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SPOTLIGHT

Keeping a stiff upper lip: p120ctn and tissue fusion

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In this issue, Teng et al. (https://doi.org/10.1083/jcb.202503070) use genome editing, classical tissue-specific gene knockouts, and live cell imaging to show that the interaction of p120ctn with the juxtamembrane domain of classical cadherins is necessary for tissue integrity during the cellular zippering required for upper lip formation in mammals.

One of the most surprising things about human embryos to many beginning students of developmental biology is that structures on the ventral surface of the embryo start out as bilateral primordia that only later meet at the ventral midline. A well-known example is the heart. Another is the formation of the face. Bilateral islands of tissue known as "prominences" must undergo a multistep suturing process to complete formation of the face. Both overlying epithelial cells of the ectoderm and underlying mesenchymal cells participate in this process: the epithelium forms the fleshy parts of the face—including the lips—and the underlying mesenchyme forms palatal "shelves." Failure of fusion of the palatal shelves leads to cleft palate, and failure of the corresponding fusion of the overlying ectoderm leads to cleft lip. These defects are among the most common among live births in humans. While life-transforming corrective surgery can repair such defects, the underlying cellular causes of cleft palate and cleft lip remain somewhat unclear.

Crucial to upper lip formation is the zippering together of the epithelial cells of the medial and lateral nasal prominences (MNP and LNP, respectively); the "zipper" initiates caudally (posteriorly) and progresses rostrally (anteriorly; Fig. 1 A) (1). The advent of routine whole-genome sequencing of individuals with craniofacial defects has been a boon for identifying mutations associated with defects in this process. A notable example is a non-myosin II (*MYH9*); human mutations are associated with cleft lip (e.g., [2]). What is often missing in the human genetics literature, however, is mechanistic drilling down to the underlying cellular basis for human diseases.

The work by Teng et al. fills an important gap in this regard in the case of cleft lip. Other zippering events, including dorsal epidermal closure in the *Drosophila* embryo (reviewed in [3]), ventral epidermal enclosure in the *C. elegans* embryo (4), and closure of the dorsal epidermis over the neutral tube in vertebrates (5, 6), display dynamic actomyosin accumulation at zipper sites. Using a tissue explant system to image the dynamics of the zippering process in vitro using high-resolution microscopy, Teng et al. found similar F-actin accumulation at zipper sites. Conditional deletion of two non-muscle myosins in the upper lip ectoderm leads to cleft lip, confirming the role of actomyosin contractility during upper lip zippering.

Teng et al. next focused on cadherin-dependent cell-cell adhesion. There was good reason to do so: mutations in genes encoding E-cadherin (*CDH1*) and p120-catenin (*CTNND1*) had previously been implicated in cleft lip (7, 8). *p120ctn* binds the cytoplasmic tail of cadherins very near their transmembrane domain (a region known as the juxtamembrane domain; Fig. 1 B). Work over many years, much of it by the Reynolds laboratory, has characterized numerous potential functions for p120ctn. These include regulating endocytic trafficking of cadherins, interactions with the transcription factor Kaiso in a transcriptional network that interacts with canonical Wnt signaling, and modulation of actin recruitment via Rho family GTPases, especially the negative regulator p190RhoGAP (9). Much of this work suggests that however *p120ctn* regulates developmental events, it largely does so as a modulator. Indeed, in both *Drosophila* (10) and *C. elegans* (11), *p120ctn* is dispensable under laboratory conditions. Significantly, however, in both cases, *p120ctn* loss predisposes embryos to cadherin complex-dependent mechanical failure. In *Xenopus* (12), *p120ctn* appears to modulate morphogenesis as well, but additional functions of *p120ctn* (e.g., as it relates to Kaiso) may also affect other aspects of development (13).

Given its multifaceted potential roles, and the pragmatic difficulty of generating high enough *n* values to establish statistical significance in a mammalian explant system, one of the impressive aspects of the work by Teng et al. is that it narrows down how p120ctn regulates epithelial zippering during upper lip formation. The fusion of the MNP and LNP is associated with cessation of cell division and high levels of apoptosis (14). Surprisingly, there was no significant change in rate of apoptosis in *p120ctn* knockouts at the time of zippering. To confirm that apoptosis plays a minor role, Teng et al. generated conditional knockouts of pro-apoptotic factors in lip tissue and showed no effect on lip development.

Next, Teng et al. used a creative genetic approach to home in on which functions of p120ctn might be involved by creating compound heterozygous mice carrying one copy of a floxed allele of *p120ctn* and another with a point mutation predicted to abrogate specific p120ctn binding interactions using a CRISPR/Cas9-based technique known as i-GONAD. The only point mutant that led to cleft lip in this assay was one predicted to

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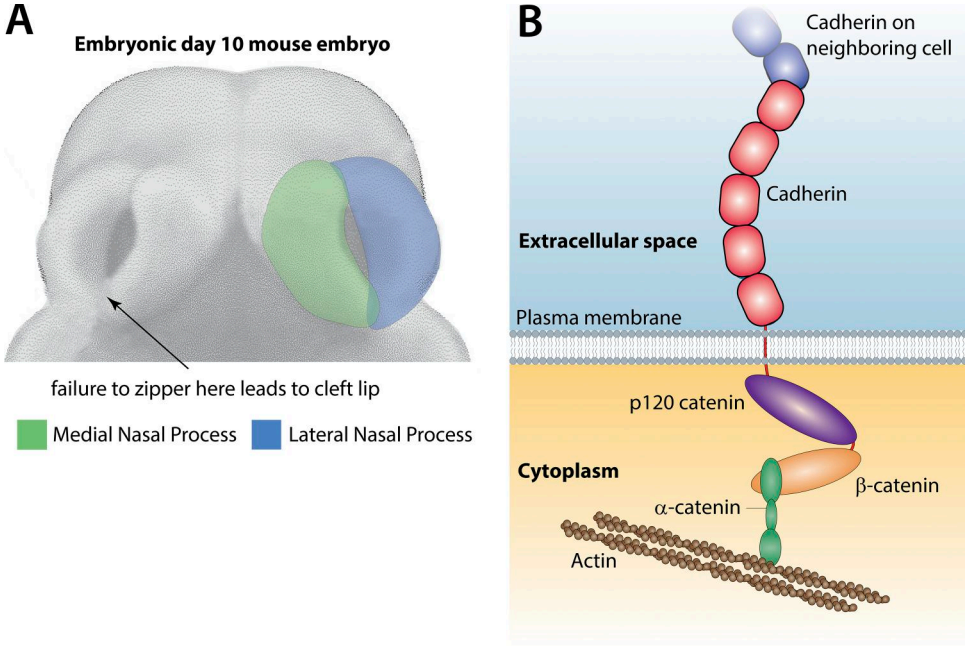

Figure 1. **Stiffening the mammalian upper lip requires p120ctn function. (A)** Failure of the medial and lateral prominences to undergo zippering leads to cleft lip during later craniofacial development. **(B)** p120ctn binds the juxtamembrane domain of classical cadherins.

block p120ctn binding to cadherins, not those that abrogate p190RhoGAP or Kaiso binding.

These experiments implicated *p120ctn* in directly regulating cadherin levels in the lip primordium. To investigate this possibility further, Teng et al. used the *i*-GONAD system to make mouse strains with various combinations of loss of two cadherins, E- and P-cadherin (*CDH1* and *CDH3*, respectively), in the lip primordium, and showed that these two cadherins act redundantly in the upper lip primordium. Finally, Teng et al. showed that cells at zippering sites in *p120ctn* conditional knockouts are more rounded than in controls. While they accumulate actin, they display reduced junctional cadherin, and such mice are more sensitive to tissue dissolution via pharmacological activation of myosin contractility.

The work of Teng et al. makes significant contributions to our understanding of how *p120ctn* contributes to epithelial zippering during craniofacial development. Several interesting questions remain, however. While the hypothesis that stabilization of cell contacts mediated by multiple classical cadherins during myosin-dependent zippering seems sensible, the explant system Teng et al. developed could be used to interrogate the mechanics of MNP/LNP zippering sites more directly (e.g., through laser ablation or additional genetic manipulations). The explants could be used to examine cadherin dynamics and/or trafficking in more detail, since the available evidence suggest p120ctn mainly functions at this level in this system. Finally, a major question in all systems in which epithelial zippering occurs is what leads to initiation of zippering at very specific sites. It will be interesting to see what developmental signals act upstream of zipper initiation in this tissue. Whatever the outcome of these future studies, the work of Teng refines our understanding of the roles of a key interacting partner with classical cadherins and establishes a powerful model system for leveraging genome editing, classical molecular genetics, and live imaging to study a morphogenetic event in mammals with major clinical relevance.

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