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Morphogenesis: Shroom in to Close the Neural Tube

Paul Martin

A novel actin-binding protein, Shroom, localises to precisely those cells that will constrict during cranial neural tube closure and appears pivotal in regulating the apical constrictions that drive epithelial foldings in vertebrate embryos.

Animal embryos are shaped during development by a series of morphogenetic episodes which frequently involve the tugging, bending, folding and sculpting of epithelial sheets. One such morphogenetic process in vertebrates, neural tube closure, is well studied and of clinical importance because when it goes wrong the consequences are dire — failure of cranial neural tube closure results in anencephaly and death at birth, while if the caudal neural tube fails to fully zipper closed, then the infant will be born with spina bifida.

Several years ago, Shroom, a novel molecular regulator of this process was identified in a gene trap mutagenesis screen. Homozygous *shroom* mutant mice generally exhibited anencephaly, which made their developing brain bulge out like a wild mushroom; much less frequently they also had spina bifida [1]. When the gene was cloned, Shroom turned out to be a novel actin-binding protein. A study published recently in *Current Biology* [2] shows how ectopic Shroom can direct naive strips of *Xenopus* ectoderm, or even sheets of confluent epithelial cell lines, to constrict and fold. Shroom localises to the apical edges of cells destined to constrict in the *Xenopus* neural plate and appears to mediate this constriction via the small GTPase switches, Rap and Ras.

Most, if not all, of the epithelial foldings and bendings that underlie morphogenesis of a vertebrate embryo involve concerted contraction of the apical surfaces of groups of cells. These cells convert their shape from cuboidal to wedge-like, and this forces the epithelium to contort. In this way, for example, patches of ectoderm will invaginate on either side of the embryonic head to convert otic placodes sequentially into otic cups and then otic pits, which finally bud off as otic vesicles to form the left and right inner ears [3]. Similarly, cells of the neural plate constrict to varying degrees to drive the neural lips upwards and toward one another until they meet and fuse in the midline to form the neural tube, which will eventually become the organism's brain and spinal cord.

Besides apical constriction of cells within the neural epithelium, at least two other forces appear to collaborate to fold the neural plate — one of these forces derives from the pushing pressure of adjacent dorsolateral ectoderm, and another is due to proliferative pressures within the neurepithelium [4]. In the trunk and tail end of the embryo, these other forces seem dominant and tube closure is not disrupted by exposure of cultured mouse embryos to cytochalasins, but cranial neural tube closure is exquisitively sensitive to these actin microfilament dissolving drugs which generally cause anencephaly [5,6], just as seen in *shroom* mutant embryos.

In the mouse, at least, the location of the chief 'hingepoint' cells of neural tube closure varies along the length of the embryo, such that, at the head end, constriction is mainly in the midline, driven by median hingepoint cells and resulting in 'V-shaped' bending, while further down the spine, paired dorsolateral hingepoints take over, giving a 'C-shaped' appearance to cross-sections of the folding neural plate [6].

In regions of the neural tube that are sensitive to the actin-inhibiting cytochalasins, it is assumed that constriction of actin networks just beneath the apical plasma membrane of hingepoint cells, and possibly their neighbors also, must be largely responsible for supplying the contractile forces that drive the tube to fold. But the signals that direct cells to assemble this machinery and to constrict in a concerted fashion have long been a mystery. The new work on Shroom [2] provides some of the first clues as to the molecular basis of such signals in vertebrates.

Shroom is a PDZ-domain-containing protein which binds F-actin and localises to the stress fibers of fibroblasts *in vitro*. In heterozygous mice carrying a β-galactosidase reporter gene under the control of the *shroom* promoter, expression is seen in the neural plate just prior to neural tube closure [1]. The *Xenopus* Shroom homolog is expressed at fairly low levels throughout the neural plate, except for two strong expression stripes corresponding to where the hinge cells will be [2]. Neural tube closure is not identical in mouse and frog but, just as in the mouse mutant, inactivation of Shroom in *Xenopus*, either by morpholino knockdown or by expression of a dominant-negative form of the protein, leads to failure of anterior neural tube closure. In embryos injected unilaterally with dominant-negative Shroom constructs, actin fails to accumulate in the hingepoint cells on the injected side of the embryo, and consequently that side of the neural tube fails to bend upwards [2].

Haigo *et al.* [2] have now reported that ectopic production of Shroom in naive *Xenopus* blastular epithelium dramatically triggers concerted apical constriction of these cells too, providing supporting evidence that this single protein may indeed be a card-carrying master-regulator for some *in vivo* epithelial folding events. This assay also serves as a superb test-bed for investigating what might be the downstream effectors of Shroom. Useful clues as to what molecules to test come from *Drosophila*, where gastrulation, like neural tube closure in vertebrates, is

Departments of Physiology and Biochemistry, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK. E-mail: paul.martin@bristol.ac.uk

partially dependent on concerted constrictions of a group of epithelial cells. In *Drosophila*, such constrictions generate the ventral furrow, which invaginates to deposit future mesoderm cells, initially as a tube, inside the fly embryo [7].

Several mutations disrupt the process of fly gastrulation, besides those in key dorsoventral patterning genes such as *twist*, which is necessary to define the ventral epithelium. One of the post-patterning gastrulation genes is *Folded Gastrulation* (*Fog*), which encodes a short-range diffusible factor apparently necessary to synchronise all of the constrictions of ventral epithelial cells [8]. Downstream of Fog is Concertina (Cta), a G protein which is presumably coupled to the Fog receptor [9]; and downstream of Cta appears to be the small GTPase Rho, as mutants defective in the Rho guanine nucleotide exchange factor RhoGef2 also fail to gastrulate properly [10]. Another small GTPase, Rap, is also implicated in these gastrulation movements, as fly embryos lacking a maternal complement of Rap fail to gastrulate normally [11].

No obvious vertebrate homolog of Fog has been identified, although presumably there must be some functional equivalent to coordinate neighboring cell constrictions. Frogs do, however, have both Rho and Rap, and it is clearly tempting to think that these molecular switches might be players in the apical constrictions driven by Shroom in the *Xenopus* neural plate. Curiously, for Rho this turns out not to be the case: dominant-negative forms of Rho fail to block Shroom-activated constrictions. But blocking Rap or Ras does prevent apical constriction, suggesting that these small GTPases are either downstream of Shroom or in some other way are necessary for transduction of the Shroom signal [2].

So what directs *shroom* expression in just those key cells that will be instructed to constrict? This is a patterning question and may relate to a recent study showing that Sonic hedgehog (Shh) expression is pivotal in regulating the formation and action of hingepoint cells in mouse. Shh appears to positively regulate the formation of median hingepoint cells and suppress dorsolateral hingepoint formation, so that in Shh knockout mouse embryos, the whole length of the neural tube is now closed by paired dorsolateral hingepoints [12]. Another candidate regulator of Shroom expression is Lmx –1, which is expressed by hingepoint cells in the chick embryo [13]. It should be revealing to determine the pattern of expression of Shroom in mice where Shh or Lmx expression is disrupted. Not only is Shroom production and function regulated within the plane of the epithelium, but its function also clearly depends on which epithelial cell layer a cell occupies; curiously, Shroom can only trigger assembly of contractile actomyosin machinery in the most superficial cells that have an already established apicobasal polarity [2].

In sum, the latest Shroom data clearly tell us that vertebrate embryos have a very powerful tool at their disposal for bending tissues at will. In all probability this tool, or related tools, will be re-used many times more during development, but their roles in processes other than cranial neural tube closure may be largely hidden by compensatory mechanisms and gene redundancy. At present, very little is known about Shroom relatives, but these may be important wherever Shroom is expressed but does not appear to be essential — as during otic vesicle invagination — or early in development. Shroom itself is not even zygotically expressed until neural plate stages, which probably rules out a role in earlier morphogenetic movements, some of which also involve cell shape changes. Hopefully, now that the first *in vivo* master regulator of morphogenetic foldings has shown his face, his partners and back-up-team may soon begin to reveal themselves also.

But a word of caution — recent live imaging studies of gastrulation in the *Drosophila* embryo show that even a simple cell constriction becomes more complex the more carefully you analyze it. Time-lapse movies of fly embryos expressing α-catenin fused to the green fluorescent protein show that initially a synchronous wave of gentle constrictions spreads throughout the patch of epithelium destined to invaginate, and this is followed by a more potent set of non-synchronous constrictions which appear to be the true drivers of gastrulation [14]. It is likely that the epithelial contortions in vertebrate embryos will prove to be more complex still. Morphogenesis is still a long way from being resolved.

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