Apical constriction and invagination: A very self-reliant couple

A fundamental phenomenon in development is the capacity of sheets of cells to bend inward, thereby positioning some of the cells from these sheets below the surface on which they were originally placed. This process, known as invagination, generates folds or depressions in a previously uniform surface. Eventually, these groups of cells separate from their original neighbors and generate new internal organs. Invagination is at the origin of the formation of germ layers, such as the mesoderm and endoderm (Shook and Keller, 2008 and Solnica-Krezel, 2005), which have allowed the morphological complexity of the animal kingdom. In more general terms, cell invagination allows the formation of three-dimensional structures out of a cell monolayer.

The collective phenomenon of invagination is very often accompanied by an individual cell behavior known as apical constriction, by which cells of columnar morphology shrink their apical surface and perimeter and become wedge-shaped. Indeed, it is widely accepted that the changes in shape produced by apical constriction, or the forces associated with this process, drive the collective invagination process. Along these lines, simulation models in collaboration with mathematicians and physicists have been put forward to account for the coupling of apical constriction and invagination and have even been used to study whether a causality linkage could lead apical constriction to invagination.

However, several experimental evidences indicate that there is neither a causal relation nor a strict requirement between these two phenomena. In this context, here we discuss the relationship between cell apical constriction and cell invagination and more precisely on how the former contributes to collective cell invagination and why these two processes are so often together.

Active apical constriction and passive shape changes

Here we refer to apical constriction as the active narrowing of the cellular apex leading the cell to adopt a bottle- or wedge-like shape (Sawyer et al., 2009). Two features are associated with this notion. First, apical constriction is an active process, and second, the forces driving this process are generated inside the cell. Commonly linked to apical constriction is the specific organization and activity of an actomyosin network thought to provide this active force. However, the way in which myosin activity leads to apical constriction varies depending on the cell type. Similarly, diverse regulators and mechanisms are involved in controlling the activity of the actomyosin network, which underlies this process (Bertet et al., 2009, Martin et al., 2009 and Sawyer et al., 2009). Furthermore, it has been proposed that different morphogenetic events can be induced by different subcellular regulation of actomyosin contractility in different body regions (Bertet et al., 2009). We emphasize that apical constriction is usually employed to designate active reorganization events that occur inside the cell. However, this does not exclude that cells can acquire specific shapes as a passive result of the morphological changes that neighboring cells actively undergo (Sawyer et al., 2009). In this regard, we could distinguish between primary or bona-fide apical constriction and a secondary type, in which cells constrict as a result of other coincident morphogenetic movements.

Apical constriction is neither necessary nor sufficient for invagination

As mentioned earlier, it is widely accepted that changes in the shapes of individual cells generated by apical constriction, or associated forces, drive the tissue or organ behavior of collective cell invagination. Many examples in nature support this correlation (Sawyer et al., 2009), as apical constriction and invagination lie at the basis of many morphogenetic events, such as the formation of tubes, organs, and germ layers. However, an increasing amount of data indicate that these often concurrent phenomena can be uncoupled as, on the one hand, groups of cells can invaginate in the absence of individual cell apical constriction and, on the other hand, apical constriction does not necessarily lead to invagination.

Accumulating evidence shows that cells can invaginate in the absence of cell apical constriction. For example, the acquisition of wedge-shaped cells and the bending of a cell sheet can be driven by the expansion of the basal surface (Sawyer et al., 2009 and Smith and Schoenwolf, 1988). Even more striking is the finding that invagination processes usually accompanied by apical cell constriction can still proceed in several mutant conditions in which apical constriction is impaired. For instance, there are well-known cases where mutants show impaired apical constriction of cells, and nevertheless, these cells end up making, for example, the mesoderm furrow (Leptin and Grunewald, 1990) or a tracheal tube (Brodu and Casanova, 2006 and Nishimura et al., 2007; see below). Thus, in these examples other mechanisms induce invagination even in cells instructed to invaginate with apical cell constriction in normal development, thereby suggesting that apical constriction is just a mechanism favoring invagination. This notion has very recently been illustrated in the case of the vertebrate lens pit invagination in the Shroom3Gt/Gt mutant mouse, in which the cylindrical-to-conical shape transition is defective. In this case, invagination is thought to proceed as a result of the filopodia that span the interepithelial space between the lens pit and the developing retina and that would transmit the invagination forces from the optical cup (Chauhan et al., 2009 and Plageman et al., 2010).

In addition, apical constriction is not sufficient for invagination. Thus, for example, apical constriction during eye morphogenesis is linked to the formation of the morphogenetic groove, where a depression is formed apically but the basal surface of the cells does not move inward but remains in the original plane (revised in Sawyer et al., 2009). Similarly, ectopic apical constriction in mutants for the Jak/Stat pathway interferes with germ-band extension but is not reported to cause cells to invaginate (Bertet et al., 2009). Therefore, given that there is neither a causal relation nor a strict requirement between individual cell constriction and collective cell invagination, the question arises as to why these two processes are so often together. In other words, how does apical constriction contribute to invagination?

Contribution of apical constriction to invagination

At the theoretical level, invagination can occur by several mechanisms. Indeed, Davidson et al. (1995) proposed up to four alternative models, in addition to apical constriction, to account for cell invagination. In particular, these authors analyzed how cell invagination is induced by the following: (1) cell tractoring toward the invagination point; (2) cell contraction along the apicobasal axis; (3) cell contraction by a pluricellular circumferential bundle surrounding the invagination area; and (4) differential swelling between the apical lamina and the hyaline layer. Although these modes of invagination have been modeled, *in vivo* evidence shows that most invagination processes analyzed to date are accompanied with apical constriction. This observation suggests, as several authors have already noted, that apical constriction is the best mechanism to facilitate invagination (Bertet et al., 2009 and Martin et al., 2009). Indeed, simply by the physical outcomes imposed by apical constriction, cells are prone to bend (Jones and Chapman, 2009), which clearly favors invagination. However, additional aspects of apical constriction could be instrumental in facilitating this process. Below we discuss three of them.

Apical constriction and changes in apicobasal cell shape appear to occur independently

Apical constriction is usually linked to changes in the apicobasal cell shape. Thus for example, in the bottle cells of Xenopus, apical constriction is linked to the lengthening of the apicobasal surfaces, although treatments inhibiting invagination abolish apical constriction but not apicobasal cell lengthening (Hardin and Keller, 1988 and Lee and Harland, 2007). In *Drosophila* mesoderm invagination, cell apical constriction is also linked in a first step to cell lengthening along the apicobasal axis, which is followed by shortening to their original length (Leptin and Grunewald, 1990 and Sweeton et al., 1991). However, in the Ascidian endoderm, invagination is associated with cell basolateral shortening. Interestingly, this shortening is not simultaneous with apical constriction but instead happens soon after the latter process. In this case, it has been proposed that this two-step mechanism accounts for the dynamics of cell shape change and tissue deformation observed during invagination (work by F. Robin and K. Sherrard as described in Bellaiche and Munro, 2009). Thus, while apical constriction is often coupled to changes in apicobasal cell shape, the actual changes and their contribution to invagination appear to differ depending on the tissue or cell contexts and like in the case of apical constriction and invagination, these two phenomena are very often coincident but not interdependent. In some cases, apicobasal cell changes could be active cell changes coordinated with apical constriction while in other cases they could be passive responses to apical constriction and mechanical constraints depending on the contact with surrounding cells (see Sawyer et al., 2009 and below).

Apical constriction controls spatio-temporal ordered invagination

Many invagination processes occur in a precise pattern, both in space and time; in these cases, not all the cells invaginate the same way and at the same time. In this regard, we would like to note here that apical constriction correlates with the orderly aspect of invagination. Mutations that impair apical constriction do not hinder invagination *per se* but completely disorganize the precise pattern of the process. For instance, in *twist* embryos, where the apical cell surfaces do not contract, cells invaginate later and in a less orderly way (Leptin and Grunewald, 1990). Indeed, very recently it has been proposed that *twist* would be involved in the translation of the intracellular forces occurring in apical constriction into tissue-wide epithelial tension (Martin et al., 2010), which could account for its role in coordinating the whole process of invagination. A similar case occurs for the invagination of tracheal placodes. In wild-type development, concentric rows of cells enter invagination and generate finger-like structures. However, in mutants with abolished apical constriction, tracheal invagination still occurs but instead gives rise to a loose depression (Brodu and Casanova, 2006 and Nishimura et al., 2007). In those mutant cases, even in the absence of a robust cell shape change some tension could be generated across the tissue, which would account for the disorganized invagination. Indeed, in all those cases analyzed, while apical constriction is not necessary for invagination to occur, it exerts a critical effect on the spatio-temporal ordering of the process.

Apical constriction affects other parallel mechanisms impinging on invagination

Apical constriction could also facilitate invagination by an associated reorganization of the cytoskeleton. Apical constriction might modify the activity of the cellular domains harboring the signaling receptors and/or transducer mechanisms or the cell sorting and trafficking machineries and thus lead to modifications in the composition or activity of cytoskeleton effectors. We have mentioned earlier that, although diverse in their mechanisms, cells that constrict apically do so by apically localizing the components of the actomyosin complex. We propose that the process that leads to this reorganization could be instrumental in other mechanisms also postulated to be involved in cell invagination, for instance differential cell adhesion, cell shortening, or the response to forces exerted by neighboring cells (Conte et al., 2009, Gustafson and Wolpert, 1963 and Sawyer et al., 2009). Some results support this notion. For instance, adherens junctions are required to link actomyosin contraction to cell apical constriction (Dawes-Hoang et al., 2005). This observation emphasizes the interplay between the mechanisms required for apical constriction and those involved in cell adhesion. Or as mentioned above, cell resistance to apicobasal elongation is important to translate cell apical constriction into cell sheet bending (Keller et al., 2003), thus suggesting that a linkage between intracellular components acting in apical constriction and those providing apicobasal stiffness also confers an advantage for cell invagination. In other cases, shrinking of the apical membrane would be just a first step in cell invagination and would succeed only when apical constriction is followed by apicobasal shortening (Keller et al., 2003).

In summary, the phenomena of individual cell apical constriction and collective cell invagination are not always linked. A number of factors, probably including interaction with the non-invaginating neighboring cells, can determine whether apical constriction acts as a driving mechanism for invagination or for other morphogenetic events. However, although these two processes are often associated, it is also clear that groups of cells can invaginate without apically constricting. We would like to propose that a combination of the physical effects directly promoted by apical constriction, the functional links generated by the intracellular reorganization associated with apical constriction, and the potential of regulated apical constriction to generate elaborate patterns of invagination would explain the association between these two phenomena.

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