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Cellular morphogenesis in ascidians: how to shape a simple tadpole

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Ascidians are invertebrate chordates that form tadpole larvae with a surprisingly small number of cells. Recently, the emergence of powerful molecular tools to study cell fate determination in ascidians has been complemented by studies, often at cellular resolution, of morphogenetic processes. These studies point to a complex interplay among mechanisms that control cell fate and polarity and those that govern cell shape change and morphogenesis. The relative simplicity and stereotypy of ascidian development suggests that it will be possible to understand, and possibly to mathematically model, this dynamic coupling between cell fate and shape change.

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Introduction

The past several years have seen rapid and spectacular advances in our understanding of cell fate determination mechanisms in the early ascidian embryo. In addition, ascidians present many obvious charms as models for understanding cellular morphogenesis. The formation of a simple ascidian larval tadpole (Figure 1) involves all of the morphogenetic movements that shape vertebrate embryos, including convergent extension, invagination, cell migration, and oriented cell divisions, but these movements occur within populations of only dozens of cells, in an embryo of fewer than 1000 cells, and against a background of highly stereotyped early development. The major cell types are specified before gastrulation begins, so that mechanisms of cellular morphogenesis can be studied at single cell resolution in the context of known and immutable cell fates. The size and relative transparency of several commonly studied species make them ideal for live-imaging studies. Finally, most of factors comprising the known metazoan machinery responsible for the regulation of cell movement and shape change are present in the ascidian genome as single copy genes [1,2].

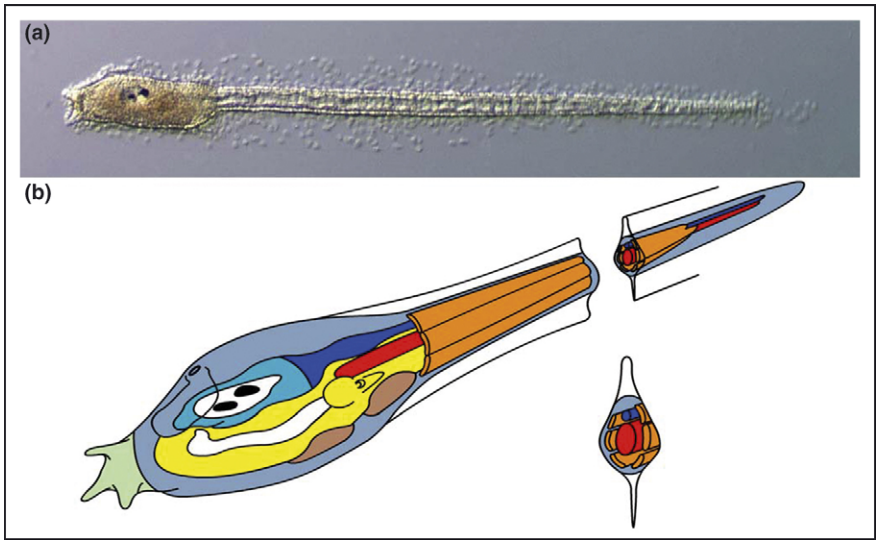
Here, we summarize what is known about ascidian morphogenesis, highlighting some key questions and emerging approaches. For clarity, we organize the review into three main sections: the first deals with the interplay among the morphogenetic forces that determine cell shape and relative positions during early cleavages and the mechanisms of cell fate determination; the second deals with the establishment of the body axes during gastrulation; and the third deals with axis elongation during the neurula and tailbud stages.

Cross-talk between fate allocation and the position and geometry of cells during cleavage stages

In contrast to the blastomeres of vertebrates, those of most ascidians are fate-restricted — they will give rise to a single cell type — by the beginning of gastrulation, shortly before the 110-cell stage. In most cases, these cell fates are also determined (i.e. irrevocably committed to making that cell type) [3]. As in *Caenorhabditis elegans*, specification mechanisms operate in the context of an invariant cleavage pattern, in which the timing of cell division, the orientation of cleavage planes, and the relative size of the daughter cells are precisely defined for all cells (Figure 2a). Unlike nematodes, however, in which the cleavage pattern is highly variable across species, the early ascidian cleavage pattern is remarkably conserved, even between the distantly related species *Ciona intestinalis* and *Halocynthia roretzi*. Thus, ascidians provide a simple model for studying fate allocation in the absence of the complex cell-rearrangements of gastrulation.

In ascidians, early cell-fate determination involves a series of essentially binary cell-fate decisions that combine inductive signals between neighboring cells and asymmetric divisions that segregate maternal determinants to one of two daughters, often of different size (Figure 2a) [3]. Some of the induction events present similarities with the vertebrate situation. For example, fibroblast growth factor (FGF) signaling induces development of the anterior neural tissue [4] and the notochord [5,6]. By contrast, early cell-intrinsic asymmetric divisions might represent an adaptation to development with a small number of cells. The best example of this asymmetry is the markedly unequal cleavages observed in the

Figure 1



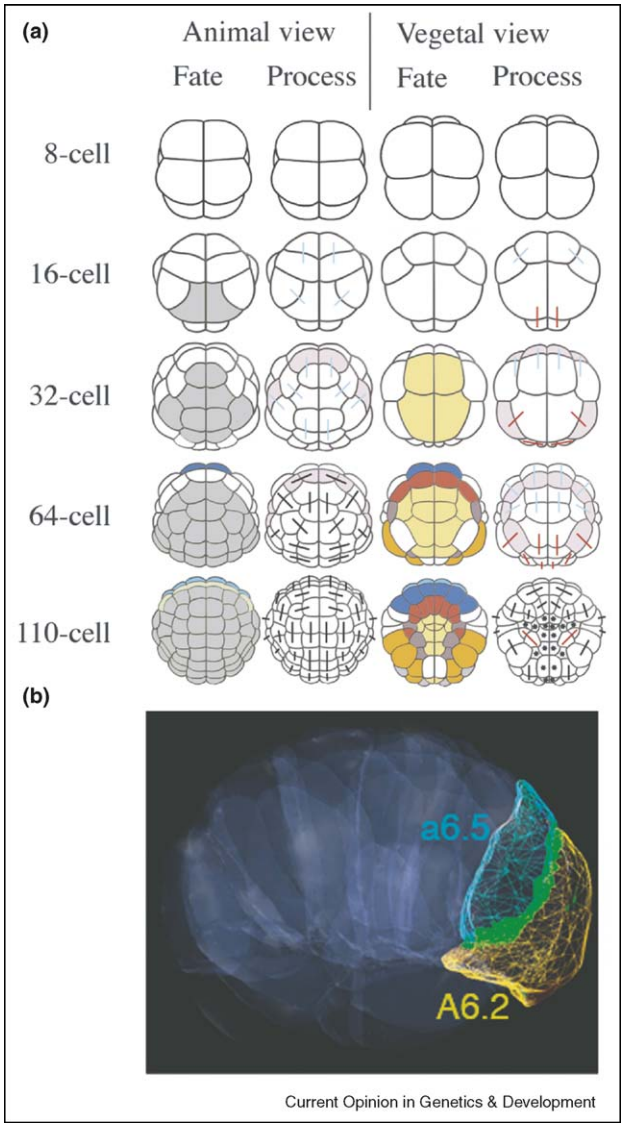
The simple *Ciona* tadpole. **(a)** Photograph of a hatched *Ciona intestinalis* tadpole. **(b)** Schematic overview of the major tissue types. The colour scheme used here and in Figures 2–4 is as follows: brown, mesenchyme; dark blue, nerve cord; green, palps; gray, epidermis; light blue, brain; orange, muscle; red, notochord; yellow, endoderm.

posterior-most vegetal blastomere at each division between the 8- and 64-cell stages. This asymmetry is due to the cell-autonomous action of an actin-rich structure called the centrosome-attracting body (CAB), named for its ability to attract the centrosome to the posterior cortex [7]. A recent study showed that members of the evolutionarily conserved Par-3–Par6–aPKC (atypical protein kinase C) complex localize to the CAB and interact closely with a population of astral microtubules that emanate from the centrosome, suggesting a role for the Par-3–Par6–aPKC complex in spindle capture and asymmetric division [8•]. However, inductive events and asymmetric divisions are not mutually exclusive strategies. In addition to partitioning intrinsic determinants, the timing, orientation and size inequality of cleavages determines the geometry of contact among inducing cells and those competent to respond. Conversely, inductive signals can shape the timing and orientation of cleavages [6], thus paving the way for subsequent fate decisions. Because of this interplay, the study of the molecular regulation of fate decision is best done in the context of a careful description of cellular geometry and arrangement.

To this aim, Tassy and colleagues [9••] designed 3D Virtual Embryo, a software framework for quantifying cell geometry and arrangement in relation to data on molecular regulatory networks. Its application to reconstruct 3D models of early ascidian embryos from stacks of 2-photon confocal images [9••] revealed that the invariance in the position and orientation of the cleavage planes extends to the geometry of individual cells. Accordingly, most early blastomeres can be characterized by a unique geometrical signature. In addition, analysis of wild type embryos or explants revealed that cell shape is surprisingly dynamic, developmentally regulated and, at least partially, under intrinsic control. This approach also revealed that unequal cleavages are more common than initially thought. In particular, novel asymmetric divisions were detected in the posterior vegetal cells that do not inherit the CAB, suggesting the existence of alternative control mechanisms. This study also reinforced the idea that inductive events act at very short ranges and are controlled by the precise geometry of the embryo. During the induction of the anterior neural fate, the measure of the area of contact between inducing vegetal cells and competent anterior animal cells (Figure 2b) appeared as a crucial, evolutionarily conserved determinant of the choice of the competent cells that are induced. Below a certain surface of contact, cells are rarely induced; above this threshold, cells are induced in most cases. This strong threshold effect provides an elegant control mechanism for the binary decision to pursue either an epidermal or a neural fate.

The development of the 3D Virtual Embryo framework opens the way to a rigorous analysis of cell shape, position and cell cycle status, in relation to rapidly emerging descriptions of molecular regulatory networks. It becomes possible, for instance, to quantify the affect on individual cell morphology of injecting morpholinos for crucial regulators of cell fate, cytoskeletal dynamics or cell division. Also, as 3D Virtual Embryo is interfaced with an ascidian model organism database, Aniseed (http://aniseed-ibdm.univ-mrs.fr), it is possible to look for putative regulators

Figure 2



Cleavage stages in ascidian embryos. **(a)** Patterns of induction and cleavage that accompany early fate specification in ascidian embryos. For each hemisphere, the left column illustrates the progressive fate-restriction of each blastomere, and the right column illustrates corresponding patterns of induction and asymmetric cell division. In the left columns, fate-restricted blastomeres appear in the colour of the corresponding tadpole tissue of Figure 1b. Blastomeres giving rise to two or more tissue types are not coloured. In the right columns, blastomeres that are induced are coloured in pink; blue lines link sister blastomeres with equal volume; red lines link sisters with unequal volumes; black lines link sisters whose volume has not been determined. In the vegetal view of the 110-cell stage, cells marked by an asterisk have not undergone cleavage since the 64-cell stage. **(b)** Visualisation in green of the surface of contact between the a6.5 and A6.2 blastomeres; made using the 3D Virtual Embryo module [9••].

expressed in cells that show a specific behavior. This should contribute to the establishment of links between the emerging transcriptional regulatory networks and the control of cell geometry.

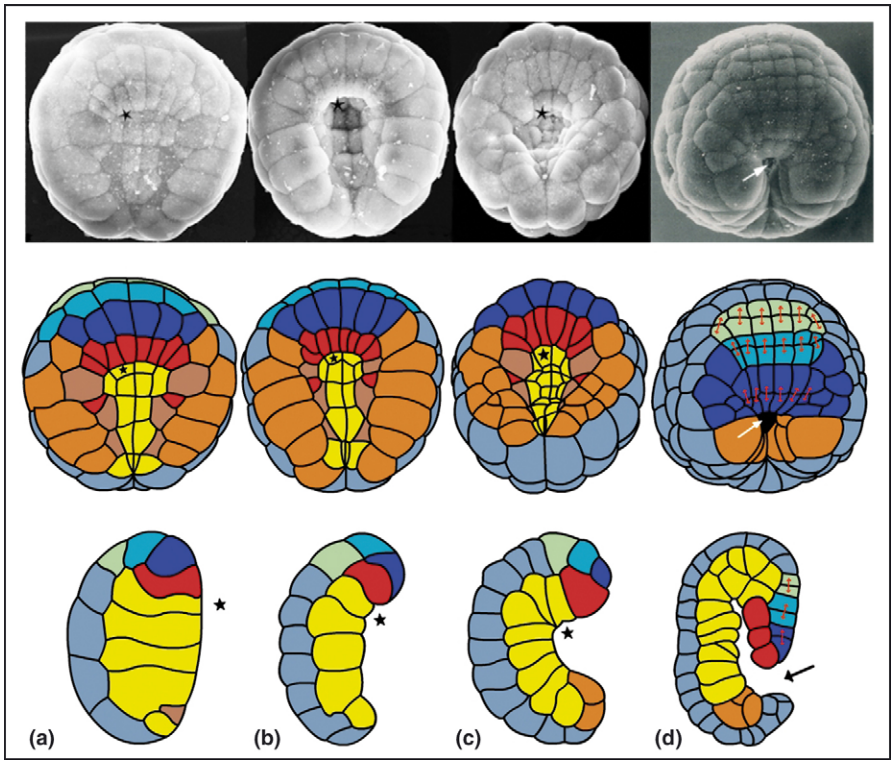
Gastrulation involves cell shape change and oriented cell divisions but only limited cell rearrangements

Early gastrulation in ascidians begins with a flattening of the vegetal plate at the 76-cell stage (Figure 3a). Apical constriction of the vegetal endoderm cells, beginning with the central A7.1 cells, accompanies the formation of a shallow invagination (Figure 3b) that subsequently deepens to form a cup-shaped structure with a large open blastopore (Figure 3c). Presumptive mesoderm cells — notochord at the anterior; mesenchyme, trunk lateral cells and muscle at the sides — fold into the depression made by the invaginating endoderm to form an asymmetric horseshoe shaped blastopore rim. This initial invagination occurs largely in the absence of cell division or rearrangement. Cell and tissue isolation experiments (K Sherrard and E Munro, unpublished) suggest that the vegetal plate has the autonomous potential to invaginate. However, the animal half of the embryo also spreads during invagination as the cells within it divide and then flatten (Figure 3b and c). It remains to be determined whether this spreading is a passive consequence of forces generated within the vegetal plate, an active force-generating process, or both.

Little is known to date about the molecular control of early gastrulation. Both vegetal cell fates and the intrinsic potential to invaginate appear to be specified by maternal determinants and are closely associated with nuclear localization of β-catenin. Endodermal cell fate and invagination potential were reported to be separable in some species [10,11] but not in others [12]. The initial asymmetry of blastopore formation correlates with restricted expression of the central blastopore regulator Brachyury within the notochord lineage [13]. Both early blastopore formation and *Brachyury* expression are negatively controlled from the posterior vegetal cytoplasm, by the maternal determinant Macho-1 [14,15].

The anterior–posterior (AP) asymmetry that emerged during early gastrulation develops further during mid–late gastrulation: in the anterior, two rounds of cell division, oriented along the AP axis, transform the notochord and neural plate precursors into an elongated bi-layer plate that extends between the lateral edges of the posterior muscle rudiment (Figure 3c and d) [16,17]. In contrast to later movements that occur during tail elongation (see below), this initial axial extension doesn't involve cell migrations, intercalation movements or the planar cell polarity (PCP) pathway, whereas involvement of all three processes has been described for vertebrates [18,19]. As the plate extends, the apices of individual cells that line the anterior blastopore gradually shrink and then vanish as these cells recede into the interior of the notochord and neural plates (the arrows in Figure 3d indicate this movement). By contrast, the posterior half of the embryo undergoes little overall external shape

Figure 3



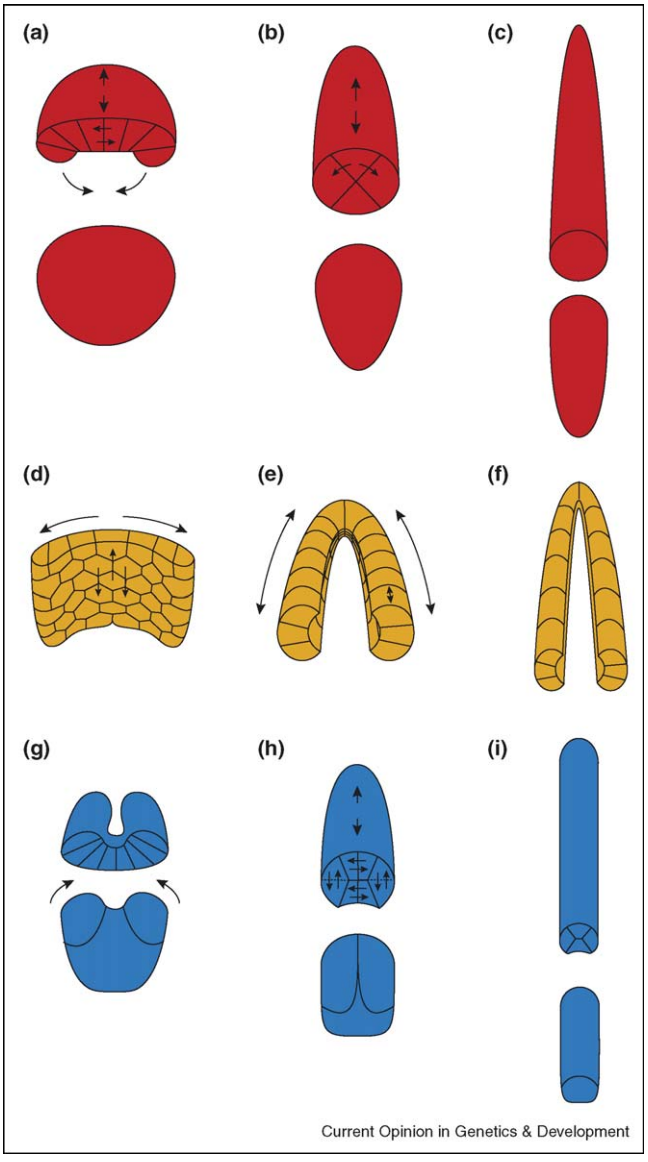
Phases of gastrulation in *Ciona*. The upper panels show vegetal views of the embryo, made by scanning electron microscope (SEM), adapted from the study by Nicol and Meinertzhagen [17]. Middle panels shows coloured tracings of the same SEMs. Bottom panels shows tracings of single parasagital confocal sections taken at corresponding stages. Colour scheme is the same as in Figure 4b. Anterior is up in all panels, and animal is left in the bottom panels. (a,b,c) First phase: formation of cup-shaped embryo. **(a)** 76-cell stage: the vegetal plate first flattens. **(b)** 110-cell stage: apices of the vegetal cells (indicated by asterisks) constrict as the vegetal half of the embryo bends inwards to form a shallow cup; the animal-half spreads as the animal cells divide once and then flatten. **(c)** Mid-gastrula: the cup deepens asymmetrically as anterior and lateral mesoderm cells fold inwards, while endoderm, muscle and epidermal precursors execute a single round of cell divisions. **(d)** Second phase: extension of anterior tissues, and asymmetrical blastopore closure produces a completely asymmetrical embryo. Red arrows indicate AP-oriented cell divisions in the neural plate and notochord lineages that drive extension of the anterior plate. Along the anterior lip of the blastopore, apices of neural plate cells [white arrow in middle panel of (d) and notochord cells (not shown)] constrict as the blastopore closes.

change during the second phase of gastrulation, although lineage studies indicate extensive local cell rearrangements in this part of the embryo.

Tail extension involves tissue-specific morphogenetic mechanisms, coupled mechanically and through cell–cell signaling

By the end of gastrulation, the general body plan of the ascidian embryo has been established and most fates have been specified, but the tail rudiment remains a compact structure. During the neurula and tailbud stages, this rudiment reorganizes to form an elongated tail with characteristic chordate axial structures — a notochord, a dorsal neural tube, and lateral rows of muscle cells — all surrounded by an epidermal layer (Figure 4). Thanks to the small cell numbers and stereotypy typical of ascidian development, the underlying cell shape changes and movements in the notochord, muscle and neural plate have been described in detail [16,20–22], and experimental analyses have revealed the joint contributions of tissue-autonomous and non-autonomous mechanisms [23,24]. In the best-studied case of notochord morphogenesis, invagination and mediolateral cell intercalation transform a monolayer plate of post-mitotic cells into an extended 'stack of coins' 40 cells long (Figure 4a–c) [16,20]. Detailed analysis of the underlying cellular behaviors showed that notochord morphogenesis is driven by the active crawling of individual notochord cells across the surfaces of neighboring notochord cells [16,20]. Notochord cell motility is polarized both within the plane of the epithelium and along the apico–basal axis of each cell; in particular, a mediolateral bias in crawling drives mediolateral intercalation and axial extension, similar to what has been described for the vertebrates [25]. Genetic perturbations and micromanipulation experiments have revealed that that although notochord cells express motile behaviors autonomously cues from neighboring tissues are required to polarize these behaviors and to produce organized morphogenesis [23,24,26••]. Cell intercalation also transforms the muscle primordium — a posterior

Figure 4



Cellular processes that accompany tail elongation during neurula and tailbud stages. (a,d,g) Early neurula; (b,e,h) early tailbud; and (c,f,i) late tailbud stages. The view is of the dorsal side, and posterior is up for all panels. Black arrows indicate whole-tissue deformations; white arrows and outlined cells indicate local cell shape change and rearrangements. (a–c) Notochord: during neurulation, **(a)** the monolayer notochord plate invaginates to form a cylindrical rod, while mediolateral intercalation within the plate drives AP extension. During tailbud stages, **(b)** intercalation about the circumference of the rod drives AP elongation of the notochord. (d–f) Muscle: during neurulation, **(d)** cell rearrangements transform a roughly 6 × 6 array of cells into an array 3 cells high and 12 cells long. During tailbud stages, **(e)** the initially isodiametric cells elongate individually along the AP axis as the tail further extends. (g–i) Neural plate and tube: during neurulation, **(g)** invagination turns the neural plate into a tube, beginning at the posterior and progressing anterior. During tailbud stages, **(h)** mediolateral intercalation (at the top and bottom of the tube), and oblique divisions (not shown), followed by mediolateral shearing (along the sides of the tube), accompany elongation of the tube.

'cap' of post-mitotic cells, roughly six cells high and six cells wide — into two lateral arrays, each three cells high and six cells wide (Figure 4d and e). All muscle cells subsequently elongate along the AP axis as the tail extends (Figure 4e and f). Direct observations and micromanipulation experiments suggest that the initial intercalation is active and autonomous, whereas the subsequent elongation of individual cells is a passive consequence of forces generated by the neighboring notochord [23]. Finally, the neural plate becomes an elongated hollow tube through a combination of several distinct processes (Figure 4g–i) [17,21]: first, beginning at the posterior, and proceeding anteriorly, the flat neural plate 'rolls up' to form a hollow tube (Figure 4g and h); second, mediolateral intercalation at the ventral midline, and obliquely oriented cell divisions, followed by mediolateral shearing of daughter cells, accompany axial extension of the neural tube (Figure 4h and i). Whereas muscle extension requires notochord extension, neural tube formation and extension does not [24]. In summary, distinct tissue-specific cellular mechanisms contribute to the extension of the ascidian tail, and these are coordinated mechanically, and possibly through inter-tissue signaling.

Regulation of notochord morphogenesis downstream of Brachyury

Early screens identified some forty gene products expressed downstream of Brachyury, exclusively within the notochord lineage [27,28]. These include many known regulators of cytoskeleton and cell–cell adhesion, but the analysis of their roles in notochord morphogenesis is still in its infancy. One of these genes is an orthologue of the PCP gene *Prickle*, and indeed several recent studies suggest that the PCP pathway controls polarized cell motility during ascidian notochord morphogenesis [24,26••]. Cell-autonomous expression of a dominant negative form of PCP member *Disheveled* in the notochord abolished polarized cell movements and convergent extension without affecting the specification of notochord cell fates [24]. More recently, Jiang *et al.* [26••] identified a *Prickle* mutation that severely disrupts notochord morphogenesis. Detailed phenotypic analysis of the mutant showed that development is essentially normal until the early neurula stage, when wild type notochord cells first express polarized motility. In *Prickle* mutants, as in the wild type, notochord cells initially extend motile processes in all directions, but in the mutant this fails to resolve into the bipolar protrusive activity that drives convergent extension. A similar defect is observed in the *Xenopus* chordamesoderm when the PCP is disrupted [29].

A Prickle-like phenotype was also observed in isolated ascidian notochord primordia; in this case, polarized cell motility was restored by contact with any of several different neighboring tissues [23]. Thus, the PCP

pathway might be involved either in transmitting a polarizing signal from neighboring tissues or in making the notochord cells competent to polarize in response to external cues. Finally, and perhaps most surprisingly, given that Prickle is a crucial component of the PCP pathway, the fact that Ci-Prickle is a single copy gene expressed specifically within the notochord lineage [30] suggests that this pathway is unlikely to be involved in polarized extension of other components of the ascidian tail.

Cell migrations are the exception in ascidian embryos

As described above, nearly all embryonic morphogenesis in ascidians involves cell shape change and local neighbor exchange. However, two notable examples of cell migrations have recently been described. One is the identification, in the colonial ascidian *Ecteinascidia turbinate*, of neural crest-like cells that leave the anterior neural tube during tailbud stages and migrate into the body wall and siphon primordia, where they differentiate as pigment cells [31•]. The second is bilateral pairs of heart precursors that are specified during gastrulation and whose progeny migrate towards and fuse at the ventral midline [32•]. Recent studies have identified a single paralog of the vertebrate Mesp genes required for both specification and migration of heart precursors [32•,33•,34]; this could become an ideal system for studying cellular mechanisms of, and regulatory control over, a simple cell migration.

Conclusions

Detailed descriptions of cell movement and shape change, as reviewed above, form the essential starting point for a more detailed analysis of morphogenesis, by defining what must be explained and by constraining the range of possible underlying mechanisms. Thus far, in ascidians, such descriptions have relied on detailed analysis of fixed, staged specimens to complement time-lapse analysis of live embryos, using transmitted light optics. However, recent work demonstrates the feasibility of using green fluorescent protein-based reporters and tissue-specific promoters to follow the temporal dynamics of cell shape change and rearrangement in 3D *in situ* at single-cell resolution [22]. Even more important will be to follow subcellular dynamics of the conserved machinery (e.g. the actomyosin cortex, and adhesion complexes) whose actions lie at the heart of the generation and transmission of cellular force.

Another lesson from recent work is that *Ciona* emerges as an ideal system to study the dynamic coupling among gene networks, cell fate allocation and cell geometry. In several instances in this review, molecular regulatory networks that control cell fate or polarity also appeared to determine patterns of force generation that in turn changed the cellular context in which those same networks operate. The integration of software such as 3D Virtual Embryo within existing model organism databases [9••] will provide an ideal framework to study morphogenesis with a cellular resolution and to mathematically model and simulate the dynamic coupling of molecular regulation and cellular mechanics. This phenomenon is probably typical of developmental mechanisms but has received little attention, because of its complexity. The tiny but simple *Ciona* embryo might point the way.

Update

While this manuscript was processed, a striking illustration of the power of ascidians to reconstruct whole-embryo gene regulatory networks was published by Imai *et al.* [35••]. It will be of great interest to assess how perturbing this network affects morphogenesis as well as cell fates.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Satou Y, Satoh N: **Genomewide surveys of developmentally relevant genes in *Ciona intestinalis***. *Dev Genes Evol* 2003, **213**:211-212.

2. Dehal P, Satou Y, Campbell RK, Chapman J, Degnan B, De Tomaso A, Davidson B, Di Gregorio A, Gelpke M, Goodstein DM *et al.*: **The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins**. *Science* 2002, **298**:2157-2167.

3. Nishida H: **Specification of embryonic axis and mosaic development in ascidians**. *Dev Dyn* 2005, **233**:1177-1193.

4. Bertrand V, Hudson C, Caillol D, Popovici C, Lemaire P: **Neural tissue in ascidian embryos is induced by FGF9/16/20, acting via a combination of maternal GATA and Ets transcription factors**. *Cell* 2003, **115**:615-627.

5. Imai KS, Satoh N, Satou Y: **Early embryonic expression of *FGF4/6/9* gene and its role in the induction of mesenchyme and notochord in *Ciona savignyi* embryos**. *Development* 2002, **129**:1729-1738.

6. Darras S, Nishida H: **The BMP signaling pathway is required together with the FGF pathway for notochord induction in the ascidian embryo**. *Development* 2001, **128**:2629-2638.

7. Hibino T, Nishikata T, Nishida H: **Centrosome-attracting body: a novel structure closely related to unequal cleavages in the ascidian embryo**. *Dev Growth Differ* 1998, **40**:85-95.

8. • Patalano S, Pruliere G, Prodon F, Paix A, Dru P, Sardet C, Chenevert J: **The aPKC/PAR-6/PAR-3 cell polarity complex localizes to the centrosome attracting body, a macroscopic cortical structure responsible for asymmetric divisions in the early ascidian embryo**. *J Cell Sci* 2006, **119**:1592-1603.
This is the first study to suggest a role for the PAR proteins in regulating the intrinsically asymmetric cell divisions that partition cell fate potential in the early ascidian embryo. The authors show that members of the conserved PAR complex Par3–Par6–aPKC localize to the centrosome-attracting body (CAB) in an actin-dependent fashion during each of a sequence of highly asymmetric posterior divisions; the authors also demonstrate that astral microtubules emanating from the centrosome nearest the CAB contact the PAR-rich cortical domain.

9. •• Tassy O, Daian F, Hudson C, Bertrand V, Lemaire P: **A quantitative approach to the study of cell shapes and interactions during early chordate embryogenesis**. *Curr Biol* 2006, **16**:345-358.
This study introduces new approaches and a new software framework for quantifying the dynamics of cell shape change and rearrangement, in relation to rapidly growing data on the expression and function of

molecular regulatory networks. Analysis of cell shape change dynamics, asymmetric division and geometry of cell contact in relation to early cell-fate determination highlights the power of integrating quantitative descriptions of cell geometry with known details of molecular regulation to understand embryological processes.

10. Jeffery WR: **Ultraviolet irradiation during ooplasmic segregation prevents gastrulation, sensory cell induction, and axis formation in the ascidian embryo**. *Dev Biol* 1990, **140**:388-400.

11. Jeffery WR: **A gastrulation center in the ascidian egg**. *Dev Suppl* 1992:53-63.

12. Nishida H: **Vegetal egg cytoplasm promotes gastrulation and is responsible for specification of vegetal blastomeres in embryos of the ascidian *Halocynthia roretzi***. *Development* 1996, **122**:1271-1279.

13. Yasuo H, Satoh N: **Function of vertebrate *T* gene**. *Nature* 1993, **364**:582-583.

14. Kobayashi K, Sawada K, Yamamoto H, Wada S, Saiga H, Nishida H: **Maternal macho-1 is an intrinsic factor that makes cell response to the same FGF signal differ between mesenchyme and notochord induction in ascidian embryos**. *Development* 2003, **130**:5179-5190.

15. Nakatani Y, Nishida H: **Induction of notochord during ascidian embryogenesis**. *Dev Biol* 1994, **166**:289-299.

16. Munro EM, Odell GM: **Polarized basolateral cell motility underlies invagination and convergent extension of the ascidian notochord**. *Development* 2002, **129**:13-24.

17. Nicol D, Meinertzhagen IA: **Development of the central nervous system of the larva of the ascidian, *Ciona intestinalis* L. II. Neural plate morphogenesis and cell lineages during neurulation**. *Dev Biol* 1988, **130**:737-766.

18. Ewald AJ, Peyrot SM, Tyszka JM, Fraser SE, Wallingford JB: **Regional requirements for dishevelled signaling during *Xenopus* gastrulation: separable effects on blastopore closure, mesendoderm internalization and archenteron formation**. *Development* 2004, **131**:6195-6209.

19. Ulrich F, Concha ML, Heid PJ, Voss E, Witzel S, Roehl H, Tada M, Wilson SW, Adams RJ, Soll DR *et al.*: **Slb/Wnt11 controls hypoblast cell migration and morphogenesis at the onset of zebrafish gastrulation**. *Development* 2003, **130**:5375-5384.

20. Miyamoto DM, Crowther RJ: **Formation of the notochord in living ascidian embryos**. *J Embryol Exp Morphol* 1985, **86**:1-17.

21. Nicol D, Meinertzhagen IA: **Development of the central nervous system of the larva of the ascidian, *Ciona intestinalis* L. I. The early lineages of the neural plate**. *Dev Biol* 1988, **130**:721-736.

22. Rhee JM, Oda-Ishii I, Passamaneck YJ, Hadjantonakis AK, Di Gregorio A: **Live imaging and morphometric analysis of embryonic development in the ascidian *Ciona intestinalis***. *Genesis* 2005, **43**:136-147.

23. Munro EM, Odell G: **Morphogenetic pattern formation during ascidian notochord formation is regulative and highly robust**. *Development* 2002, **129**:1-12.

24. Keys DN, Levine M, Harland RM, Wallingford JB: **Control of intercalation is cell-autonomous in the notochord of *Ciona intestinalis***. *Dev Biol* 2002, **246**:329-340.

25. Keller R: **Shaping the vertebrate body plan by polarized embryonic cell movements**. *Science* 2002, **298**:1950-1954.

26. •• Jiang D, Munro EM, Smith WC: **Ascidian prickle regulates both mediolateral and anterior–posterior cell polarity of notochord cells**. *Curr Biol* 2005, **15**:79-85.
This study identifies a mutation in the *Ciona* orthologue of the planar cell polarity component prickle, which disrupts notochord morphogenesis. Detailed analysis of the phenotype using techniques developed by Munro and Odell [16,23] revealed that although notochord cells become motile with normal timing they fail to express *polarized* motility, and this leads to a defect in both mediolateral cell intercalation and convergent extension.

27. Takahashi H, Hotta K, Erives A, Di Gregorio A, Zeller RW, Levine M, Satoh N: **Brachyury downstream notochord differentiation in the ascidian embryo**. *Genes Dev* 1999, **13**:1519-1523.

28. Hotta K, Takahashi H, Asakura T, Saitoh B, Takatori N, Satou Y, Satoh N: **Characterization of brachyury-downstream notochord genes in the *Ciona intestinalis* embryo**. *Dev Biol* 2000, **224**:69-80.

29. Wallingford JB, Rowning BA, Vogeli KM, Rothbacher U, Fraser SE, Harland RM: **Dishevelled controls cell polarity during *Xenopus* gastrulation**. *Nature* 2000, **405**:81-85.

30. Hotta K, Takahashi H, Ueno N, Gojobori T: **A genome-wide survey of the genes for planar polarity signaling or convergent extension-related genes in *Ciona intestinalis* and phylogenetic comparisons of evolutionary conserved signaling components**. *Gene* 2003, **317**:165-185.

31. • Jeffery WR, Strickler AG, Yamamoto Y: **Migratory neural crest-like cells form body pigmentation in a urochordate embryo**. *Nature* 2004, **431**:696-699.
This study of colonial ascidian embryos identifies a population of neural-crest-like cells that migrate from the anterior neural tube into the body wall and siphon primordia, where they differentiate as pigment cells. This suggests an evolutionary origin for the neural crest in a primitive chordate ancestor.

32. • Davidson B, Shi W, Levine M: **Uncoupling heart cell specification and migration in the simple chordate *Ciona intestinalis***. *Development* 2005, **132**:4811-4818.
Together with the study by Satou *et al*. [33•], this study identifies the basic helix–loop–helix transcription factor Mesp, a paralog of mouse Mesp-1 and Mesp-2, as a conserved regulator of heart precursor specification and migration in ascidians. Their analysis of heart precursor migration in living embryos, and the identification of a Mesp mutant that inhibits cell migration downstream of cell fate specification, suggests that this is a promising system for studying cellular mechanisms of, and lineage-specific control over, a simple migration event.

33. • Satou Y, Imai KS, Satoh N: **The ascidian *Mesp* gene specifies heart precursor cells**. *Development* 2004, **131**:2533-2541..
See annotation [32•].

34. Davidson B, Levine M: **Evolutionary origins of the vertebrate heart: specification of the cardiac lineage in *Ciona intestinalis***. *Proc Natl Acad Sci USA* 2003, **100**:11469-11473.

35. •• Imai KS, Levine M, Satoh N, Satou Y: **Regulatory blueprint for a chordate embryo**. *Science* 2006. in press.
In this work, the authors used morpholino antisense oligonucleotides to knock down with the activity of 26 early, putative regulators of *Ciona* development. They then analysed the effect of each individual perturbation on the expression of more than 50 other regulatory genes. This breathtaking work led to the establishment of the first wholeembryo gene regulatory network for a metazoan.