The Mechanics and Genetics of Embryonic and Tumour Development team studies the role of mechanical strain and deformation of macroscopic biological structures at the cell or multi-cellular level, into the regulation and the generation of active biochemical processes at the microscopic molecular level, including gene expression, \textit{in vivo}. The group focuses on the coupling between mechanical strains and biochemical signalling in developmental and cancer biology.

Our findings chronologically goes from the mechanical modulation of the endocytosis of signalling proteins as a mechanotransductive underlying molecular mechanism of cell trans-differentiation (early 2000’s), to its role in the involvement of mechanical cues in the trigger of early Drosophila embryos mesoderm invagination (late 2000’s). It additionally goes from our finding of the mechanosenstivity of the beta-catenin pathway as involved in the mechanical induction of early Drosophila embryos endoderm differentiation (from early to late 2000’s), most recently found as at the probable evolutionary origins of mesoderm emergence in the common ancestor of bilaterians, a process anomalously reactivated as a tumorigenic signal in compressed healthy epithelial tissues in response to tumour growth pressure \textit{in vivo} (2010’s).

From latest to earliest research:

\textbf{Gastrulation is mechanotransductively triggered by soft internal fluctuations of cell shape}

Gastrulation consists in the formation of large domains of tissue that internalize into the early embryo often like tubes, and which will develop as the internal organs of the adult animal, like the digestive tracks, or the heart, muscles and the kidney lung for most complex animals. In the Drosophila embryo, the first tube to form is the mesoderm, from which will derive all internal organs of the adult organism, except the digestive track. It forms thanks to the apical
stabilisation of the molecular motor Myo-II at the external embryonic surface of the cell, which has the function of constriction the external surface of the embryonic tissue, thereby inducting the inward curvature of the tissue leading to the internalisation of the mesodermal tube. This constriction follows two phases. During the first phase, cells constrict in an erratic and unstable way, due to the erratic and unstable formation of Myo-II spots at the mesoderm cells apexes. Then, cells constrict in a stable and coordinated way, due to the stabilisation of the Myo-II spots progressively reaching cell apexes.

We have demonstrated that the mechanical constraints developed by the stochastic fluctuations of shape of the apexes activate the apical stabilisation of Myo-II, thereby triggering the active process of mesoderm invagination.

To do so, we have used a mutant in which mesodermal cells do not fluctuate anymore, and which does not show any mesoderm invagination. We have mimicked apex shape fluctuations with the amplitude of 500 nm only, by magnetic means. Effectively, we have injected magnetic liposomes inside mesodermal cells and have approached at a few microns a network of micro-magnets which individual size, of 10 microns, is on the order of magnitude of the individual cell size. The specificity of the local magnetic field produced by these magnets was to vary with time, controlled by the experimentalist, so that we made oscillate the local micrometric magnetic fields in such a way cells apex began to pulsate exactly like in the non mutated embryo (Figure 1-left). In response to this stimulation, we have observed the stabilisation of Myo-II and the trigger of mesoderm invagination (Figure 1-right). This stimulation is due to a mechanical activation of biochemical reactions, which we have identified as the activation of the Fog signalling pathway.

In addition, we have shown, by magnetic means again, that the mechanical deformation, this time induced by the mesoderm invagination on the cells of the endoderm of the posterior pole of the embryo (the future embryonic posterior gut track), triggers the apical stabilisation of Myo-II and initiate the posterior gut track formation.

The mechanical constraints of gastrulation cause the opening of the major site Y654 of beta-catenin interaction with E-cadherin that initiates its phosphorylation by Src42A and the activation of the downstream transduction pathway, then leading to the expression of beta-catenin target genes, like twist.
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Figure 2. **a** Simulation of the complex β-cat-E-cad under mechanical stress of 6pN. **b** Application of a mechanical stress mimicking the initiation of mesoderm invagination by magnetic means on a defective embryo in gastrulation (sna- twi-) and observation of the 1nm stretching around the Y654 site β-cat by fluorescence transfer between the alpha helixes of β-cat and E-cad connected by the site. **c** Increased accessibility to site Y654 under the constraints of invagination to the Y654-β-cat antibody.

Simulations predict that under the effect of a 6pN force, the two alpha helixes connected by the interaction of Y654-β-cat with D665-Ecad expand by 1nm, with the site Y654 having a 15% chance of opening (Fig.2a). Such dilation was confirmed quantitatively experimentally in FLIM in response to mesoderm invagination (not shown) or to associated mechanical stresses mimicked by magnetic means in embryos defective in gastrulation (Fig. 2b). The site Y654 is then indeed made more accessible, by about 20% under stress, to its specific antibody (Fig. 2c) – and this consistently even more in the absence of Src42A responsible for its phosphorylation under mechanical stress and its release into the cytosol for transcription. This favours its release of junctions (not shown) (Röper et al, e-LIFE 2018), and stimulates the maintenance of twist gene expression during mesoderm invagination (see section “Evo-Devo” and “Developmental Biology” below, Desprat et al, Dev. Cell. 2008, Brunet, Bouclet et al, Nature Communications 2013).

**Tumourigenesis: mechanical induction of tumourigenesis in compressed healthy cells, in response to the mechanical strains developed by tumorous growing tissues**
We found β-catenin dependent mechanical induction of oncogenes expression and tumour initiation in both pre-tumorous and wild type mice colon healthy epithelia, in response to tumour growth pressure in vivo ([M-E Fernandez-Sanchez, S. Barbier et al, *Nature* 2015], – Figure 3).

To do so, we mimicked the 1kPa tumour growth pressure in vivo by magnetically loading the mesenchymal conjunctive tissue with ultra-magnetic liposomes, which we submitted to a permanent magnetic field gradient due to a millimetric magnet subcutaneously localized in front of the colon. Such mechanical strain activated the phosphorylation of both the Y654-beta-catenin leading to the release of a junctional pool into the cytoplasm. It additionally led to the phosphorylation of Ser9-GSK3b allowing the nuclear translocation of the cytoplasmic beta-catenin into the nucleus and the expression of its tumorigenic target genes. The same responses are observed in the non-tumorous crypts compressed by neighbouring Notch-hyperproliferative crypts.

**Figure 3. Mechanical induction of the β-catenin tumorigenic pathway in healthy epithelia in response to tumour growth pressure, in vivo.** Left- Magnetic loading of mesenchymal cells conjunctive of epithelial crypt colonic cells (in orange), submitted to a millimetric magnetic field gradient, generates a permanent 1kPa pressure quantitatively mimicking tumour growth pressure on weeks to months, in vivo. Right- Resulting mechanical activation of the phosphorylation of the Y654 site of β-catenin, leading to its release into the cytoplasm and nucleus, and leading to the expression of its tumorigene target gene c-Myc.
Evo-Devo: a mechano-transductive origin of mesoderm emergence in the common ancestor of bilaterian complex animals

We found that the mechanical activation of the beta-catenin pathway, anomalously activated in the process of tumour development, is an ancestral property, having been probably involved in the emergence of first differentiation patterns in ancient organism embryos, such as in the evolutionary emergence of the mesoderm in the last common ancestor of bilaterians. We effectively demonstrated the conservation of mechanical induction as involved in early mesoderm differentiation in both the zebrafish and Drosophila embryo, initiated by the mechanotransductive phosphorylation of the Y654 site of beta-catenin impairing its interaction with E-cadherins, leading to its release from the junctions to the cytoplasm and nuclei, and subsequently to the brackury and twist earliest mesoderm target genes expression, respectively (Figure 4).

The evolutionary origin of mesoderm emergence remains a major persisting opened question of Evo-Devo. Our results allow to suggest mechanotransductive Y654 phosphorylation in response
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Developmental Biology: mechano-genetic and mechano-proteic reciprocal coupling in the regulation of gastrulating embryos development

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. We recently revealed reversed control of the Twist developmental differentiation patterning gene expression (Figure 5) and of Myosin-II active relocalisation (Figure 5) by the mechanical strains developed by morphogenetic movements at Drosophila gastrulation, through mechanotransduction processes involving the Armadillo/beta-catenin and the down-stream of Fog signalling pathways (due mechanical inhibition of Fog endocytosis in this case, see next paragraph), respectively.

We used experimental tools (genetic and biophysical control of morphogenetic movements, Figure 5,6), and theoretical tools (simulations integrating the accumulated knowledge in the

Figure 5. Mechanical induction of Twist by convergence extension in the early anterior endoderm determination. A Ectopic mechanical induction of Twist-lacZ expression in response to uniaxial global deformation of about 10% of the Drosophila embryo dorso-ventral size. B Mechanical rescue of the Twist protein expression by an indent of the anterior endoderm lacking Twist expression associated to its defect of compression in a bcd, nos tsl mutant defective in convergent-extension. C Up- Magnetic loading with super-paramagnetic nano-particles to quantitatively rescue physiological compression, of wild-type photo-ablated embryos lacking endoderm cells compression. Down- Rescue of the strong expression of the Twist protein by the magnetically induced rescue of the anterior endoderm compression in the photo-ablated embryo lacking both compression and the strong expression of Twist. Such high level of Twist expression is vitally required for anterior mid-gut functional differentiation of the larvae (Desprat et al, Dev Cell, 2008).

to first embryonic morphogenetic movements at the origin of mesoderm emergence in the 570 millions years ago last common ancestor of bilaterians (Bouclet, Brunet et al, Nature Comm. 2013).
genetics of early embryonic development and morphogenesis) (Figure 7), to uncouple genetic inputs from mechanical inputs in the regulation of Twist meso-endoderm gene expression and Myosin-II active reallocation. Specifically, we set-up an innovative magnetic tweezers tool to measure and apply physiological strains and forces in vivo, allowing to mimic morphogenetic movements from the inside of the tissue in living embryos (Figure 4). Farge, Curr. Biol., 2003; Desprat et al, Dev Cell, 2008; Pouille et al Phys. Biol. 2008; Ahmadi, Pouille et al, Science Signalling, 2009).

Figure 6. Mechanical trigger of mesoderm invagination in sna defective mutants. a- Indent of a mutant of snail that does not invaginate (of 5 microns), 5 minutes after the end of cellularisation. b- Rescue of both the apical accumulation of Myo-II and mesoderm invagination wild-type phenotypes, lacking in the mutant of snail, after the indentation of the mutant of snail mesoderm.
Figure 7- Hydrodynamic simulation of embryonic gastrulation in response to the apical constriction of mesoderm cells. a- Before gastrulation (red arrows delimit the mesoderm domain). b- Gastrulation response to apical constriction into the mesoderm, regulated by membrane-cortical elasticity, and the hydrodynamic flow inside and outside the embryo.

Endocytosis: vesicle budding driving force; mechanical modulation of endocytosis as a mechanotransduction process triggering transdifferentiation

Historically, the first main thematic studied in the team was the motor role of biological membrane soft matter elasticity into the budding driving force of vesiculation initiating plasma membrane endocytosis (Rauch et al, Bioph. J, 2000), as well as the role of mechanical inhibition of morphogene endocytosis in mechanical induction of cell transdifferentiation (Figure 8, Rauch et al, Am. J. Cell Phys, 2002).

Figure 8. Mechanotransductive cell trans-differentiation by mechanical inhibition of signalling proteins endocytosis due to tension induced membrane flattening. A Membrane tension flatten membranes, leading to the inhibition of endocytosis of secreted signalling proteins. In the case of an involvement of endocytosis in the inhibition of downstream signalling, mechanical blocking of endocytosis leads to an enhancement of signalling. B This is the case for mechanical inhibition of BMP2 (a,b) which leads to the enhancement of C2C12 myoblast-osteoblast transdifferentiation initiated by junB expression (c,d).
Key publications

Year of publication
2018

Röper Jens-Christian, Mitrossilis Démosthène, Stirnemann Guillaume, Waharte François, Brito Isabel, Fernandez-Sanchez Maria-Elena, Baaden Marc, Salamero Jean, Farge Emmanuel (2018 Jul 19)
The major β-catenin/E-cadherin junctional binding site is a primary molecular mechanotransductor of differentiation in vivo
eLIFE : 7:e33381. DOI: https://doi.org/10.7554/eLife.33381

Broders-Bondon Florence, Nguyen Ho-Bouldoires Thanh, Fernandez-Sanchez Maria Elena-Farge Emmanuel (2018 May 17)
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on in tumor progression: The dark side of the force.
Journal of Cell Biology : 217(5):1571-1587 : DOI : 10.1083/jcb.201701039
Year of publication 2017
Nature communications : 13883 : DOI : 10.1038/ncomms13883
Year of publication 2015
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Evolutionary conservation of early mesoderm specification by mechanotransduction in Bilateria.
Nature communications : 2821 : DOI : 10.1038/ncomms3821