
CHAPTER 12

Mechanical Induction in Embryonic Development and Tumor Growth: Integrative Cues Through Molecular to Multicellular Interplay and Evolutionary Perspectives

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Abstract

Embryonic development is a coordination of multicellular biochemical patterning and morphogenetic movements. Last decades revealed the close control of myosin-II-dependent biomechanical morphogenesis by patterning gene expression, with constant progress in the understanding of the underlying molecular mechanisms. Reversed control of developmental gene expression and of myosin-II patterning by the mechanical strains developed by morphogenetic movements was recently revealed at *Drosophila* gastrulation, through mechanotransduction processes involving the Armadillo/ β -catenin and the downstream of Fog Rho pathways. Here, we present the theoretical (simulations integrating the accumulated knowledge in the genetics of early embryonic development and morphogenesis) and the experimental (genetic and biophysical control of morphogenetic movements) tools having allowed the uncoupling of pure genetic inputs from pure mechanical inputs in the regulation of gene expression and myosin-II patterning. Specifically, we describe the innovative magnetic tweezers tools we have set up to measure and apply physiological strains and forces *in vivo*, from the inside of the tissue, to modulate and mimic morphogenetic movements in living embryos. We discuss mechanical induction incidence in tumor development and perspective in evolution.

I. Introduction

The most ancient known scientific report on embryonic morphogenesis is by Aristotle, four centuries BCE (Aristotle). In his report, Aristotle emphasizes that the different parts of the body of the chicken embryo form in a sequential process rather than all at the same time. This seminal observation led to the so-called “epigenetic” conception of embryogenesis, through which the existence of the structure of the embryo of a given stage conditions the emergence of the structure of the next stage.

This conception does not require a preexistent body plan, in opposition to the earlier Platonic preformationist conception of morphogenesis. Much later, the progresses of optical microscopy from the 17th to beginning of the 20th century allowed increasingly accurate observation of embryonic development. If preformationists initially thought that they could detect the existence of small preformed human shapes in the head of the male spermatozoid (called the “homunculus” by Leeuwenhoek (1683), the epigenetic view of development was rapidly confirmed by the observation of morphogenetic movements of tissues that correlate to growth, which progressively shape the embryo from ovoids to complex body shapes in a step-by-step sequential process. At that time, the privileged observable feature of embryogenesis was thus the morphogenetic movements, which appeared as hydrodynamic fluid movements. After the Newtonian evolution of physics, these observations were naturally interpreted by many of the embryologists of that period, like His and Leduc (His, 1875; Leduc, 1912), as passive flows exclusively governed by the Newtonians laws of hydrodynamics. One of these embryologists, however, D’Arcy Thompson, suspected the existence of still unknown hidden underlying additive physiological factors driving the morphogenetic processes (Fox-Keller, 2003; Thomson, 1917).

The discovery of the genome and the emergence of molecular biology and the genetics of developmental biology in the middle of 20th century revealed the nature of these factors, both genetic and biochemical in nature. From this evolution of biology, developmental biology focused most of its efforts on the study of the genetic control of the elaboration of the biochemical differentiation of the tissues designing the body plan of the future organism (Garber *et al.*, 1983; Lewis, 1978; Nusslein-Volhard and Wieschaus, 1980; St Johnston and Nusslein-Volhard, 1992). However, the end of 20th century (at the onset of the 1990s) was marked by a return to geometrical morphogenetic considerations, with the discovery of master developmental genes regulating the generation of morphogenetic movements in embryogenesis (Sweeton *et al.*, 1991). Today, our understanding of the molecular mechanism linking patterning gene expression to the production of mechanical forces that shape the embryo increasingly progresses (Martin, 2009). In the beginning of the 21st century, reverse signals were discovered showing the mechanical control by the morphogenetic movements of the expression of patterning and developmental genes, based on biochemical mechanotransduction processes (Brouzes *et al.*, 2004).

Here, we will describe the up-to-date state of the art in this emerging field, reciprocally coupling genetics to mechanical physics. The study of such coupling necessarily requires the establishment of methods allowing the uncoupling of pure genetic inputs from pure mechanical inputs in the regulation of patterning gene expression. After a first part describing our knowledge on the genetic control of morphogenetic movements in embryogenesis, we will review today’s knowledge of the mechanical control of patterning and developmental gene expression, and the distinct genetic and biophysical methods that have been set up to uncouple mechanical inputs from biochemical inputs in the control of developmental gene expression *in vivo*. Specifically, we will describe the innovative tools we have set up to measure and apply physiological strains and forces *in vivo*, from the inside of the tissue, to

inhibit or quantitatively mimic morphogenetic movements. We will report our understanding of the underlying molecular mechanisms that translate mechanical strains applied to cells and tissues *in vivo* into the activation of transduction pathways connected to major developmental biochemical events during embryogenesis. Next, we will describe the influence of such mechanotransduction processes in medicine, and more specifically in carcinogenesis. Finally, we will then rapidly speculate on evolutionary perspectives potentially related to the emergence of such mechanotransduction processes.

II. Genetic Control of Morphogenetic Movements: Underlying Molecular Mechanisms and Evaluation of Forces

A. Genetics of Morphogenesis

Embryogenesis is composed of two major morphogenetic processes: the biochemical patterning of the embryo and the mechanical morphogenetic movements that geometrically shape the embryo. Since 20 years ago, experiments initiated in early *Drosophila* embryos have shown that the morphogenetic movement sequence is tightly controlled by patterning gene expression (Sweeton *et al.*, 1991). For instance, embryonic mesoderm invagination requires the expression of the Fog (expressed under the control of Twist) and Snail zygotic proteins in the mesoderm (Seher *et al.*, 2007), whereas the germ-band extension movement (i.e., the anterior–posterior elongation of the invaginated embryo) requires the expression of the Bicoid, Nanos, and Torso-like maternal proteins, which control the anterior–posterior polarity of the embryo (Irvine and Wieschaus, 1994) (Fig. 1). However, the elucidation of the relationship between gene expression and generation of strains leading to the tissue shape changes remained until recently largely unknown. The key role of cell polarities, in terms of the cortical (subplasma membrane) concentration of the molecular motor myosin-II protein (Myo-II), in generating multicellular morphogenetic movements was first found in the generation of invaginations during *Drosophila* embryo gastrulation. In this process, the apical concentration of myosin-II leads to the constriction of cell apices and generates the trapezoidal cell shape changes leading to posterior pole cell invaginations (Young *et al.*, 1991). More recently, the role of the dorsoventral and anterior–posterior patterning genes was established, which induce the embryo polarities at the multicellular embryonic scale, in the generation of apicobasal and planar polarities in Myo-II concentration leading to morphogenetic movements (Bertet *et al.*, 2004; Dawes-Hoang *et al.*, 2005).

B. From Genes to Shape: Molecular and Cell Biology of *In Vivo* Force Generation

1. Patterning Planar and Apicobasal Polarities in Contractile Actomyosin Concentration

Regarding germ-band extension, the Bicoid, Nanos, and Torso-like genes establishing the anterior–posterior polarity of the embryo regulate the concentration of Par-3 in plasma

membrane surfaces parallel, but not perpendicular to the axis, through a still poorly understood mechanism. As Myo-II interaction with the subcellular cortex is impaired by Par-3, this establishes a high cortical concentration of Myo-II on membranes perpendicular to the axis (Zallen and Wieschaus, 2004). As a consequence, the surface tension increases and relaxes through a decrease of the cell–cell surface contact perpendicular to the axis, which leads to cell intercalation and extension of the germ band (Fig. 1) (Bertet et al., 2004). Understanding of this process was reinforced by successful *in silico* simulations mimicking germ-band extension with only these ingredients (Rauzi et al., 2008). Interestingly, planar polarity genes also appear to be critical in the generation of convergent extension morphogenetic movements in the embryos of other species, including zebrafish and *Xenopus* (Heisenberg and Tada, 2002; Keller, 2002; Roszko et al., 2009).

Regarding mesoderm invagination, the Fog signaling pathway has been demonstrated to involve the apical attraction of Myo-II through the activation of a Rho signaling pathway. Fog is a secreted signaling molecule that is expressed under the control of Twist in the mesoderm and in the posterior pole, activating apical redistribution of Myo-II (Dawes-Hoang et al., 2005). *T48*, another gene acting downstream of Twist, cooperates with Fog in triggering the apical attraction of RhoGEF2, a protein required for apical redistribution of Myo-II and for mesoderm invagination. In addition to the Twist-dependent activation of the RhoGEF2 apical redistribution process, Snail is also necessary for stable apical redistribution of Myo-II and mesoderm invagination, through a still unknown molecular mechanism (Fig. 1).

Different simulations were developed to test whether the apical surface tension increase induced by redistribution of Myo-II would be the only genetically controlled active perturbation necessary for mesoderm invagination, or the invagination would require additive active movements, such as cell shortening. Whereas simulations describing cells as a continuous viscoelastic medium suggest the necessity of an active shortening of mesodermal cells to accomplish invagination (Conte et al., 2008), hydrodynamical simulations describing the tissue as composed of individual cells with individual plasma membranes characterized by an actomyosin cortical tension and contractile apical rings connected by intercellular junctions suggest that the Myo-II-dependent increase of apical surface tension of mesodermal cells is sufficient to trigger the movements observed during invagination (Pouille and Farge, 2008). Simulations of mesoderm invagination with apical constriction only were also performed in sea urchin embryos, which have an extracellular matrix that should be specifically compliant to allow gastrulation (Davidson et al., 1995). However, the Myo-II activity was proposed to not be the only player of early embryonic morphogenesis. Effectively, recent simulations proposed the involvement of the microtubule network within *Caenorhabditis elegans* epithelial cells in redistributing the stress originally produced by actomyosin-oriented actin filaments, thus leading to the elongation morphogenetic movement of the embryo (Ciarletta et al., 2009).

2. Cell Genetic Identity and Cell Sorting: Actomyosin Versus Adherence Surface Cortical Tensions

In addition to the genetic control of myosin-II cell polarities, the origin of multicellular morphogenetic movements was proposed to be driven by the difference of adhesive surface tensions between cells of different differentiation states. In analogy with the physics of liquid mixing, cells characterized by a higher adhesive surface tension with their homologous cell types than with other cell types will have a tendency to aggregate, with the higher surface tension cells inside and the lower outside. Such proposal was first

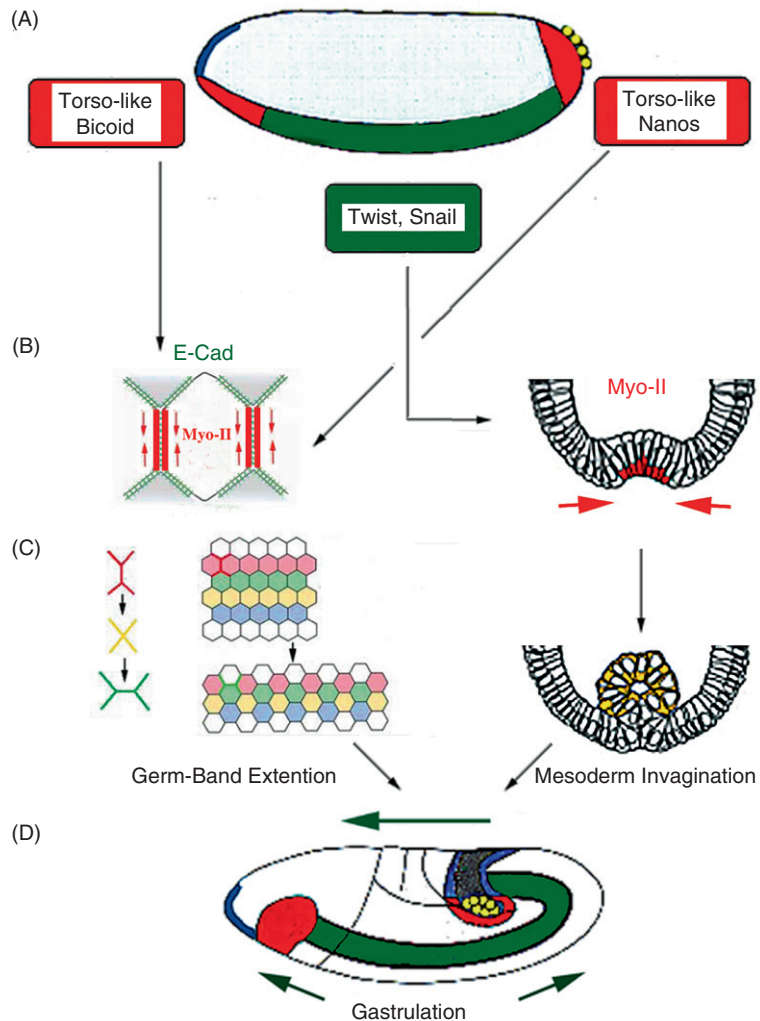


Fig. 1 (Continued)

experimentally tested *ex vivo* by mixing two types of cells with differential adhesive properties (Foty and Steinberg, 2005). Interestingly, the adhesive surface tension of different cell types was measured by the application of a uniaxial deformation on mono-differentiated aggregates of cells. The pressure applied to the aggregate being controlled led to the evaluation of the tension by measuring the deformation of the aggregate “droplet” as a function of the applied force. Interestingly, the cells characterized by a higher surface tension adhesive interaction were effectively found in the core of the aggregate, when the two types of cells were mixed (Foty and Steinberg, 2005). The dynamics of associated demixing behavior could be simulated in the case of the hydra demixing process preceding the onset of regeneration morphogenesis (Graner and Glazier, 1992). Such a demixing mechanism was proposed *ex vivo* to be at the origin of the sorting between mesendoderm and ectoderm cells in the process of blastopore involution in zebrafish (Schotz *et al.*, 2008). More recently, the involvement of the cytoplasmic cortical tension of cells associated to the cortical actomyosin tension was taken into consideration in addition to the adhesive surface tension and was proposed to drive demixing of endoderm, mesoderm, and ectoderm cells and the movement of the blastopore *in vivo* (Krieg *et al.*, 2008). To ensure the stability and integrity of the tissue, the adhesive surface tension and cortical tension should be on the same order of magnitude, with slight differences in between the two governing the geometry of demixing and cell rearrangement (Lecuit and Lenne, 2007). For instance, simulations combining the effect of the cortical tension to the adhesive surface tension generated the shape of the *Drosophila* retina cells observed experimentally, in contrast to simulations privileging the adhesive surface tension (Kafer *et al.*, 2007).

C. Measuring the Active Forces Developed in Morphogenetic Movements *In Vivo*

In the preceding case, the origin of the multicellular morphogenetic movements of the embryonic tissue is the anisotropy of Myo-II concentration within individual cells,

Fig. 1 Control of multicellular morphogenetic movements in gastrulation of *Drosophila* embryos, via genetically controlled intracellular polarities in Myo-II concentration. *Germ-band extension* (A) Before gastrulation, the pattern of expression of developmental genes determining the anteroposterior polarity of the embryo is controlled by the expression of the maternal gene products Bicoid in the anterior and Nanos in the posterior (in red). (B) This combines to the expression of the terminal patterning genes controlled by the maternal gene Torso-like product, to establish the planar polarity of Myo-II submembranar concentration (in red, left). The origin of the underlying molecular mechanism linking anteroposterior patterning gene expression to planar polarity remains to be fully understood. (C) The consequence of the polarity is an increase of tension in membranes perpendicular to the anteroposterior axis, leading to a decrease of these surface areas, then to the dorsoventral cell intercalation (adapted from Bertet *et al.*, 2004) extending the anteroposterior length of the tissue *at* gastrulation (D, green arrows). *Mesoderm invagination*. (A) Before gastrulation, the pattern of expression of developmental genes determining the dorsoventral polarity of the embryo is controlled by the expression of the maternally induced nuclear translocation of the transcription factor Dorsal, which activates the expression of the ventral mesodermal genes *twist* and *snail* (in green). (B) These genes are necessarily together to induce the submembrane apical accumulation of Myo-II (in red, right) that increases the apical surface tension. (C, D) This leads to the decrease of apical surface area compared to basal surface areas, triggering the inward curvature and invagination of the mesoderm at gastrulation. The understanding of the underlying molecular mechanisms linking the expression of the patterning genes *twist* and *snail* to apical attraction of Myo-II are better and better understood (see Fig. 4). (See Plate no. 6 in the Color Plate Section.)

leading to both cell migration intercalation movements in response to polar planarity anisotropies and cell shape changes in response to apicobasal polarities. Even though the link between the patterning of gene expression and the generation of a three-dimensional embryonic morphology begins to be understood, the evaluation of the forces developed by these morphogenetic movements has until today been very rarely measured *in vivo*. For instance, looking at *Xenopus* embryonic explants, the measurement of the deflection of the beam emerging from an optical fiber, of which the bending elastic constant has been calibrated and applied to the tissue submitted to convergent extension, leads to a maximum force of 1 μN (Moore, 1994). Such an apparatus is able to measure forces in the range of 50 nN to 10 μN , but necessitates working *ex vivo*, on tissue explants (Davidson and Keller, 2007).

Another extensively studied morphogenetic movement is the *Drosophila* embryo dorsal closure, which combines the action of a closing purse string surrounding the amniosera tissue and constricting it, with contractile amniosera cell oscillations, extrusion of cells inside the embryo, and cell apoptosis in the amniosera (Jacinto *et al.*, 2000; Solon *et al.*, 2009; Tokoyama *et al.*, 2008). In this case, fine mathematical analysis of the geometry of the tissue elements of dorsal closure, combined with the quantitative analysis of tissue relaxation photoablations of specific domains of the dorsal closure, lead to the evaluation of the ratio between the purse string and the amniosera tissue tensions collaborating in the driving of dorsal closure (Hutson *et al.*, 2003). Relative forces can be evaluated directly by studying tissues dynamics but only a local mechanical deformation allows access to the constraints field and the absolute forces.

Measuring the forces associated to morphogenetic movements *in vivo*, within the developing embryo, requires the use of magnetic nanotechnologies to mechanically manipulate the living multicellular mechanical medium of the embryo from the inside. Injection of ferrofluids composed of 7-nm magnetic particles into the cytoplasm of *Drosophila* embryos at the end of cellularization allows the magnetization of a condensed pack of 50 μm cells, on which a force of 60 nN was applied by using a calibrated magnetic tweezer to quantitatively mimic the rate of deformation of anterior pole stomodeal cells of the embryo normally due to the convergent extension of the mesoderm at gastrulation (see protocol details in Section III-B-2-ii) (Desprat *et al.*, 2008). The difference of two orders of magnitude of these forces is coherent with the fact the *Drosophila* embryo is typically 10 times smaller than the *Xenopus* embryo, which develops 1 μN forces, as the forces developed are generally proportional to the section of the tissue involved, which is square the size.

III. Mechanical Control of Gene Expression: Testing Mechanical Induction by Application of Endogenous Forces from the Inside of the Embryo

Developmental genes control both the biochemical patterning and the generation of morphogenetic movements that geometrically shape the embryo. How does the

genome control the state of elaboration of the patterns and shapes it is charged to develop? Regarding biochemical patterning, the genome is constantly testing the pattern of expression of developmental proteins through classical biochemical induction: the pattern of expression of the RNAs of a given stage of development is triggered in response to the pattern of expression of the proteins of the previous stage. Because multicellular morphology is not biochemical in nature, the existence of such feedback cannot be based on classical biochemical inductive cues. We proposed that it was rather due to mechanical cues associated to tissue deformation.

A. Twist: A Master Mechanosensitive Gene in Early *Drosophila* Embryos

The existence of mechanosensitive patterning genes making the expression of the genome sensitive to the biomechanical shape of its tissues was first postulated and demonstrated in early *Drosophila* embryos, just before gastrulation (Farge, 2003). A simple device composed of a tensed semipermeable membrane (BioFOLIE 25 Heraeus) clipped in between two homemade plexiglass concentric rings and a coverslip controlled by x,y,z micromanipulators coupled to a piezoelectric device (Physics Instruments M-UMR5,16 micromanipulators and P-762-1L Polytec PI piezoelectric, respectively) is used to softly deform embryos laterally by a uniaxial pressure applied to the entire embryo. In order to do so, embryos are first deposited on the membrane after dechoriation. They are then immersed in a clearing oil permeable to oxygen (halocarbon oil 27, Sigma) (Wieschaus and Nusslein-Volhard, 1998). The embryos are stably orientated laterally, and deformed by the micromanipulated coverslip in order to increase the dorsoventral size of the embryo on the order of 10% and of 10 min, which are the orders of magnitude of the length and time scales of *Drosophila* embryo morphogenetic movements at gastrulation. The semipermeable nature of the membrane allows the diffusion of the oxygen even in the presence of the coverslip, preventing hypoxia. The expression of the first zygotically expressed master genes of dorsoventral and anterior–posterior patterning can thus be screened during late stage 5 of development, just before gastrulation (stage 6 designates the initiation of gastrulation, namely of morphogenetic movements). These expressions were first checked mostly by using LacZ reporter genes, as the β -galactosidase product of LacZ is particularly stable compared to endogenous proteins within embryonic contexts (Bradshaw *et al.*, 1996). While the anterior–posterior patterns were found to be unchanged (the hunchback gradient and the even-skipped stripes remained unperturbed), the dorsoventral patterning was profoundly modified, with a repression of expression of the dorsal genes *zen* and *dpp*, and an ectopic expression of the ventral genes *snail* and *twist* all around the embryo, including the dorsal tissues (Farge, 2003).

Strikingly, looking at endogenous morphogenesis, the expression of the Twist protein is found to be strongly amplified in anterior pole stomodeal cells, after 10–20 min of compression of these cells by the morphogenetic movement of germ-band extension, suggesting that Twist expression might be mechanically induced in the stomodeum at the onset of gastrulation (Fig. 2) (Desprat *et al.*, 2008; Farge, 2003). Before beginning experiments of control of the deformation of anterior pole stomodeal

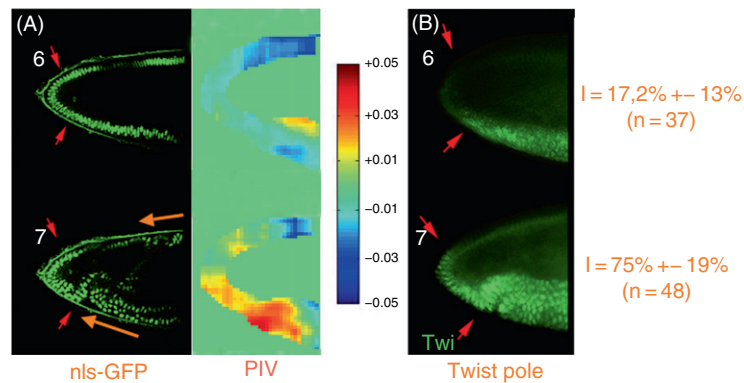


Fig. 2 Increase in Twist expression correlates with compression by GBE in stomodeal cells at the onset of gastrulation. (A) Nls-GFP nuclear labeling shows the compression of stomodeal cells (between red arrows) in between early stage 6 and late stage 7 due to GBE movements (orange arrows). Quantitative Particle imaging velocity analysis shows a $2\% \text{ mm}^{-1}$ dynamics of compression during the first 10 min of GBE (encoded in red). (B) Twist is overexpressed at late stage 7 in stomodeal compressed cells (quantitative mean values combine Farge (2003) and Desprat *et al.* (2008) data, and figures adapted from the same references). (See Plate no. 7 in the Color Plate Section.)

cells, the expression of the functional protein Twist had to be checked in response to the uniaxial global deformation of the embryo using specific antibodies, instead of the LacZ constructs, which only signal the initiation of transcription in mechanical response to deformation. The classical 30 min at 20°C protocol of fixation of embryos (during which the exact time of fixation is not defined) being longer than the physiological 10 min characteristic time scale of stomodeal compression, the embryos were systematically fixed at 4°C in the 4% PFA fixative for 1 h, after 1 min–15 min of deformation, in order to immediately stop any active biochemical process at the onset of the fixation time. Within these time scale-controlled physiological conditions, the Twist protein was found to be expressed ectopically all around the embryo in response to mechanical deformation after typically 12 min, which is the dynamics of the stomodeal compression correlated to the overexpression of Twist at the onset of gastrulation (Farge, 2003). Interestingly, we subsequently found that embryos classically fixed at 20°C lose most of the ectopic dorsal expression of Twist (but not in anterior stomodeal pole cells, see below), in contrast to *twi-lacZ* embryos stably expressing β -galactosidase within the same conditions, possibly suggesting the existence of a highly dynamical negative feedback repressing any accidental mechanical induction of Twist in tissues not pre-patterned to stabilize Twist mechanical induction (unpublished data). Consistent with this, the use of highly sensitive DAB peroxidase substrate amplification tools (DAB Vectastain ABC Elite kit, Vector) and good contrast camera with related imaging treatment program (Hamamatsu C4-742 95 and Hi-Pic) were necessary to detect ectopic expression of the Twist protein in whole-mount embryos, in contrast to anterior pole cell experiments in which immunofluorescence was sufficient (see below).

B. Mechanical Induction of Twist in the Future Anterior Gut Cells, in Response to Myo-II-Dependent Germ-Band Extension Compression

Testing the existence of mechanical cues leading to Twist mechanical induction in stomodeal cells in response to the endogenous morphogenetic movements of germ-band extension requires the elaboration of tools allowing the inhibition and rescue of the Myo-II-dependent germ-band extension morphogenetic movement *in vivo*.

1. Genetic Tools to Control Morphogenetic Movements: Mechanical Rescue of Twist Expression After Inhibition of Germ-Band Extension

Taking advantage of the well-characterized genetics of early *Drosophila* embryo morphogenesis, one can use the anterior–posterior apolar triple mutant *bicoid nanos torso-like* to block germ-band extension (Irvine and Wieschaus, 1994). In these mutants, anterior stomodeal cells are found to not be compressed, with no amplification of Twist expression in these cells (Farge, 2003). A 50 μm micromanipulated needle was then used to compress stomodeal anterior pole cells in these mutants, with a physiological order of magnitude of deformation. In response to deformation, the amplification of the expression of Twist was rescued in these mutants, suggesting a mechanical induction of Twist by stomodeal cell compression in response to their compression by germ-band extension in wild-type embryos (Farge, 2003). The rescue of the strong expression of Twist in stomodeal cells was also triggered in response to the rescue of germ-band extension by using the *bicoid torso-like* double mutant, in which the expression of the posterior gene *nanos* allows the first 20 min of germ-band extension (Irvine and Wieschaus, 1994), without affecting the genetic background of anterior pole cells (Farge, 2003). Altogether, these results suggest that the endogenous morphogenetic activity of Myo-II, leading to germ-band extension (Bertet *et al.*, 2004), mechanically induces the activation of Twist gene expression at the onset of *Drosophila* gastrulation.

2. Biophysical Tools in Morphogenetic Movement Control: Magnetic Tweezers Mechanical Rescue of Twist Expression After Mechanical Inhibition of Germ-Band Extension by Two-Photon Local Ablation

The ability to use genetics and simple micromanipulated needles to control stomodeal cell deformation suggests endogenous mechanical induction of Twist in stomodeal cells. However, working with non-wild-type embryos using nonphysiological forces of tissue deformation prevents the establishment of a definitive conclusion, which would require experiments performed within fully physiologic genetic and biomechanical conditions. One has thus to develop new biophysical tools allowing control of the deformation of stomodeal cells, with finely controlled physiological forces and within the wild-type genetic context.

i. Inhibiting Stomodeal Cell Compression by Photoablation Because the germ band largely extends posteriorly, leading to a strong dorsal wave of compression from

the posterior to the anterior pole of the embryo (Fig. 1), the ablation of the most dorsal tissue of the embryo was inhibited to prevent anterior pole stomodeal cell compression in wild-type embryos (Fig. 3A). In fact, the ablation of the most dorsal part of the embryo blocks the germ-band extension on a time scale largely higher than the characteristic time of stomodeal cell compression during normal development, inhibiting both the posterior extension and the anterior extension which efficiently compress stomodeal cells (Farge, 2003; Supatto *et al.*, 2005).

The ablation uses high-power two-photon femtosecond microscopy. The two-photon technology allows the generation of a powerful irradiation only at the point of focus of the infrared incident beam. At this point only, the probability of condensing two photons at the same place and time becomes high, leading to a destructive beam whose energy is square the energy of the nondestructive original infrared beam. As a consequence, the incident beam can cross tissues without destroying them and will be destructive only at the point of convergence. In addition, because destructive ablation effects are proportional to the power of the energy of the laser (namely the energy deposited by unit of time), the fact that the femtosecond laser impulsions are very short (10^{-15} s) means that the energy deposited in the impulses can remain very small in order to trigger a power high enough to generate destruction. Indeed, the heating of the embryos after ablation was evaluated to be on the order of 0.1°C only (Supatto *et al.*, 2005).

As a result of the laser treatment, wild-type ablated embryos showed an inhibition of stomodeal cell compression Fig. 3B, with an inhibition of the amplification of Twist expression in these cells Fig. 3C (Farge, 2003; Supatto *et al.*, 2005).

ii. Quantitative Rescue of Physiological Stomodeal Cell Compression: Ferrofluid Injection and Magnetic Manipulation

To rescue the compression of stomodeal cells with physiological dynamics, a magnetic ferrofluid is injected into the anterior dorsal cells neighboring the stomodeal cells at the end of cellularization, after the photoablation of middle dorsal cells Fig. 3A (Desprat *et al.*, 2008). The ferrofluid (composed of a 5 mol l^{-1} volumic concentration of superparamagnetic monodisperse 5 nm maghemite $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles stabilized with sodium citrate (Mayer *et al.*, 1999)) is injected in front of the basal side of the cells still open to the yolk. As simple uncalibrated electromagnet (composed of a cylindrical pole piece of diameter 5 mm ended with a sharp tip and surrounded with 700 turns of coiled copper wire of diameter 0.7 mm) is applied perpendicularly to the tissue surface, to attract the ferrofluid into the cytoplasm of the cells, after successive 1 s pulses of 1 A. Once cells are magnetized by ferrofluid insertion, the electromagnet is removed, and the cells are subsequently attracted by a calibrated magnetic tweezers made of two conical small pieces of AFK1 pole pieces (length 6 mm; Imphy Alloys, France) at the tip of the two magnets (diameter 5 mm, length 10 mm; Binder Magnetic, gift of V. Croquette) so as to set a 1 mm interspace between the two magnetic poles (Fig. 3A).

The position of the magnetic tweezer is systematically explored in order to quantitatively tune the dynamics of compression rescue of the ablated embryos to

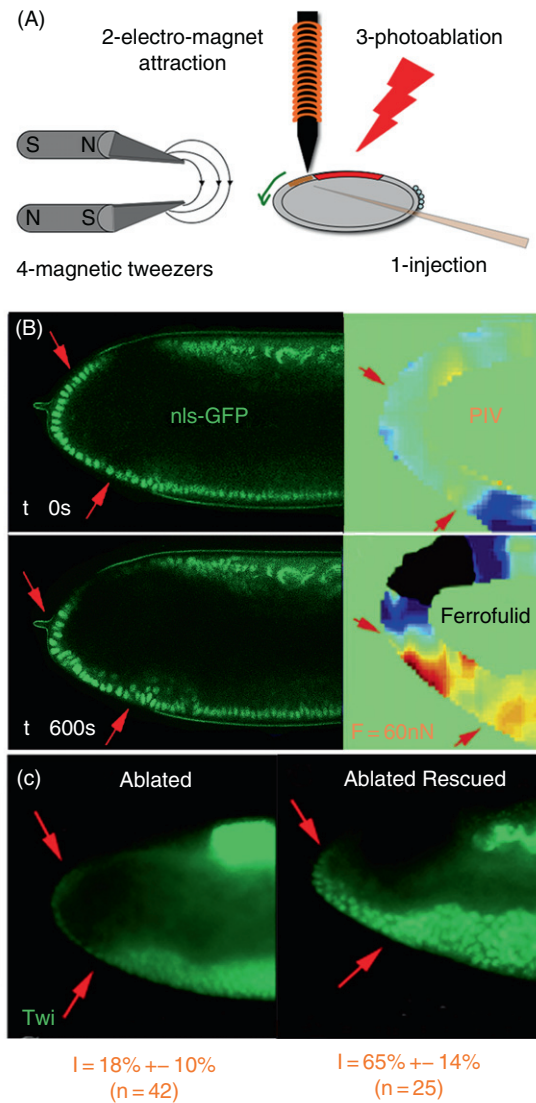


Fig. 3 Testing Twist mechanical induction in controlling stomodeal cells compression photoablation and magnetic manipulations. (A) The dorsal domain of the embryo is photoablated to block GBE, after injection and concentration of a calibrated ferrofluid of magnetic nanoparticles into anterodorsal cells. Calibrated magnetic tweezers are positioned in order to attract magnetized dorsal cells to compress anterior pole stomodeal cells with a deformation rate that mimics GBE endogenous compression dynamics. (B) Dynamics of stomodeal cells compression (in between red arrows) induced by magnetic manipulation mimicking the first 10 min of compression due to GBE during normal development, observed in nls-GFP and in PIV. A force of 60 nN is applied to stomodeal cells to quantitatively phenocopy the endogenous compression measured by PIV in Fig. 2A. (C) Inhibition of Twist overexpression in noncompressed stomodeal cells into the ablated embryo (in between red arrows), and recovery in the stomodeal cells of the ablated embryos in which the physiological compression is rescued by magnetic manipulation. Adapted from *Desprat et al. (2008)*. (See Plate no. 8 in the Color Plate Section.)

the value of the endogenous compression of the nonablated wild-type embryos. Such comparison can be performed by using particle imaging velocimetry, a program adapted from hydrodynamics to embryogenesis, in order to evaluate the local field of velocity as well as deformation of multicellular tissues (Desprat *et al.*, 2008). After injection of 30 fl of ferrofluid, the endogenous dynamics of stomodeal compression of $2\% \text{ min}^{-1}$ (namely $2 \mu\text{m min}^{-1}$) is rescued for a distance between the tweezers and the magnetized cells leading to a gradient of magnetic field of 120 T m^{-1} (measured by means of a small Hall probe KS14, Siemens) (Fig. 3B). Applied to the ferrofluid characterized by a magnetization of $2.1 \times 10^5 \text{ A m}^{-1}$ (determined by a custom-made vibrating sample magnetometer according to Foner, LI2C, University Paris 6), this corresponds to the application of a force of 60 nN by magnetized cells onto stomodeal cells (Desprat *et al.*, 2008). This compression strain was applied for 10 min in order to mimic endogenous stomodeal cell compression, with immediate subsequent fixation of the embryos and classical labeling with anti-Twist antibodies.

As a result, the strong expression of Twist was rescued in stomodeal cells in response to compression. Quantitative analysis revealed a level of expression rescue of Twist in stomodeal cells normalized to mesoderm cells of $65 \pm 14\%$, which is amplified compared to the $18 \pm 10\%$ characterizing the ablated embryos (ablated and injected without magnetic field application) and comparable to the value of expression after compression by the endogenous movement of germ-band extension of $71 \pm 19\%$ (Fig. 3C) (Desprat *et al.*, 2008). Student test confidence values of these analyses were $p < 0.001$.

Thus, rescuing stomodeal cell compression from ablated noncompressed embryos by using the physiological biomechanical deformation of $2\% \text{ mn}^{-1}$ (representing slow movement of $2 \mu\text{m mn}^{-1}$), within the physiological wild-type genetic background, quantitatively rescues the high level of Twist expression in the stomodeal cells compressed by germ-band extension, which is lost after inhibition of compression due to dorsal cell photoablations. This demonstrates that Twist overexpression is mechanically induced by stomodeal cell compression due to germ-band extension during endogenous development.

C. Underlying Molecular Mechanism of Mechanotransduction and Physiological Function in Development

The underlying molecular mechanism of Twist mechanical induction is the mechanically induced release of Armadillo/ β -catenin from the junctions into the nuclei. Armadillo/ β -catenin is the cotranscription factor of TCF. Dominant negative mutations of TCF and overexpression of Axin (which traps Armadillo/ β -catenin in the cytoplasm, preventing any nuclear translocation) are both characterized by a lack of Twist mechanical induction, showing the necessity of the transcriptional activity of the β -catenin in Twist mechanical induction (Farge, 2003). Such release belongs to a

mechanotransduction process that is dependent on Src42A, a protein triggering the inhibition of the interaction of β -catenin with E-cadherin in *Drosophila* embryos. Because Src42A is already activated (phosphorylated) before compression, and not overactivated in response to stomodeal cell mechanical strains, Src42A appears to be permissive in Armadillo/ β -catenin released from the junctions, and is not directly involved in the mechanotransduction pathway (Desprat *et al.*, 2008). One possibility, among others, is that mechanically induced conformational changes of junctional Armadillo/ β -catenin would open sites of phosphorylation with activated Src42A, leading to the inhibition of its interaction with E-cadherin and to its release into the cytoplasm. Such activated Src-dependent mechanical activation of p130Cas was demonstrated *in vitro*, in response to mechanical induction of p130Cas changes of conformation (Sawada *et al.*, 2006).

Interestingly, mechanical activation of Armadillo/ β -catenin was also found to be involved in mouse bone development, through muscle contractions inducing mechanical shocks in between the bone synovial joints. The pluripotency of synovial bone cells, which control both bone growth and synovial joint differentiation, is indeed maintained by such mechanically induced nuclear translocation of β -catenin (Kahn *et al.*, 2009). In addition, other pathways were suggested to be mechanically activated during development, like the MAL-D pathway during border-cell migration in oogenesis. The deformation due to the movement of these migrating cells in between static cells was suggested to activate the nuclear translocation of MAL-D, an event necessary to build a robust actin cytoskeleton in migrating cells (Somogyi and Rorth, 2004). Furthermore, one of the major problems to be solved in embryonic and animal development is what tells an achieved organ or tissue to stop growing. This issue is especially crucial to prevent tumor development where growing is often unrestrained. Whereas a consensus exists about the fact that morphogen gradients control such processes, it has been recently proposed in the case of the *Drosophila* imaginal disc that a mechanical stress, arising in the tissue from the nonuniformity of morphogen, could induce the arrest of cell proliferation (Hufnagel *et al.*, 2007). This alternative scenario comes from a numerical simulation but could be tested precisely by the novel experimental approaches described here.

Regarding the physiological function of Twist mechanical induction, the high expression of Twist at stage 7, which is mechanically induced by germ-band extension, was found to be necessary for midgut cell functional differentiation at late embryonic stages 14–16 (11–15 h of development) through the control of Dve expression, as well as for survival of 4- to 5-day larvae. Effectively, genetically controlled defects in Twist overexpression to the level of expression of noncompressed stomodeal cells (due to patterned and staged expression of Twi-RNAi interfering with Twist expression in stomodeal cells only and during compression only) lead to the loss of Dve expression in anterior midgut cells, as well as to lethality in typically 80% of cases (Desprat *et al.*, 2008). Therefore, in *Drosophila* embryos, mechanical induction of Twist expression in stomodeal cells during their compression appears to control vital functional differentiation of the anterior midgut cells of the embryo.

IV. Mechanotransduction in the Control of Posttranslational Morphogenetic Events: Mechanical Activation of the Myosin-II Apico-Basal Polarity Triggering Mesoderm Invagination

In addition to controlling the state of expression of the genome, mechanotransduction processes in development can also control posttranslational events, involving, for instance, myosin-II intracellular behaviors at the onset of gastrulation. In *Drosophila* embryos, gastrulation begins by a Snail- and Twist-dependent apical redistribution of Myo-II that leads to a constriction of apical cell surfaces. Such constriction generates a trapezoidal shape change of individual cells, leading to the decrease of the apical surface area of the mesoderm compared to the basal surface area, which induces the inward bending constraints of mesoderm invagination (Sweeton *et al.*, 1991).

A. Mechanical Activation of Myo-II Apical Redistribution Hypothesis Emerging from the Theoretical Analysis of the Genetics of *Drosophila* Mesoderm Invagination

Interestingly, there exist two phases of apical constriction. The first 4 min one is stochastic, randomly involves the uncorrelated reversible pulses of constriction and relaxation of individual cells, and is unable to trigger mesoderm invagination (Sweeton *et al.*, 1991). These pulses are associated with reversible pulses of apical spots of Myo-II (Martin *et al.*, 2009). The second one is collective and involves the constriction of all mesodermal cells (Sweeton *et al.*, 1991), through a process of pulsatile constrictions, including a ratchet process progressively stabilizing cell apices into more and more constricted states. This is associated with the progressive stabilization of the apical spots of Myo-II, leading to apical Myo-II coalescence and redistribution (Martin *et al.*, 2009). Because mutants of *twist* only show the stochastic phase, the collective phase is Twist dependent. In fact, the Fog secreted factor, which is expressed under the control of Twist, is the key signaling protein triggering the collective phase (Costa *et al.*, 1994). However, the *snail* mutants are defective in both the stochastic and collective phases, indicating that the stochastic phase is indeed Snail dependent, but also that the two phases interact (Fig. 4A). Strikingly, a purely biochemical interaction between the Snail and Twist/Fog underlying genetic and biochemical networks can be excluded by the following observations. In mutants of Snail, in which Fog is still expressed in the mesoderm (Morize *et al.*, 1998), no apical redistribution of Myo-II can be observed in the mesoderm (Martin *et al.*, 2009; Pouille *et al.*, 2009). In addition, the ectopic expression of Fog all around the embryonic tissue does not rescue apical constriction in *snail* mutants mesoderm, whereas it does in *twist* mutants (Morize *et al.*, 1998). Thus, Fog alone is not sufficient to trigger apical redistribution of Myo-II and mesoderm invagination, but Fog and Snail together are necessary for Myo-II apical redistribution (Seher *et al.*, 2007). On the other hand, the ectopic expression of Fog all around wild-type embryo shows an apical redistribution of myosin-II in all tissues of the embryo, including in

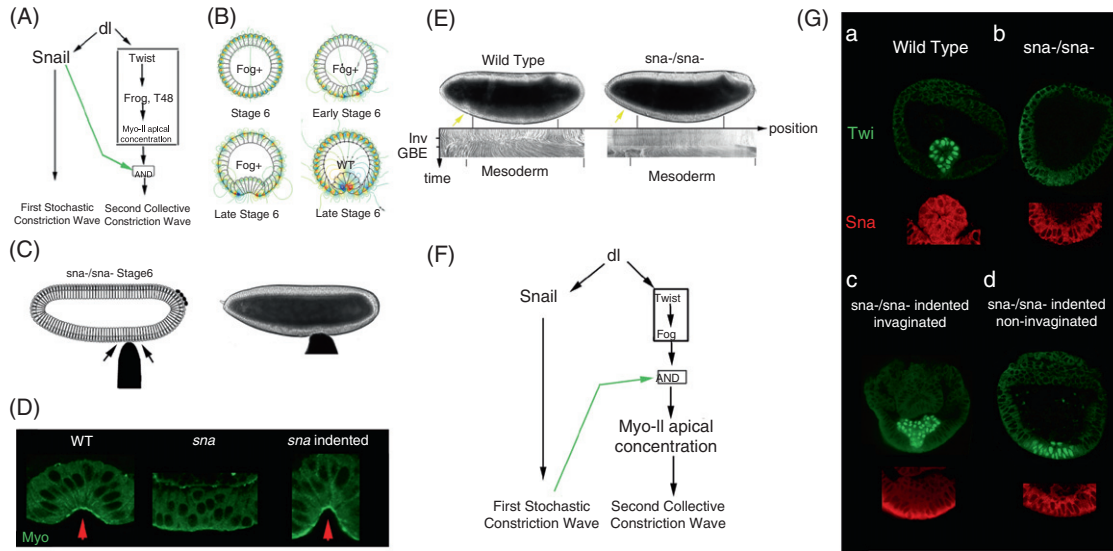


Fig. 4 Mechanical induction of Myo-II apical redistribution leading to mesoderm invagination. (A) The genetic network controlling mesoderm invagination. The mesoderm Snail and Twist/Fog,T48 pathways cooperate in triggering the apical constriction leading to mesoderm invagination (green arrow). (B) Simulating an embryo in which Fog is expressed ectopically, and responding to mechanical strain by mechanical activation of the Fog signaling pathway, phenocopies the experimentally observed propagation of Myo-II apical redistribution and flattening from mesoderm to lateral and dorsal tissues (see text). (C) Indenting *sna* mutants to rescue the lack of mechanical strain of *snail* mutants and to test the mechanical reactivation of the Fog signaling pathway controlling both apical redistribution of Myo-II and mesoderm invagination. (D) Rescuing apical accumulation of Myo-II and mesoderm invagination, lost in the *sna* mutant, after soft indent of the mesoderm of *sna* mutants, in a Fog-dependent process. (E) Kymographs of the mesoderm constriction movements at earliest stage 6 in the wild type, which is not observed in earliest stage 6 nonconstricting *sna* mutant embryos, as a phenotype criteria to select *sna* mutant embryos *a priori*, before indent (see text). The *sna* mutants are doubled checked through the delay in anterior midgut formation of *snail* homozygous compared to wild-type and heterozygous mutants (yellow arrow). (F) The mechano-genetic network controlling mesoderm invagination. Snail initiates apical constriction fluctuations, which activates the Fog signaling pathway (through the mechanical blocking of Fog endocytosis, see text), leading to the coordinated Myo-II apical stabilization and coordinated apical constriction of mesoderm cells necessary for mesoderm invagination. Adapted from Pouille *et al.* (2009). (G) Strong expression of Twist at late stage 8 1 h after mesoderm invagination into wild types (A) is lost in homozygous mutants of *snail* (B), 84%, $n = 6$) and rescued after indentation of the mesoderm in the complete pool of both the embryos having invaginated (C, $n = 7$) or having not invaginated (D, $n = 9$) after indent. Green is Twist labeling (Alexa 488 emission following (Desprat *et al.*, 2008) labeling procedure). *Snail* homozygous were determined following the phenotype criteria described in Pouille *et al.* (2009), or using Snail labeling in red (anti-snail made in guinea pig was a generous gift of Yutaka Nibu and was used at 1:200 with Alexa 546 anti-guinea pig from Molecular Probes at 1:100). Note that the nuclear expression of Snail in the wild-type mesoderm is low at stage 8, as described in Alberga *et al.* (1991) and inexistent in *snail* mutants. (See Plate no. 9 in the Color Plate Section.)

ectoderm cells in which Snail is not expressed (Dawes-Hoang *et al.*, 2005). So, contrary to the previous observations, this would suggest that Fog alone is able to trigger the apical redistribution of Myo-II.

This apparent contradiction can be solved by considering that the interaction between the two phases is not mediated biochemically, but by cell–cell mechanical interactions via a Fog-dependent mechanotransduction process leading to apical redistribution of Myo-II (Pouille *et al.*, 2009). Effectively, considering that the mechanical strains (mean surface tension or mean cell pressure) developed by the stochastic phase of constriction could trigger a Fog-dependent mechanical activation of apical redistribution of Myo-II in the mesoderm, this would mean that stable apical redistribution of Myo-II would require mechanical strains plus Fog expression. Such mechanical strains being absent in the mesoderm in a *snail* mutant, no mesodermal apical redistribution of Myo-II is observed in the *snail* mutants, even in the presence of Fog. On the other hand, if Snail is expressed in the mesoderm, the mechanical strains would activate the Fog-dependent mechanotransduction pathway and lead to the apical redistribution of Myo-II, then to mesoderm invagination. In turn, mesoderm invagination mechanically strains ventrolateral cells. In the wild type, in the absence of Fog in these cells, the apical redistribution of Myo-II is not induced in nonmesoderm cells and is thus restricted to the Fog expressing mesoderm. On the other hand, in embryos in which Fog is expressed ectopically all around the embryo, the apical redistribution of Myo-II will be first activated in ventrolateral cells in response to stretching by mesoderm cells, in which the apical stress will increase. In turn, these cells will stress more lateral cells and activate apical redistribution of Myo-II, a process that will propagate from ventral cells to dorsal cells after the initiation of mesoderm invagination of stage 6.

B. *In Silico* Physical Tools to Test Theoretically the Viability of the Mechanotransduction Hypothesis

Such a scenario can be predicted by simulations of mesoderm invagination in which the Snail-dependent stochastic phase is introduced with a Fog-dependent mechanical activation of stable apical redistribution of Myo-II. The Fog-dependent mechanical activation is first tuned in the mesoderm to mimic mesoderm invagination in the wild type. It is then added all around the embryo to mimic the genetic background associated with the ectopic expression of Fog. The output of the simulation is the production of an embryo characterized by apical flattening in lateral ectoderm and dorsal cells, leading to a lateral tension preventing the formation of a complete mesoderm invagination (Pouille *et al.*, 2009) (Fig. 4B), a phenotype characteristic of embryos overexpressing Fog ectopically (Morize *et al.*, 1998). This suggests, but yet does not prove, the possibility of an underlying mechanotransduction mechanism of interaction between the Snail (initiating deformations) and Fog (actively responding to the strain by apical stabilization of Myo-II) networks, necessary for early *Drosophila* embryo mesoderm invagination.

C. Coupling of Mechanical and Genetic Tools to Experimentally Test Mechanotransduction in Myo-II Apical Redistribution

The core of the model is that Snail expression in itself is not necessary with Fog to trigger the apical redistribution of Myo-II, but the mechanical strains developed by Snail expression with Fog are. Thus, if the model is correct, rescuing the existence of a mechanical strain in the mesoderm of *snail* mutants should rescue both the apical redistribution of Myo-II and the mesoderm invagination, both of which are missing in these mutants. The *snail* mutant embryos are thus indented 5 μm (namely 30% of the thickness of the ectoderm and 2% of the thickness of the embryo) locally in the middle of the mesoderm, with a micromanipulated needle, precisely 2–3 min after the end of cellularization, which signals the initiation of the stochastic phase in wild-type embryos (Fig. 4C). As a result, both apical redistribution of Myo-II and invagination were rescued in 67% of the indented *sna* homozygous mutant embryos (Fig. 4D), not only in the indented tissue, but throughout the complete mesoderm, suggesting a propagation of the contractile wave prepatterned by Twist expression (Pouille *et al.*, 2009). This percentage decreases to 38% when the indent is realized 10 min after at the onset of Germ-Band Extension (GBE), probably because of a competition with the GBE morphogenetic movement (Pouille *et al.*, 2009). Interestingly, *twi sna* double mutants do not show any response of the mesoderm to mechanical indent, showing that the mechanotransduction pathway is dependent on the expression of Twist in the mesoderm. On the other hand, indenting a *twi sna* double mutant, in which Fog has been additionally expressed only in the mesoderm, rescues the apical redistribution of Myo-II and mesoderm invagination. In contrast, in the absence of indent, Fog expression alone does exhibit any rescue within the *sna twi* context (Pouille *et al.*, 2009). Thus, Fog expression alone, without Snail, does not induce the apical redistribution of Myo-II, but rescues the apical redistribution of Myo-II in response to mechanical strains.

Such genetic manipulation can be realized by crossing a *sna twi* double mutant with a *twi PE-Fog* transgenic mutant (Seher *et al.*, 2007), in which Fog is expressed under the control of the proximal element of the promoter of Twist (PE) which is known to control the expression of Twist in the mesoderm only. Thus, Fog is expressed in the mesoderm only, within a *sna twi* context (because the single mutation of *snail* adds to the fact that Snail expression is highly deficient at stage 6 within the *twi* mutant context (Leptin, 1991), as confirmed by no effect of mesoderm invagination of the *twi sna/twi; PE-Fog* nonindented embryos (Pouille *et al.*, 2009)).

The determination of the *snail* homozygous mutant phenotype is realized *a posteriori* just before the indent, based on the fact that the Snail-dependent stochastic phase of constriction is signaled by a contraction of the mesoderm initiating precisely at the end of mesoderm cellularization, 10 min before the initiation of germ-band extension (precise timing is allowed by sagittal observations of the embryos), which can be quantified by using kymographs (Fig. 4E) (Pouille *et al.*, 2009). Thus, waiting for typically 2–3 min after the end of cellularization without a constriction ensures that the embryo is *sna* homozygous, allowing the indent of the embryo at 3 min, which is sufficient to rescue the apical redistribution of myosin-II in all cases and the mesoderm invagination in nearly 90% of cases (and in nearly 40% of cases for an indent at 10 min, probably because late mesoderm invagination enters into competition with germ-band extension) (Pouille *et al.*, 2009). The *snail* homozygous are

double checked *a posteriori* by the fact the timing of the anterior midgut formation compared to GBE initiation is delayed of 10 min in *sna* homozygous mutants compared to wild-type and heterozygous mutants (Pouille *et al.*, 2009).

D. Mechanical Modulation of Fog Endocytosis: A Potential Underlying Molecular Mechanism of Mechanotransduction

Fog is a secreted signaling protein that activates the Rho pathway through its interaction with its putative receptor Cta (Costa *et al.*, 1994). The activation of the pathway leads to the apical attraction of Myo-II, possibly through its release from microtubules, and its attraction by Cta, helped by T48 (Kolsch *et al.*, 2007). One of the underlying mechanotransduction mechanisms involving secreted signaling proteins is the mechanical modulation of endocytosis. Cell culture experiments have already shown the possibility of enhancing or triggering the activation of transduction pathways due to the increase of membrane mechanical tension, leading to the flattening of the membrane, and thus to the inhibition of endocytosis of signaling proteins (Rauch *et al.*, 2002). Membrane tension can be activated by the increase of the volume pressure in the cells (for instance, due to a mechanical deformation by pressure applied to the cells). Generally, the endocytosis of signaling proteins involves the degradation of the interaction with its specific receptor inside the endosomal compartments and the arrest of downstream signaling pathway inhibition. Therefore, the mechanical inhibition of signaling protein endocytosis leads to the enhancement of the activation of the downstream transduction pathway. Under sub-threshold concentrations, the signaling protein is unable to activate the pathway within normal conditions. Within these conditions, the mechanical blocking of endocytosis is also able to rescue the activation of the pathway (Rauch *et al.*, 2002).

Fog being a secreted signaling protein, the role of the pressure developed in the mesoderm by the Snail-dependent stochastic phase of constriction, triggering the inhibition of Fog endocytosis, leading to the activation of the downstream Rho transduction, can also be investigated by coupling mechanical with genetic tools. Building a double *shi sna* mutant allows blockage of endocytosis, thanks to the temperature-sensitive *shi* mutation of dynamin which inhibits endocytosis within 2 min. Labeling Fog with a specific antibody confirmed the accumulation of Fog at the plasma membrane under conditions of inhibited endocytosis, as compared to the permissive temperature. Such plasma membrane accumulation of Fog is specifically observed in indented mutants of *sna*, showing that the indent of the mesoderm mechanically induces the blockage of Fog endocytosis. This plasma membrane accumulation is also observed during the first 4 min of *Sna*-dependent stochastic constrictions in wild-type embryos, showing that Snail induces the inhibition of Fog endocytosis. Finally, the blocking of endocytosis in the *sna shi* double mutant rescues the apical redistribution of Myo-II and mesoderm invagination, both of which lack in the *sna shi* mutants in endocytosis permissive temperature conditions.

Together these observations suggest that the mechanical strains developed by *Sna*-dependent stochastic constrictions in the mesoderm lead to the inhibition of Fog endocytosis, in turn leading to the activation of the downstream Rho pathway (Fig. 4F) (Pouille *et al.*, 2009).

E. Mechanical Induction of Twist Expression into the Mesoderm

Interestingly, Twist expression into the mesoderm is known to dramatically decrease at late stage 8, 1 h after mesoderm invagination (Leptin, 1991) in *sna* mutants lacking the morphogenetic movement of mesoderm invagination (Fig. 4G), suggesting a possible participation of mechanical strains associated with mesoderm invagination at stage 6 into Twist expression at stage 8 (Brouzes *et al.*, 2004). Consistent with this, after labeling indented *sna* homozygous mutants at late stage 8 by using classical procedures (Desprat *et al.*, 2008), we found a rescue of Twist expression into the mesoderm, both in the pool of embryos having responded by mesoderm invagination rescue and in the pool having not responded that show a typical *snail* mutant non-invagination phenotype at late stage 8 (Fig. 4G, see Section IV-C regarding the conditions to generate the two pools).

F. Incidences of the Mechanical Induction of Apical Redistribution of Myosin-II in Developmental Biology

Interestingly, Myo-II dynamics was also found to be regulated by tension in actin cables, proposed to be maintained by another positive feedback mechanism to generate efficient germ-band extension tissue elongation in *Drosophila* embryos (Fernandez-Gonzalez *et al.*, 2009). In *Xenopus*, the correct spatiotemporal assembly of the fibronectin matrix, a key process in the morphogenesis of the embryo, was suggested to be regulated by a tension integrin-dependent process (Dzamba *et al.*, 2009). A role of mechanical strains in the regulation of microtubules orientation during meristem development was also suggested (Hamant *et al.*, 2008). Such positive mechanical feedback from strains to tensile or structural molecule redistribution could also be at work in processes of tissue reactive contraction resistance to stress having been proposed to be involved in *Xenopus* embryogenesis (Belousov *et al.*, 2006).

Here, the finding of Fog signaling as a mechanotransduction pathway specifically possesses two potential distinct implications in developmental biology.

1. Long-range and Rapid Cell–Cell Interactions Through Mechanical Cues

The first implication, very directly addressing the understanding of the respective roles of Snail and Fog in the apical redistribution of Myo-II, indicates the existence of mechanical cues allowing rapid and long-range interactions between nonadjacent cells mediated by mechanical cues and mechanotransduction. Effectively, the fact that Myo-II is redistributed apically within the mesoderm in the presence of Fog and Snail, but all around the embryo when expressing Fog ectopically despite the expression of Snail remaining restricted to mesodermal cells, shows that the expression of both Snail and Fog is necessary, but not necessarily within the same cells, to trigger the apical redistribution of Myo-II. In other words, Snail and Fog interact across very distant cells (mesodermal to dorsal, (Fig. 4B), through the lateral propagation of mechanical strains initiated by the stochastic pulses of the apical constriction generated by Snail in the mesoderm (Dawes-Hoang *et al.*, 2005).

2. Coordinated Constrictions Integrated by Mechanical Cues and Mechanotransduction Leading to Mesoderm Invagination?

The second implication addresses the question of the intimate mechanisms leading to the coordination of apical constriction which is necessary for mesoderm invagination. It indicates the existence of processes triggering collective ordered cell behaviors directly by the increase of the stochastic fluctuations of behavior of individual cells. Effectively, these experiments suggest that the Snail-dependent fluctuations feedback to individual cell states through Fog-dependent mechanotransduction, leading to the activation of a strong constriction of individual cells which is coordinated via mechanical interactions between cells that propagate very rapidly through the mesoderm, via cell surface deformations or possibly via internal cells hydrostatic pressure (Pouille *et al.*, 2009). In contrast to physical system behaviors, in which fluctuations at a given scale fight against ordered collective behaviors at the same scale, here fluctuations would trigger ordering and coordination restricted to mesoderm, because of mechanotransduction patterned by Fog expression. Such collective coordination determines the very efficient multicellular morphogenetic movement of mesoderm invagination.

V. Incidences of Mechanical Induction in Tumor Development

The activation of tumor genetic programs has long been proposed to possibly be associated with the anomalous reactivation of embryonic programs in adult tissues (Brabletz *et al.*, 2005). Strikingly, the nuclear translocation of β -catenin is a signature of tumor initiation and progression in many tissues, and especially in human and mouse colon tumors (Kirchner and Brabletz, 2000; Morin *et al.*, 1997). Because the nuclear translocation of β -catenin from the junctions mediates mechanical activation of Twist in early *Drosophila* embryos (Desprat *et al.*, 2008; Farge, 2003), we asked the question of a putative mechanical activation of β -catenin nuclear translocation in response to the strains developed by the mechanical pressure associated with tumor growth in the tissue surrounding the tumor (i.e., the stroma) (Brouzes *et al.*, 2004; Whitehead *et al.*, 2008). Following our *Drosophila* embryo protocols, we began to deform mouse tissues with uniaxial pressures applied to colon tissue explants. These experiments were designed to test the potentiality of an oncogenic biochemical response of the tissue in response to artificial mechanical strains. However, in this specific case, the pressure can be controlled at the level of the intestinal transit pressure of the mice, the colon being submitted to such natural pressure daily. Wild-type tissues did not exhibit any response at all to the β -catenin, or to the two target genes Twist-1 (involved in invasivity) and Myc (involved in cell division and tumor growth). Interestingly, regarding the β -catenin pathway, a major difference between the wild-type mouse tissue and the early *Drosophila* embryo tissue is the state of expression of adenomatous polyposis coli (APC) collaborates with the GSK-3 system to send the

cytoplasmic β -catenin into a degradation pathway, preventing β -catenin translocation into the nucleus. APC is not expressed in the early *Drosophila* embryo (Hayashi *et al.*, 1997), but is expressed in mouse colon tissues. To test that the expression of APC in wild-type colon tissues could prevent the nuclear translocation of β -catenin after mechanical strain, we strained heterozygous mutants of APC. In these tissues, some of the β -catenin is translocated into the nuclei, with the observation of the expression of Twist and Myc target genes and protein products (Whitehead *et al.*, 2008). Thus, loss of 50% of APC expression leads to a defect of the degradation of the β -catenin released from the junctions to the cytoplasm in response to mechanical strain and to the translocation of a certain pool of β -catenin into the nuclei, where it is able to trigger the activation of oncogene transcription.

Eighty percent of human colon cancer tumors carry APC mutations, of which 10% are hereditary mutations. In these cases, the question of the sensitivity of such pretumoral colon tissues to intestinal transit is potentially addressed by our observations. Should *in vivo* studies confirm such behavior, adopting an alimentary regime regulating the stiffness of the food might decrease the probability of developing tumors in the APC^{+/-} context. The other 90% of cases first develop a sporadic mutation in one cell, leading to the natural growth of a clonal APC^{+/-} domain. Inside this domain, a second sporadic event involving loss of the second allele of APC in one cell is thought to trigger the transition to cancer (a cell with complete loss of APC is no longer able to prevent the nuclear translocation of the β -catenin which is constantly produced by the cells). Thus, an APC^{-/-} clonal domain grows within the APC^{+/-} pretumoral domain. Our observations thus ask the question of a potential activation of β -catenin nuclear translocation and target oncogene expression in the APC^{+/-} pretumoral tissue domain in response to the pressure developed by APC^{-/-} tumor growth, which might amplify tumor progression.

Mechanical activation of cytoskeletal elements in response to the variation of the stiffness of cell substrates (related to tumor rigidity) was already systematically studied in breast cancer (Bissell *et al.*, 2005). A correlation has also been established between prostate tumorigenicity *in vivo* and disorganized growth in a laminin-rich matrix gel determined by the dysregulation of vimentin and β 1-integrin (Zhang *et al.*, 2009). Furthermore, a novel both chemical and mechanosensitive signaling pathway that controls angiogenesis has been found, whose deregulation contributes to development of many diseases, including cancer, involving a direct regulation of p190RhoGAP by growth factors, integrin-dependent Extra Cellular Matrix (ECM) binding, and mechanical distortion of the cytoskeleton, which in turn controls VEGFR2 (vascular endothelial growth factor receptor 2) expression by modulating the balance between two mutually antagonistic transcription factors, TFII-I and GATA2 (Mammoto *et al.*, 2009).

Here, we ask the question of the activation of signaling pathways connected to oncogene expression directly, with future investigations designed to probe the involvement of the mechanical induction process in response to tumor growth during tumor progression (Alexander *et al.*, 2008).

VI. Mechano-Genetics Network in Perspective of Evolution: Reactivation of a Primitive Feeding Response of Ancient Embryos Recapitulated in Embryonic Morphogenetic Invagination? Mechanical Induction in First Multicellular Organism Emergence?

Coming back to Myo-II apical redistribution mechanosensitivity in the early *Drosophila* embryo, a last implication belongs to evolutionary speculation addressing the question of the emergence of the feeding reflex of ancient embryos in response to touch, and its evolution by integration of the underlying mechanisms regulating embryo morphogenesis. The idea that ancient embryos must have developed primitive motor sensorial responses of multicellular phagocytosis in response to touch has long been suggested and developed (Jaegerstem, 1956; Wolpert, 1992). Such phagocytosis was thought to be a response of tissues to touch due to contact of embryos with the ground after gravity-driven migration. The contact was proposed to activate a primitive motor sensorial response of invagination to touch, leading to the phagocytosis of sediments. Strikingly, *sna* mutant embryos react by an active generation of invagination in response to touch in Fog expressing domains (Fig. 4C and D). We thus proposed that we might have reactivated in early *Drosophila* embryos an ancient feeding reflex response to touch (Farge, 2003; Pouille *et al.*, 2009). In other words, we suggest that the emergence of the Fog/Myo-II mechanosensitive pathway, or a primitive equivalent, might have been at the origin of the generation of a transient primitive gastric organ in response to external stimuli of touch. Strikingly, this would mean that the emergence of such mechanotransduction pathway would have been the key event, leading to the emergence of the first organisms (by definition, a multicellular system with an organ) from the earliest embryos defined as an aggregation of cells without collective functional cell behavior. Following this view, the “cell aggregate” to “first organism” transition could be thought of as the consequence of the emergence of a mechanosensitive Myo-II apical redistribution in response to external strains. With regard to the polarized mechanical rescue of Twist expression in *sna* mutants (see paragraph IV-E and Fig. 4), it would also be tempting to speculate that the local response of earliest embryos tissues to mechanical contact with the ground after gravity sedimentation could have participated in the determination of the primary axis formation of earliest embryos through mechanotransduction.

Then, we speculated that the generation of mechanical strains due to Snail-dependent stochastic oscillations developed from the inside of the mesoderm tissue has replaced the external stimuli, in such a way that a permanent primitive gastric organ evolved to develop independently of the external stimuli. This might have initiated the process of morphogenesis by co-opting a favorable response of the embryo to external mechanical stimuli for use in response to the internal mechanical stimuli, leading to the generation of a gastric organ (Pouille *et al.*, 2009).

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