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Nail mesenchyme: Tipping the hand on regeneration

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Digit tip regeneration rebuilds amputated structures in some mammals if the nail organ is preserved. In recently published *Cell Reports* papers, Castilla-Ibeas et al., Johnson et al., and Mahmud et al. define the patterning function and regenerative capacity of the dorsal nail mesenchyme in this process.

The ability to regrow functional parts of a lost body structure, as during epimorphic regeneration, is a superpower in several vertebrate species. For example, adult salamanders can regenerate a limb following amputations and adult zebrafish can regrow clipped fins. In comparison, mammals have more limited regenerative capabilities. However, it was more than 50 years ago when surgeons realized that young patients with amputated fingertips can regenerate the entire tip when treated with simple dressing changes.[1] This rare example of multi-tissue regeneration in humans is conserved in other mammals, including mice, which as a model helped demonstrate that preservation of the nail organ is critical for successful regeneration.[2] How then does the nail, a dorsal ectodermal derivative, and its associated mesenchyme enable the regeneration process? Conversely, how is the regenerating tissue patterned along the dorsal-ventral (DV) axis to restore tissue organizations and morphologies, allowing, for instance, the nail to regrow only dorsally?

In recent issues of *Cell Reports*, studies by Castilla-Ibeas et al., Johnson et al., and Mahmud et al.[3–5] tackled these questions using mouse genetic experiments to perturb the formation of DV structures and to investigate the roles of the nail mesenchyme (Figure 1). Their results showed that dorsal limb structures formed during development are necessary for adult digit tip regeneration, but the DV patterning of the new digit tip utilizes a non-developmental program. The nail-associated mesenchyme also directly gives rise to progenitor cells in the blastema, which is a transient structure formed below the wound epithelium at the regenerative amputated stump. As blastema cells differentiate to reconstruct all lost tissues, a functional blastema is required for any epimorphic regeneration.

By amputating mouse digits at different proximal-distal (PD) levels, past studies examined how the amount of nail preserved affects blastema functions. While blastema-like cell mass could still form when amputation removed a significant portion of the nail, its regenerative capacity was reduced, and bone regrowth was incomplete.[6] Transcriptional profiling of non-regenerative digit tip mesenchyme at single-cell resolution also revealed that they failed to acquire the same transcriptional state of a regenerative blastema.[7] Dermal fibroblasts that didn't initially express blastema markers were shown to obtain blastema signatures when transplanted to amputated digits that preserved nails, but not when done so to those without.[7] Therefore, the nail structure confers an environment that induces and/or maintains the blastema function. This notion was supported by results from conditional ablation of β-catenin and Wnt signaling in the nail epithelium, which inhibited bone regeneration.[8] While the signaling mechanism mediating the epithelial-mesenchymal induction remains unclear, these studies pointed to the potential requirement of the nail organ during digit tip regeneration. However, assessing the role of nails by amputating at different levels is an indirect approach and may introduce confounding factors.

To more explicitly test the nail's requirement in regeneration, Castilla-Ibeas et al. used the nail-less *ΔLARM1/2* mouse line that lacks limb-specific expression of *Lmx1b*, encoding the LIM-homeodomain transcription factor 1b.[3] During normal limb development, *Lmx1b* is expressed in the dorsal limb bud mesenchyme and patterns the limb dorsally.[9] *ΔLARM1/2* limbs are thus double-ventral, with only a few nail characteristics retained on the dorsal surface.[3] When amputated at the same PD level that permitted regeneration in adult control mice, *ΔLARM1/2* digits failed to regenerate. In contrast, the *Del(27)* mutants that develop double-dorsal limbs due to loss of the ventral patterning gene Engrailed 1 (*En1*) could still regenerate the double-dorsal digit. The same result was obtained by Johnson et al. when *Lmx1b* and *En1* were conditionally deleted in the embryonic limb buds.[4] The nail and its associated dorsal dermis are therefore required for regeneration. Correct DV patterning, however, is dispensable. Notably, amputated *ΔLARM1/2* digits still formed a small blastema, but it failed to progress to the differentiation phase and significantly downregulated dorsal dermis-specific genes, including *Rspo4* and *Sfrp2*, which could further modulate Wnt signaling. It is therefore plausible that ventralized tissues could not properly generate or respond to inductive signals that promote digit regeneration, which needs to be tested in the future.

Given that the adult digit maintains restricted *Lmx1b* expression in the dorsal dermis,[4] Johnson et al. asked if the embryonic DV patterning program is re-employed during digit tip regeneration. However, *Lmx1b* expression became uniformly distributed in the blastema upon amputation,[4,5] thus contradicting a role in assigning DV pattern. To determine a requirement for patterning the regenerating digit, Johnson et al. deleted *Lmx1b* and *En1* in the adult digit fibroblasts and

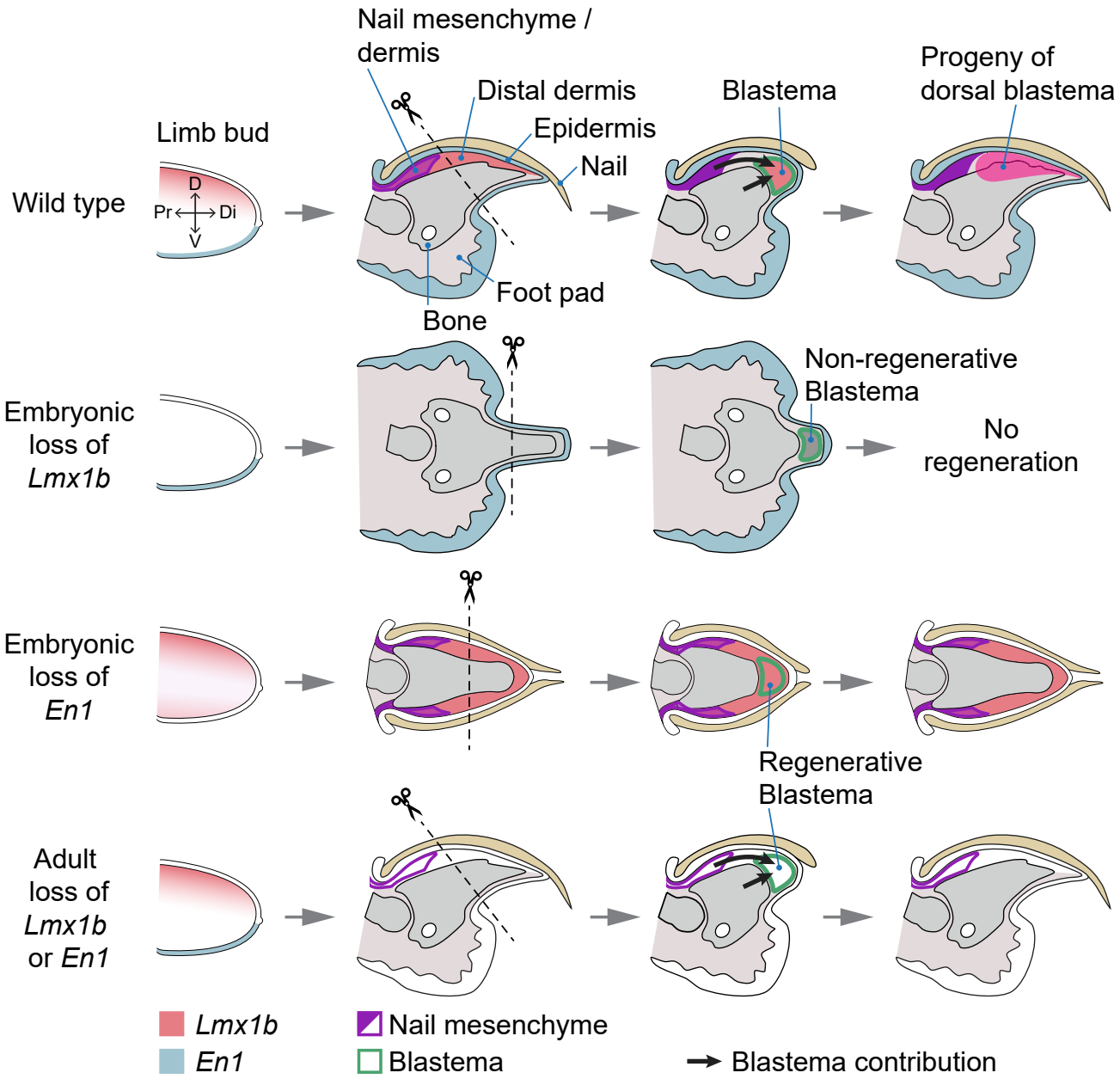

Figure 1. *Lmx1b* and *En1* determine the DV axis of the developing limb
Dorsal structures, including the nail mesenchyme, are required for adult digit tip regeneration. Pr, proximal; Di, distal.

epithelium, respectively, using tamoxifen-inducible Cre recombinases prior to amputation. Then, they performed analysis of the regenerated phalangeal bone morphology and found that neither *Lmx1b* nor *En1* were required for DV patterning, although they do play a role in promoting bone regeneration. These results resonate with previous studies showing that blastema cells are transcriptionally different from embryonic limb mesenchyme[7] and challenged the old posit that regeneration recapitulates developmental events.[10] Identifying patterning factors based on new hypotheses is an important next step.

Also intrigued by how nail organ contributes to blastema formation, Mahmud et al.[5] reasoned that because the blastema is primarily mesenchymal and the nail mesenchyme expresses markers of mesenchymal precursors,[11] the nail mesenchyme may function as a source population for the blastema. Using *Lmx1b-CreER* to label and lineage trace the nail mesenchyme, the authors demonstrated that following amputation nail mesenchymal cells gave rise to the dorsal blastema and later regenerated the dorsal dermis and bone. On the surface, this

dorsal restriction suggested that dorsal blastema is a distinct population. But congruent with the uniform blastemal *Lmx1b* expression, single-cell RNA-sequencing found that the nail mesenchyme-derived dorsal blastema is transcriptionally similar to the more scattered bone-derived blastema, indicating that blastema cells are deprived of their original identities and positional values. Instead, spatial constraints or post-transcriptional differences may have governed such a restriction.

Collectively, these studies have advanced our understanding of digit tip regeneration and have provided insights on how nail and dorsal mesenchyme contribute to digit regeneration. New questions are also raised. What are the intrinsic differences between the dorsal and ventral dermis that permit the dorsal mesenchyme to support regeneration? Can the ventral dermis functionally substitute the dorsal dermis if dorsal and/or wound epithelial signals are kept intact? What are the inductive and pro-regenerative signals between the epithelium and the mesenchyme? How are the regenerated tissues patterned if blastema cells are proven transcriptionally equivalent? Addressing these questions and disentangling the roles of nail epithelium and mesenchyme will expand our knowledge of epimorphic regeneration and help derive regenerative cells for translational applications.

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DECLARATION OF INTERESTS

The author declares no competing interests.

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