

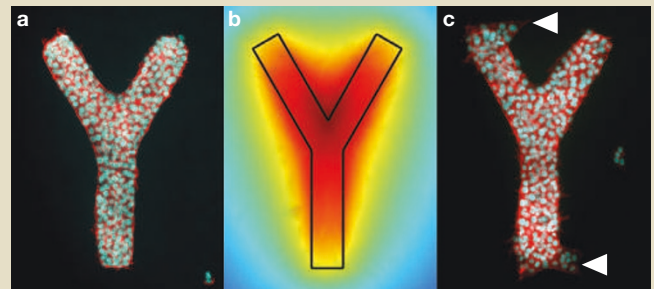
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## Shaped to split

During development, many organs with branched tubule-based structures, including the mammary glands, initially grow in straight lines and subsequently undergo reiterative splitting of the growth activity of the ducts in two directions — a process known as branching morphogenesis. Branching morphogenesis requires tight temporal and spatial integration of a multitude of signals, but analysis of individual factors involved in this process has been difficult. A recent study by Nelson *et al.* (*Science* **341**, 298–300; 2006) shows that the spatial organization of cells in the mammary gland has an essential role in determining a gradient of growth factor concentration that, in turn, shapes organ growth.

Mouse mammary epithelial cells embedded in cavities of collagen gel generate hollow tubules of defined geometry. When these tubules were treated with growth factor, branching into the surrounding collagen was observed that mimicked *in vivo* development, but only at the ends of the tubules. Branch initiation requires the invasion of epithelial cells into mesenchymal tissue. Nelson *et al.* demonstrated that this invasive phenotype is specified before branching by showing that mammary epithelial cells expressed a reporter gene that was under the control of a mesenchymal promoter before branching occurred. The activation of the reporter was spatially restricted to cells at the ends of the tubules, which was confirmed in an elegantly designed series of experiments that analysed tubules with different initial geometries.

Given that the site of bifurcation depended on the initial structure of the tubule, and that this specifically localised branching was observed in an *ex vivo* model system that did not include mesenchymal tissue, the group proposed that the signal for branching was secreted locally by the epithelial cells themselves.



**Figure 1** Immunofluorescence staining of actin (red) and nuclei (blue) in a bifurcated tubule (a). The calculated concentration profile of a diffusible inhibitor produced uniformly by the cells in the tubule predicts that the local concentration of inhibitor will be lowest at the distal ends of tubules

A computational three-dimensional model of diffusion of a hypothetical inhibitory molecule showed that cells organized in a tubular shape and secreting an inhibitor will induce a concentration gradient around the tubule structure, with the lowest concentrations located at the ends of the tubules where branching was induced (Fig. 1). Examining the extracellular distribution of transforming growth factor  $\beta$  (TGF $\beta$ ), which is secreted by the epithelial cells in the tubule, confirmed that this computationally generated concentration profile was accurate. Disrupting the signalling pathway induced by TGF $\beta$  in the epithelial cells lead to uniform branching events across the entire surface of the tubule, demonstrating its role as an inhibitor of morphogenesis.

This study shows that tissue geometry can determine the location of branching and the simple *ex vivo* system the authors have developed will allow further analysis of the role of other morphogens in this process.

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