Interplay of mechanical deformation and patterned gene expression in developing embryos

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The shaping of the early embryo requires pattern formation as well as geometric and topological morphogenesis of the developing tissues. The morphogenetic movements that lead to geometric shape changes are controlled by patterned gene expression. How particular movements are related to patterning genes, and which underlying molecular and cellular mechanisms lead to coordinated macroscopic movements that induce morphogenesis, remain the challenging questions of embryonic development. How morphogenetic movements could modulate the expression of developmental genes is an emerging question, potentially opening new horizons in developmental biology. This question instigates the task of characterizing the molecular and cellular mechanisms underlying these mechano-transcription events.

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Abbreviations
BMP bone morphogenetic protein
MAPK mitogen-activated protein kinase

Introduction

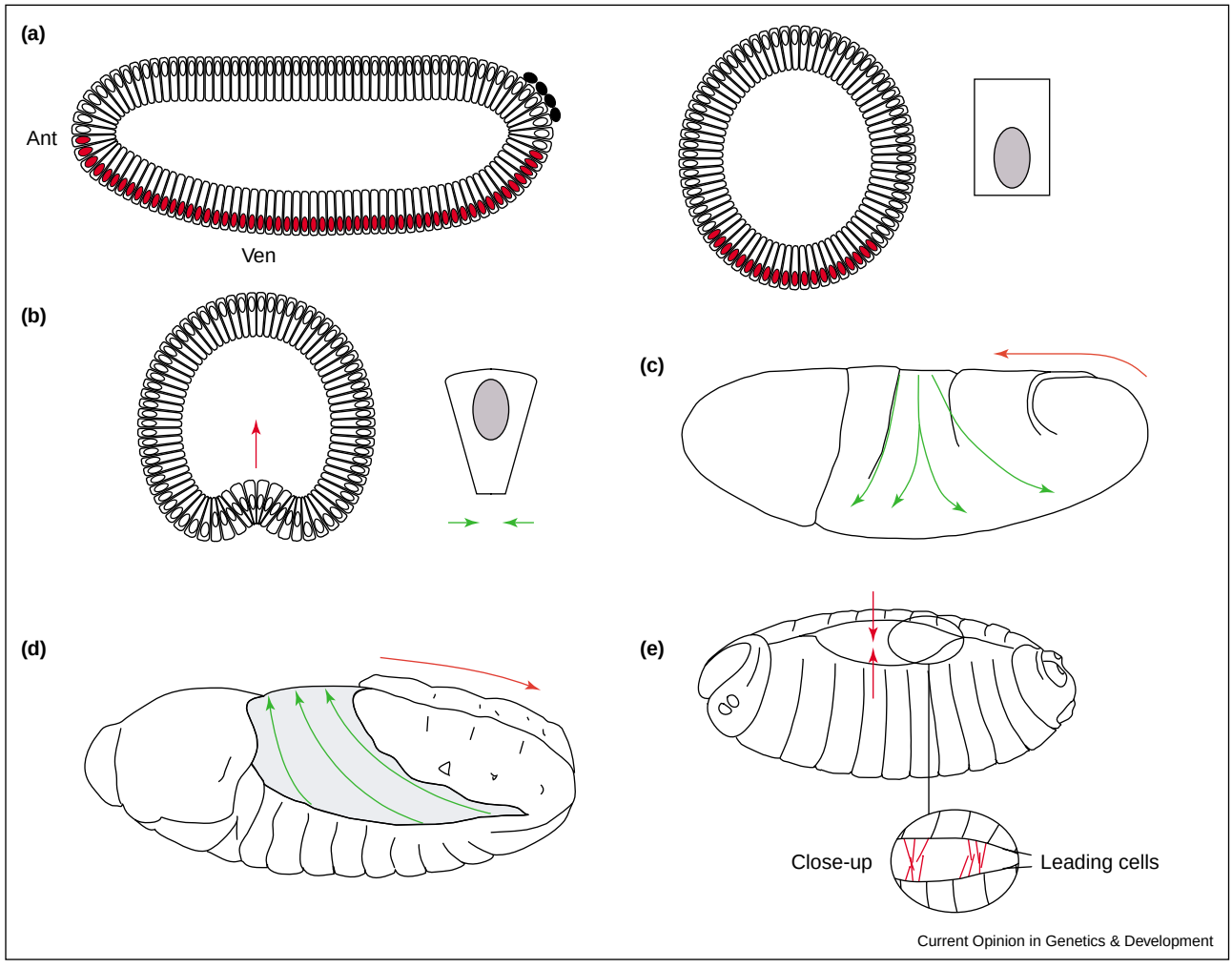
The biochemical pattern of the embryo is induced by cascades of gene expression that lay down the body plan [1]. The morphology of the embryo is shaped by the generation of forces that lead to tissue-specific deformations [2]. Genetic analyses have already demonstrated the close control of the morphogenetic movements by patterned gene expressions [3]; but how does a physical shape emerge from a biochemical pattern? Can the expression of some developmental genes be mechanically controlled by changes in the physical shape of the embryo? Could this feedback loop assure the robust coordination between pattern formation and morphogenesis? Using examples from both vertebrate and invertebrate development, we illustrate recent findings addressing these fascinating questions that relate the biochemical processes to the morphological phenotype of the embryo. Recent progress in understanding the relationship between morphogenesis and biochemical patterning will be reviewed, as will emerging evidence for a role of mechanical forces in modulating the expression of developmental genes. Finally, the deciphering of the mechanical interplay between gene expression and mechanical deformation will require investigation of the underlying molecular mechanism of mechano-transcription.

From patterns to movements: the genetic control of morphogenesis

One of the most thoroughly understood examples of genetically controlled morphogenetic movements is the mesoderm invagination that initiates *Drosophila* embryo gastrulation (Figure 1a,b) [4]. In this case, both the *twist* and *snail* ventral genes are required for complete and proper mesoderm invagination [5]. *snail* is thought to initiate or 'allow' random stochastic cell deformations, and *twist*, to subsequently trigger the collective cell-shape changes. Both genes, together, are required for the simultaneous cell apical constrictions that induce the bending force necessary for mesoderm invagination (Figure 1b) [6]. Although the *snail*-dependent part of this process remains poorly understood, much is known about the mechanism related to the *twist*-dependent apical constriction. The collective shape changes are induced by the *twist*-dependent expression of *folded gastrulation* [7], a secreted factor that activates the concertina G-protein [8], thought to lead to the re-organization of the actin-based cytoskeleton, via dRhoGEF2 activation [9,10]. Moreover, the re-localization of the non-muscle myosin to the apices of mesodermal cells correlates with the apical constriction of the individual cells [11]. Recently, it has been shown in *C. elegans* embryos that non-muscle myosin activity is essential for apical constriction and ingression of the endodermal precursor [12•]. Interestingly, the asymmetrical localization of PAR-3 in these embryos is proposed to apically concentrate the actomyosin complex during invagination [13•]; however, the underlying molecular mechanisms that produce the active forces have not yet been fully elucidated. The development of explant assays of *C. elegans* embryos that recapitulate gastrulation will probably enable this issue to be addressed [14].

Preceding the morphogenetic movements of gastrulation, the cellularization of the *Drosophila* embryo involves a cytoskeleton-regulated membrane growth around each

Figure 1



The morphogenetic movements of the *Drosophila* embryo, from gastrulation to dorsal closure. **(a)** Cellular blastoderm, at the end of cellularization (longitudinal section, left). A ventral–dorsal gradient of nuclear concentration of the transcription factor Dorsal triggers the expression of the *twist* and *snail* mesodermal genes into the most ventral cells of the embryo (transversal section, center; in red, into the nuclei). Cell shape before apical constriction (individual cell, right). **(b)** Gastrulation. Mesoderm cells contract their apex under the control of *twist* and *snail* (green arrows), initiating mesoderm invagination (red arrow). **(c)** Gastrulation: germ-band extension (lateral view). Lateral tissues are submitted to a convergent-extension movement, under the control of the antero-posterior patterning genes, via the planar polarity gene *PAR-3*/*Bazooka*. This leads to dorso-ventral cell movements (green arrows), that trigger the macroscopic movement of germ-band extension (red arrow). **(d)** Germ-band retraction follows germ-band extension. A RhoA-dependent dorso-ventral contraction of the lateral amnioserosa cells (green arrow) contributes to the force that drives germ-band retraction (red arrow). **(e)** Dorsal closure follows germ-band retraction, and entails the closure of a dorsal gap, made of the amnioserosa tissue. It is driven by the contraction of actin cables at the leading edge of epidermal tissues and by the contraction of the amnioserosa itself (red arrows). The zippering process that completes dorsal closure is finely tuned by filopodial protusions (shown in inset) that mediate the correct apposition of the segment halves. (Ant, anterior side; ven, ventral side of the embryo.) Drawings adapted from [3].

nucleus of the blastoderm, followed by the basal closure of the cell. The membrane furrow invaginates along microtubules and a contractile actin ring forms at its leading edge. The actin rings of individual cells are connected to each other by the Bottleneck protein [15]; hence, the actin–myosin contraction that is activated by src64 is counter-balanced by the intercellular physical links [16]. Such mechanical resistance is thought to be released at the end of cellularization, after the rapid shut-off of Bottleneck expression, thereby leading to the basal contraction that closes the cells [16]. However, by contrast to previous assumptions, actin ring contraction does not seem to be the major driving force of membrane growth [17]. It is suggested that membrane invagination relies primarily on the lateral addition of golgi-derived membrane vesicles [18–20], although basal closure crucially depends on myosin II activity [17].

Convergence–extension, another classical morphogenetic movement that occurs during gastrulation, has been

studied in *Xenopus* and zebrafish. In vertebrate embryos, convergence–extension depends on Wnt genes, and is further elicited by molecular pathways that share several components with the non-canonical wingless pathways that direct planar cell polarity in *Drosophila* (reviewed in [21]). This homology has been further demonstrated by the characterization of the role in vertebrate gastrulation of the *Drosophila* planar polarity genes *strabismus* [22–24], *prickle* [25–28], *Winderbost* [29], and RhoGTPases [30–33]. The establishment of cell bipolar morphology that results from extension of cytoplasmic protrusion in a medio-lateral direction appears to be essential for the directed cell intercalations that drive convergence–extension [34,35]. Recently, it has been shown in zebrafish that *silberblick*/*Wnt11*, which is required for proper convergence–extension movement, controls the orientation of the cellular processes that might facilitate or stabilize cell movements [36•]. These results correlate with the fact that Rok2 in zebrafish and RhoGTPases in *Xenopus* are downstream components of Wnt [30–33]. The functions of the different RhoGTPases in cytoskeleton dynamics during vertebrate gastrulation will help to define the cellular events that are triggered by the different non-canonical *wingless* pathways [30,37]. In *Drosophila*, the germ-band extension, driven by dorsal-to-ventral cell intercalations, is a convergence–extension movement (Figure 1c) [38]. Recent data demonstrate a role for PAR3/Bazooka in defining planar polarity at the cellular level in this system [39••]. PAR-3 (part of the PAR family of proteins that associate asymmetrically with the cell cortex), which is required for correct germ-band extension, accumulates at the dorso-ventral cell borders, whereas non-muscle myosin II is distributed at the reciprocal antero-posterior cell borders. However, these polarized distributions depend on the pair-rule genes *even-skipped* and *runt*, associated with the specification of the antero-posterior axis of the *Drosophila* embryo, and are not linked to the non-canonical *wingless* pathway [39••]. The underlying cellular mechanisms that interpret cell planar polarity into intercalation movements remain unknown.

Interestingly, during germ-band retraction of the *Drosophila* embryo, which follows convergent-extension, cells in the lateral germ-band do not intercalate. Thus, the movement is not a reversal of convergence–extension, but correlates with the dorso-ventral shortening of the amnioserosa tissue (Figure 1d) [40•]. Amnioserosa cells form lamellipodia that migrate over the caudal end of the retracting germ-band. These lamellipodia might direct migration of the amnioserosa and, therefore, maintain its overlap over the retracting germ-band [40•]. The $\alpha_{1,2}$ laminin and the αPS3βPS integrin were found to be required for both lamellipodia formation and amnioserosa adhesion to the posterior pole of the germ-band and to the underlying yolk sac [41].

More directly accessible, from the mechanical point of view, is the *Drosophila* embryo dorsal closure event that follows germ-band retraction. This movement involves closure of a dorsal gap, made of the extra-embryonic tissue amnioserosa, by drawing lateral epithelium from the two sides up to the midline of the embryo (Figure 1e). The epithelium movement seems to be driven by the contraction of an actin cable that lies at its leading edge [42] and its completion seems to be finely controlled by dynamic filopodial protrusions [43]. However, the amnioserosa is not simply a passive tissue or substratum, but participates directly in the morphogenetic movement [44,45]. An elegant mapping of force distribution by laser micro-dissection and mathematical modeling has quantified the relative contributions of the different cellular processes to the dorsal closure [46••] and has further confirmed the motor role of the amnioserosa in the morphogenetic movement.

Testing of these models and emerging ideas will require the set-up of new methods allowing measurement and local perturbation of the mechanical forces and deformations that are involved in endogenous morphogenesis, *in vivo*. Up until now, convergence–extension in *Xenopus* explants is the only morphogenetic movement in which a force has been measured and quantified — this was found to be in the order of 1 μN [47].

From movements to gene expression: the morphogenetic control of patterned gene expression

Morphogenetic movements are closely controlled by the expression of developmental genes. Do morphogenetic movements, in turn, regulate the expression of developmental genes? Morphogenetic cell migrations or changes in the embryonic topology (e.g. at gastrulation) are already known to play an important role through classical induction. These movements cause changes in the cell environment, enabling gene induction via new local interactions. For instance, in the *Drosophila* embryo, the segmented expression of the homeogene *labial* in the endoderm is induced by its proximity, with the visceral mesoderm that expresses *ultrabithorax* in the para-segment 7. This leads to the perfect and precise projection of the mesodermal segment onto the originally un-segmented endoderm [48]. Also, in the chick embryo, the migratory routes that are followed by the primitive streak cells are known to determine their cell fate [49]. This indicates the existence of inductive signals that are encountered by migratory cells during their movements, in particular during their migration across the Hensen node (the organizer center of the chick embryo, corresponding to the Spemann's organizer in amphibians).

Whether developmental gene expression is directly controlled by mechanical forces that develop within a tissue during morphogenesis is an emerging question of

developmental biology. In the early *Drosophila* embryo, the application of artificial cell-shape changes just before gastrulation leads to a ventralized phenotype [50••]. In particular, the mesodermal protein Twist, which is normally expressed in the most ventral cells of the embryo, is expressed in all tissues after cell deformations. Interestingly, Twist mechanical induction appears to be triggered in the compressed cells of the stomodeum by endogenous morphogenetic movements at gastrulation. Stomodeal cells at the anterior pole undergo an important compression, due to the mechanical squeezing of the extending germ-band, and exhibit a strong amplification in Twist expression (Figure 2a). The use of mutants and biphoton microsurgery inhibited germ-band extension and subsequently prevented the expression of Twist in these cells. In these conditions, Twist expression was recovered in response to the local compression that was exogenously applied with a microneedle [50••]. These data indicate that Twist expression at the anterior pole during gastrulation is sensitive to changes in the cellular physical geometry. In other words, the expression of some developmental genes appears to be induced by changes in the physical shape of the embryo. Such data reveal a reciprocal interplay between patterning genes and morphogenesis and underline the possible role of this feedback loop in regulating development.

In light of these observations, several experiments that have been performed on vertebrates might further support a putative role for mechanical strains in the control of developmental gene expression. For instance, the *Xegr-1* mesodermal gene was found to be anomalously expressed in *Xenopus* embryo cap explants and to correlate with MAPK (mitogen activated protein kinase) pathway activation (Figure 2b) [51]. Two further studies have shown that the MAPK pathway is activated by dissection and even by pricking of the embryo with a microneedle [52,53]. The explanations that have been proposed for these results range from the induction of the MAPK pathway by the release of soluble factors [51,52], to an inducing effect of tissue relaxation [53]. This second hypothesis, of a direct coupling between the cytoskeletal tension and the signal transduction pathway, is in agreement with data demonstrating that the MAPK pathway is induced by wounding in intestinal epithelium, independently of soluble factors [53,54]. Another experiment indicates that tissue tension in explants of gastrula *Xenopus* embryos is necessary for the development of a normal set of rudiments and for differentiation [55]; or, alternatively, that tension might be important for maintaining the tissue cohesion rather than for differentiation [55]. Finally, mechanical contraction waves were reported to correlate with primary neural induction during development in axolotl embryos [56].

Such mechanical induction might well play a coordinating role during development. First, mechanical forces can promote rapid and long-range interactions between non-adjacent tissues, on a larger scale than those permitted by soluble factors. These long-range inductive interactions could permit the synchronization of different biochemical events across the whole embryo at critical stages of development. On a smaller spatial scale, this mechanical induction might coordinate individual cell behaviors to drive macrosocopic morphological changes and synchronize these morphological changes with cell-fate determination.

Flows as master regulators of organogenesis

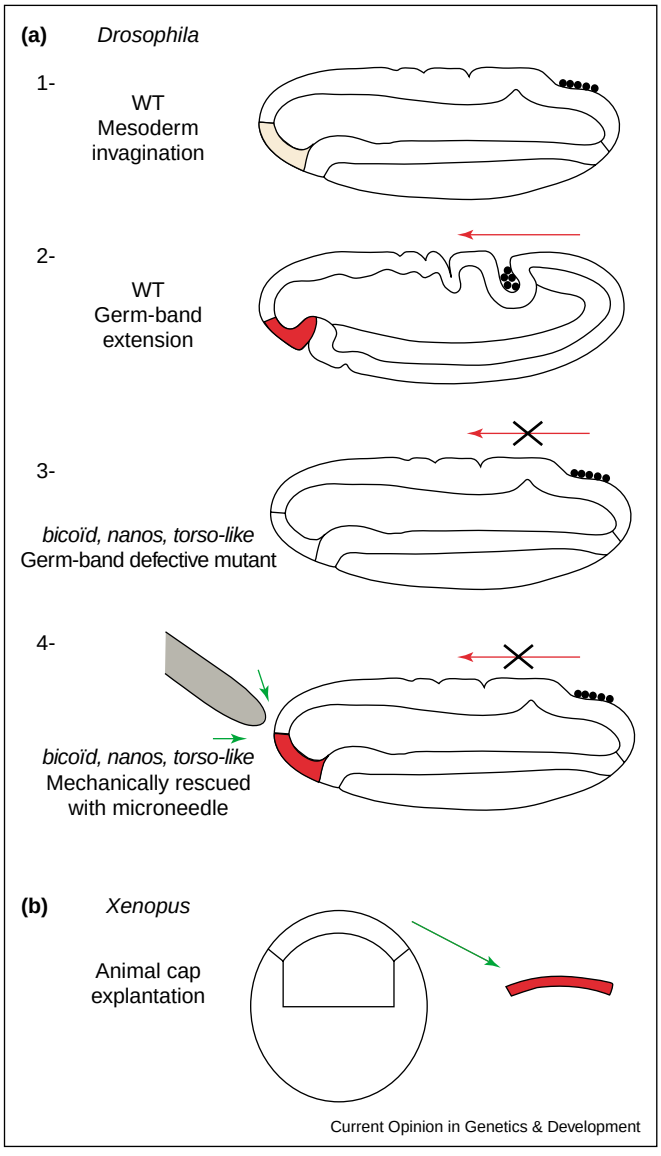
Mechano-transcription effects may also be relevant to the later stages of development that involve organogenesis. Indeed, mechanical forces related to hydrodynamic flows have long been proposed to play a significant role in organogenesis, such as in lung and cardiac morphogenesis. Fetal breathing movements, peristaltic airway contractions and lung fluid necessarily exert physical forces onto the developing lung. They have been proposed to play a role in balancing proliferation and apoptosis, and therefore to control lung growth *in vivo* [57]. Furthermore, intra-cardiac fluid forces in the zebrafish embryo heart were demonstrated recently to be necessary for proper development of the heart [58••]. The implantation of beads in the vicinity of the heart, to block the blood efflux or influx, led to severe phenotypic defects in heart formation, such as the complete omission of the third chamber. Reminiscent of these macroscopic phenotypic observations, vascular flow was also found to be necessary for glomerular assembly during kidney morphogenesis [59••], as well as for arterial–venous differentiation in the chick embryo yolk sac [60]. More significantly, kidney development depends on the expression of metalloproteinase 2, already known to be mechano-sensitive in other systems [59••]. In contrast to the forces that develop within the tissue throughout the course of the morphogenesis that shapes the embryo, hydrodynamic flows couple the physiological function to the development of the specialized organs. In other words, the phenotypic structure adapts to the function through the inductive properties of the fluid it has to process.

These examples underline the difficulties of interpreting phenotypes for a process that implies the interplay between gene expression and mechanical induction. Indeed, taking into account these inductive mechanical forces in development or organogenesis might help the re-evaluation of the role of some genes in certain phenotypes [60]. For example, some mutants for genes that are involved in vascular development also exhibit impaired hemodynamic flows. In this case, what is the primary effect that results in the defective vascular development?

In search of molecular morpho-sensors

How does a cell translate a mechanical signal into a transcriptional event? Mechano-transduction and/or

Figure 2



Gene mechanical induction and gene expression initiated by wounding. **(a)** Mechanical induction of the mesodermal gene *twist* into stomodeal cells at the anterior pole. During mesoderm invagination, Twist expression is weak in stomodeal cells (pink) (a1), and is strongly amplified as the cells are compressed by the extending germ-band (red) (a2). In the antero-posterior mutant *bicoid nanos torso-like*, defective in germ-band extension, stomodeal cells remain uncompressed and the expression of Twist fades to background levels (a3). In these conditions, strong Twist expression is rescued by mechanical compression, applied with a micro-needle (a4). **(b)** Explantation of the ectoderm cap of *Xenopus* embryo blastula leads to anomalous expression of the mesodermal gene *Xegr-1*.This gene expression correlated with the activation of the MAPK pathway. These effects were proposed to result from the release of soluble factors upon wounding. Alternatively, they might also be associated with mechanical changes in shape, such as tension relaxation of the cap after explantation. WT, wild type. Drawings adapted from [3].

mechano-transcription events occur in many cell types, such as cardiac myocytes, vascular smooth muscle, bone and endothelial cells. Therefore, several *in vitro* studies have focused on this issue. However, it is beyond the scope of this paper to review this domain exhaustively and so we will focus primarily on the opening questions that have been raised by the study of endothelial cells submitted to shear-stress. Endothelial cells are constantly submitted to the shear flow of blood and adapt by expressing numerous genes that remodel its structure. *In vitro* studies aimed at understanding how endothelial cells respond to such stimuli provide useful insights and a paradigm for mechano-transcriptional processes [61]. Several genes have been shown to be differentially expressed under static and shear-stress conditions, and promoter studies have identified different shear-stress responsive elements (SSREs), thought to mediate some shear-stress transcriptional responses [61,62]. Furthermore, different

transcription factors, including NF-κB, Egr-1, Sp1, fos, jun and SREBP1 (sterol regulatory element-binding protein 1), were found to be activated by laminar shear-stress and were able to bind to SSREs for some of them. Finally, potential shear-stress receptors, like integrins αVβ3, FAK (focal adhesion kinase), c-Src and the VEGFR2 (vascular endothelial growth factor receptor)–VE-cadherin–β-catenin complex, were shown to be involved upstream of these transcription factors or transcriptional activation events [61]. However, the activation cascades of these transcription factors that are triggered by laminar shear-stress remain elusive.

The identification of mechano-sensors that are able to transduce mechanical stimuli into a biochemical activity has retained much interest. A first model proposes that transduction events take place in the vicinity of the plasma membrane [63]. These models suggest that mechano-sensitive ion channels, tyrosine kinase receptors, caveolae and G proteins work as mechano-sensors [61,63]. Interestingly, mechanical membrane tension can enhance transcriptional events by blocking the endocytosis and preventing the degradation of the receptor–ligand complex into endosomes. For example, the blockade of BMP2 endocytosis induced by plasma membrane mechanical tension dramatically enhanced the BMP2-dependent myoblastic/osteoblastic trans-differentiation of murine cultured cells [64]. Other localized models propose that mechanical transduction occurs at the sites of cell–cell junctions or cell–matrix interactions, through the activation of PECAM-1 (platelet/endothelial cell adhesion molecule-1), of the VEGFR2–VE–cadherin–β-catenin complex or integrins [61]. In agreement with this view, β-catenin/armadillo seems to be involved in Twist mechanical induction during *Drosophila* gastrulation [50••]. Finally, a delocalized model of mechano-transduction suggests that forces applied at the cell surface are transmitted, through integrins and via the cytoskeleton, to other locations of the cells and potentially to the nucleus, where they could trigger transcriptional events [65]. Such a model has been speculated to explain how the contraction waves could trigger neuronal differentiation in axolotl embryos [56]. However, it is not clear whether these putative mechano-sensors act independently and whether the different molecular pathways they activate are integrated to elicit a transcriptional response. In addition, the growing number of molecules or structures that are involved in mechanical transduction highlights the need to definitively distinguish between molecular complexes that are activated by mechanical stimuli and the cellular mechano-sensor(s) that triggers subsequent responses [63].

Conclusions

The studies discussed in this review highlight the diversity and the conservation of the cellular mechanisms that are involved in morphogenetic movements in vertebrate and invertebrate embryos. Moreover, an increasing number of examples point to the existence of a reciprocal interplay between expression of some developmental genes and the mechanical forces that are associated with morphogenetic movements or with hydrodynamic flows during development. Challenging issues that remain to be addressed include the deciphering of the underlying molecular mechanisms of this interplay and the uncovering of the physiological significance of these mechano-transcription events during development.

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