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Mechano-regulation of germline development, maintenance, and differentiation

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ABSTRACT

Biochemical signaling arising from mechanical force-induced physical changes in biological macromolecules is a critical determinant of key physiological processes across all biological lengths and time scales. Recent studies have deepened our understanding of how mechano-transduction regulates somatic tissues such as those in alveolar, gastrointestinal, embryonic, and skeleto-muscular systems. The germline of an organism has a heterogeneous composition - of germ cells at different stages of maturation and mature gametes, often supported and influenced by their accessory somatic tissues. While biochemical signaling underlying germline functioning has been extensively investigated, a deeper interest in their mechanical regulation has been gaining traction in recent years. In this review, we delve into the myriad ways in which germ cell development, maintenance, and functions are regulated by mechanical forces.

Introduction

By virtue of its ability to transmit genetic information accurately to the next generation, the germline ensures the perpetuation of a species and is therefore subjected to rigorous scrutiny. It comprises mature gametes and stem/progenitor cells at various stages of development. Surrounding somatic cells transduce mechanical and biochemical signals and provide nourishment to the germline [1,2]. The germline often segregates from somatic lineages early in the development of an embryo. The nature and function of the germline have been of particular interest for several decades, mostly due to its ability to mature into gametes as well as maintain a regenerative potential throughout the organism's reproductive period [1].

In addition to genetic, biochemical, and electrophysiological signals, the ability of cells to sense mechanical stimuli and transduce them into downstream signals plays a major role in various cellular and physiological processes, such as the fate determination in stem cells, cell differentiation, polarity establishment processes in differentiated cells, and embryonic gastrulation [3,4]. While the mechanical regulation of germ cells has been observed for decades, the role of mechano-transduction in germline development and homeostasis has only recently been explored [1,5–8]. These mechanical cues may arise from the surrounding microenvironment of the cell, including the extra-cellular matrix (ECM), inter-cellular (germ cell-germ cell or germ cell-somatic cell) contacts, or from intrinsic cellular states, such as membrane composition, cytoskeleton architecture, organelle dynamics, or various trafficking processes in the cell [9]. These stimuli are sensed by various mechanosensitive proteins like integrins, cadherins, stretch-activated ion channels, growth factor receptors, and G protein-coupled receptors, amongst others [10,11]. Here, we review our current understanding of mechanical regulation in germline development and gamete formation in adult reproductive systems, across diverse model systems. We focus on specific examples from primordial germ cell (PGC) development to fertilisation, and leave out the role of mechanics in embryogenesis, which has been extensively discussed in recent reviews [12–14].

Germline development and specification

The germline development in an organism begins early in the embryonic stages, originating from the pluripotent precursor – the Primordial Germ cells (PGCs). During early embryogenesis in vertebrates, PGCs actively migrate from the outer surface of the embryo to the endodermal layer [15]. PGCs in *Drosophila melanogaster* (*Drosophila*) and *Danio rerio* (zebrafish) are specified by cytoplasm (germplasm) containing maternally derived RNAs and proteins. In Drosophila, PGCs arise at the posterior of the embryo, migrate collectively into the midgut pocket during gastrulation, and finally move individually through the posterior midgut into the mesoderm where they interact with the

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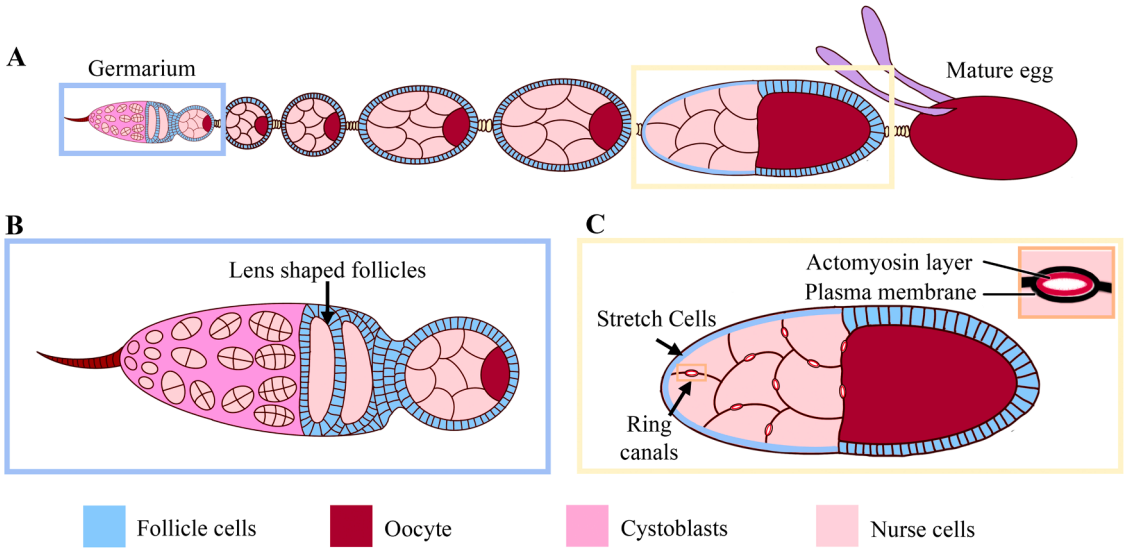

Fig. 1. *Drosophila* egg chamber development is associated with changes in cell number, cell shape and an overall structural morphogenesis. (A) The follicles at different stages of maturation from the initial germarium to the oocyte at the end. (B) The germarium with developing follicles at different stages and characteristic shapes. (C) A matured follicle with the nurse cells, oocyte and the surrounding follicle cells. The nurse cells and oocyte are connected by cytoplasmic bridges called ring canals (inset) formed of an outer rim of plasma membrane (black) and inner rim of actin-myosin network (red).

somatic gonad precursors (SGPs) to form the gonad [15]. A similar migration pattern is seen in the case of PGCs in Zebrafish and mice, however, the formation of PGCs in mammalian embryos depends on cell-cell induction [15] .

In the soil nematode, *Caenorhabditis elegans* (*C. elegans*), asymmetric segregation of P granules and activity of the PIE-1 protein during early embryonic divisions specifies the P blastomere as the germline founder cell [16]. The segregation of P granules is facilitated by the cytoplasmic flow from the anterior to the posterior end of the one-celled zygote while cortical actomyosin flows in the opposite direction [16]. Spatial segregation of PAR proteins at the cell surface also contributes to the selective stabilization of these ribonucleoprotein complexes at the posterior region [17]. Subsequently, the designated P-blastomere undergoes four rounds of asymmetric divisions giving rise to the germline founder cell, P4, which subsequently gives rise to Z2 and Z3, the PGCs. These PGCs start elongating symmetrically in the anterior-posterior axis of the worm body post-hatching, finally giving rise to the two U-shaped gonad arms in the adult hermaphrodite (Fig. 3A) [18].

Cell-cell interactions constitute a major source of mechanical signals

Inter-cellular interactions are mediated by a diverse array of cell surface assemblies such as cell junction proteins, receptor-ligand complexes, cytoplasmic bridges, and ion channels. The *Drosophila* germline cyst consists of an oocyte and 15 nurse cells originating from a single cystoblast through four rounds of incomplete cell divisions [19,20]. The cyst is in constant physical contact with the surrounding pre-follicle cells. E-cadherin-dependent intracellular adhesion between the follicle cells and the nurse cells drives the directed movement of the follicle cells, whose controlled proliferation and migration form the somatic epithelium [21,22]. As a result, the somatic epithelium encases the cyst. The differential expression of E-cadherin between the oocyte and the posterior follicle cells defines the positional fate of the oocyte as well as the future anterior-posterior axis of the embryo [23]. In addition to cell-cell junctions, the maturing oocyte and the nurse cells are connected by intercellular bridges called 'ring canals' (RC). These RCs are composed of an inner actin ring attached to the outer electron-dense membrane rim, which in turn remains connected to the plasma membrane of the nurse cells (Fig. 1C). During vitellogenesis, exponential germline proliferation stretches the nurse cell membrane. The increased membrane tension resulting from the stretching directs the trafficking of E-cadherin, mediated by the clathrin adaptor protein 1 (AP-1) and the recycling endosome marker Rab11. An increase in E-cadherin stabilizes the RC and ensures its sustained anchorage to the nurse cell membrane thereby preventing multinucleation in nurse cells [19].

Likewise in mammalian ovaries, cadherin (N- and E-cadherins) mediated inhibition of granulosa and germ cell apoptosis promotes follicular growth and viability. Temporal dynamics of intercellular adhesions mediated by cadherin-mediated cell-cell adhesions have a continued influence on the gonad, gamete development throughout their developmental stages, and viability of the mature gametes [24–27]. Filopodial extensions probe the cellular environment for mechanical and biochemical cues. Recent reports have highlighted the role of specialized filopodia, extending from the granulosa cells to the oocyte surface, called transzonal projections (TZPs), in mediating oocyte-granulosa cell interactions (Fig. 2). Incidentally, SMAD4 transcription factor-activated through the TGF beta signaling pathway promotes TZP growth via N-cadherin and Notch2 [28].

Like cadherins, intercellular contacts mediated by desmosomal junctions are necessary for germ cell survival in mammalian fetal testis and ovaries. This is evidenced by the decreased number of germ cells and follicles in these tissues due to the loss of Desmoplakin (DSP) [29]. In adult testis, desmosome-mediated adhesion within Sertoli cells and between Sertoli cells and the developing germ cells is necessary for morphogenesis, differentiation, and maturation of the spermatids (Fig. 2C) [30]. Pharmacological perturbation of adhesion junctions between spermatids and Sertoli cells by Adjudin induces detachment of spermatids, resulting in low sperm count. This identifies Adjudin as a potential male contraceptive agent [31].

Seasonal breeders like the mink species *Neovison vison*, and the Armadillo species *Chaetophractus villosus*, modulate the strength of adhesion between Sertoli cells and germ cells to maintain periodic regression of the seminiferous epithelium. During regression, a loss in Sertoli-germ cell adhesion leads to a possible regulation of post-meiotic shedding of germ cells and massive germ cell death [32,33]. Similar to mammals, Sertoli cells in fishes are connected by basic cell-cell junctions like adhesive, tight, and gap junctions. Adhesive junctions are also present between germ cells and in the germ cell-sertoli cell interface. These interactions in the seminiferous epithelium are speculated to be

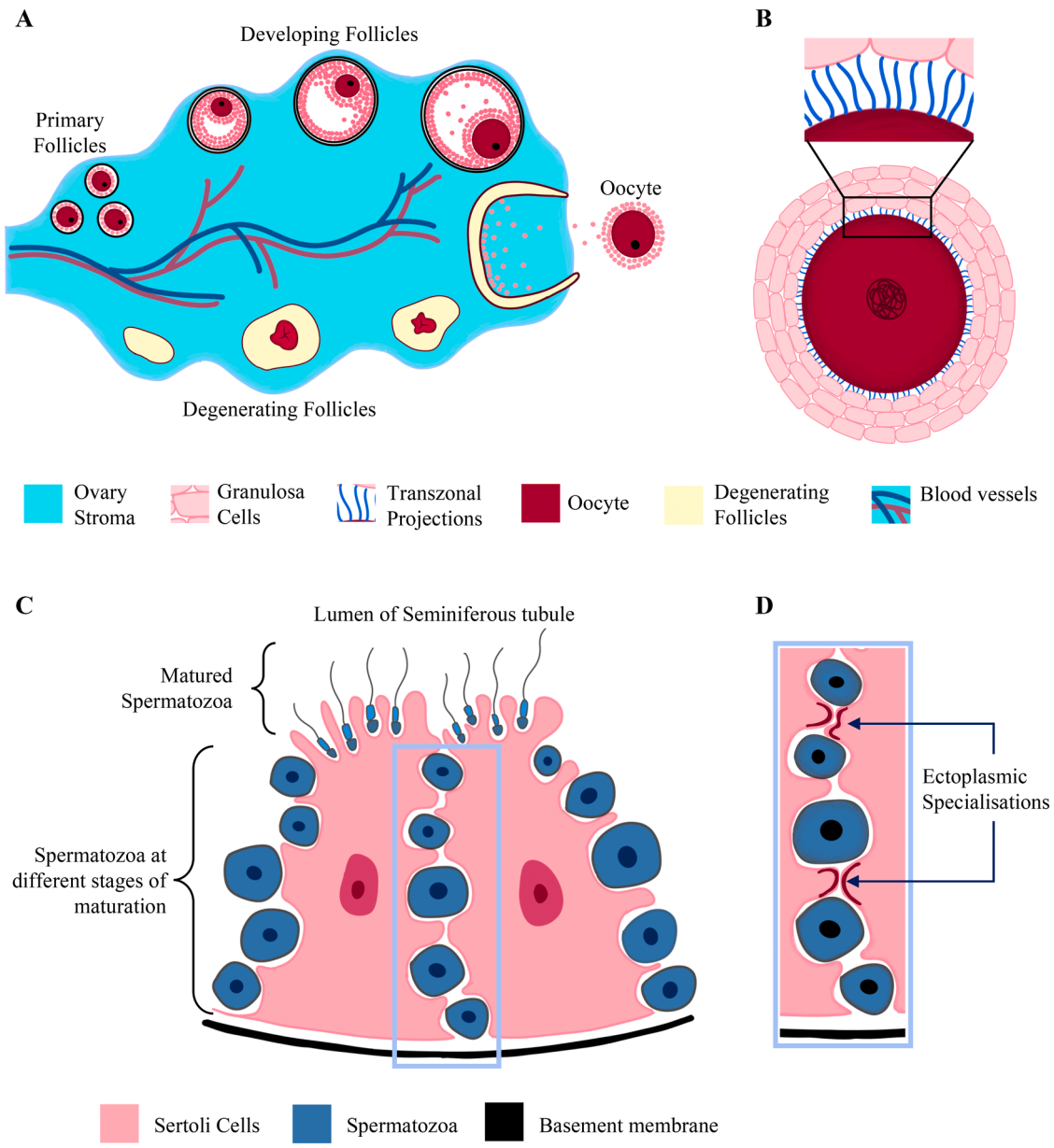

Fig. 2. Mammalian Germline. (A) The mammalian ovary with follicles at different stages of development and degeneration arranged on a common stroma. (B) The matured oocyte is surrounded by a few layers of granulosa cells, that act as nurse cells. These cells are in constant contact with the oocyte by cytoplasmic extensions called Transzonal projections (TZP). (C) In the mammalian testis, seminiferous tubules are lined with larger sertoli cells (pink) and spermatozoa at different stages of maturation (blue) attached to the Sertoli cells. (D) Specialised cell-cell contacts are found in the interfaces of germ-germ cell or germ-sertoli cell junctions.

important for the development and survival of the germ cells [34].

Local mechanics govern germ cell fate

In addition to cell-cell interactions, germ cells interact with and get influenced by their surrounding matrix elements. The development of the *C. elegans* hermaphrodite gonad is initiated post-larval hatching, with the emergence of two Distal Tip cells (DTCs) from the pair of somatic gonad precursor cells, Z1 and Z4. Z1 and Z4, and the associated basement membrane, flanks the PGCs Z2 and Z3. DTCs lead proliferating germ cells in opposite directions into the U-shaped reproductive organ (Fig. 3) [7,35]. While movement of germ cells and elongation of the gonad is propelled, at least in part by the mechanical forces generated due to germ cell proliferation behind the DTC, the differential degradation of the basement membrane determines the direction of DTC migration [36,37]. In addition to the cortical actomyosin contractility, microtubule pushing localizes the nucleus to the leading edge of the DTC. These complementary mechanical forces ensure the polarity, shape, and integrity of the DTC to ensure the development, architecture, and maintenance of the germline [38,39]. The L4 stage of larval development witnesses rapid proliferation and gametogenesis. A dynamic, 3D in-silico study suggested that the mechanical stretching of the proximal gonad during larval stage 4 (L4) promotes normal gonadogenesis. Additionally, the cell cycle stage of the germ cells in the adult proliferative zone is determined by their packing density (Fig. 3A) [40]. The stretching and compressive packing forces thus modulate cell fates at various developmental stages implying the significance of local mechanical feedback in cellular decision making.

Similar mechanical influence on development is demonstrated during *Drosophila* egg chamber undergoing elongation from a spherical to an ellipsoid shape (Fig.1). The elongation is accompanied by a microtubule-dependent circumferential rotation of the follicular

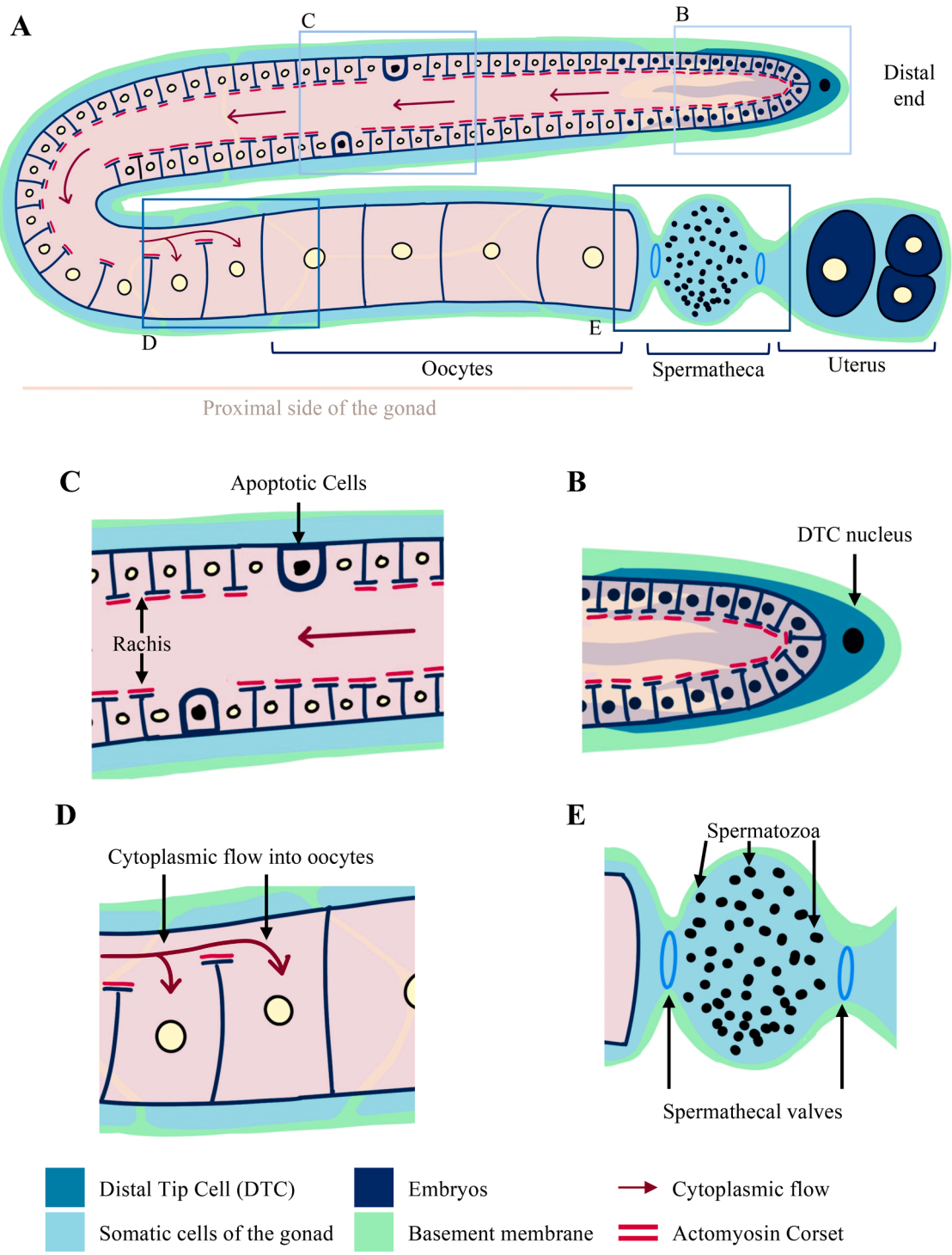

Fig. 3. A schematic showing germ cells in the adult *Caenorhabditis elegans* hermaphrodite gonad. (A) The gonad consists of two U-shaped arms flanking a common uterus. Each arm consists of a somatic DTC and sheath cells that envelope the germ cells. This structure is encased by a basement membrane. (B) DTC, at the distal edge of the gonad migration, maintains the germline stem cells. (C & D) The syncytial gonad at various stages of meiotic development surrounds a common rachis with a cytoplasmic flow from distal to proximal end (red arrows). Germline cells that undergo physiological apoptosis support oogenesis by contributing their cellular contents to the growing oocytes. (E) The Spermatheca houses a number of matured spermatozoa. Fertilisation takes place upon entry of a matured oocyte from the distal side and its exit into the uterus post-fertilisation. This process is supported by the opening and closing of the spermathecal valves.

epithelium along the A-P axis [41]. This rotation results in the formation of a polarised corset of actin bundles in the epithelium and extracellular matrix (ECM) fibrils surrounding the egg chamber. The organization of these ECM fibrils mirrors that of the epithelial actin bundles [42,43]. The Dystrophin associated protein complex (DAPC), a complex that connects the ECM to the intracellular actin cytoskeleton, affects the deposition of the ECM fibrils and orientation of actin bundles in the egg chamber. The stiffness gradient along the A-P axis exhibited by the ECM-based basement membrane confers differential resistance to the growing follicles, thus affecting their direction of elongation. Elongation process, therefore, is augmented by the collective contractility of the circumferential corset as well as the mechanical anisotropy around the

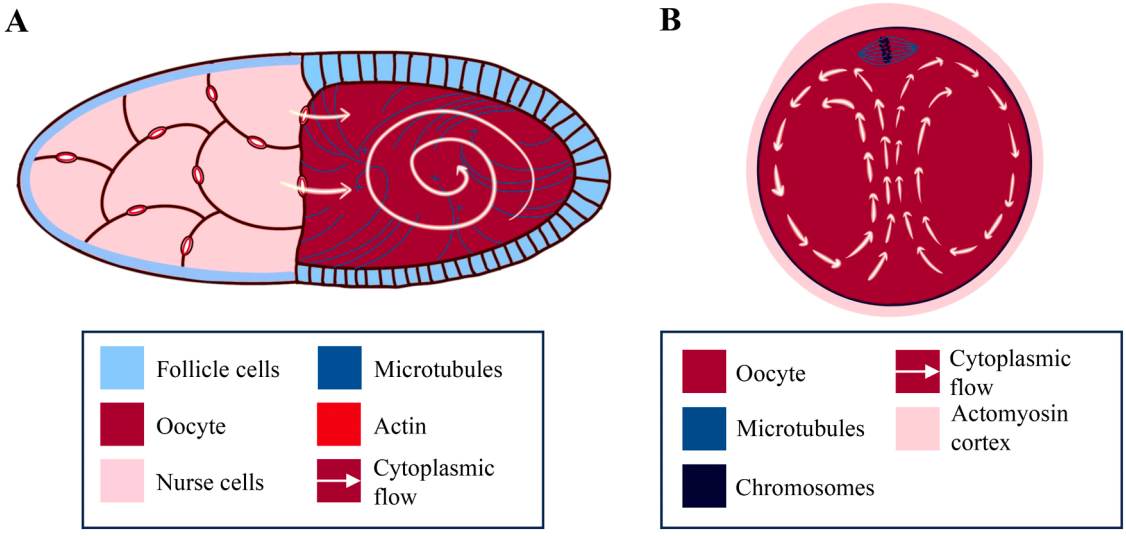

Fig. 4. Cytoplasmic streaming. (A) A matured *Drosophila* follicle showing microtubule arrangement and cytoplasmic streaming in the oocyte. (B) A Meiotic II stage mice oocyte showing the actomyosin cortex, asymmetrically placed spindle assembly and cytoplasmic streaming direction.

follicles [44].

In mammals, the periodic development and maturation of oocytes from a pool of primordial follicles ensures the longevity of the female reproductive life. Developmentally advanced follicles demonstrate stiffer regions in the tissue compared to regions enriched in stromal cells [45]. The compressive stiffness of the extracellular matrix (ECM) surrounding the oocytes maintains the dormancy of primordial follicles at the edge of the ovary, finally resulting in their nuclear rotation which is involved in the maintenance of the dormant state and inhibiting germline differentiation [46]. In addition to directly influencing cell fate dynamics, both these external interactions, - cell-cell and cell-matrix adhesions also regulate a number of intracellular determinants and processes. Some of these processes are discussed in sections below.

Germline regulation through flows and waves – role of cytoskeleton

In *C. elegans*, much of the adult germline is a syncytium around a common rachis with cytoplasmic flow away from the DTC, and into the developing oocytes (Fig. 3A and 3C). This flow is driven by the pulling forces from the asymmetric organization of the actomyosin cytoskeleton in maturing oocytes and is sustained via Major Sperm Protein (MSP) Signalling in the proximal arm of the gonad and GLP-1/Notch Signalling in the distal arm [47–49]. The flow-induced shear stress in the gonad is further intensified by the ovulation-associated periodic contraction of the surrounding sheath cells. Actomyosin corset that envelops the rachis counters the cytoplasmic pressure on the gonad architecture (Fig. 3A) [50]. The scaffold protein Anillin (ANI-2) is enriched in the intercellular bridges of the germ cells and stabilizes the rachis. This provides elasticity to the germ cell walls against continuous mechanical stress. Perturbing the rachis structure through ANI-2 depletion or disruption of actomyosin results in multinucleate germ cells [51]. Loss of ANI-2 also results in smaller germ cells and displays a concomitant increase in apoptotic cells [52]. Thus, in addition to regulating the architecture of the *C. elegans* gonad, mechanical forces also regulate the survival and fate of cells in the germline (Fig. 3A). Hydraulic instability originating from the differences in the cytoplasmic flow across individual germ cells results in variable cell sizes. While cells that receive more material grow, others shrink and undergo apoptosis (Fig. 3C). This size-driven induction of cell fate is further demonstrated by the depletion of ANI-2 [52].

Interestingly, while the cytoplasmic flow in the C. elegans gonad is largely unaffected by the microtubule disruption, microtubule-dependent cytoplasmic streaming is essential for *Drosophila* oogenesis. Kinesin-1 and Dynein-mediated cargo transport along with Kinesin-1-driven microtubule sliding at the ooplasm, and F-actin dynamics at the ring canals, causes this cytoplasmic streaming [53]. Subsequently, a precise level of cytoplasmic streaming, maintained by an actin mesh, orients and organizes microtubules with their growing ends towards the posterior end of the oocyte [54]. A slower streaming during mid-oogenesis drives the polarised localization of *oscar (osk)* and *gurken (grk)* mRNAs, while a faster streaming in later stages of oogenesis aids in the process of cytoplasm dumping of the nurse cells into the oocyte (Fig. 4A) [55–60]. Acetylation on the conserved lysine (K40) residue of alpha-tubulin has been shown to stabilize microtubules [61]. The loss of *Drosophila* CG17003/leaky (lky), required for alpha-tubulin acetylation in early germ cells, also results in incomplete egg chamber separation in older flies, often resulting in fused cysts in the ovaries [62]. Apart from the microtubular network, an actin motor Myosin V also contributes to the streaming-driven oocyte polarisation in *Drosophila* [63]. Similarly, in mouse oocytes, Arp2/3 mediated actin polymerization results in cytoplasmic streaming away from the actomyosin-rich cortical cap of the oocyte. Although the source of activation for Arp2/3 during Meiosis I (MI) is not yet known, Arp2/3 activation during Meiosis II depends on the Meiotic II (MII) chromosomes. This flow, in turn, creates a net pressure on the MII spindle, keeping it positioned near the cortical cap, thus maintaining the spindle position asymmetry necessary for oocyte quality maintenance and fertility in the organism (Fig. 4B) [64,65].

Intrinsic co-ordination between biochemical signaling and mechanotransduction in germline homeostasis is also evidenced in the *Drosophila* ovarian follicle. In these tissues, the tension generated on the follicle cells by germline proliferation has been proposed to stimulate epithelial growth [66,67]. The pushing force emanating from the growing cyst creates tension in the surrounding epithelium which is balanced by myosin activity within the actomyosin cytoskeleton on the apical surface [67]. Reciprocally, forces exerted by germline proliferation induce spatiotemporal variability in follicular cells' cortical actomyosin contractility, leading to altered cellular viscoelasticity and shape deformation [68]. The process of encapsulation of the germ cells by the follicle cells is coordinated by periodic and contractile actomyosin waves at the cortex of the germ cells. An adequate balance between the cortical stiffness and adhesive forces of the germline and constricting forces from the somatic cells is required for individual sorting of the germline cysts. Disruption in this mechanical crosstalk leads to compound or incomplete egg chamber formation [69].

In Zebrafish oocytes, actin and non-muscle myosin II localizes to the cell cortex [70]. Here, cell cycle mediates bulk actin polymerization

waves in the animal-vegetal axis. The resulting segregation of the ooplasm and yolk granules facilitates the establishment of the body plan axis [71]. Apart from the bulk actin network, columnar actin structures at the animal pole anchor cell-cycle regulators such as cyclin B1 mRNAs as granules and prevent their precocious translation activation [72].

Mechanostimulation of cultured oogonial stem cells promotes their differentiation into oocytes through a Yes-associated protein (YAP), Rho-associated protein kinase (ROCK), and Filamentous actin (F-actin) pathway [73]. Additionally, an actin nucleating factor formin-like 2 (FMNL2), regulates spindle migration and organelle distribution during oocyte meiosis, essential for their maturation [74].

Mammalian Sertoli cells have an elaborate cytoskeleton, comprising primarily of actin, vimentin-type intermediate filaments, and microtubules [5,6,75]. A unique actin-rich cell-cell junction, known as Ectoplasmic specializations (ESs), is found in the cytoplasm of these cells. These ESs maintain adhesion among adjacent Sertoli cells or between Sertoli cells and Spermatozoa. The turnover of cytoskeletal components in these junctions mediate the basal to apical movement of Spermatozoa as well as the release of matured spermatozoa from the apical surface of Sertoli cells [5,6,76].

While the roles of actomyosin in germline mechano-transduction have been well documented, the functions of microtubules and intermediate filaments (IFs) are largely unexplored and are beginning to be understood. During spermatogenesis, IFs integrate endogenous and exogenous forces resulting in their redistribution. This leads to changes in the shape and maturation of the spermatozoa [77]. Loss of IFs by Interleukin 1 alpha (IL1) affects the gap junction communication between Sertoli cells and spermatozoa, due to the aggregation of stress fibers and disruption of the exogenous F-actin by the Sertoli cells. This eventually affects the blood-testis barrier [78].

Mammalian fertility depends heavily on sperm health and motility. A group of coiled-coil filamentous proteins, the tektins, originally purified from sperm flagella of the sea urchin *Strongylocentrotus purpuratus*, have been seen to stably associate with the microtubular lattice. Tekt4 is a member of the tektin family of proteins expressed exclusively in the mice testis and highly abundant in spermatozoa of humans. Loss of Tekt4 in mice disorganizes the flagellar ultrastructure and severely depletes ATP levels. This reduces male fertility by impairing the forward progressive motility and defects in the flagellar motion of spermatozoa [79].

Nucleus

Organelle dynamics is a key intrinsic stimulus, that co-regulates germline homeostasis in close association with cytoskeletal reorganization. At the nuclear envelope, the Linker of nucleoskeleton and cytoskeleton (LINC) complex mediates the trans-nuclear mechano-transduction. In *C. elegans*, the assembly, maintenance, and function of this complex in a growing oocyte is dependent on a nuclear lamin component, OOC-5 (abnormal oocyte formation 5), a homolog of torsinA in worms. A coordinated activity of the LINC complex, OOC-5/torsinA, and nuclear lamin architecture ensures the nuclear integrity of early meiotic germ cells and promotes proper insertion of the nuclear pore complex proteins into the growing nuclei of interphase germ cells [80].

Homologous chromosomes in pachytene stage of the early meiotic cells assemble the synaptonemal complex. Disruption in the assembly of this complex leads to PLK-1-dependent phosphorylation of the nuclear lamina. Disassembly of phosphorylated lamins destabilizes the nuclear envelope. This instability is sensed by the mechanosensitive Pezo-1 channels on the nuclear envelope, resulting in the LINC complex-mediated induction of apoptosis [81]. Additionally, the conserved nuclear envelope membrane protein 1 (NEMP1), through its association with Emerin, maintains nuclear envelope stiffness in the germlines of metazoans. The loss of NEMP1 function in *Drosophila, C. elegans,* zebrafish, and mice results in a number of fertility defects such as immature gametes, reduced brood size, and an increased number of dead eggs [82].

Mechanics mediated metabolism and transcriptional regulation during germline maintenance

In the *Drosophila* ovary, during its process of differentiation, the rounded cyst is deformed to a characteristic one-cell-thick lens shape, and eventually becomes spherical in the budding egg chamber (Fig. 1B). The mechanical stress resulting from these shape changes facilitates the maintenance of cytosolic Ca^{2+} in the germ cells via the Transmembrane Channel like (TMC) channel. A sustained cytosolic Ca^{2+} concentration triggers the transcriptional activation of Oxidative Phosphorylation (OXPHOS) in the differentiating cysts [83]. The switch from glycolysis to OXPHOS, as a source of major ATP production, coincides with the transition from stem/progenitor state to a more differentiated state [84,85]. The developmentally arrested mature oocytes undergo egg activation and a concomitant increase in the levels of intracellular Ca^{2+}. During *Drosophila* egg activation, environmental calcium enters the cell via the Transient receptor potential M (TRPM) family of channels in response to mechanical stimuli. These channels are distributed equally all along the oocytes, but are specially activated in the poles- the sites of mechanical stimulus, initiating calcium waves at these sites [86,87]. A similar regulation in human ovarian cells is mediated through the transient receptor potential vanilloid 2 (TRPV2), a channel that is known to be activated by mechanical stimuli via the transport of Ca^{2+} [88].

The YAP/TAZ (Yes-associated protein/Transcriptional Activator with a PDZ domain) proteins are known to act as sensors and relays of mechanical cues in *Drosophila* and mammalian cells [89,90]. The germline growth-induced stretching of the surrounding follicle cells also induces nuclear localization of Yki, the single homolog of the Hippo signaling pathway effector YAP/TAZ in *Drosophila*. Similarly, physiological mechanical strain causes the flattening of a group of follicle cells, called the 'stretch cells' (Fig. 1C). This shape change leads to reduced dimerization of Hippo in the apical region of these follicle cells, resulting in the nuclear localization of Yorkie (Yki). This promotes cell flattening of the stretch cells [91]. The nuclear localization of Yki, along with nutritionally induced insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS), activates the Scalloped-mediated transcription. This, in turn, promotes proliferation in the ovarian follicle cell epithelium [92].

Reproductive processes and behaviour as an outcome of germline mechano-transduction

Apart from germline maintenance and function, mechanical regulation is also observed in ovulation, fertilization, and egg-laying behaviours. The *C. elegans* spermatheca is a myoepithelial tubular bag lined with circumferential actin bundles and two valves opening into the oviduct (distal valve) and uterus (spermathecal-uterine valve/sp-ut valve) on both sides of the structure. The entry of oocytes into the spermatheca via the distal valve stretches its surrounding layer and the fertilized embryo is pushed out into the uterus via the sp-ut valve due to spermathecal contraction. This stretching and contraction of the spermatheca is driven by intracellular calcium release and actomyosin contractility involving RHO-1, RhoGAPs, RhoGEFs, and actin cross-linkers like Filamin [93–95]. Pezo-1/Piezo-1, a mechanosensitive ion channel, expressed in the sheath cells and spermathecal valves is responsible for synchronizing the opening/closing of the valves with the contraction of the sheath cells, during processes of ovulation and fertilization. [96]. Accumulation of embryos in the *C. elegans* uterus stretches the uterine wall. The muscles and neurons in the vulval tissue modulate the egg-laying behaviour through a feedback mechanism in response to this mechanical stretching and contraction [97,98]. Abdominal neurons expressing Piezo-1 channels regulate the post-mating behaviour of *Drosophila melanogaster* females. These

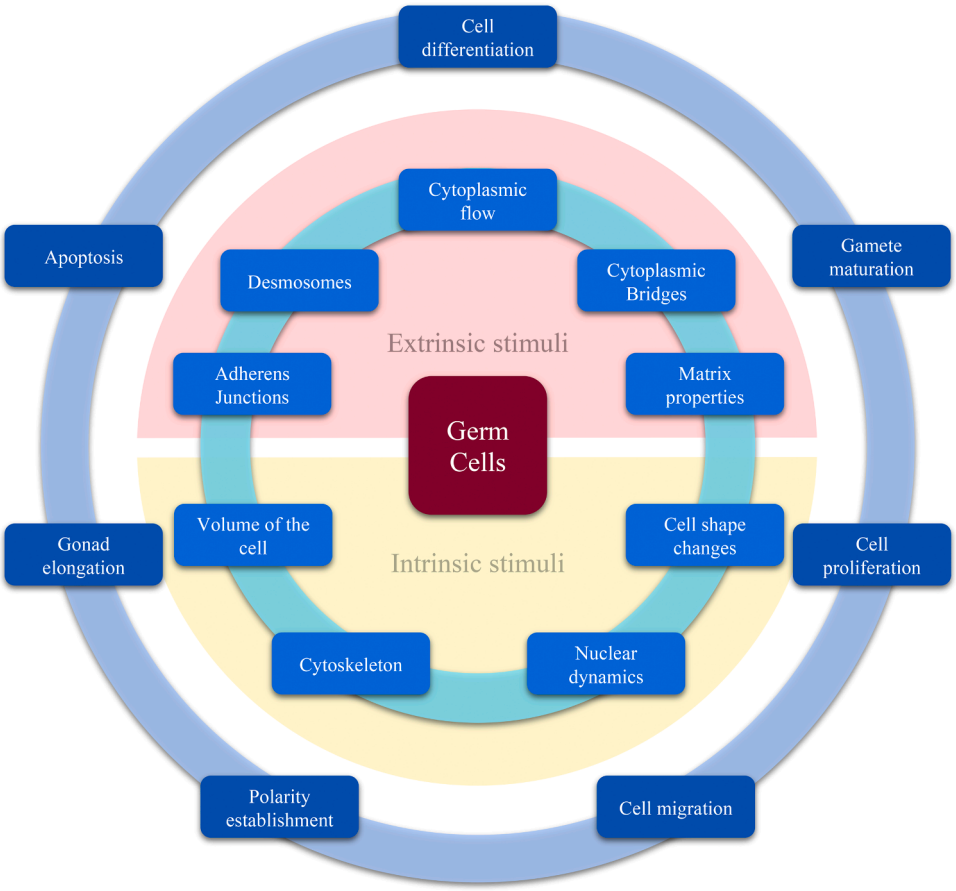

Fig. 5. Mechano-regulation of the Germline has similar mechanisms across organisms. The mechanical stimuli arise from either extrinsic (pink) or intrinsic sources (yellow). These stimuli are transduced by mechanotransducers shown along the inner green ring. Feedback reinforcement of these mechanical stimuli brings about modified cellular behaviour as depicted in the blue outer circle.

neurons sense mechanical stretch of the reproductive tract and the signal is then relayed to the central nervous system of the flies that triggers certain characteristic reproductive behaviours, like their attraction to acetic acid [99].

Concluding remarks and future perspectives

Material properties and mechanical forces play essential physiological roles across the entire biological length scale - from biomacromolecules to organ systems. Although widely investigated in the context of somatic tissues and embryonic development, our understanding about their impact on germ cell development and maturation remains limited. Despite adopting distinct mechanisms, mechano-regulation of the germline across organisms exhibit fundamental similarities. We highlight few key similarities below.

Cytoplasmic continuity: Cytoplasmic continuity is a common feature observed in germline tissues across organisms. Shared cytoplasm through specialized structures, such as cytoplasmic bridges or common cytoplasmic corridors, enables material movement between cells, including organelles and fate determinants. Cytoskeletal organization maintains these specialized structures and is the primary force generator driving this cytoplasmic flow. Besides the existence of germline syncytium, organisms also exhibit similarities in how the bulk cytoplasmic flows determine cell fates. For example, in *Drosophila* and *C. elegans* germlines, enlargement of a germ cell due to material accumulation through directed cytoplasmic flow results in its maturation into viable gametes, while the shrinkage of the cells that donate their materials results in apoptosis.

Feedback reinforcement: Changes in intrinsic physical properties (such as cell stiffness, cortical tension, elasticity, etc.) of germ cells or extrinsic stimuli from the surrounding cells lead to cytoskeletal remodeling. This modifies cellular behaviour, which in turn alters the mechanical properties of the cell, creating a feedback loop. At the tissue scale, actomyosin cytoskeleton in the germline may be organized into specialized structures such as the contractile corset, actin columns, or incomplete cytokinetic bridges. In most cases, these higher-ordered structures lead to associated tissue behaviour, like the fluid flow in the rachis of *C. elegans*, or the egg chamber rotation in *Drosophila*, reinforcing the maintenance of the actomyosin organization.

Local mechanics: The majority of forces sensed by germ cells arise from their immediate niche consisting of somatic cells and the ECM. Change in the strength and kind of interactions, between germ cells and somatic cells, drive processes that determine germline development and maintenance. Moreover, changes in the local matrix properties influence germ cells either directly or indirectly through modification of their somatic niche.

Not surprisingly, local mechanics governing cell fate is commonly observed in tissue morphogenesis, as well as in cancer. Tumor cell growth, for instance, is influenced by the mechanical properties of their microenvironment, such as variable matrix stiffness, shear stress from fluid flow, or compressive stress due to proliferation, very similar to the kinds of mechanical stimuli encountered by germ cells. Cell-cell adhesion complexes and cytoskeleton networks act as mechano-transducers and affect downstream cell fates and metabolic states in tumors. Tumorigenesis can thus be seen as a dysregulation or non-adaptive continuation of developmental processes resulting in pathological abnormalities. In *C. elegans,* for example, teratomas formed by unfertilized oocytes are commonly found in aging worms, etiologically similar to

ovarian teratomas found in mammals, both arising from embryogenetic quasi-programs. Therefore, investigation into the development and homeostasis maintenance of germline can provide insights into such pathologies [100–102].

Besides understanding pathological disorders, studying germline regulation can improve reproductive medicine techniques. The mechanical properties of germ cells (oocyte or spermatozoa) change continuously throughout development. These properties can be measured non-invasively (e.g. Atomic Force Microscopy) and used as a proxy to estimate the developmental stage or viability of the germ cells [103–108]. Enhanced reliability of such measurements can increase the efficiency of Artificial Reproductive Techniques. Additionally, the conservation of many fish populations, especially those with long developmental cycles like Sturgeons, currently relies on artificial reproduction. This process requires cryopreservation of reproductive cells, in particularly spermatozoa. Improving the quality of frozen spermatozoa requires them to be exposed to different factors, including an optimal level of acoustic-mechanical stimulation leading to improvement of the reproductive capacity [109].

It is evident that mechanics play a significant role in the development of the reproductive tracts, maintenance of the germline as well as all reproductive processes, including but not limited to, gamete maturation. However, direct measurement of mechanical forces in germline systems is technically challenging at present. This area promises to be an intriguing avenue for future exploration.

Across species, a similar trend arises regarding the types of mechanical stimuli experienced by germ cells and the responses elicited by these cells. Extrinsic stimuli faced by germ cells arise either from the surrounding matrix, or through cell-cell interactions such as, adherens junctions, desmosomes, or cytoplasmic bridges, with the surrounding somatic or follicular cells. Additionally, intrinsic factors to germ cells, such as the shape, size and/or the state of different cellular components, like the cytoskeleton or the nucleus, serve as significant sources of mechanical stimuli regulating the germ cell growth and function. Beyond the mechanical stimuli, the resulting phenotype exhibited by the germ cells display remarkable similarity. For instance, polarity establishment in early gonad development and subsequent gonad elongation are major outcomes of mechanical stimuli in the germline precursor cells. Similarly, the fate determination of the early germ cells, whether and how they will proliferate, differentiate, mature, or undergo apoptosis are also conserved across species. Thus, across species, despite differences in the morphology and function of the reproductive systems, a consistent trend exists in the type of mechanical forces driving germ cell development and homeostasis. Furthermore, homologous groups of proteins functioning as mechanotranducers are observed across species (Fig. 5). Looking forward, further research in this field should reveal molecular details of conserved mechanisms underlying germline maintenance through mechanical regulation.

CRediT authorship contribution statement

Ishani Sharma: Writing – review & editing, Writing – original draft, Conceptualization. **Anup Padmanabhan:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Anup Padmanabhan reports article publishing charges, equipment, drugs, or supplies, and travel were provided by Wellcome Trust DBT India Alliance. Anup Padmanabhan reports travel was provided by India Ministry of Science & Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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