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Building a Stable Relationship: Ensuring Homeostasis among Cell Types within a Tissue

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Many processes controlling cell growth and death are well characterized for individual cell lineages, but how ensembles of different cell types in a tissue regulate collective size and composition remains unclear. In this issue of *Cell*, Zhou et al. employ experiments and theory to uncover design principles of tissue homeostasis arising from cross-talk between fibroblasts and macrophages.

Tissues contain multiple types of cells that must collectively orchestrate their respective proliferation and apoptosis rates in order to regulate the size and composition of tissue as a whole (Penzo-Méndez and Stanger, 2015). This process of tissue growth and homeostasis is critically regulated throughout development, in the inherent safeguarding against cancer, and in the response to perturbations from disease states, including tissue injury, infection, and expansion and contraction of immune cell populations during the course of infection (Varelas, 2014) (Jameson, 2002). We lack, however, a deeper understanding of how the proliferative behaviors of individual cell types give rise the emergent properties of the tissue. In this issue, Zhou et al. combine experimental and theoretical tools to elucidate design principles underlying multi-lineage population homeostasis (Zhou et al., 2018).

To do this, the authors distilled the notion of the tissue into a fundamental cellular circuit consisting of two archetypal classes of cells. Some cell types in tissues are highly abundant, such as fibroblasts, and their numbers are primarily bounded by extrinsic properties of the tissue, such as physical (e.g., spatial, mechanical) or energetic (e.g., oxygen, pH, glucose) constraints. These limitations are analogous to the concept of "carrying capacity" from evolutionary biology, which quantifies the maximum number of individuals that can inhabit an environment based on available resources and scale (Korolev et al., 2014). These "host" cells are in coexistence with smaller "accessory" populations that provide critical support functions but exist in small numbers far from the carrying capacity. The authors use this host-accessory relationship as a powerful reductionist conceptual model for understanding some of the basic dynamics of tissue homeostasis (Figure 1A).

Here, fibroblasts and macrophages—two cell types that exist in most tissues—were used as the host population and accessory population, respectively (Davies et al., 2013). The authors screened for molecular channels of communication between the two types by looking for lineage-restricted growth factors whose receptors are preferentially expressed on the opposite type. The found that fibroblasts can provide growth signals to macrophages via Csf1 (sensed by its cognate receptor Csf1r), while, reciprocally, macrophages can provide growth signals to fibroblasts via PDGFs (sensed by their cognate receptors PDGFrs). When the two cell types are co-cultured, they form a signaling circuit that remarkably tends to maintain the two populations in a fixed ratio in culture, largely independent of starting ratio.

How do these individual, seemingly simple signaling components assemble to create a stable circuit in tandem? There could potentially be a large space of different feedback and regulatory loops (Figure 1A) that dynamically modulate the effects of these signals. The authors leveraged quantitative mathematical modeling to find inroads into

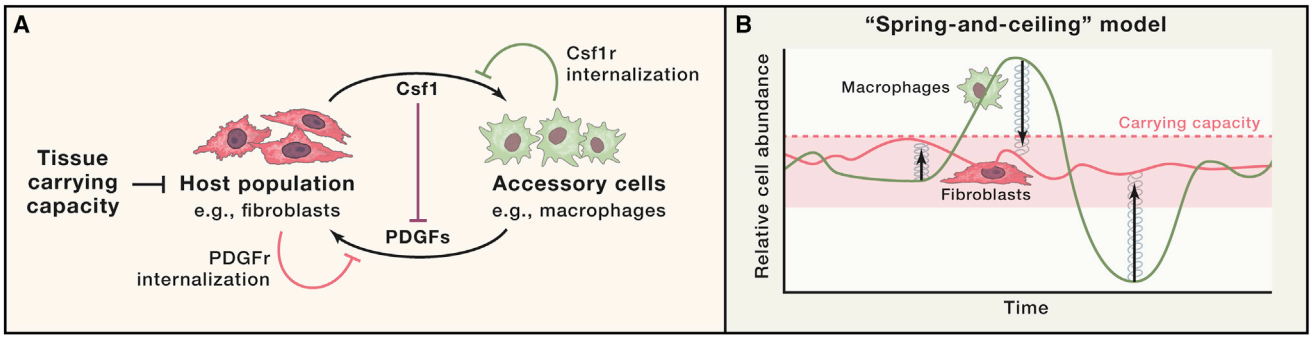

Figure 1. Design Principles of Tissue Homeostasis

(A) Conceptual model of homeostasis between two archetypal cell types in a tissue. "Host" populations (red) are abundant and limited by tissue carrying capacity, such as fibroblasts. These coexist with various smaller populations of cells with support functions, or "accessory" cells (green), such as macrophages. The two types interact through secretion of growth factors, such as Csf1 and PDGFs, controlled by self- and cross-regulation.

(B) "Spring-and-ceiling" mechanism predicted by theory and confirmed with experiment. Populations of host cells like fibroblasts are pinned to the carrying capacity of the tissue ("ceiling"). They tether the size of accessory populations which fluctuate ("spring").

possible mechanisms of stability (Barillot et al., 2013). They created a variety of circuits *in silico*, representing all possible permutations of self- and cross-regulation, both positive and negative, on each signaling component and screened these circuits for their ability to lead the behavior described above, wherein mixed populations tended to approach a fixed coexisting state as long as a critical starting density existed. This property was only found in roughly a third of configurations.

Taking these successful configurations together, it became clear that stability can only be maintained when the host population is at carrying capacity and serves to negatively regulate the accessory population size. Metaphorically, the host is anchored to the "ceiling" that is the carrying capacity, and the accessory population is in turn tethered to the host population by a dynamic "spring" mediated by negative feedback on growth factor availability. This "spring-and-ceiling" design principle can generally describe how two such populations can maintain a stable circuit when growing together (Figure 1B).

Importantly, the model generated testable hypotheses regarding the requirements for population stability. For example, the observed stability requires a mechanism that restrains the effect of growth factors on the macrophages. This could either arise from self-regulating behavior of each cell type or from cross-regulation—the action of one cell type on the other. The authors investigated this experimentally by measuring the dynamics of receptor expression and growth factor abundance after transient stimulation with each growth factor type. They determined that both receptor types are internalized rapidly on stimulation, and the cognate growth factors were consequently depleted as well, indicating self-regulation. Furthermore, Csf1 had a secondary effect on macrophages aside from eliciting growth: to downregulate expression of PDGFs, leading to cross-regulation (Figure 1A). These findings were consistent with the model's predictions of the requirements for stability.

By making many measurements of growth rates at different starting levels of cells, the authors were able to experimentally reconstruct the theoretical "phase portrait" of the system, i.e., its evolution from a variety initial conditions. This recapitulation confirmed that the spring-and-ceiling model is biologically relevant for this simplified tissue system. The use of Csf1-deleted fibroblasts further confirmed the dependency on this growth factor. Interestingly, microscopy revealed that these signaling exchanges between cells types is contact mediated despite involving diffusible factors, suggesting that spatial effects and local concentrations may play a decisive role in tissue dynamics.

Highly simplified experimental systems can be instructive for the uncovering of fundamental circuit behaviors that underlie tissue organization (Velazquez et al., 2018). This approach brings to bear not only the wealth of cellular *in vitro* methods but also quantitative modeling techniques. By continuing to apply such approaches, it may be possible to further dissect tissue level homeostasis at the same level of detail as seen in intracellular studies.

Still, this reductionist approach is not without limitations. *In vitro* culture systems naturally have some shortcomings in recapitulating native multicell-type environments: culture media may not reflect the panel of growth factors and inhibitors present in the cells' native milieu; setting the experimental carrying capacity, whether it be from physical or energetic constraints, is not well defined. Finding an animal system for studying these dynamics in a quantitative manner will be difficult with currently available techniques. The approach of having two cell types is only a stepping stone to more sophisticated experimental systems with more cell types.

There are other broad questions that could be explored in multicellular circuits that are not focused only on population size homeostasis. How do multiple cell lineages work together in a tissue environment to allow phenotypic changes in cells, such as during patterning or the maturation of stem cells in specific tissue niches (Ehninger and Trumpp, 2011)? And perhaps most importantly, how do these dynamical properties shape the final function of the tissue for the organism? Answering these questions will require a meeting in the middle of traditional phenomenological top-down and newer quantitative bottom-up

approaches, such as those elegantly deployed in this study.

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Sort Your Self Out!

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Discrimination between viral and self-derived nucleic acid species is crucial in maintaining effective antiviral immunity whilst avoiding autoinflammation. Ahmad et al. and Chung et al. delineate the consequences of MDA5 gain of function and loss of ADAR1 activity, highlighting the blurring of the concept of self and non-self when considering endogenous retroelements.

Aicardi-Goutières syndrome (AGS) is the name given to a severe inflammatory neurological disease first described in 1984. While there is a robust association between enhanced type I interferon signaling and affected status, AGS is genetically heterogeneous and can occur due to mutations in proteins involved in RNA sensing (MDA5) or DNA and RNA processing (TREX1, the RNase H2 complex, SAMHD1, and ADAR1). As AGS's clinical phenotype is reminiscent of *in utero* acquired congenital infection, and given the central role of viral nucleic acid in interferon induction, it has been hypothesized that the aberrant recognition of self-derived nucleic acids as non-self may underlie disease pathogenesis. However, the precise nature of self DNA and RNA that might trigger an inappropriate interferon response in AGS has remained unclear (Stetson et al., 2008; Crow and Manel, 2015).

In this issue of *Cell*, Sun Hur and colleagues utilize knowledge of the effects of AGS-causing gain-of-function (GOF) mutations in MDA5 to explore the biology of this cytosolic double-stranded RNA (dsRNA) sensor. First, they show that mutation-associated enhanced interferon signaling is dependent on the ability to bind an endogenous ligand, not due to constitutive activation of the receptor (Ahmad et al., 2018). As MDA5 binding can protect agonist dsRNA from RNase digestion, they could then sequence MDA5 ligands and demonstrate that Alu-Alu inverted repeats (IR-Alus), largely derived from the 3′ UTR of retrotransposition-incompetent RNA polymerase II (pol II) transcripts, are the primary endogenous ligand of mutant MDA5. Alu is a ~300-nucleotide-long retroelement that constitutes ~10% of the human genome. In a series of elegant experiments, Ahmad et al. show that up to 25% of cytosolic Alu RNA is in the form of Alu-Alu hybrids. However, the reason for Alu being the primary ligand for MDA5 is not just because it is abundant, but also because of its high level of sequence conservation, making IR-Alu more likely to assemble in a hairpin structure based on complementarity.

Importantly, Alu:Alu hybrids are post-transcriptionally modified by ADAR1, which converts adenosine to inosine (A-to-I)—the most common type of RNA editing in humans—thereby weakening dsRNA integrity. Ahmad et al. show that A-to-I edits render IR-Alus immunologically inert with respect to wild-type (WT) MDA5 due to mismatches and bulges within the duplex (Figure 1). In contrast, mutant MDA5 is indifferent to these modifications. Importantly, AGS has been shown to result from either MDA5 GOF or hypomorphic mutations in ADAR1. Using ADAR1-KO (knockout) cells, Hur and colleagues demonstrate that WT MDA5