Mechanical regulation of early vertebrate embryogenesis

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Abstract | Embryonic cells grow in environments that provide a plethora of physical cues, including mechanical forces that shape the development of the entire embryo. Despite their prevalence, the role of these forces in embryonic development and their integration with chemical signals have been mostly neglected, and scrutiny in modern molecular embryology tilted, instead, towards the dissection of molecular pathways involved in cell fate determination and patterning. It is now possible to investigate how mechanical signals induce downstream genetic regulatory networks to regulate key developmental processes in the embryo. Here, we review the insights into mechanical control of early vertebrate development, including the role of forces in tissue patterning and embryonic axis formation. We also highlight recent in vitro approaches using individual embryonic stem cells and self-organizing multicellular models of human embryos, which have been instrumental in expanding our understanding of how mechanics tune cell fate and cellular rearrangements during human embryonic development.

Rheology
The structural properties of a material that predict its dynamical behaviour when subject to a defined stress.

How much is the genetic programme leading to embryonic morphogenesis constrained by physical laws? This question has been rooted in developmental biology for decades and explored in a thousand pages by D'Arcy Thompson in his seminal book *On Growth and Form*, an encouragement to look at the variety of shapes through the prism of physics and mathematics. The conventional picture of morphogenesis in developing embryos usually links patterning and axis specification to signalling via morphogens[1]. The discovery that cells across vertebrate tissues can sense and transduce mechanical signals and that the mechanosensitive pathways tune developmental events has enriched the discussion[2,3], leading to the question of to what extent mechanical cues instruct specific aspects of patterning and increase the robustness of development. What we refer to as mechanical cues are physical signals such as a change in the rheology of the environment, a change in the osmolarity of the extracellular medium or active stretching and/or compression of the cells.

That mechanical forces are likely to play an important role from the earliest stages of development is supported by ample experimental evidence. Frog eggs can be fertilized artificially by a simple poke of a needle, entirely bypassing the requirement for sperm entry. Later on, embryonic tissues exhibit highly dynamic rearrangements of cell–cell contacts and permanent deformation, allowing patterning and morphogenesis, and ultimately the segregation of discrete cell types within each organ.

In this Review, we discuss the current understanding of how mechanical forces regulate embryonic development, from in vivo studies of whole embryos to in vitro synthetic models of human embryogenesis. We use physical concepts to approach several processes conserved in vertebrate embryos: shape generation, biomechanical patterning, symmetry breaking and boundary formation. These concepts can then be used to design new in vitro studies that deconstruct morphogenesis into more controllable experiments.

In this regard, we review to what extent knowledge from these model systems can be transferred to human development. Notably, even though universal organizational principles of development can be identified through comparative embryology, these principles coexist with particular solutions specific to each organism. The recent development of systems that model human gastrulation is an opportunity to study mechanics in morphogenetic processes specific to human embryogenesis. Overall, we envision that more accurate knowledge of the mechanical determinants of morphogenesis will eventually lead to a new generation of synthetic embryo models to dissect developmental processes with even greater molecular precision.

This Review is designed to deliver a broad scope on developmental tissue mechanics across vertebrates and we reference more detailed reviews on key points whenever possible. Owing to the impossibility of here addressing findings outside the vertebrate phylum, we also redirect the reader to excellent work regarding mechanical influences in invertebrate systems[4–7].

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https://doi.org/10.1038/s41580-021-00424-z

Bottle cells
In the early *Xenopus laevis* gastrula, elongated epithelial cells undergoing apical constriction at the site of the blastopore formation.

Blastopore
A circular and transient morphological structure located on the dorsal side in amphibians, through which the mesoderm is internalized during early gastrulation.

Convergent extension
Tissue-scale deformation, whereby the tissue stretches in one direction and narrows in the orthogonal axis owing to the radial intercalation of cells into the elongating layer.

Blastomeres
Cells produced by the early cleavages of the zygotic egg.

Force fields
Continuous force descriptions that represent as a vector field the physical interactions in a given system. For instance, in a developing embryo, a coarse-grained representation in which each individual force to which a cell is submitted within a tissue is computed.

Trophectoderm
An extra-embryonic tissue forming the outer layer of the mammalian blastocyst that envelops the embryo proper and supplies nutrients.

Durotaxis
A mode of cellular migration in which cells follow stiffness gradients as a long-range cue.

Jamming–unjamming
A special type of phase transition where increasing (jamming) or decreasing (unjamming) the packing of particles in a granular material leads to a macroscopic viscosity change. This theoretical framework has been used to describe the packing of cells in a biological tissue.

Wetting
The spreading of a liquid phase on top of another phase owing to favoured heterotypic interactions between the constituents of the two phases.

Shaping of embryonic tissues

Morphogenesis is a multiscale process: forces generated at the single-cell level and averaged at the tissue scale contribute to the patterning taking place during the whole course of development. In this section, we provide selective examples of how single embryonic cells generate mechanical forces in vertebrate embryos, before focusing on the interplay between these forces and signalling.

Force integration at the tissue scale. Embryonic cells generate forces on their surroundings using motor proteins linked to the cytoskeleton[8], leading to single-cell morphogenetic movements such as wound healing in *Xenopus laevis* oocytes[9], which happens by constriction of an actomyosin ring under the oocyte membrane. In a multicellular context, during early gastrulation of *X. laevis*, the apical constriction of bottle cells supports tissue involution around the blastopore[10–12]. Embryonic cells also exert forces on their neighbours through cellular protrusions called filopodia, which are required for medio-lateral intercalation in *X. laevis* gastrulae, a cell behaviour supporting convergent extension[13] (FIG. 1A). Filopodia traction is also likely involved in the compaction of the mouse embryo at the eight-cell stage, during which initially spherical blastomeres flatten against each other — although the importance of this mechanism is still debated[14,15].

Forces acting in embryos thus emerge either from the integration of individual cell behaviours to the tissue scale or from external force fields such as the gravitational field[16,17]. We focus here on forces emerging from the mechanical interactions developed between the cells.

Many morphogenetic processes involving epithelial and non-epithelial cells in embryonic development are driven by the contraction of a supra-cellular actin cable[9,18–21], emerging through the coordination of individual cytoskeletons at the level of adherens junctions. Tension generated by these supra-cellular structures can mediate cell intercalation, required, for example, for convergent extension[12]. Additionally, the molecular structures that generate forces, the cytoskeleton and its associated proteins, exhibit mechanosensitivity themselves. Myosin recruitment is tension-dependent, which may lead to a reinforcement and amplification of the contraction generated at the site of mechanical stimulation[22–24].

Epithelial tissues are also shaped by the formation of embryonic cavities during early development, partially built through the accumulation of internal hydrostatic pressure. After compaction, the mouse blastocyst cavitates and forms the blastocoel, building up the internal hydrostatic pressure through the activity of ion pumps (FIG. 1B). Water accumulates at the interface between cells and breaks part of the cell–cell contacts — a process called hydraulic fracturing — forming multiple microlumens that eventually coalesce into a single one[25]. The pressure inside the blastocyst reaches a few hundred pascals at the end of this process. During this steady growth, the external trophectoderm is stretched and its cortical tension increases[26] until it reaches a threshold at which epithelial junctions start to break. At this late blastocyst stage, the ultimate size reached by the embryo is ruled by a mechanical feedback between the internal pressure and the stress that the trophectoderm can sustain.

Mechanical regulation of tissue dynamic properties. The shaping of embryonic tissues is mechanistically complex, mostly because of regulative loops between cell-autonomous behaviour (apical constriction, polarization, crawling, migration) and the generation of forces that span fields of cells. To accurately describe morphogenesis at the multicellular scale, various techniques have been developed, probing in situ the forces and the rheology of embryonic materials (BOX 1).

The most precise study of the mutual crosstalk between collective mass motion and force generation is the phenomenon of convergent extension that occurs during *X. laevis* gastrulation. During this process, cells from the medio-lateral area of the early gastrula converge towards the dorsal side, generating the anteroposterior axis (FIG. 1A). Collective cellular motion combined with the rearrangement of polarized apical junctions and basolateral protrusive activity generates the forces responsible for this tissue shape change[27]. In turn, active stretching of *X. laevis* ectodermal explants can induce a collective migratory pattern similar to the stereotypical movements associated with convergent extension in the embryo[27,28].

Changes in the viscoelastic properties of the embryonic tissues accommodate the important cellular rearrangements, collective motion (for example, durotaxis[29]) and shape changes happening during development[30,31]. Fluid-like tissues tend to have a high rate of cellular rearrangements, low packing and low cell density whereas solid-like tissues are denser and more organized[30]. Embryonic tissues exhibit a mixture of solid and fluid-like behaviours, allowing different types of deformations and shape changes. Because of this adaptation in their viscoelastic properties, they can be deformed permanently and remain stable upon small mechanical perturbations[30,32]. Morphogenesis can thus be described using the formalism of complex fluid mechanics and phase transitions, such as jamming–unjamming[32] or wetting[33]. Jamming stabilizes the newly formed shape whereas unjamming gives freedom to the embryonic tissue to expand and elongate, as shown for the elongation of the zebrafish anteroposterior axis[32]. Wetting transitions could drive the spreading of the zebrafish blastoderm during epiboly[34].

This coarse-grained approach describes complex morphogenesis using only a very limited number of parameters. It can then be linked to the cellular identities and biologically relevant pathways using, for instance, the link between cadherin concentration, cell–cell adhesion, cell connectivity and, eventually, blastoderm viscosity[35]. Eventually, such a framework can enable species to species comparison of morphogenetic events regardless of molecular disparities to find morphogenetic homologies; that is, a conserved way to make a neural fold or to internalize the mesoderm. Furthermore, by imaging developmental tissue dynamics, it is possible to provide a continuous description of cellular identities that are traditionally considered discrete when evaluating single-cell gene expression profiles. This is particularly interesting in the case of

epithelial–mesenchymal transition occurring during gastrulation, where cells move while adopting progressively new fates[36].

Biomechanical tissue patterning

Patterning of tissues involves the establishment of genotypically different domains from initially identical cells. During embryogenesis, an important cue for patterning is the generation of morphogen gradients, which then guide cell behaviour and fate. However, embryonic cells not only read and interpret morphogen gradients but are also capable of decoding mechanical information[37]. Accordingly, in vertebrate embryos, mechanical instabilities and mechano-transduction provide important inputs into tissue patterning.

Contribution of mechanical instabilities. In his seminal work published in 1952 (REF.[38]), Turing uses the abstract problem of spatial patterning as a way to formalize morphogenesis. He thus described the emergence of a typical

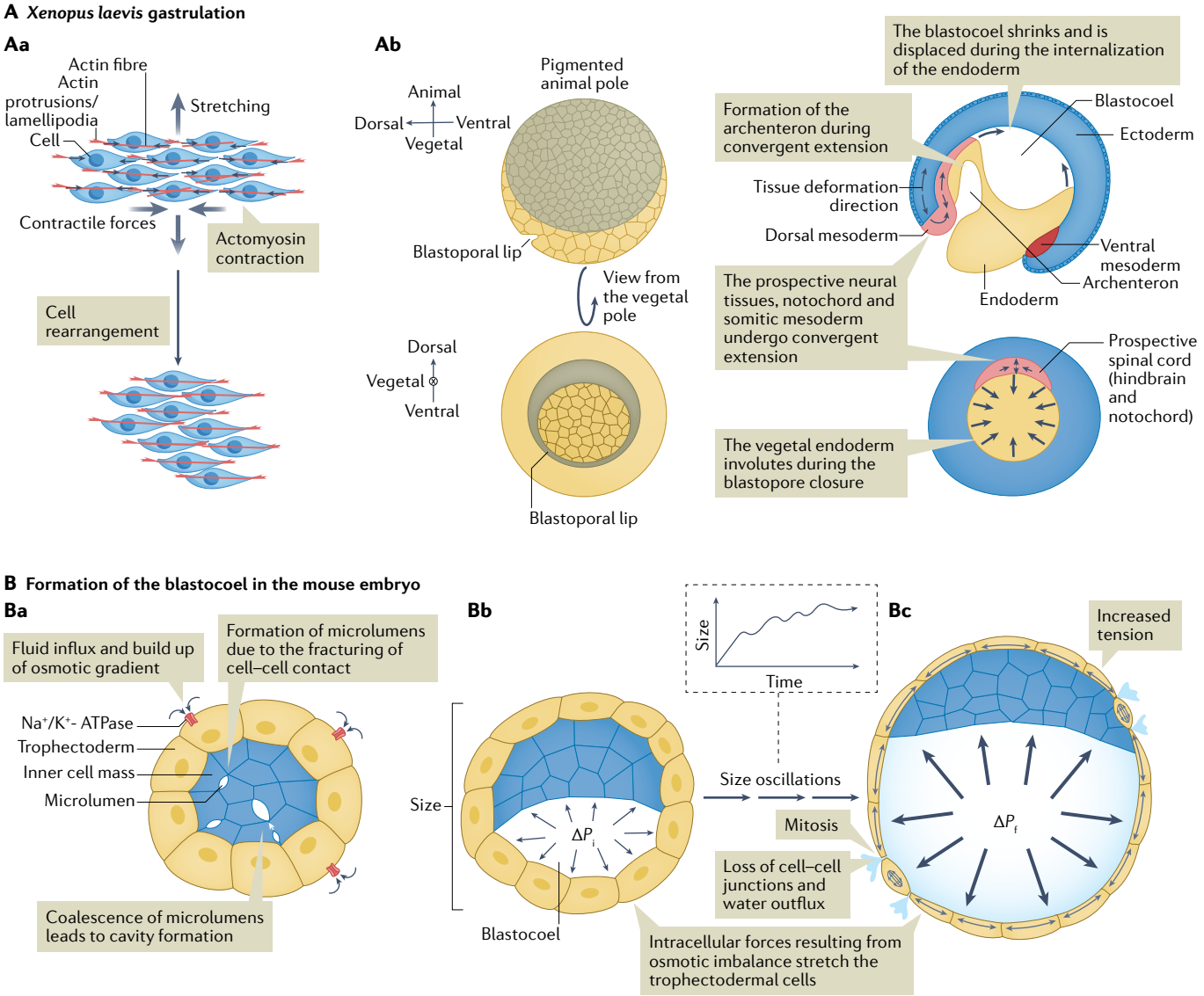


Fig. 1 | **Forces acting in animal embryos. A** | Convergent extension in *Xenopus laevis* gastrulation. **Aa** | Forces generated individually by cells lead to cellular rearrangements and tissue-scale morphogenesis. Cells intercalate medio-laterally by exerting forces on their neighbours through actin protrusions[27]. These local forces result in convergent extension on the dorsal side of the embryo, with extension in one direction and shrinking in the perpendicular axis. **Ab** | Representation in two orientations of *X. laevis* gastrula in a realistic style (left). The different tissues responsible for convergent extension are represented in simplified colour-coding (right) on a side view of the *X. laevis* gastrula. Movements of convergent extension in the mesoderm — attached to the vegetal endoderm — contribute to closure of the blastopore by pulling the tissue inside (top)[20,36]. View from the vegetal pole of the mesenchymal cells with a cut exposing the deeper layers of the marginal zone (bottom). **B** | Hydraulic fracturing of the mouse blastocyst[25,26]. **Ba** | Formation of the blastocoel by building up an osmotic gradient. Fracturing of cell–cell contacts by fluid accumulation leads to formation of microlumens, which coalesce into a single one. The blastocoel segregates the polar trophectoderm and the inner cell mass cells on one side, and the mural trophectoderm on the other. **Bb** | Activity from ion pumps keeps increasing internal pressure (P_{int}) in the blastocyst with respect to the external pressure (P_{ext}), whereas cell divisions of trophectoderm cells result in weakening of cell–cell junctions and fluid leakage out of the blastocyst cavity, inducing cycles of swelling and discharge. **Bc** | These cycles terminate when the pressure difference $\Delta P = P_{int} - P_{ext}$, initially ΔP_i, reaches its final value ΔP_f, with epithelial junctions between trophectodermal cells at their maximal tension and the blastocyst reaching its final size.

Box 1 | **Force measurements in biological specimens**

Different techniques have been developed to study force generation, both in in vivo (animal models) and in vitro, in a developmental context[174,175].

Atomic force microscopy
Atomic force microscopy relies on the physical interactions between the probe (a cantilever) and a surface to assess the forces developed by the system. It can be used to study the rheology of a tissue[176] or the forces developed by the cells[177], or to exert active and localized mechanical perturbations[75]. This low-throughput and high-resolution technique can be calibrated and has been used both ex vivo (in explants) and in vitro. It is particularly efficient to measure the forces developed by an individual cell but is not suited to study the force field at the tissue scale, owing to the time necessary to perform multiple measurements.

Fluorescence resonance energy transfer-based molecular force sensors
In this molecular approach, the deformation of a stretched protein is used to produce a fluorescence resonance energy transfer signal. Molecular motors, extracellular matrix proteins or cytoskeleton components can be genetically modified for this purpose[178,179]. This technique can be used in vitro to study the forces developed at the subcellular level but its in vitro calibration is difficult, even in a well-controlled chemical environment, which has not yet made this technique reliable in living embryos[180].

Laser ablation
Laser ablation is a destructive measurement technique that consists of severing a tissue with a laser and measuring the wound recoil (displacement and velocity) to estimate the initial tension. This technique can be performed easily in vivo and adapted to many types of microscopy[175]. It can be used more qualitatively to determine which specific areas of a developing tissue are under tension[18]. It cannot be used to monitor tensional or strain changes during a specific morphogenetic process owing to its destructive nature.

Traction force microscopy
This technique is used in in vitro approaches to convert the deformations exerted by the cells on their substrate. It may use either beads embedded in a soft substrate or elastic micropillars for force readout. In the first case, cells are cultured on a gel with embedded beads, from which displacement can be mapped computationally to a force field[49]. In the second case, the substrate is micropatterned with pillars[181], but the principle remains the same. This technique is computationally expensive but can be calibrated. It provides multiple measurements at the same time and is adjusted to the spatial scale of interest, which allows one to derive a force field.

Optical tweezers
This spectroscopic technique can be used to assess the tension of intercellular junctions in a non-destructive way (in contrast to laser ablation)[182]. The intercellular junction is deformed with the laser-generated optical trap and the resulting force balance is used to deduce the tension. The other alternative requires injecting beads into the embryo that will be trapped in the same way[175]. Both methodologies require an optically transparent embryo, which has limited this application mostly to the zebrafish[183] embryo among other vertebrate models.

Micropipette aspiration
A micropipette is applied on the cell or the embryo membrane and a pressure difference is applied to suck it inside the micropipette. Measuring the resulting surface deformation gives an estimate of the membrane tension (provided by the underlying membrane cortex)[184]. This method is used in vivo mostly, and in vitro with cellular aggregates such as spheroids. It can be calibrated and is reserved for bulk assays of the surface mechanical properties.

Magnetic beads
A magnetic force field can be applied to mechanically indent parts of the embryo, seeded with magnetic beads[45] that can be functionalized with extracellular matrix proteins to target cellular integrins and promote cell–bead adhesion. As for optical tweezers, this technique still requires the embryo to be transparent enough to be able to track the beads and measure their displacement upon application of the force[185].

Liquid droplets
This recent technique is very useful to map the stresses experienced by embryonic epithelial tissues[114] and is the in vivo counterpart of traction force microscopy approaches. Magnetic oil microdroplets are injected into the extracellular space and their deformation is a readout of the forces exerted by the cells. Contrary to magnetic bead methods, a single droplet conveys information on the stress anisotropy that is experienced at different points of the tissue.

Epithelial–mesenchymal transition
A cellular transition whereby polarized epithelial cells lose adhesion with respect to one another and become independent (mesenchymal) and mobile. There is a spectrum of epithelial–mesenchymal transitions: that is, not all epithelial–mesenchymal transitions lead to fully mesenchymal cells.

Morphogen
As defined historically by Turing, a molecule secreted by a group of cells and acting from a distance on another group of cells, eliciting different outcomes in a dose-dependent manner.

length scale in embryonic tissues with a set of chemical equations modelling the transport and reaction of an informative chemical signal — called morphogen. Turing's initial proposition relied on chemical instabilities generated through reaction–diffusion processes, although he already knew the prominent role played by mechanics in embryonic patterning[1,38]. Indeed, mechanical instabilities can also lead to patterning with fundamental differences to morphogen-generated patterns[39]: their first manifestation is a physical signature (wrinkling, buckling) instead of a chemical gradient and their spatial range extends from the subcellular scale up to several hundred microns[37,40,41], whereas diffusive length scales are usually of a few microns. Besides, the transmission of a mechanical stimulation can be almost instantaneous whereas the diffusion of a molecule takes longer to operate[39,40], leading to faster patterning through mechanical cues.

Mechanical instabilities shape the morphogen landscape by altering the physical environment. As an example, during the formation of the mouse gut, the cells at the tips of the intestinal villi have more neighbours secreting the morphogen Sonic hedgehog (SHH) than the cells residing at the villi base. The geometry of the folded epithelium leads to asymmetrical signalling that restricts WNT activity — another morphogen — to the base of the villi. These changes generate a niche for intestinal progenitors at the base of the villus where progenitor cell fate is being retained[42]. This mechanism could be generalizable to other morphogen pathways in developmental processes in which the shape of a tissue is significantly impacted by mechanics. For example, in chick feather follicle specification, the regular pattern of feathers is produced by a mechanical instability derived from local cellular contractility and long-range stiffness[43].

Interplay of mechano-transduction and morphogens. In vertebrate development, most secreted signalling molecules can act as morphogens. These include members of the TGFβ superfamily of ligands (classified into the BMP and ACTIVIN/NODAL branch), FGFs, WNTs and Hedgehog factors. Mechano-transduction has been linked to the WNT pathway via mechanically responsive

Mechanical instabilities
Instabilities that appear when small fluctuations around an unstable equilibrium position drive the system towards two very different outcomes. The wrinkling or buckling of epithelial sheets is an example of a mechanical instability.

Mechano-transduction
Processes that convert extrinsic mechanical signals (for example, environmental stiffness or a force from a neighbouring cell) into an intrinsic change in the intracellular biochemical, transcriptional or bioelectrical activity of the cell.

Planar cell polarity (PCP) pathway
The signalling pathway that regulates the polarization of a field of cells within the plane of a cell sheet. This process is involved in various developmental events, including convergent extension.

Hippo pathway
The signalling pathway associated with cell proliferation, tissue and organ growth, size determination and mechanosensation.

elements downstream of WNT ligands, in both canonical and non-canonical signalling. In early vertebrate development, this pathway is of critical importance for gastrulation, as it is implicated in mesoderm specification, neurulation and specification of the blastopore lip.

In the canonical branch of the WNT pathway, cells receiving the WNT ligand stabilize the β-catenin protein in the cytoplasm, enabling its nuclear translocation where it activates downstream target genes. This process is further mechanically regulated in epithelial tissues where β-catenin is bound to E-cadherin-based cell–cell adhesion junctions and, hence, is unable to translocate to the nucleus. Tissue deformation such as stretch/strain causes mechano-transductive events that can allow β-catenin release from this membrane-bound pool, increasing its transcriptional activity. This mechanical regulation of β-catenin localization was first identified in the fly[44–47] and remains to be studied in the context of vertebrate embryos, although roles for mechanical inputs into the β-catenin signalling have been shown in stem cells[48,49].

WNTs can also signal in a β-catenin-independent manner through the planar cell polarity (PCP) pathway[50], which can also be mechanically regulated. The PCP core components define polarization and orient proliferation in the plane of the epithelium, in addition to the apico-basal polarity that is already present. The PCP pathway thus regulates some aspects of convergent extension in vertebrate embryos[27,51,52] by either defining an axis (medio-lateral or proximodistal) or a polarized direction. In the former case, an atypical cadherin (CELSR) builds up preferentially on cell junctions perpendicular to the selected axis. In the latter case, heterotypic cell–cell interactions polarize each cell by setting the localization of the receptor Frizzled (FZD) on one side of the cell, and of its partner Van Gogh Like (VANGL) on the other[53]. This asymmetrical tissue patterning, in turn, directs the orientation of subcellular structures, such as cytoskeletal elements and cellular adhesions, which then dictate polarized cell behaviours. External mechanical cues such as morphogenetic shape changes or fluid flow have been shown to modulate PCP[54]. For example, tissue strain regulates PCP establishment in *X. laevis* embryonic epidermis during gastrulation[55] and in mammalian skin[54,56,57], whereas cilia orientation and beating of multiciliated cells (one of the key read-outs of PCP) is regulated by fluid flows both in *X. laevis* gastrulae and in developing mammalian brain ventricles[58,59]. In addition, cell-intrinsic regulation of cortical tension may act synergistically to propagate PCP, which has been shown to be important for orienting convergent extension in mammalian embryos[27,60].

Mechanical control of growth and differentiation. The ability of the WNT pathway to respond to mechanical inputs, facilitating the interplay between morphogens and mechanics in vertebrate embryonic development, raises the question of whether this is also true of other signalling pathways. The core components of the Hippo pathway[61], a key regulator of tissue and organ size, regulate growth and cell proliferation in development (FIG. 2) and in cancer[62]. The Hippo pathway relies on the shuttling of YAP (Yes-associated protein) and TAZ (Transcriptional co-activator with PDZ-binding motif) proteins between the cytoplasm and the nucleus[63,64]. A double phosphorylation cascade involving Hippo kinases maintains the cytoplasmic localization of phosphorylated YAP/TAZ when the Hippo pathway is activated. If the Hippo pathway is inactive, YAP/TAZ are dephosphorylated and shuttled to the nucleus where they trigger TEAD-dependent transcription of target genes[64] (FIG. 2A).

Mechanical tension promotes myosin recruitment and actin fibre polymerization, which regulate YAP/TAZ activity[64–66]. The link between the cytoskeleton, HIPPO signalling and mechanical forces was first described in *Drosophila melanogaster*[67], and was subsequently shown to be conserved in mammalian cells[66] (FIG. 2B). In vivo studies of zebrafish fin regeneration also suggest that this specific regulation is conserved in vertebrates[68]. Independently of this actin-based regulation, developmental studies in vertebrate embryos indicate that size homeostasis is, at least partially, under YAP/TAZ control. This mechanism provides size control of organs[69] such as the heart[70] and shape regulation up to the organism scale[17]. The regulatory loop between mechanics, cytoskeleton recruitment, YAP/TAZ nuclear translocation, cell proliferation and, eventually, tissue growth has been investigated via both theoretical and experimental studies in the fly[71–73]. This multiscale picture of mechanical regulation of tissue growth and, hence, embryonic development through the intermediary of YAP/TAZ activity seems to be conserved in vertebrate development. Medaka fish embryos carrying a YAP mutation (*hirame* mutant) show reduced actomyosin tissue tension. When embedded in a gel in different orientations, their body flattens in the same direction as the gravitational field[17].

It was first shown in vitro that cytoskeletal tension is required for YAP/TAZ localization to the cell nucleus[63], independently of the classical Hippo cascade. The role of YAP/TAZ in determining individual cellular fates through the integration of mechanical information has been studied more extensively in cell culture[64,74], where additional mechano-regulative mechanisms of YAP/TAZ have been isolated — in particular in the context of nuclear stretching[75], which changes the physical structure of nuclear pores and the rate of nuclear import and export.

Force-induced symmetry breaking

The vertebrate body plan is asymmetrical, featuring anteroposterior, dorsoventral and left–right axes. Mechanical forces can contribute to this patterning through their role in early symmetry breaking events. On a molecular level, actin filaments and microtubules can become polarized, and the direction of the motors linked to them may be predetermined, thereby directing cellular asymmetry[76]. Here, we outline how this molecular determination is integrated at the level of the embryo to break symmetry, first in the establishment of the dorsoventral axis in *X. laevis* and then in left–right patterning in the mouse. In both cases, we highlight how intracellular and extracellular mechanical forces pattern vertebrate embryos.

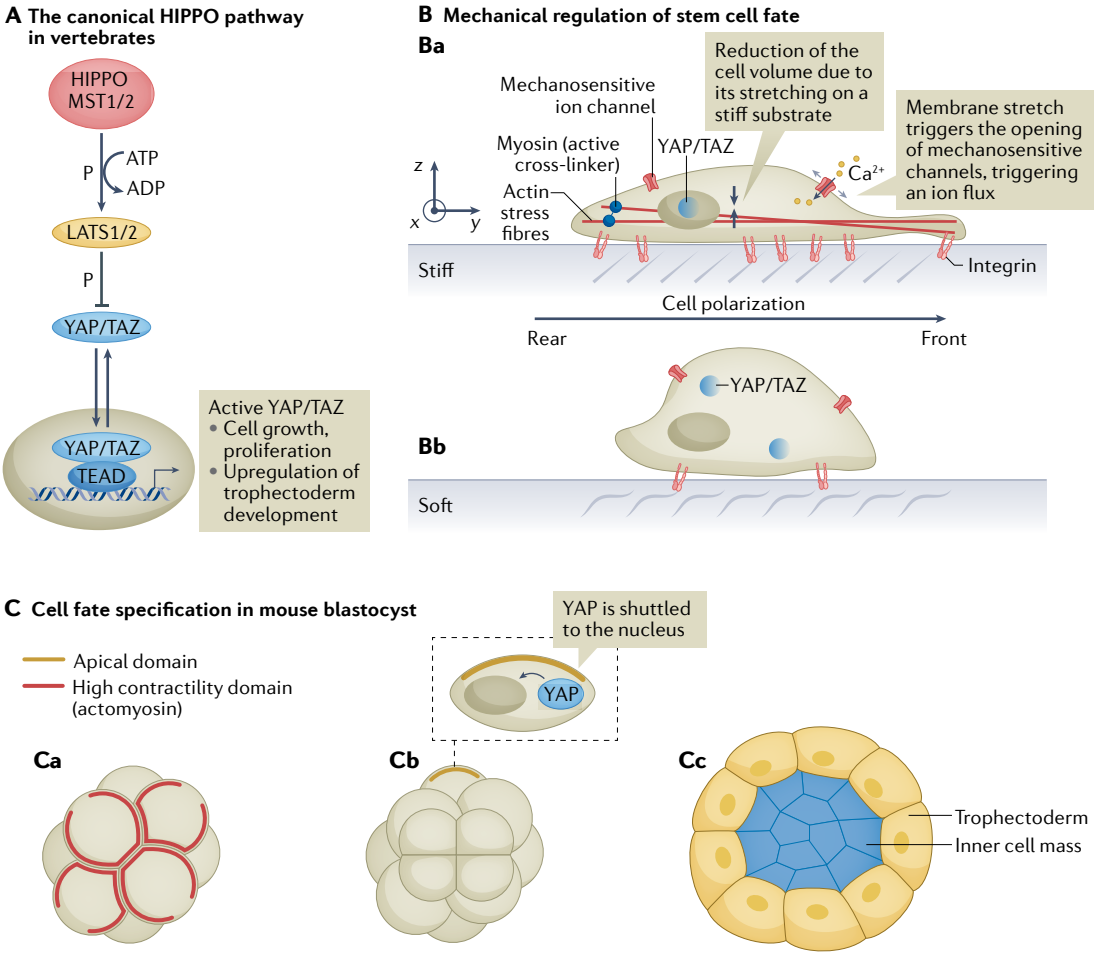


Fig. 2 | **Mechanical regulation of cell fate. A** | Canonical Hippo pathway in vertebrates[64]: a double phosphorylation (P) cascade inhibits YAP (Yes-associated protein)/TAZ (Transcriptional co-activator with PDZ-binding motif). When the Hippo pathway is activated, MST1/2 kinases (HIPPO in the fly) phosphorylate LATS, which phosphorylates YAP and TAZ, leading to their accumulation in cytoplasm followed by their degradation. When the Hippo pathway is inactivated, YAP/TAZ are translocated to the nucleus and activate TEAD transcription factors. **B** | Mechanical regulation of stem cell fate. **Ba** | At single-cell level, cells on a stiff substrate experience reduction in their overall volume, increase in their spreading area, compression of the nucleus[75,167] and polarized organization of the actin cytoskeleton with the formation of ventral stress fibres[168]. Stiffness-induced stretching of the cell opens mechanosensitive PIEZO ion channels[169,170] and modulates calcium influxes. YAP/TAZ localize to the nucleus in cells cultivated on a stiff substrate[64]. **Bb** | On a soft substrate, cells adopt a round shape, the actin cytoskeleton exhibits a radial symmetry and no ventral stress fibres are observed[168]. YAP/TAZ are inactivated and present in the cell cytoplasm. **C** | In mouse blastocyst, mutual inhibition between contractility and apical polarization directs cell fate through positional information[14], with outer cells being directed to the trophectoderm lineage. **Ca** | At the 8-cell stage, the blastocyst compacts with increased actomyosin contractility at the interface between blastomeres. An apical domain develops at the contact-free interface. **Cb** | During divisions between 8-cell stage and 16-cell stage, cells inheriting the apical domain remain at the outer surface of the blastocyst whereas more contractile cells are internalized. Inheritance of the apical domain leads to YAP/TAZ shuttling to the nucleus[171,172]. **Cc** | Nuclear YAP then activates TEADs, leading to trophectoderm specification in outer cells.

Cortical rotation in dorsoventral axis specification. The first axis that arises in the *X. laevis* embryo is the dorsoventral axis, which is determined through cortical rotation[77,78] (FIG. 3A). The *X. laevis* egg initially displays a single cylindrical symmetry axis extending from the pigmented top — the animal pole — to the unpigmented bottom — the vegetal pole. Shortly after fertilization, the minus end of microtubules nucleates at the sperm entry site. Microtubules then polymerize and extend towards the opposite pole, where less organized microtubules rest in the cortex layer. The organized microtubule polymerization induces a rotation of the cortex of the egg relative to its cytoplasmic core, shearing in the process the cytoplasm between the membrane and the cortical structure at the vegetal pole. Microtubule plus ends growing and sliding over the actin cortex align the disorganized microtubules into a parallel array, which supports the transport of dorsal determinants — β-catenin stabilizing proteins — away from the vegetal pole to the side diametrically opposed to the sperm entry site. In this process, the initial animal–vegetal symmetry axis is broken, and dorsoventral territories are established, with the dorsal side being specified by the accumulation of β-catenin inducing WNT activity. The relocation

Nodal
A morphogen belonging to the super TGFβ family, initially named because of its association with the node structure in the developing mouse embryo.

Node
A small structure at the anterior part of the primitive streak.

of dorsalizing molecules involves both active transport via kinesins on a microtubule polarized network in the cytoplasm and transient binding to the moving egg cortex[77,78].

Blocking cortical rotation by preventing microtubule polarization leads to ventralized embryos lacking dorso-anterior structures[78]. Low-speed centrifugation can override the location of the axis initially specified by the point of sperm entry[79], experimentally demonstrating that ectopic forces can modify the embryonic architecture. This physical shearing of the *X. laevis* egg sits at the top of a hierarchy of events leading to the induction of all of the molecular pathways that are necessary and sufficient to generate a complete body axis. Vertebrate embryos are thus plastic and their competence for responding to inductive signals[80] is at least partially mechanically regulated.

Left–right patterning. Vertebrate embryos specify the left–right axis during gastrulation, which manifests in the positioning of the inner organs (for example, liver or pancreas) and in their development (for example, gut and heart looping). Left–right patterning is traditionally thought to occur immediately after the apparition of the anteroposterior and dorsoventral axes. It consists of a pattern of differential gene expression starting with the biased expression of Nodal on the left side of the anteroposterior axis. Cells expressing *Nodal* activate downstream *Pitx2* and *Lefty1/2* transcription regulators, which in turn antagonize *Nodal*[81] (FIG. 3B). In vertebrates, the textbook model describes an asymmetrical flow generated by the beating of cilia in the mouse node (gastrocoel roof plate in *X. laevis* and Kuppfer's vesicle in zebrafish)[81]. We focus here on the biophysical mechanisms underlying left–right patterning in mouse embryos,

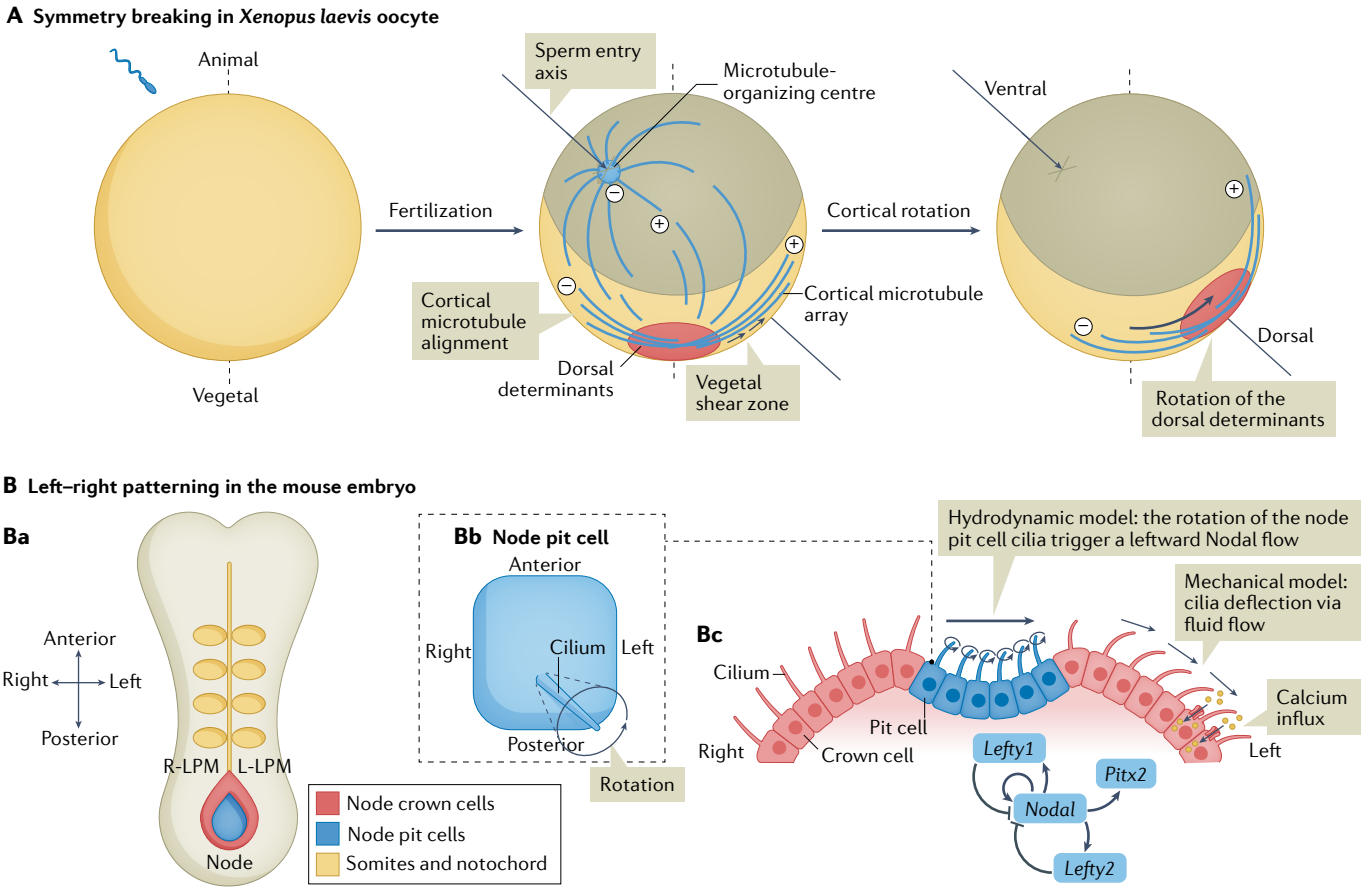


Fig. 3 | **Force-induced symmetry breaking. A** | *Xenopus laevis* egg cortical rotation[77,78]. Sperm entry positions the microtubule-organizing centre. These microtubules then polymerize and extend towards the opposite pole, generating forces that shift non-organized microtubules layering the cortex with respect to the initial animal–vegetal axis, shearing the cytoplasm between cortex and egg core. This shearing induces cortical rotation, whereby dorsal determinants are transported towards the (plus) end of the microtubule array — either together with the moving cell cortex or, additionally, via active transport on microtubules involving kinesins — which defines the dorsoventral axis in the embryo. **B** | Left–right patterning in the mouse embryo[81]. **Ba** | The node is a small cavity on the ventral side of the embryo. It is lined by a monolayer of ciliated epithelial cells; cells at the centre of the monolayer (pit cells) harbour motile cilia, whereas cells at the periphery (crown cells) harbour immotile cilia. **Bb** | Pit cells have rotating motile cilia implanted at the posterior end of the apical side[81], which leads to asymmetrical stroke with respect to the anteroposterior and left–right axis and results in leftward hydrodynamic flow. **Bc** | This flow can transport morphogens (as nodal vesicular parcels, carrying Sonic Hedgehog (SHH) and retinoic acid) to the left side of the node where they deliver their cargo in a manner not yet elucidated (chemical model). Additionally, or in parallel, this flow is also the source of hydrodynamic forces that deflect immotile cilia of crown cells, leading to opening of mechanosensitive Ca^{2+} channels and Ca^{2+} influx inducing Nodal production specifically on the left side of the node (mechanical model). In both cases, the final result is induction of a regulation loop on the left side where Nodal auto-activates its production and activates *Lefty1/2* and *Pitx2* production. *Lefty1/2* inhibits, in turn, Nodal production and prevents Nodal diffusion on the left side of the embryo, initiating left–right patterning. L-LPM, left lateral plate mesoderm; R-LPM, right lateral plate mesoderm.

Basal body
A protein structure found at the basis of eukaryotic cilia that serves as a nucleation site for microtubules.

Chiral molecules
Molecules that cannot be superposed to their mirrored image by any combination of rotations. Chiral molecules often have different reactivities.

which have been elucidated through genetic approaches and led to the Nodal flow model. In this model, Nodal morphogen is produced around the node, a leftward hydrodynamic flow is generated by the rotating cilia of the node pit cells during gastrulation and left–right asymmetries in signalling are established with increased Nodal activity in the left lateral plate mesoderm[82–86] (FIG. 3B).

Epithelial cells layering the node cavity exhibit a single cilium[87]. The apical side of these cells is convex, and the ciliary basal body is asymmetrically positioned in the intracellular space, leading to a biased stroke and a leftward hydrodynamic flow[81,87]. The PCP pathway is thought to orient the shift in implantation of these cilia, as confirmed by left–right patterning defects in Van Gogh (*Vangl*) and Dishevelled (*Dsh*) mice mutants[84–86]. The read-out of Nodal molecular concentration in the extracellular space by the perinodal crown cells occurs by two mechanisms. The first involves actual hydrodynamic molecular transport, either in the form of extracellular vesicles containing morphogens (SHH and retinoic acid[88]) secreted by pit cells upon FGF activity or as direct autocrine–paracrine Nodal communication[89]. The second model relies on the mechanical detection of the flow through the polycystin calcium channels PKD1 and PKD2 (REF.[90]). In both cases, the mechanism generating and amplifying symmetry breaking relies on the generation of the hydrodynamic flow mediated by ciliary beating.

The Nodal flow model of left–right symmetry breaking cannot be transposed to some other vertebrate species, such as pigs or chicks, which do not exhibit motile cilia in the node homologous region. Alternative theories based on the directional asymmetries induced early in development by chiral molecules, such as the ion flux model, can also explain the left–right segregation of molecular determinants[91,92]. In this alternative hypothesis, ion pumps are asymmetrically distributed between left and right owing to early asymmetries in the cytoskeletal architecture. This generates an ion flux, leading to the segregation of small molecules (for example, accumulation of serotonin on the right[93,94]) and asymmetrical expression of *Nodal*.

Mechanics at tissue boundaries

Patterning of entire embryos requires establishment of defined tissue boundaries and specification of embryonic territories with different lineage identities. During mammalian development, the first lineage bifurcations specify the tissues that will become extra-embryonic versus those that will constitute the embryo proper. At the onset of gastrulation, the cells that contribute to the embryo proper sort into the three major germ layers. Ultimately, the contractility differences arising from the different emergent cell types ensure that tissues segregate and boundaries remain stable throughout these consecutive developmental programmes. Below, we discuss these events in more detail.

Specification of embryonic versus extra-embryonic tissues. The first fate decision in the mammalian embryo is the specification of the inner cell mass and trophectoderm, which relies partially on the mechanical interactions between the cells. Blastomeres produced by the early cleavages will segregate between these two populations and form the blastocyst, with the trophectoderm epithelium enveloping the inner cell mass and a fluid-filled cavity called the blastocoel[14]. This process begins at the eight-cell stage and cell fate allocation is dependent on the position of blastomeres within the embryo[95]. Although the cells keep dividing, the embryo compacts: cells on the surface of the embryo become trophectoderm and the inner cells acquire the inner cell mass fate.

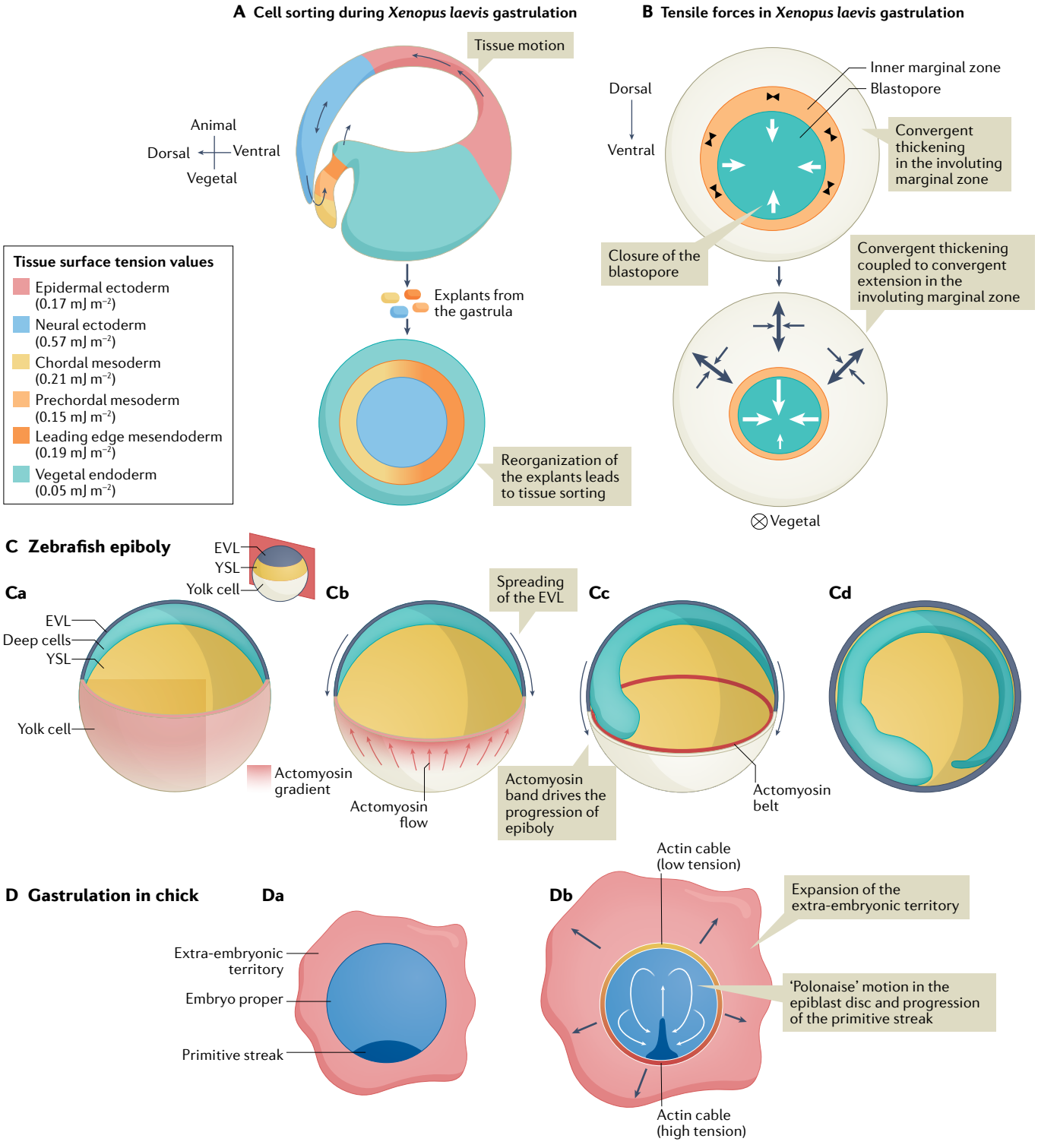
During compaction, actomyosin accumulates at the contact interface of the blastomeres (FIG. 2C). Cells become polarized and express an apical and a basal domain. This polarization initiation is cell-autonomous and can occur as long as the cell membrane has its surface partly free from neighbour contact[96]. The emergence of this apical domain inhibits cell contractility. During the divisions occurring between the 8-cell stage and the 16-cell stage, its inheritance is sufficient for trophectoderm specification[97]. The apical domain activates the YAP/TAZ pathway, which in turn is required for the lineage restriction between the trophectoderm and the inner cell mass[98]. CDX2, a transcription factor expressed specifically in the developing trophectoderm, becomes restricted to the outer cells whereas the pluripotency factor SOX2 marks the inner cell mass lineage. Trophectodermal fate is thus specified by positional information that emerges from blastocyst compaction.

Fig. 4 | **Mechanics of cell rearrangements. A** | Cell sorting during *Xenopus laevis* gastrulation[102] and surface tensions of different prospective embryonic tissues (0.05–0.5 mJ m^{-2}) measured in several explants. As a comparison, surface tension between air and water is much higher (~ 70 mJ m^{-2}). When combined ex vivo, these explants sort and reproduce spatial ordering witnessed in the embryo, with the most cohesive tissue, the ectoderm, at the centre. **B** | Tensile forces during *X. laevis* gastrulation[20]. During early stages of gastrulation, blastopore closure is driven by convergent thickening in the involuting marginal zone, which produces a tensile force around the blastopore (top). Later in the process, involuting marginal zone cells continue to undergo convergent thickening but this motion is coupled to convergent extension, leading to asymmetrical repartition of tensile forces, which drives blastopore closure (bottom). **C** | Zebrafish epiboly[99]. At the onset of epiboly, the zebrafish embryo is composed of blastoderm (enveloping layer (EVL) and deep cells underneath), of the yolk cells and of the yolk syncytial layer (YSL) between blastoderm and yolk cell. Actomyosin flows between the vegetal and animal poles (part **Ca**) when epiboly reaches 40% of coverage. This flow produces a friction force necessary for epiboly progression (part **Cb**), eventually coalescing into an actomyosin band in the YSL, close to the EVL border (part **Cc**). Tension in the band results in additional force pulling the EVL in the direction of the vegetal pole, reaching the bud stage (part **Cd**). **D** | During chick gastrulation, extra-embryonic territory expands to cover the yolk whereas the embryo proper (or epiblast tissue) maintains its area constant (part **Da**). The boundary between the two tissues is made of a supra-cellular actin cable exerting tensile forces all around the embryonic disc[18]. Asymmetrically distributed tension generates vortex flows ('polonaise' movements) that extend and deepen the primitive streak to allow ingression of presumptive mesendodermal precursors further in the anterior part (part **Db**).

Primitive streak
A transient embryonic tissue acting as a signalling centre and a point of conversion to mesendoderm. Morphologically, an elongated depression that progresses from the posterior to the anterior part of the epiblastic disc.

Morphogenesis of gastrulation. In vertebrate embryos, gastrulation leads to the establishment of the antero-posterior axis, morphologically manifested by a primitive streak in amniotes or a blastopore in amphibians[20]. Recent studies show that the cellular rearrangements occurring during gastrulation are driven by forces arising at the border between different embryonic cell populations[18,20] (FIG. 4). These studies illustrate how complex morphogenetic movements can be dissected at the mechanistic level using force-based measurements (macroscopic force measurement in *X. laevis*[20] or laser ablation and stress inference from motion in the chick embryo[18]).

The first studies of gastrulation motion in *X. laevis* focused on deconstructing different cellular behaviours into a series of simple morphogenetic modes — collective cellular motion categories — that can be combined to describe complex morphogenesis and find homologies between gastrulating organisms. For instance, blastopore closure is described as a combination of involution, convergent thickening and convergent



Involution
Specific morphogenetic event happening during gastrulation that involves the collective motion of cells that spread inwards by rolling around a boundary imposed by bottle cells, leading to basal to basal juxtaposition of the internal and external cells.

Convergent thickening
A process whereby cells rearrange themselves and leave the surface of the tissue to invade the space in-between, leading to shortening and thickening of the tissue.

Epiboly
The partial enveloping of one cell population by another in early developmental processes, owing to proliferation differences between the two tissues.

Neural crest
A transient cell population in vertebrates that originates from the ectoderm.

Surface tension
Modelized as a force per unit of length or as an energy per unit of area, tension that results from the cost of maintaining an interface between two fluid-like systems having a homotypic interaction (between molecules or cells of the same type) more favourable than the heterotypic interaction (between molecules or cells of a different type).

Ephrin signalling pathway
A cell–cell signalling mechanism involving, on one side, Eph receptor kinases (transmembrane proteins) and, on the other, their membrane-tethered ligands called ephrins. This signalling has been intensely investigated in the context of the nervous system development and is involved in cell sorting, positioning and migration.

extension[2,20]. The mesoderm around the blastopore site — called the involuting marginal zone — undergoes convergent thickening through non-polarized cell motion, producing a resulting symmetrical tensile force around the margin[20] (FIG. 4B). Convergent thickening pushes the involuting mesoderm symmetrically around the blastopore whereas medio-lateral intercalation acts on the dorsal side to close the blastopore. Similar examples of force integration at the tissue scale resulting in a supra-cellular tensile force can be found in other organisms, such as epiboly progression in the zebrafish embryo[21,99] (FIG. 4C), migration of the posterior neural crest population in zebrafish and *X. laevis*[19], and primitive streak development in the chick[18]. We will describe this last example in detail (FIG. 4D).

The chick primitive streak is a structure homologous to the blastopore[100], formed through a massive cell reorganization called 'polonaise movement'. The epiblast, which will later develop into all three germ layers, is positioned above the endoderm layer — called the hypoblast in the chick (primitive endoderm in the mouse). Cells from the epiblast start to detach from the undifferentiated epithelial monolayer, migrating between the epiblast and the hypoblast to first produce the embryonic endoderm and then the mesodermal precursors. At the same time, the epiblast undergoes symmetrical cellular rearrangements on both sides of the delamination axis and starts to specify into ectoderm. Different scenarios have been proposed to explain the scale of these cellular rearrangements in the chick, based on local rules first (medio-lateral intercalation or asymmetrical rate of cell division) and then on long-range cues such as chemotactic attraction[101]. A force-based model has been recently proposed, grounded by the experimental observation that an asymmetrical tensile margin between the epiblast and the extra-embryonic tissue powers the whole reorganization of the embryonic disc[18]. Actin cable contraction in the posterior region drives the progression of the primitive streak (FIG. 4D). A fluid-mechanical model — whereby the epiblast is modelled as a viscous fluid, and forces at the supra-cellular scale are inferred from the velocity field and validated experimentally — recapitulates the polonaise movement observed in vivo in chick embryos. This elegant approach also brings forth a new mechanism to explain the primitive streak formation.

Although forces generated by actin cables are prominent in large yolky embryos such as the frog, chick and zebrafish, they have not been reported in the mouse. The mechanical underpinnings of primitive streak formation in the mouse are still unknown.

Surface tension in tissue sorting. The emergence of specific embryonic territories requires cell sorting and the establishment of sharp boundaries. An example of this process is the segregation of the primitive endoderm from the epiblast in the mammalian blastocyst, or of the three embryonic germ layers — the ectoderm, the mesoderm and the endoderm — during *X. laevis* gastrulation. We discuss what we know of the forces underlying tissue sorting in the amphibian embryo.

In amphibian embryos, tissue sorting can be linked to differences between cadherin surface concentrations[102]. Specifically, as first theorized by Holfreter, dissociated embryonic cells can self-organize and reconcile into their original embryonic germ layers owing to differential cell-adhesive affinities between embryonic tissues. This was later formulated in a thermodynamic framework by introducing surface tension to describe the observed sorting[103,104] (FIG. 4A). An analogy can be made with the demixing of fluids or the motion of an immiscible liquid phase on top of another. In vitro, a cell population with a higher concentration of cadherins has a higher surface tension and tends to get enveloped by populations with a lower amount of surface cadherins, in order to minimize the free adhesive energy of the system[103]. As a consequence, in vitro, the forces driving tissue segregation scale with the cadherin concentration[104]. In embryos, contractility (actomyosin-dependent cell cortex tension) and repulsive heterotypic interactions — encoded in the Ephrin signalling pathway[105,106] — also participate in the observed sorting[107–110]. Of note, Ephrin signalling is more complex than cadherin-based signalling and has not been described as a mechanical surface interaction[105,111]. The contact surface and, hence, adhesive energy that a cell develops with its neighbours decreases when its cortical tension increases[110,112]. Molecular interactions at the interface between cells thus produce forces at the supra-cellular scale that maintain the different fates and tissues properly segregated.

From in vivo to in vitro

As detailed in the previous sections, mechanical cues are important signals and drivers of early vertebrate development. Hence, the investigation of forces acting in early embryos, including their source, distribution and function, in individual cells and at the tissue scale, is essential to understanding the mechanisms of embryogenesis. For this purpose, in vitro models of development have gained much attention in recent years. In particular, they provide unique means for reconstituting parts of early mammalian development ex utero, thereby providing insights into mechanisms specific for developmental processes in mammals.

Mechanical patterning in explants. In vitro models of development can be provided by whole embryonic tissue explants or self-organizing aggregates of embryonic stem cells.

In the first case, one or several embryonic tissues are separated from the whole animal to study their mechanical properties independently of the surrounding tissues. This technique was used repeatedly in *X. laevis* to determine the relative surface tensions between the different germ layers[113] and provide a mechanism for the spatial ordering of these tissues in vivo during gastrulation[20]. It is possible to make macroscopic force measurements with *X. laevis* explants linked to a newtonmeter (tractor-pull assay) to understand the origin of cellular collective rearrangements in the internalizing mesoderm[20]. Such force measurements at the tissue scale are difficult to make in vivo in frog embryos, even with recently developed techniques[114], owing to the depth of the tissues involuting at gastrulation.

Blastoderm
Usually, the initial population of embryonic superficial cells on top of the yolk (in a yolky embryo such as the chick or the zebrafish).

Competence
For an embryonic cell, being competent means being able to respond to inductive signals. As mechanics shape the potential of cells to be responsive in a specific time and space window, it is likely that competence is also affected by mechanical forces.

Gastruloids
In vitro assembled 2D or 3D aggregates of pluripotent embryonic cells that model some aspects of in vivo gastrulation, whereby cells self-organize to generate patterns of embryonic and extra-embryonic tissues.

Fish explants — yolk-less blastoderm explants sometimes called pescoids — were developed initially from zebrafish embryos[115,116] and extended to other teleosts[117]. In these models, fish embryonic cells are isolated prior to the specification of germ layers and reaggregated in vitro[116]. Such aggregates can then elongate to specify an anteroposterior axis, even when they are mixed and devoid of extra-embryonic cues, although this artificial developmental patterning is less robust than in vivo. In a different experiment[115], separating the embryo proper from the extra-embryonic tissues showed that morphogenesis might be encoded autonomously in the separated blastoderm. Whether this encoding, or priming, is genetic or mechanical[118] is still to be determined.

Finally, some experimental approaches have focused on expanding the frontier of ex utero culture of mammal embryos[119–124]. In vitro culture of mouse embryos post embryonic day 3.5 (prior to implantation) unveiled the mechanics of post-implantation morphogenesis — formation of the epiblast rosette and hollowing — that is induced by the deposition of a basal membrane[122]. This extracellular matrix polarizes the localization of the basal integrin receptors of the epiblastic cells, which induces cavitation through homogeneous polarization and actomyosin contraction. Older embryos need active recirculation of their culture medium to develop: the improvement of rotating cultures in past decades[119–121,124] now allows direct manipulation and visualization of ex utero embryos, including electroporation and confocal imaging. With these new approaches, both mechanical measurements and perturbations can be utilized to study the robustness of post-implantation mammalian morphogenesis.

Stem-cell based models of mechano-patterning. The use of isolated embryonic stem cells to study developmental mechanobiology has uncovered some aspects of the mechanical basis of embryonic lineage specification. These in vitro experiments combine control of environmental mechanics (gel stiffness, osmolarity, microstructuration) with the application of a specific developmental programme instructed by a combination of growth factors. The concept of matrix stiffness-directed lineage specification, initially established with human mesenchymal stem cells[125], has been extended to stem cells representative of embryonic tissues[48,74,126,127].

YAP/TAZ activation through cytoskeletal recruitment[63,64] and fate specification via mechanosensitive channel activation[128,129] (inducing, in turn, changes in intracellular calcium signalling[130,131]) are two prominent examples of mechanisms related to the mechanical determination of cellular identity that have been characterized in vitro (FIG. 2B).

Aside from designing synthetic substrates, implementing active mechanical stimulation to cultured cells allows one to probe in vitro their mechanical competence. Mechanical compression or stretching can induce remodelling of intercellular junctions in epithelial monolayers. Active devices have been used to study the response of human adult epithelial cells[132–134] and stem cells[49,127]. An isolated study also demonstrated a relationship between osmotic pressure change and cell fate[135].

Embryoids and organoids are 3D stem cell aggregates modelling, respectively, early embryonic and organ development. While proliferating and adopting different fates, the stem cells also develop a 3D structure at the supra-cellular scale (for example, lumen expansion or vilification). These models can be used to study the impact of environmental mechanics on the emergence of these 3D structures, as this question cannot be addressed quantitatively in vivo or ex vivo for human development.

It is now well established that spatial organization of stem cell colonies impacts the outcomes of biochemical stimuli on the specification of cellular identities[136], but the repartition of intercellular stresses and contractility differences leading to mechanical patterning is not a parameter usually taken into account in stem cell assays, apart from a few exceptions[49,137,138]. Thus, most of the mechanosensitive assays that have been performed with embryonic stem cells to date have ignored the consequences of mechanical patterning. This lack of control for in vitro mechanical fields is exemplified by our inability to customize the programming of specific morphogenetic events in stem cell cultures (for example, choosing the spatial localization of folds and crypts), and we rely instead on pre-encoded developmental programmes (a morphogenetic 'default model') when growing organoids or embryoids. In that latter case, the 3D structure emerges from the spatial heterogeneity of cell fate specification.

Mechanics in human embryo models

A fundamental process of early vertebrate development — gastrulation — can be partially recapitulated in in vitro cultures of embryonic stem cell aggregates, known as gastruloids. These cultures have been particularly important in addressing questions regarding how geometrical confinement impacts patterning[136,139–142]. Owing to ethical considerations, extrapolation of the knowledge gained from animal models to human embryos requires the complementary use of human embryonic stem cells (hESCs) and human induced pluripotent stem cells. In this last part, we describe the existing models addressing human gastrulation in vitro and what we know of their mechanics.

Blastocyst cultures. There are two types of in vitro platform that model human early development. The first is in vitro implantation of natural embryos, where blastocysts can be cultured for up to 14 days[143,144] (human) or 21 days[145,146] (non-human primates). Blastocyst cultures isolate the embryo from maternal tissues. Culture of human[143,144] and non-human primate[145,146] blastocysts ruled out the role of mechanical inputs from the mother endometrium up to 14 days post fertilization in the human case[144]. Accordingly, it has become clear that the embryo has the minimal information required to start the implantation programme even without the mechanical and biochemical signalling coming from the endometrium. These findings echo the recent debate regarding the role of the uterine wall in specifying the orientation of the embryonic axis in mouse development[147,148].

Synthetic model embryos. Even without the time limit enforced by ethical considerations, natural blastocysts are a precious biological material that is too limited to build robust statistical studies. Synthetic embryos provide a unique opportunity to study instructive mechanical cues and the biomechanics of early human development in a simple and highly quantitative platform[149].

Comparative studies of gastrulation highlight species-specific traits and point to their limited relevance to human gastrulation[150], both at the molecular and the cellular level. Self-organizing models of human gastrulation derived from hESCs and human induced pluripotent stem cells are thus developed to determine whether observations made in model systems hold true for human embryos as well as to discover human-specific traits.

This second platform consists of self-organizing stem cell-based embryo models that are grown in 2D (REFS[49,136,151,152]) or 3D (REFS[141,153,154]) geometrical confinement (FIG. 5). In two dimensions, cell adhesion to the

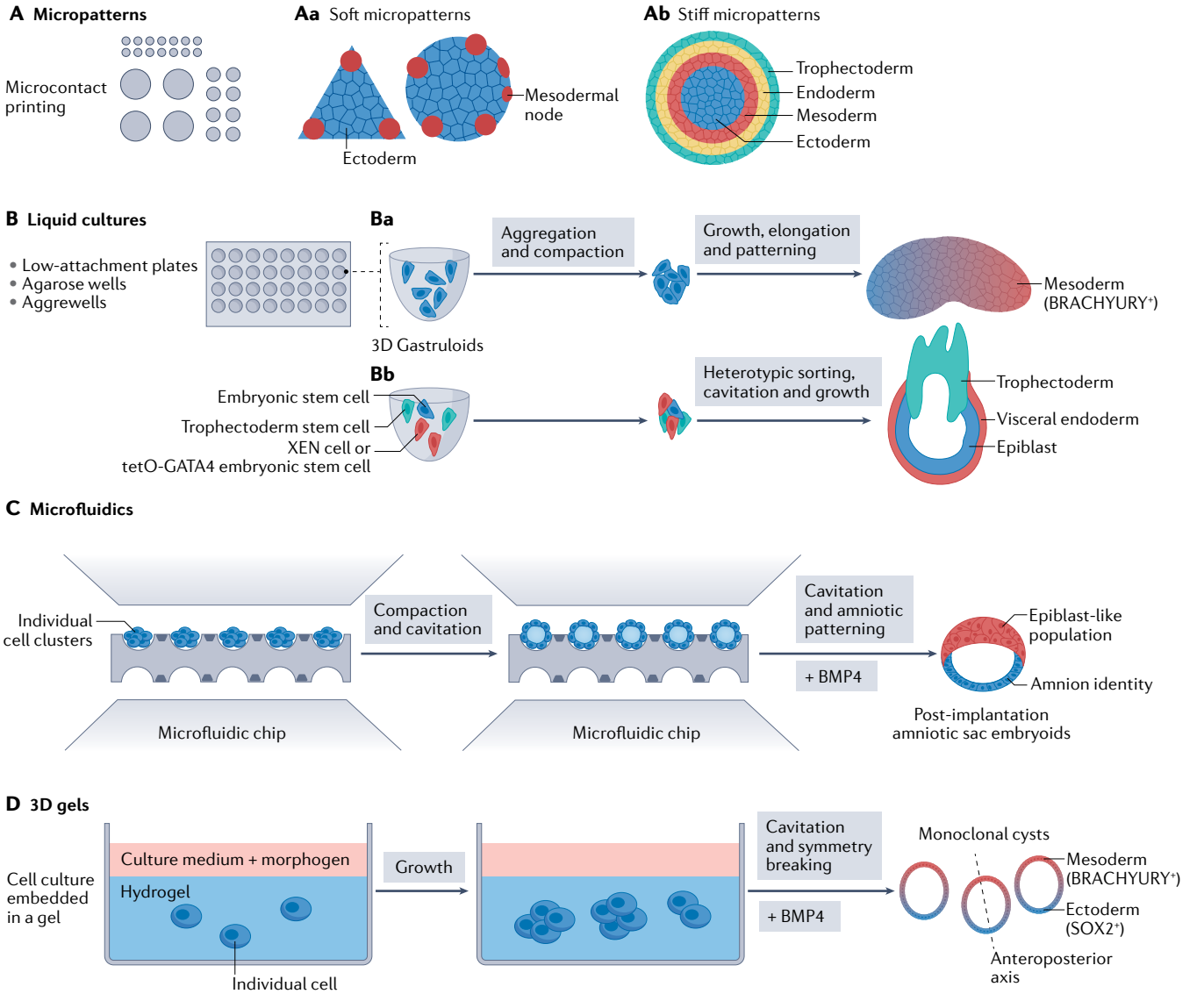


Fig. 5 | **Stem cell models of early mammalian development.** Overview of different strategies leading to symmetry breaking and patterning in vitro to recapitulate some aspects of mammalian gastrulation. **A** | Self-organization of human embryonic stem cells (hESCs) in two dimensions using micropatterns, with BMP4-induced differentiation. **Aa** | Specification of mesodermal identity in areas under tension when cells are patterned on soft hydrogel[49]. **Ab** | Expression of three embryonic germ layers and of trophectodermal fate on glass substrates[136]. **B** | 3D aggregates of stem cells can self-organize in liquid culture and model post-implantation morphogenesis, including partial gastrulation. **Ba** | When starting with a homogeneous and pluripotent population, aggregates of human or mouse embryonic stem cells elongate and specify mesodermal identity and somites[141,154]. **Bb** | Mouse stem cells representative of different embryonic and extra-embryonic tissues can be pooled together, form cysts, cavitate and specify the amniotic cavity and the ectoplacental cone[142,173] (elongated embryo). Embryonic stem cells are representative of epiblast, XEN cells of extraembryonic endoderm, tetO-GATA4 cells inducible for GATA4 represent primitive endoderm and trophectoderm stem cells model trophectoderm. **C** | Microfluidic approaches for patterning in vitro: pluripotent hESCs are seeded in a microfluidic chamber with a central gel band that confines and induces cyst formation while maintaining stable gradient of BMP4 during the culture. Cysts posteriorize and develop an amniotic cavity. **D** | 3D culture by gel embedding: a single clonal population of hESCs self-organize into a cyst when grown in biomimetic hydrogel, with spontaneous symmetry breaking under low BMP stimulation[153].

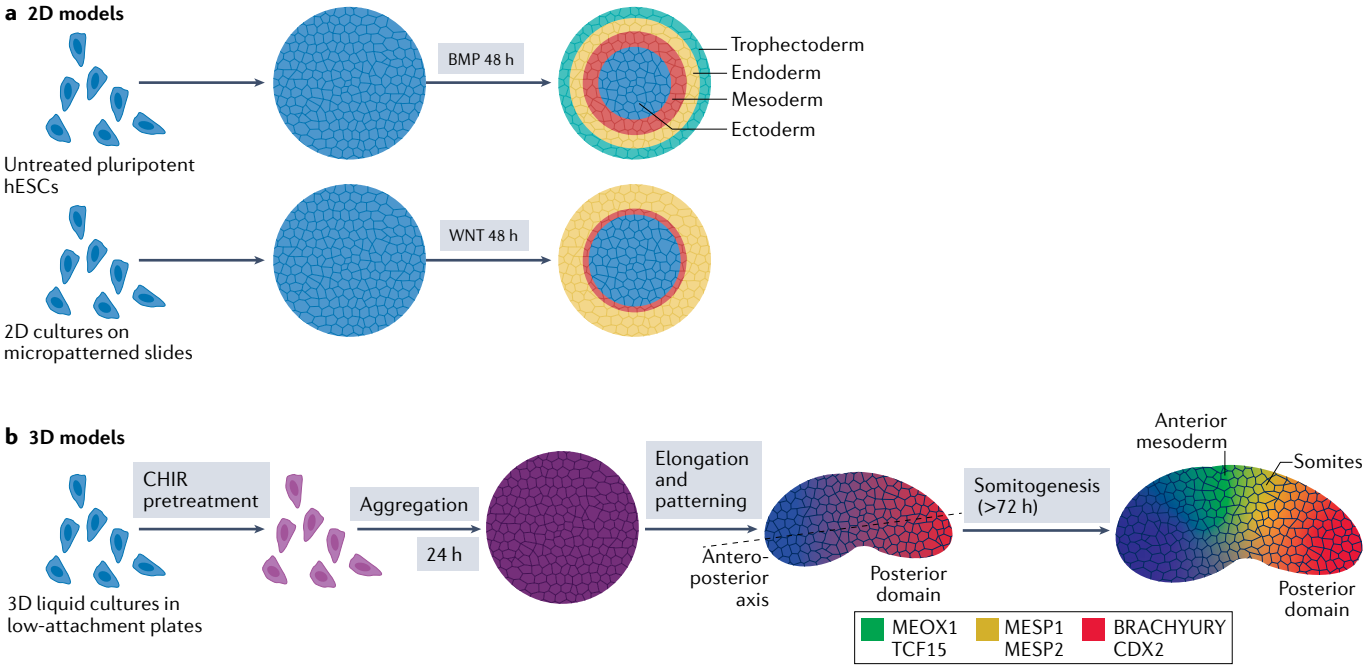


Fig. 6 | **Human gastruloids. a** | Pooling of undifferentiated population of pluripotent human embryonic stem cells (hESCs) on micropatterns. Forty-eight hours after BMP morphogen addition to culture medium, three embryonic germ layers and the trophectodermal layer are specified on circular micropatterns and their ordering matches that observed in embryos[142,173]. WNT signalling acts downstream of BMP morphogen. This pathway can be stimulated with addition of CHIR (small molecule) to culture medium and specifies mesendodermal layer with a primitive streak identity on the outer edge of the micropattern, surrounded by an endoderm ring[141,154]. These models show how a specific geometry (here, a disc) modulates accessibility of basolateral receptors to the supplied morphogens (here BMP4), inducing circular patterning. **b** | Anteroposterior patterning with elongation mimicking vertebrate axis elongation can also occur in cells pretreated with CHIR when cells are grown in low-attachment plates[142,173]. Cells cluster and develop a posterior domain with BRACHYURY and SOX2-positive cells 48 h post seeding. If maintained over 72 h, a somitogenesis signature appears (marked by MESP1 and MESP2 expression). MEOX1 and TCF15 mark anterior part (mesoderm).

surface is controlled by micropatterns of extracellular matrix proteins. In three dimensions, confinement is achieved in microwells or by embedding cell aggregates in a gel. The 2D gastruloid models have shown that mechano-transduction affects mesendodermal fate specification[49]. The mechanical activation of the pan-mesodermal marker BRACHYURY, through preferential recruitment of β-catenin and activation of the canonical WNT pathway, had already been demonstrated in embryos for the Bilateria[44,46] and Cnidaria[155] and established in standard hESC cultures on soft substrates[48]. In 2D gastruloids (FIG. 6), tension is built at the edge of the colony and specifies the domain of BRACHYURY expression[49]. Mechanical cues can thus change the self-organization undergone by hESC colonies.

In 3D models, the role played by mechanics in the self-organization of major embryonic tissues is less clear. Several approaches have been attempted in parallel to generate structures mimicking the morphology of natural blastocysts and initiating gastrulation. Some focus on the generation of form from a single homogeneous population and analyse the resulting symmetry breaking[141,153,154] (FIG. 5A,B). The end point structure can be improved if the delivery of morphogens is itself patterned, through the use of microfluidic chips[156] (FIG. 5C). Other approaches focus on mixing different cell types together[142,157–159], but this has been achieved only with mouse embryonic stem cells representative of both embryonic and extra-embryonic tissues as the generation of extra-embryonic stem cell types in the human case is still a matter of debate[160–164]. One of the limitations of human gastruloids is that, so far, their development cannot be sustained for more than 4 days[141] and the cellular motion and rearrangements have not been mapped to the expression of particular fates except in 2D studies[49,152]. The induction of epithelial–mesenchymal transition that is associated with gastrulation in these 3D models breaks down the cohesion of the cellular aggregate[153], in contrast to the in vivo situation where epithelial–mesenchymal transition is a transient state that does not impair the subsequent development of the embryo.

Human morphogenesis in a dish. To leverage 2D and 3D models to the point where mechanics can be robustly studied, several approaches have to be combined in parallel. The generation of mutant, opto-genetic and reporter lines for specific pathways known to be mechanosensitive (WNT, HIPPO) can enrich our understanding of the role played by forces in human development and lead to a new generation of gastruloids that develop more robustly or specify differently the repartition of the embryonic tissues. The development of new mechanical platforms[148] such as biomimetic gels, 3D printed cellular environments, microfluidic chambers and mechanically excitable devices could also provide more permissive environments for human morphogenesis.

Finally, although recent efforts have been understandably focused on enhancing reproducibility in the production of synthetic model embryos, development of live and high-resolution imaging is essential to understanding their morphogenesis. This technology is accessible[165,166] and would provide data that can fuel mathematical models of morphogenesis, paving the way for a description of forces directing human morphogenesis.

Overall, 2D and 3D cultures of embryonic stem cells possess considerable potential for dissecting mechanical interactions between extra-embryonic and embryonic populations, and could also be extended to other models as technologies of embryonic stem cell isolation and culture are further developed.

Conclusions and perspective

Mechanobiology has already deeply impacted developmental biology. Combining in vivo measurement of the force field at the tissue scale with live tracking of cellular identities could help us in the future analyse the mechanics of developing tissues and answer prominent and ancient — yet unsolved — questions, such as the role played by mechanics in size regulation or in the long-range patterning of vertebrate embryonic tissues.

In vitro platforms are valuable for analysing the chain of molecular relays in tissue-scale mechanics. They allow more quantitative and intensive studies that can then be validated in embryos. However, the complexity of morphogenetic events taking place in a developing organism also reveals the current limitations of stem cell-based models. If we set our goal on reconstituting human morphogenesis in a dish, we will have to understand and classify the mechanical interactions that embryonic cells develop collectively — a thought process we might call reverse engineering. This necessitates quantitative studies of mechanics in vertebrate (non-human) embryos to infer the basic principles linking these mechanics to patterning. These principles can then be tested in vitro with human stem cells, in a minimal setting, to reproduce milestones of early embryonic morphogenesis. Eventually, this dynamic in vitro simulation of human early development could be used to investigate early subtle phenotypes that lead to developmental defects.

Another tremendous advantage of in vitro approaches is that they can duplicate developmental studies in many different species — provided that stem cells can be derived in each organism — which sets the grounds for evo-devo studies. The same platform can then be used to investigate mechanically driven processes that are conserved between species, thereby shedding light on overarching themes that govern vertebrate morphogenesis.

Published online 9 November 2021

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Acknowledgements
The authors thank all members of the Brivanlou laboratory, C. LaBonne, O. Campàs and participants in the 2021 Marine Biological Laboratory (MBL) Embryology Advanced Research Training Course for useful discussions and comments. M.V. is supported by the Human Frontier Science Program (HFSP) fellowship LT000283-2020-C. E.D.S. is supported by National Science Foundation (NSF) grant No. 2013131.

Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
A.H.B. and E.D.S. are co-founders of RUMi Scientific. A.H.B. is the co-founder of OvaNova Laboratories. M.V. declares no competing interests.

Peer review information
Nature Reviews Molecular Cell Biology thanks the anonymous reviewers for their contribution to the peer review of this work.

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