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Common mechanisms regulate stem cell self-organization and symmetry breaking across various glandular epithelia

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In this issue of *Developmental Cell*, Journot et al. identify a conserved mechanism promoting the development and lineage segregation of multipotent stem cells across different glandular epithelia. p63, YAP, and Notch control symmetry breaking, cell positioning, and cell-fate decision during development and regeneration, illustrating how spatial cues orchestrate tissue self-organization.

The mammary, prostate, salivary, and lacrimal glands are bilayered, branched exocrine glands composed of basal cells (BCs) and luminal cells (LCs). They arise from multipotent stem cells (SCs) during embryonic development..[1–4] However, in adult mice, BCs and LCs are sustained by their own lineage-restricted unipotent SCs and contribute independently to tissue maintenance under homeostatic conditions.[5]

These epithelia can reactivate their multipotent program during pathological and regenerative conditions, such as transplantation of BCs, LC ablation, or oncogene expression.[5–7] The molecular mechanisms regulating multipotency, lineage commitment, and tissue regeneration remain poorly understood.

In this issue, Journot et al. developed a comprehensive approach using *in vitro* organoids, *ex vivo* embryonic explants, and single-cell quantitative imaging to unravel the molecular mechanisms driving symmetry breaking and cell-fate commitment in these four epithelia during mouse embryonic development and tissue regeneration.[6] To this end, the authors first established *in vitro* organoid models derived from single-adult BCs. After 7 days of culture, organoids were composed of two spatially distinct cell populations consisting of an external BC layer surrounding an inner LC layer, mimicking the spatial organization of these epithelia *in vivo*. These BCs and LCs expressed the markers of their specific lineages *in vivo*, demonstrating that these organoids faithfully recapitulated the binary cell decision, lineage segregation, and spatial organization of multipotent SCs during mouse embryonic development.

The authors then assessed the temporality of lineage segregation and found that after 48 h post-seeding, all cells coexpressed basal and luminal markers, suggesting that multipotent BCs consist of a homogeneous population of hybrid BCs/LCs, similar to multipotent embryonic progenitors *in vivo*[1,2] (Figure 1A). Symmetry breaking occurs 72 h following BC seeding, and basal and luminal markers begin to be segregated. Interestingly, the size of the organoids distinguished organoids composed of multipotent cells (small) from lineage-segregated organoids (large) in all four tissues.

To assess the *in vivo* relevance of their findings, the authors assessed the dynamic expression of p63, a key regulator of basal fate[1] and luminal markers in cultured explants of the embryonic

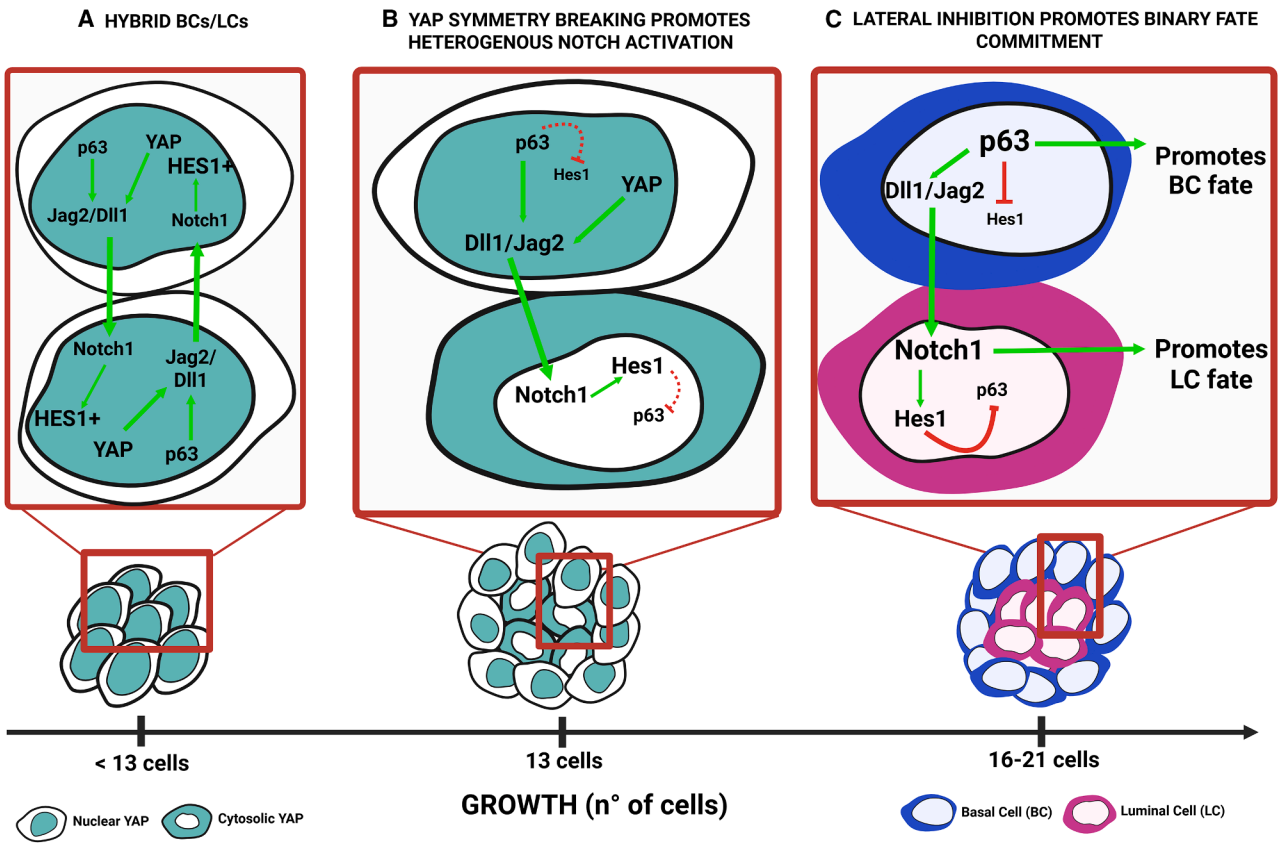

Figure 1. Lineage segregation in glandular epithelia results from YAP-mediated symmetry breaking and Notch-driven lateral inhibition
(A) Following BC seeding, cells in early organoids are relatively homogenous, expressing Notch ligands, a Notch receptor, Hes1, YAP, and p63, reflecting a hybrid BC/LC state.
(B) When organoids reach a critical size, some BCs move into the inner mass, accompanied by lower YAP activity, marking symmetry breaking. As active YAP promotes Dll1/Jag2 expression, the decrease of YAP activity in the inner cells results in a differential Notch activity between inner and outer cells. The inner cells experience high Notch activity, promoting Hes1 expression and p63 downregulation, whereas the outer cells maintain a high level of Notch ligands, YAP, and p63 activity.
(C) Notch lateral inhibition reinforces basal and luminal lineage segregation.

tissues. They show that, like their findings in organoids, the basal marker p63 is progressively downregulated in inner cells as morphogenesis proceeds, showing the conservation of p63 symmetry breaking in all four glandular epithelia.

Notch signaling has been shown to promote LC fate in adult mice, as demonstrated by LC differentiation following active Notch intracellular domain (NICD) expression in BCs.[2] Expression of NICD in BCs from organoids during the first two days in culture induced BC-to-LC conversion. Similarly, NICD expression in multipotent embryonic SCs *in vivo* promoted luminal fate. Conversely, pharmacological inhibition of Notch signaling precluded BC differentiation into LCs in organoids and in embryonic explants.

To confirm the temporal dynamic of Notch activation during lineage segregation, the authors used a green-fluorescent reporter of Hes1 (a canonical Notch target gene). Upon single-cell seeding, Hes1 is homogenously expressed in all BCs, with expression becoming restricted to the inner cells when organoids reach their critical size associated with symmetry breaking. Similarly, Hes1-GFP and p63 are initially coexpressed *in vivo* and then become segregated as lineage commitment proceeds.

To understand the mechanisms regulating the spatiotemporal activation of Notch signaling, the authors assessed the expression of Notch ligands and receptors over time. Notch ligands Dll1 and Jag2 were initially homogenously expressed but became restricted to the outer BCs alongside organoid growth, mimicking Hes1 and p63 segregation patterns, whereas Notch1 remained homogenously expressed (Figure 1B). Pharmacological Notch inhibition prevented the differential expression of Dll1 and Jag2 in the 2 layers, consistent with the notion that position-dependent cell heterogeneity is amplified by Notch signaling. p63 reinforced the spatial segregation of Notch signaling by promoting the expression of Dll1 and Jag2. This suggests that symmetry breaking is mediated by Notch lateral inhibition (Figure 1C).

In theory, two cells should be sufficient to trigger symmetry breaking via lateral inhibition. However, symmetry breaking only occurs when organoids reach a critical size of about 13 cells, suggesting that an upstream mechanism maintains uniform Notch activity until a threshold is reached. The correlation between organoid size and tissue architecture led the authors to investigate whether Hippo/YAP (Yes-associated protein) signaling[8] regulates lineage segregation. YAP was active in all the cells at the early stage of organoid growth and,

as symmetry broke, YAP remained active only in the external basal layer, supporting the notion that YAP activity is regulated by cell positioning. Continuous activation of YAP prevented lineage segregation despite the increasing size of the organoids. Sustained YAP activation in the embryonic explants increased the proportion of p63/Hes1 double-positive cells, maintaining an embryonic-multipotent-like state, and the lack of spatial YAP patterning impaired symmetry breaking. YAP promoted Jag2 expression, acting as a capacitor upstream of Notch, preventing lateral inhibition until cells became internally localized. Further studies will be needed to understand the precise mechanisms that regulate YAP activity such as cell density, matrix stiffness, or other biochemical cues.

Following LC ablation in mice, BCs reactivate multipotency and coexpress basal and luminal markers before differentiating into LCs,[6] similar to the process during embryonic development. The authors have now shown that the reactivation of multipotency upon LC ablation involves the coexpression of p63, Hes1, and nuclear-localized active YAP. Likewise, ionizing radiation-induced cell death promotes the appearance of hybrid cells coexpressing p63 and Hes1, together with a high level of nuclear YAP. These findings support the notion that regeneration induces a cell-fate switch reminiscent of early multipotent SCs during embryonic development.

In conclusion, the authors uncovered the patterning mechanisms where geometry, mechanics, and signaling pathways intersect to govern cellular organization and lineage commitment across different glandular epithelia.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Dual threat: VSIG4$^+$ macrophages use IL-11 and VSIG4 to silence T cells

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In this issue of *Developmental Cell*, Ma et al. show that embryonically derived VSIG4$^+$ macrophages suppress CD8$^+$ T cell responses across cancers. They identify IL-11 as a key effector and MEF2C as a transcriptional regulator of VSIG4$^+$ macrophages, highlighting new therapeutic avenues for targeting immunosuppressive tumor-associated macrophages to improve immunotherapy outcomes.

Macrophages are among the most heterogeneous and plastic immune populations in the tumor microenvironment. Their identity and function are shaped by both local cues and developmental origin. Over the past decade, numerous studies have demonstrated that tumor-associated macrophages (TAMs) may derive from circulating monocytes or from embryonically seeded tissue-resident macrophages (TRMs). Both tissue-resident and monocyte-derived populations coexist within tumors but differ in longevity, tissue integration, and transcriptional programs. While neither ontogeny alone nor environmental cues fully determine TAM function, both jointly shape how