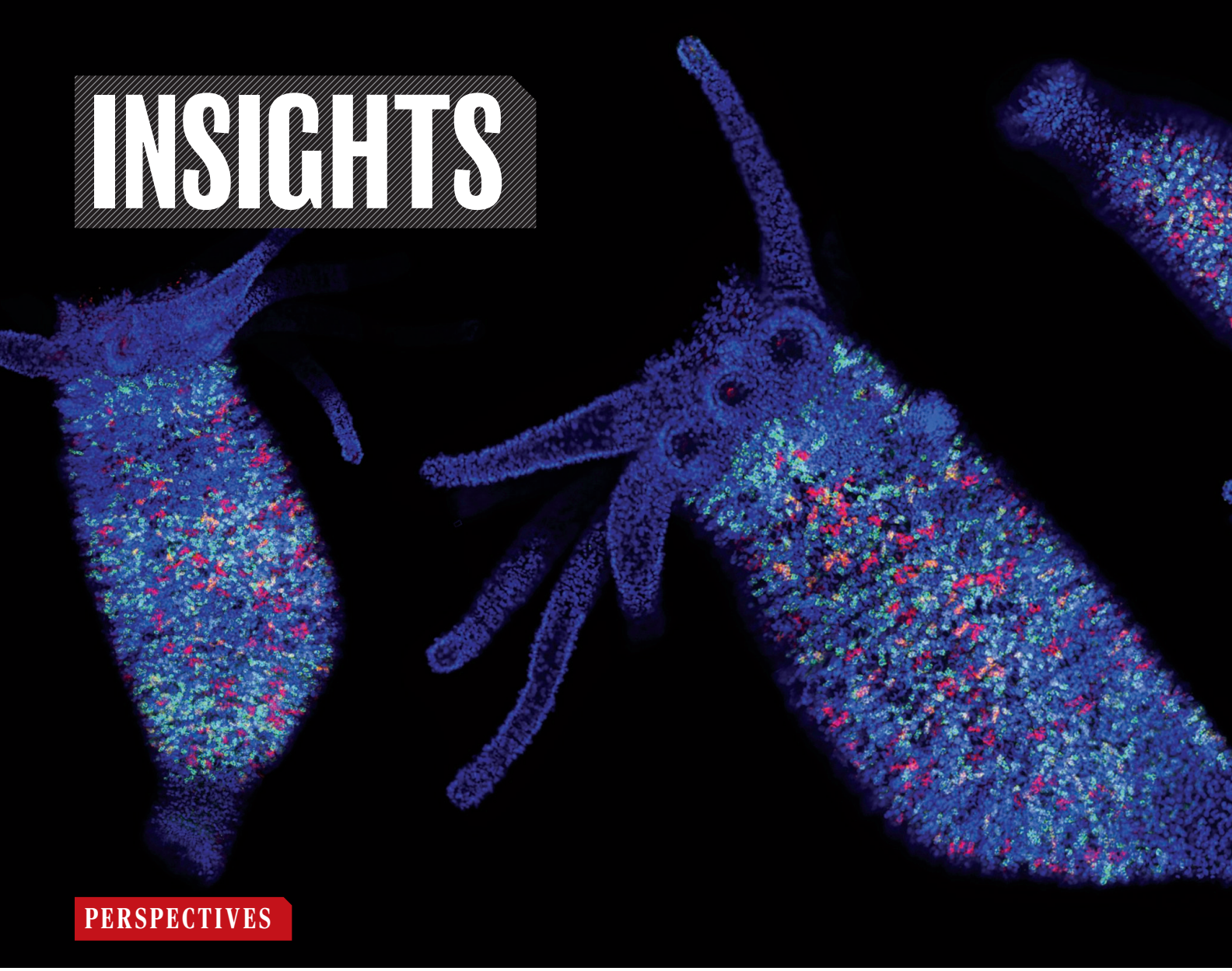


REGENERATION

The cells of regeneration

Single-cell RNA sequencing identifies cell types with central roles in regeneration

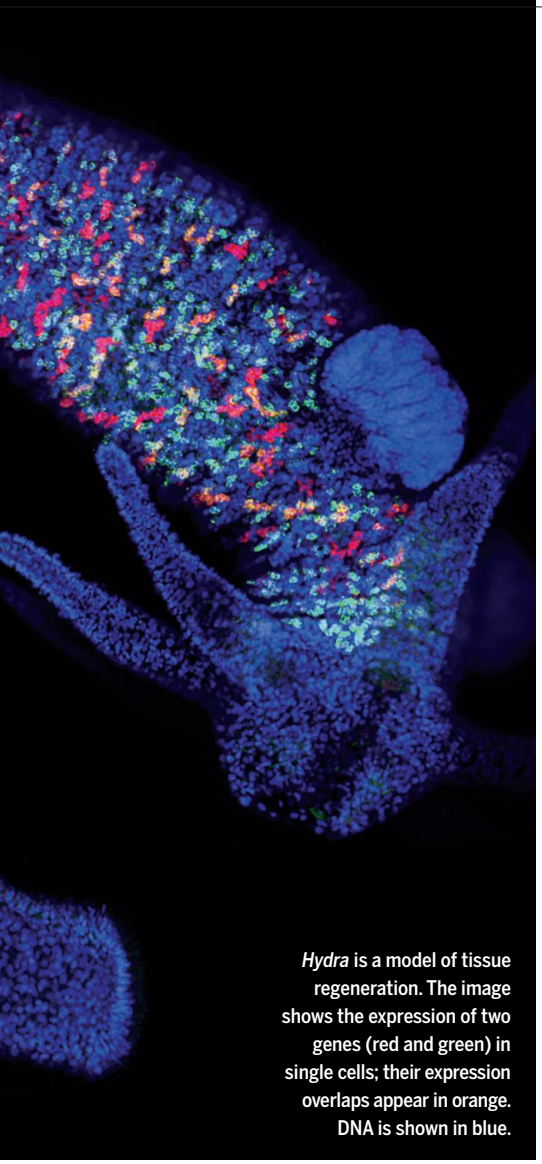
By **Peter W. Reddien**

The ability to regenerate missing body parts is a prominent feature of many animals. Investigation into the cellular and molecular basis of regeneration using highly regenerative model organisms should identify principles that explain how regeneration can occur and might clarify why such regenerative capacity is limited in humans. *Hydra* are freshwater cnidarians capable of whole-body regeneration after amputation. On page 341 of this issue, Siebert *et al.* (*1*) report a cell type transcriptome atlas of all major cell types of *Hydra*, generated by single-cell RNA sequencing (scRNA-seq). In another recent study, Aztekin *et al.* (*2*) used scRNA-seq to study *Xenopus* tadpole tail regeneration and identified a cell that regulates regeneration, which they call the regeneration-organizing cell (ROC). These sequencing approaches provide a wealth of molecular data that should enable future dissection of regeneration mechanisms.

scRNA-seq is a transformative technology that can rapidly identify the transcriptomes of thousands of cells. This approach is being applied to the many unique problems of regeneration, including the identity of adult progenitor cells that generate new tissue and the regulatory mechanisms that orchestrate the formation of correctly organized new adult tissues.

In animals with a high degree of cell turnover as well as regenerative ability, such as *Hydra*, scRNA-seq of uninjured animals can capture the transcriptomes of all differentiated cell types, the progenitor and transition states that generate these cells, and the constitutively active positional information (i.e., the genes that regulate regional tissue identity and pattern) guiding the process, all in a single animal stage. Such progenitor cells and positional information can also be involved in regeneration. This approach has been applied to planarians (a type of flatworm) (*3*, *4*). Planarians possess a population of pluripotent stem cells called neoblasts that generate all differentiated cell types in tissue turnover and regeneration.

PHOTO: STEFAN SIEBERT/YASHODARA ABEYKOON/JULIANO LAB AT UC DAVIS



Hydra is a model of tissue regeneration. The image shows the expression of two genes (red and green) in single cells; their expression overlaps appear in orange. DNA is shown in blue.

scRNA-seq defined the genes expressed in subsets of neoblasts, including those that can be functionally pluripotent (*5*) and others as they transit to differentiation (*3*–*5*). Planarian positional information is prominently harbored in muscle, and scRNA-seq identified genes regionally expressed in muscle that are associated with adult tissue patterning and regeneration (*3*, *6*).

scRNA-seq can also be applied to stages of regeneration after injury. For example, the cell type architecture of the transcriptional response to wounds in planarians was determined with scRNA-seq (*7*). Axolotls (a type of salamander) can replace lost limbs after injury. scRNA-seq in axolotl limb regeneration revealed that heterogeneous connective tissue cells change state toward a more homogeneous pool of multipotent progenitors (*8*). In zebrafish, scRNA-seq characterized transcriptomes for hair cell progenitors (*9*) and was combined with simultaneous cell-clone tracing to identify transcriptomes for lineage-restricted progenitors in fin regeneration (*10*). In mouse digit tip regeneration, scRNA-seq identified nerve mesenchymal populations that act as progenitors for connective tissues (*11*). Moreover, the transcriptomes of regulatory cells in vertebrate regeneration identified genes that can be studied to understand how such cells promote regrowth. For instance, the epidermis that covers an injury, the wound epidermis, has an essential role in regeneration and has been subjected to scRNA-seq in axolotls (*12*).

The regenerative feats of *Hydra* have been studied since the 1700s. *Hydra* cell turnover involves two populations of dividing myoepithelial cells (endoderm and ectoderm) and interstitial stem cells (ISCs). The myoepithelial cells divide in the body column and displace cells toward the oral and aboral ends, where they are shed. The multipotent ISCs produce a diversity of cell types, including neurons, gland cells, nematocytes (stinging cells that typify cnidarians), and germ cells (when depleted). Siebert *et al.* sequenced the transcriptomes of ~25,000 cells from *Hydra* polyps. In addition to enabling investigation of *Hydra* regeneration, this resource, together with recent scRNA-seq of the cnidarian *Nematostella vectensis*, a sea anemone (*13*), will enable molecular investigation of the fascinating cell biology of cnidarians, the comparison of gene roles across organisms, and study of the evolution of cell types and cell ensembles.

By arranging *Hydra* epithelial cells on the oral-aboral axis according to gene expression on a predicted trajectory, Siebert *et al.* explored constitutive regional gene expression that might constitute adult positional information. This approach identified spatially regulated gene expression patterns that matched predictions from prior work and for numerous previously unstudied *Hydra* genes. These genes, including potential Wnt, bone morphogenetic protein (BMP), and fibroblast growth factor (FGF) pathway regulators, are good candidates to regulate body plan maintenance and regeneration.

Siebert *et al.* identified a set of cells largely defined by the absence of expression of differentiation markers and proposed that these represent ISCs. ISCs are important for understanding *Hydra* regeneration and the evolution of regeneration. One ISC marker gene (*Hy-icell1*) and a gene activated in both putative transition states from ISCs (*HvSoxC*) could enable future visualization and isolation of these cell states for characterizing the molecular processes that underlie state transitions in multipotent regenerative progenitors. Analyses indicated that nematocyte progenitors and a shared neuronal and gland cell progenitor are produced from ISCs. The interpretation that gland cells and neurons share a common transitional cell state was unexpected. The proposed branched-tree trajectory for ISC-derived cell types is potentially important for understanding the mechanisms of multipotency restriction during differentiation and for understanding the origins of different cnidarian cell types. One challenge in determining cell lineage from trajectory analyses using transcriptomes is that different conclusions can be reached depending on where the trajectory is inferred to start. Siebert *et al.* supported their model by validating that a marker of the candidate common neuron/gland progenitor (*Myb*) was coexpressed with both neuronal and gland markers. This model can be further tested using cell-tracking tools and with gene function investigations.

Large-scale cell transcriptome studies harbor myriad fascinating features. For instance, *Hydra* gland cells, like epithelial cells, are proposed to undergo position-dependent gene expression changes as they transit orally or aborally during cell turnover and to transdifferentiate (e.g., from zymogen to granular mucous gland cells) (*14*). In accordance with this model, position-dependent gene expression changes in gland cells were predicted from trajectory analyses. Interestingly, some changes included genes involved in oral-aboral axis patterning (e.g., *Wnt1*, *Wnt3*), raising the question of whether gland cells contribute to axis pattern.

The African clawed frog *Xenopus laevis* is a classic model of embryology and presents an intriguing case of regeneration that shows stage specificity. Tadpole tails can regenerate in regeneration-competent developmental stages, but not in a regeneration-incompetent stage (*15*). Aztekin *et al.* used these stages and scRNA-seq to identify cells with a regulatory role in promoting regeneration.

An epidermal cell, the ROC, was found to exist at regeneration-competent tail wounds but not at regeneration-incompetent ones. ROCs also exist at the midline edge of uninjured tadpole tails. Existing tail ROCs spread over the wound after injury, as opposed to this cell state being induced in the wound epidermis after injury. Genetic ablation of ROCs blocked regeneration, supporting a model in which ROCs promote regeneration.

The identity and transcriptome of ROCs raise many questions and opportunities for future work. The localization and function of ROCs during blastema growth, after the initial 24 hours of wound repair, is not well resolved and could be a target of future

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study. The ROC transcriptome should enable exploration of the roles for the varied signaling molecules expressed in these cells in regeneration-initiating events. ROCs have molecular similarity to the apical ectodermal ridge (AER) that promotes limb growth in many organisms, and are present early in development at the tail bud tip, raising the question of whether a role for these cells in promoting tail development (analogous to the AER) is linked to their role in regeneration. Comparing the roles of ROCs versus other cells in regulating specific regenerative events will also be important. Finally, it will be important to explore how the roles of ROCs compare to the molecular and cellular roles of the wound epidermis in adult vertebrate appendage regeneration.

ROCs exist in the uninjured epidermis of tadpoles at the regeneration-incompetent stage. However, their absence from wounds 24 hours after amputation at this stage raises the possibility that failed ROC wound coverage explains failed regeneration. Additional work will be important for testing this possibility. Determining what explains the difference in the behavior of ROCs between regeneration-competent and -incompetent stages is an interesting direction. Regeneration ability is naturally regained in tadpole tails after a refractory period (*15*), and it will also be of interest to examine ROCs at this later stage.

scRNA-seq can readily be applied to traditional model systems as well as understudied organisms with important and fascinating biology. The resources generated can fuel molecular inquiry into a vast array of questions. The cells of regeneration in many organisms will continue to be profiled with scRNA-seq, opening the door to a myriad of molecular and cellular experiments aimed at revealing the logic of regeneration. ■

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IMMUNOLOGY

Immune control of the microbiota prevents obesity

Specialized T cells regulate the composition of the intestinal microbiota, with important consequences for obesity

By **Yuhao Wang**[1] *and* **Lora V. Hooper**[1,2]

Obesity is a complex disease that is influenced by a mixture of genetic, behavioral, and environmental variables, such as the ability to metabolize nutrients, how much food is eaten, and diet composition. Adding to the complexity are the diverse communities of intestinal bacteria—known as the microbiota—that can influence whether obesity develops or not. But which species of gut bacteria predispose to obesity and which protect against it? And how do these bacteria become established in the gut? On page 340 of this issue, Petersen *et al.* (*1*) show that the immune system promotes gut colonization by bacteria that protect against obesity. The findings reveal how defective immune control of the microbiota can provoke metabolic disease.

The gut microbiota helps humans digest food by breaking down complex carbohydrates that come from plants. But the metabolic functions of the microbiota go beyond simple digestion. Microbiologically sterile (germ-free) mice accumulate less body fat than conventionally reared mice that have a microbiota, indicating that the microbiota promotes the storage of body fat (*2*, *3*). Moreover, the microbiota composition is starkly different when comparing lean and obese mice (*4*), and notably, lean and obese humans (*5*). But are these differences in microbiota composition truly a cause of leanness or obesity?

Remarkably, metabolic phenotypes can be transmitted through the microbiota, similar to an infectious disease. When the fecal microbiota from an obese mouse or human is transplanted into a germ-free mouse, the recipient mouse eventually becomes obese (*6*, *7*). Similarly, the fecal microbiota from an underweight mouse or human transplanted into a germ-free mouse will cause that mouse to become underweight (*6*, *7*). These findings show that different assemblages of bacteria can indeed predispose to obesity or leanness, indicating a cause-and-effect relationship between microbiota composition and body composition.

Given that differences in microbial communities can predispose to leanness or obesity, what determines the types of bacteria that inhabit the gut? Several studies, including that of Petersen *et al.*, indicate that the immune system is a key factor. Indeed, the intestines of both humans and mice are full of immune cells, including T and B cells, agents of the adaptive immune system that confer long-term, specific immunity to microorganisms. In particular, intestinal B cells produce large quantities of immunoglobulin A (IgA) antibodies. IgA antibodies are secreted into the gut lumen where they stick to the surfaces of gut bacteria, and through mechanisms that are not yet fully understood, help to determine which bacteria colonize the gut and which are excluded (*8*).

Petersen *et al.* discovered that immune cells that promote IgA production also protect mice from obesity (see the figure). T follicular helper (T_{FH}) cells are specialized T cells that reside in lymphoid follicles–specialized regions within lymph nodes that contain developing B cells. In the follicles, T_{FH} cells directly interact with B cells and induce genetic changes, known as class-switching, which prompt the B cells to produce IgA. This same research group previously discovered that a signaling pathway, involving the myeloid differentiation primary response protein 88 (MyD88), was needed for this "helper" function of T_{FH} cells (*9*). When the *Myd88* gene was deleted in mouse T cells, T_{FH} cells failed to develop properly and very little gut IgA was produced. Consequently, the gut microbiotas of these T-*Myd88*$^{-/-}$ mice were quite different from those of wild-type mice (*9*).

Petersen *et al.* watched the T-*Myd88*$^{-/-}$ mice age and in doing so, acquired some interesting metabolic insights. Although aging wild-type mice remained lean, the aging T-*Myd88*$^{-/-}$ mice became obese. Further, the researchers could accelerate the development of obesity if they fed mice a high-fat,

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