniform internuclear distances represent a favourable arrangement, which is reached by active processes of the cytoskeletal networks, including their associated motor proteins and crosslinkers. For the return to a regular pseudo-hexagonal nuclear arrangement, F-actin serves as passive stabilizer of the nuclear movement by dampening the apparent forces that are generated by the microtubule network (Kanesaki et al., 2011). Simulations of nuclear positions show that repulsive forces elicited by F-actin can be necessary and sufficient to generate the observed reordering of the nuclear array (Koke et al., 2014). The establishment of order crucially depends on the timing of the re-establishment of the microtubule network and aster interactions, which occurs in early interphase (Koke et al., 2014). A recent theoretical model for nuclear arrangement in the *Drosophila* blastoderm embryo distinguishes two contributing factors: (1) long-ranged passive forces due to the cytoplasm being a viscoelastic matrix, and (2) the active, stochastic forces generated

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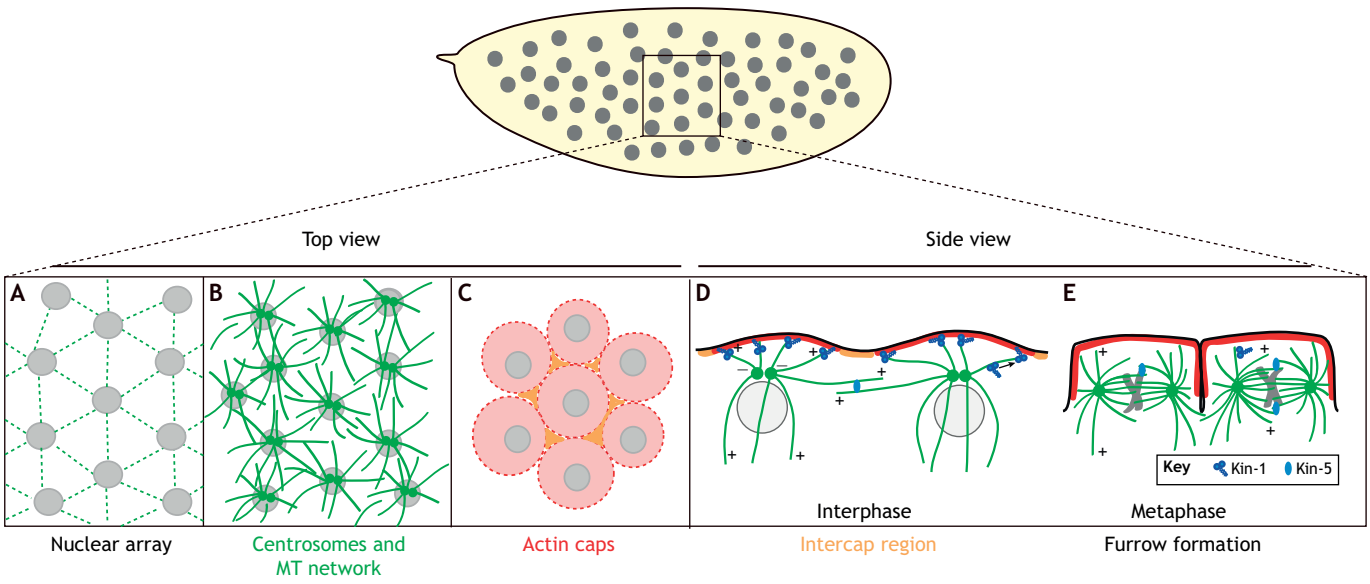

Fig. 2. Cytoskeletal organization during *Drosophila* blastoderm development. (A–C) Top view of *Drosophila* blastoderm. (A) Nuclei position at the embryo cortex in a highly ordered array, with most nuclei having six neighbours. (B) Microtubules growing dynamically from the duplicated centrosomes assemble into asters that are in direct contact with each other and generate an extended network. (C) F-actin assembles into dense areas called actin caps just above each nucleus. These caps are thought to establish a physical spacer between nuclei and so maintain their distance by antagonizing the attractive forces from microtubules. (D,E) Sagittal view. (D) In interphase, actin forms cap structures between the membrane bulges and the nuclei. Caps form localized protein-binding platforms that concentrate microtubule-binding proteins, therefore anchoring the aster and the nucleus. The plus-ends of microtubules from neighbouring asters overlap and recruit crosslinking proteins, which are thought to generate repulsion and keep nuclei separated. (E) During mitosis, while microtubules reorganize into spindles, the actin caps disappear and, instead, form furrows with periodic membrane invagination. The process of furrow formation depends on F-actin turnover, microtubules and membrane trafficking processes. Furrows are thought to separate neighbouring spindles, which are expanding in anaphase and telophase.

by motor proteins (Kaiser et al., 2018). The model suggests that the stochastic forces have to decrease with every cell cycle to maintain the positional regularity of nuclei (Kaiser et al., 2018); this means that motor proteins have to generate less force with the increasing nuclear density. Indeed, experiments show that, at higher nuclear density, the lifetime of kinesin-5 clusters bound to the spindle microtubules decreases (Kaiser et al., 2018), suggesting that the stochastic force is diluted, in support of the theoretical model.

In addition to the episodic establishment of order, mitotic waves represent a second collective behaviour. Nuclear divisions occur highly synchronous locally, but not globally, which is visible as a propagating mitotic wave sweeping over the embryo, especially during mitosis 13 (Deneke et al., 2016; Idema et al., 2013).

A third emergent feature is the stereotypical, directed nuclear movement immediately following the front of the mitotic wave (Lv et al., 2020). Following the isotropically oriented divisions, all nuclei move anisotropically a distance of several nuclear diameters away from the wave front before they return to their original position a few minutes later. Phenomenologically, this movement can be compared to a ‘yo-yo’, without implying any mechanistic or physical similarity (Lv et al., 2020). As the nuclear array remains largely intact and interactions with neighbours are maintained, the nuclei appear to move as a collective (Lv et al., 2020). The movement is driven by a combination of isotropic spindle elongation, repulsive interaction of neighbouring spindles and the mitotic asynchrony (Lv et al., 2020). The cortical F-actin, which undergoes a stereotypic remodelling linked to nuclear division cycles, stabilizes the nuclear movement. Indeed, mutant embryos with impaired F-actin display more-prominent movement, leading to larger initial displacement or loss of the return movement (Lv et al., 2020).

The ordered arrangement is not a stable state in terms of energy. When the ordered array is re-established, the nuclei and the centrosomes are not stationary at the microscopic scale, nor regarding time nor space, displaying an irregular shifting back and forth locally over time (referred to hereafter as fluctuation) (Kanesaki et al., 2011; Winkler et al., 2015). The astral microtubules growing from neighbouring centrosomes strongly interact during interphase. Motor proteins and the polymerization of microtubule filaments drive this network out of the dynamical equilibrium (Mackintosh and Schmidt, 2010). Hence, nuclei and centrosomes embedded in a cytoskeletal matrix can be interpreted as nodes of a network, where their positional fluctuations represent the dynamics of the cytoskeleton. Indeed, depletion of F-actin by Latrunculin A injection increases the fluctuation movements of the nuclei in interphase, whereas co-injection of Latrunculin A and Colcemid represses it (Kanesaki et al., 2011). Taken together, these observations suggest that microtubules are required for nuclear fluctuations (Kanesaki et al., 2011). The centrosomes display a similar fluctuation behaviour to the nuclei in interphase, as well as a similar response when cytoskeletal dynamics are targeted (Winkler et al., 2015). Cortical actin caps and the motor Kinesin-1, which is localized at the actin caps, serve to stabilize the microtubule network (Winkler et al., 2015). The four-headed crosslinker Kinesin-5 also stabilizes the microtubule network as its depletion increases centrosome fluctuation (Lv et al., 2018). Therefore, a plausible, but hitherto untested hypothesis, is that, in interphase, Kinesin-5 connects adjacent microtubule asters by serving as a crosslinker of the antiparallel-oriented microtubules, rather than a sliding motor. Whether there are any other active components remains an open question. The minus-end motor Kinesin-14 represents a promising candidate, whose function may be tested by inhibition or depletion experiments.

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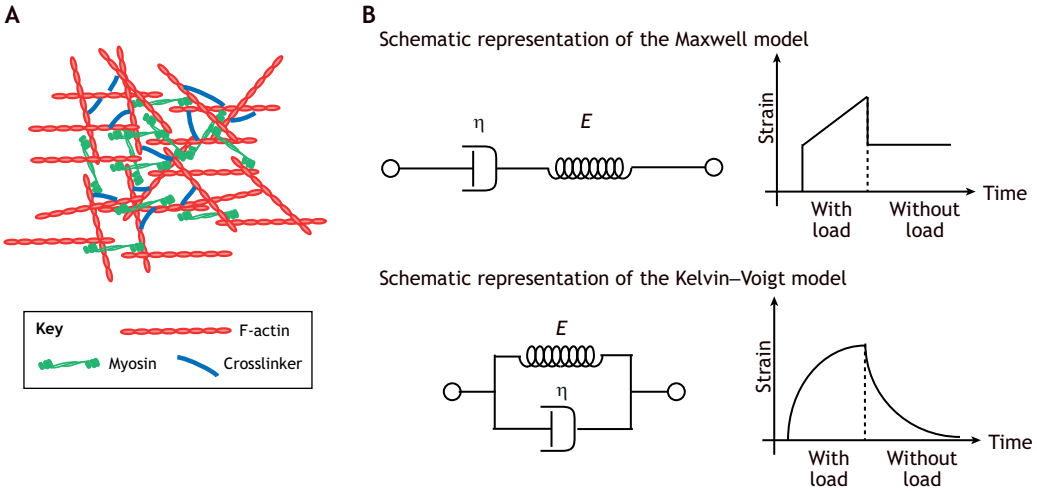

Fig. 3. Models of cell mechanics. (A) Actin–myosin networks dominate the behaviour of the tissue or cell under force. (B) A viscoelastic material is commonly described by either the Maxwell or the Kelvin–Voigt model, or a combination thereof. These mathematical models are composed of a combination of springs and dashpots, representing elastic and viscous components, respectively. When a constant tensile or compressive load is applied, the strain profile of each model is characteristically distinct. The Maxwell model does not recover the strain change when released from the load, giving rise to a history-dependent response. The Kelvin–Voigt model is characterized by smoother responses to sudden load and recovers to the original configuration after load release. Cells exhibit a combination of these responses upon force application and are viewed as partly solid and partly fluid material.

The cytoskeletal morphology determines the cellular biophysical properties

Contemporary models of tissue dynamics incorporate mechanical forces at three levels: (1) cell-endogenous forces autonomously produced by the active cytoskeleton (Pollard and Cooper, 2009), (2) non-autonomous forces elicited by neighbouring cells within the tissue (Pinheiro et al., 2017), and (3) extrinsic forces from outside of the tissue (Breau et al., 2017). Active mechanical forces inside the cell are mostly generated by motor proteins, moving on their corresponding polymers by utilizing the free energy of ATP hydrolysis. For example, forces exerted from myosin on F-actin, coupled to the membrane by members of the ezrin–radixin–moesin (ERM) protein family (Fehon et al., 2010), drive cell shape changes and membrane remodelling (Chugh and Paluch, 2018). In addition, polymerization of F-actin also generates mechanical force (Footer et al., 2007), which is mediated by nucleators and elongation factors, such as Arp2/3 and formins (Pollard and Cooper, 2009) (Fig. 3A). Kinesins and dynein move along microtubules and generate forces not only for the separation of nuclei during mitosis, but also for the positioning of the nuclei in the syncytial *Drosophila* embryo and in muscle cells (Kaiser et al., 2018; Lv et al., 2018; Metzger et al., 2012).

The forces that cells produce or experience activate mechanisms of force sensing, its transduction and response. A cell responds to forces by (1) changing shape, which is classically described by material properties; (2) altering its position relative to neighbouring cells, which depends on cell–cell adhesion; or (3) changing growth, function or fate, and these responses can be explained by a combination of intracellular properties and cell–cell adhesion (Ladoux and Mège, 2017; Vogel and Sheetz, 2006). Although cell shape change is defined to a considerable extent by the cytoskeletal organization (Pollard and Cooper, 2009), surface properties and surface tension of cell membranes also contribute to cell shape (Lecuit and Lenne, 2007). A number of micro-rheological methods, such as atomic force microscopy (AFM), and the use of magnetic beads or ferrofluid droplets, whereby inert beads are dispersed in cells or tissues, have been developed to probe the material properties of living tissues (Abidine et al., 2013; Mongera et al., 2018; Norregaard et al., 2014; Rigato et al., 2017; Serwane et al., 2017; Wirtz, 2009). Based on these analyses, living tissues and cells are considered viscoelastic materials (Fig. 3). Purely elastic solids store the applied energy when being deformed by a force; thus, when the force is removed, the material releases the energy, thereby fully reversing the deformation (Kasza et al., 2007). By contrast, a purely viscous fluid flows when an external force is applied, but it will not recover from this deformation and the energy is dissipated by the internal friction of the fluid (Kasza et al., 2007). Viscoelastic materials exhibit both behaviours. Furthermore, the response of a cell to an applied force shows temporal characteristics – the tissue displays elastic behaviour at short timescales (seconds) and viscous behaviour at a longer timescale (minutes to hours) (Forgacs et al., 1998; Foty et al., 1994; Guevorkian et al., 2010; Marmottant et al., 2009).

The *Drosophila* syncytial embryo, given its sub-millimeter size, is tractable to measurements of cell biomechanics. In a passive microrheology approach, trajectories of passive fiduciary markers, such as endogenous particles or injected fluorescent beads have been recorded (Wessel et al., 2015). The viscoelastic material properties can be derived from the fluctuations of the passive particles. Such measurements reveal the shear modulus, which describes the response to an externally applied force and which can be decomposed into an elastic modulus and viscous modulus (Xia et al., 2018). In one approach micrometre-sized beads were injected in early *Drosophila* embryos. These measurements revealed that the syncytial embryo behaved like a typical cytoplasm with the viscous component dominating over the elastic response (Wessel et al., 2015). The behaviour of probes at different depths of the embryo revealed a trend for the viscoelastic modulus to decrease from the cortex towards the yolk, which is consistent with a decrease in microtubule network density (Wessel et al., 2015). The viscous modulus is partly dependent on microtubules but not on F-actin; possibly the passive beads were too large to enter the actin cortex.

The conclusion of a dominating viscosity was confirmed by an active microrheology approach (Doubrovinski et al., 2017). Here, ferrofluid droplets were injected into the embryo at the stage of cellularization. Pulling on the droplet with a magnet led to a

measurable displacement of the ferrofluid droplet (Doubrovinski et al., 2017). The pulling force required was found to be related to the displacement and, hence, to allow the measurement of the rheological properties of the embryonic cortex by recording the movement of the ferrofluid droplet when the magnetic force was applied and removed. This method also allowed the specific investigation of the F-actin-rich cortical domain of the embryo, which is difficult to measure with passive rheology methods. This active rheology approach revealed that the cellular cortex is quasi-elastic (Doubrovinski et al., 2017). The authors quantitatively described the behaviour of the elastic cortex by means of a computational framework that allows the input of the experimentally determined parameters in order to calculate the timescale of relaxation; the embryo cortex shows an elastic response with a decay on the timescale of 4 min. In other words, the cortex behaves elastically for deformations occurring shorter than 4 min, whereas deformations lasting much longer lead to a significant viscous response (Doubrovinski et al., 2017). Importantly, cortical elasticity originates from the F-actin cytoskeleton, but not microtubules (Doubrovinski et al., 2017).

A similar study, in which magnetic beads were injected into the cellularizing *Drosophila* embryo, enabled a controlled force application (D'Angelo et al., 2019). The displacement of the beads and the deformation of the apical surface of the epithelium were analysed by means of a viscoelastic Maxwell model and Kelvin–Voigt model (Banks et al., 2011). These models are widely used in analysis of the viscoelastic response of materials when force is applied. The materials are often modelled by combinations of spring (elastic elements) and dashpot (viscous elements). The Maxwell model and Kelvin–Voigt model are two simple arrangements of springs and dashpots (Fig. 3B). Repeated application of force, which causes sequential bead displacement, resulted in a progressive increase of bead mobility. In the view of those authors, the mechanical properties of the embryo evolve and change during cellularization, and this depends on microtubule polymerization (D'Angelo et al., 2019). A similar response was also observed in the ferrofluid droplets study; however, instead the authors proposed there that the mechanical response of the cortex was history dependent (Doubrovinski et al., 2017). This implies that any applied force induces local changes in the state of the cortical network, which remain for an extended time even after release from the mechanical stress (D'Angelo et al., 2019). In addition, modelling analysis of the tissue deformation field indicated that the embryo softens during the cellularization process, while the external friction between the embryo tissue and the vitellin envelope increases (D'Angelo et al., 2019). Furthermore, embryos treated with a microtubule-depolymerising drug became more fluid, and the friction between the cells and the vitellin membrane increased further compared to control embryos (D'Angelo et al., 2019). A possible interpretation is that in addition to the actomyosin network, the microtubule cytoskeleton contributes to epithelial mechanics. The application of biophysical approaches on Drosophila syncytial embryos opens the opportunities to explore how mechanics originated from cytoskeletal components contributes to sculpting embryonic tissues.

Conclusions

It has become evident in recent years that tissues act as multicellular systems and exhibit emerging collective behaviour. A prerequisite for our understanding of these emerging properties is the quantitative and multifaceted description of the constituent cells as active elements. Thus, in addition to studying gene function, molecular interactions and cellular processes, future investigations should focus on three additional aspects: (1) spatiotemporal force maps of isolated cells, specifically how the dynamic distribution of active force generated by the cytoskeleton and motors affects individual cells; (2) mapping of material properties, such as the spatiotemporal changes in the viscoelasticity of the cell cortex and the cytoplasm; and (3) elucidating mechanical cell–cell interactions in tissues. We believe that an integration of the cellular parameters at the tissue scale could provide an explanation for the observed tissue dynamics. Although the conceptual transition from cellular to tissue systems is difficult due to the increasing complexity and the magnitude difference in spatial scales, emerging new technologies and experimental methods will provide the required information to feed the model concepts. Although the *Drosophila* syncytial embryo is a simplified tissue model, it presents unique features and requires fewer model parameters than other 'real' tissues. For example, the cytoskeleton elements of each unit such as microtubule asters can directly interact and form a network. Junctions and cell adhesion, which link the cytoskeletal units in tissues do not need to be considered. Yet, individual nuclear domains form an extended tissue-like array that retains molecularly defined interactions. Furthermore, material properties can be measured more accurately in this system, as the cell membranes in real tissues often mask the cytoplasmic response to mechanical measurements. The membrane-free, quasi-cellular domains of the syncytial nuclei display complex cytoskeletal interactions, from which collective responses emerge.

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