which is responsible for converting organic fuel molecules (e.g., glucose) and oxygen into carbon dioxide, water, and energy—a mutation in one of these enzymes, the 2-oxoglutarate dehydrogenase enzyme (*sucA*), is sufficient to not only abolish this up-regulation but also confer carbenicillin resistance. These findings reinforce that antibiotic efficacy is intimately linked to the cell's metabolic state.

These analyses suggest that rewiring of central metabolism may be a general strategy to acquire antibiotic resistance, but the scope of metabolic changes resulting from these mutations remains to be systematically unraveled. One hypothesis emerging from this study is that different metabolic mutations can converge to similar adaptive changes and provide resistance to antibiotics with largely different modes of action. Hence, although individually at lower frequency than classical resistance mutations, it is possible that different metabolic mutations can play the same role in mediating antibiotic resistance (see the figure).

Advances in technologies that enable the monitoring of dynamic metabolic changes in response to genetic and environmental perturbations combined with computational models of cellular metabolism will help researchers to investigate the common scope of diverse metabolic mutations in antibiotic resistance (*3*, *5*, *9*, *10*). Predicting how mutations affect the metabolic state of cells, alter drug action, and ultimately shape the fitness landscape of resistance mutations will be of paramount importance to leverage this understanding in the optimization of treatment regimens and the discovery of new drugs and combination therapies. Small molecules that interfere with respiratory and fermentative metabolism might improve the killing efficacy of classical antibiotics (*4*, *11*) and hamper the evolution of resistance by changing the fitness landscape of antibiotic resistance (*3*, *5*, *6*). From the data and analysis presented by Lopatkin *et al.*, mechanisms by which pathogenic bacteria can become resistant in vivo can be unraveled. ■

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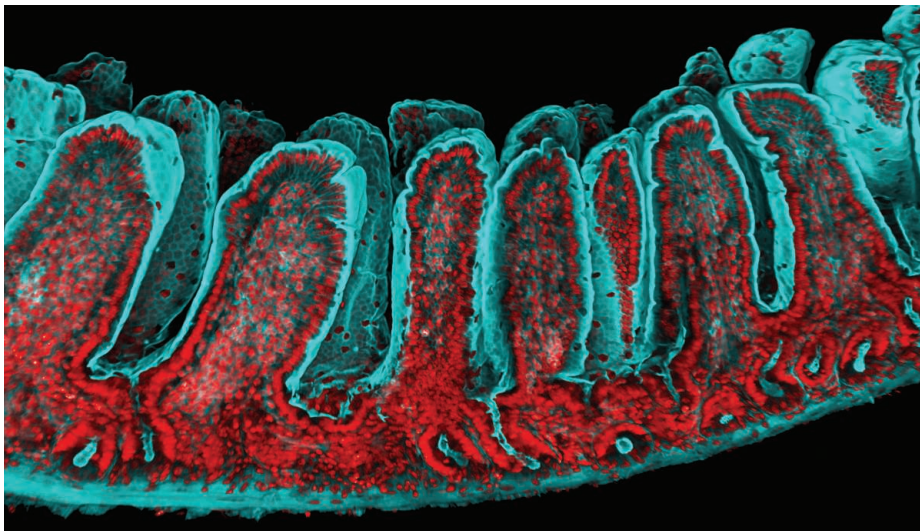
ACKNOWLEDGMENTS

I thank V. Chubukov and K. Ortmayr for helpful feedback.

10.1126/science.abf7922



A cross section of mouse small intestine, showing intestinal crypts and villi, is visualized with immunofluorescence microscopy (nuclei in red, and F-actin, which marks the cytoskeleton, in blue). Intestinal stem cells reside at the base of crypts, where they maintain cell turnover.

PHOTO: FLORIJN DEKKERS AND ANNE RIOS

STEM CELLS

Tissue regeneration: Reserve or reverse?

Stem cells recover from injury by tissue dedifferentiation, not from dedicated reserves

By **Ramesh A. Shivdasani[1,2,3], Hans Clevers[4,5,6], Frederic J. de Sauvage[7]**

Tissues with high intrinsic turnover, such as the skin and intestinal lining, rely on resident stem cells, which generate all native cell types. Intestinal stem cells (ISCs) are highly sensitive to damage, although they recover quickly. It is unclear whether this recovery (i.e., regeneration) occurs from less sensitive pools of "reserve" stem cells (*1*) or whether ISC progeny undergo "reverse" differentiation into stem cells (*2*). Recent studies in diverse organs highlight that dedifferentiation of specified cell types is a pervasive and dominant means for tissue regeneration. The findings have broad implications because all tissues experience some cell attrition over a lifetime, and knowing how tissues replenish those losses may help in preventing or treating organ failure. Moreover, it remains unclear whether incomplete differentiation, a common feature of cancer, reflects normal tissue plasticity, and it is unclear whether stem cells that arise by dedifferentiation may spawn cancers.

ISCs expressing leucine-rich repeat–containing G protein–coupled receptor 5 (*Lgr5*) lie at the bottom of small bowel crypts (*3*). In the course of homeostatic tissue turnover, their immediate progeny adopt alternative enterocyte or secretory fates, then fill the crypts with replicating progenitors that migrate away from ISCs. Cell division ceases at the crypt tops, where postmitotic cells begin a 3- to 5-day journey along intestinal villi. When ISCs sustain irreparable damage, some source in the crypt must regenerate new ISCs. Other adult epithelia—such as airways, prostate, and liver—are organized differently from the intestine and from each other (see the figure). These epithelia also restore cells lost by damage or attrition, even though at rest they turn over at least a hundred times more slowly than the intestinal lining.

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Airway epithelial structure varies from trachea to small bronchioles, and distinct progenitors in different segments produce assorted secretory and ciliated cell types. In the lining of human and mouse upper airways, flat basal cells lie beneath a layer of columnar differentiated cells and adjacent to submucosal myoepithelial glands. Stem cell activity in normal tissue turnover maps to a subpopulation of keratin 5 (*Krt5*)–expressing basal cells (*4*). The trachea and bronchi are vulnerable to diverse injuries, including targeted destruction of Krt5$^+$ stem cells and pervasive mucosal damage from noxious inhalants or viruses.

Adult human and mouse prostate glands also contain columnar luminal and flat KRT5$^+$ basal cells. Distinct unipotent progenitors maintain both populations, and castration induces massive luminal cell loss. Androgen reexposure restores prostate mass within weeks, which implies the presence of castration-resistant progenitors. However, an unequivocal stem cell pool has not been identified. The liver also has notable regenerative abilities after chemical or surgical injury. The emerging consensus is that this organ lacks a dedicated stem cell compartment and recovers from damage through dedifferentiation of mature hepatocytes and biliary cells (*5*, *6*).

Stem cell activity in vivo is demonstrated most persuasively by introducing into a tissue a permanent color or fluorescent label whose expression depends on Cre recombinase–mediated excision of a STOP cassette. When Cre activity is restricted to stem cells, all the progeny of those cells exclusively carry the label. ISCs and tracheal stem cells were thus identified because targeted Cre activity in LGR5$^+$ or KRT5$^+$ mouse cells labeled the respective full lineages (*3*, *4*). Investigation of tissue regeneration requires ablation of a stem cell compartment, followed by tracking of the restored ability to produce sufficient numbers of all native stem cell progeny. The canon of tissue repair rests heavily on such lineage-tracing experiments, but one limitation is that Cre recombinase is not often confined to a single defined cell type. This challenge lies at the heart of competing models for tissue recovery after lethal cell injuries.

Dividing cells take up labels such as [^{3}H]thymidine or fluorescent histone 2B and shed these labels as they replicate further or their daughters die. In the intestine, however, rare cells located near the fourth tier from the crypt base retain [^{3}H]thymidine for weeks. Given once-popular ideas that stem cells must be few in number and retain one "immortal" DNA strand when they replicate, +4 label-retaining cells (LRCs) were described as ISCs. In support of that idea, lineage tracing from *Bmi1*, a locus thought to be restricted to nonreplicating +4 LRCs, elicited an ISC-like response in vivo (*7*).

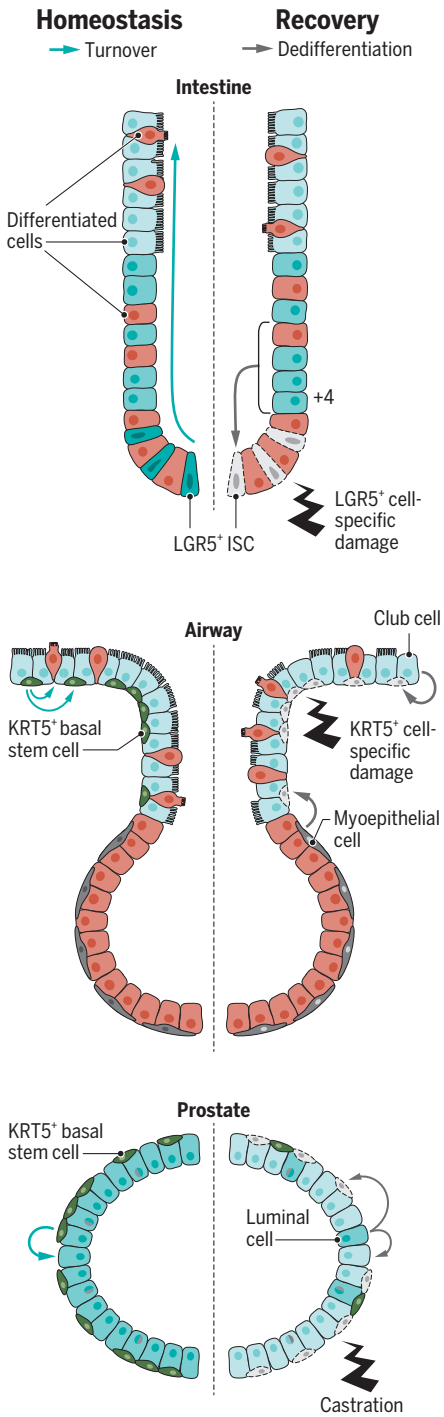
To reconcile the evidence for ISC properties in both LGR5$^+$ crypt base columnar cells (CBCs) and +4 LRCs, researchers postulated that abundant CBCs serve as frontline ISCs, whereas the smaller +4 LRC population contains dedicated reserves. Indeed, intestinal turnover is unperturbed when LGR5$^+$ CBCs are ablated because other crypt cells' progeny continue to repopulate villi and an LGR5$^+$ ISC compartment is soon restored (*1*). Multiple candidate markers of +4 LRCs that regenerate ISCs after injury have been proposed (*8*). Although these cells are too few to explain the typical scale and speed of ISC restoration, the prospect of two stem cell pools carried the additional allure of a sound adaptive strategy in a tissue that requires continuous self-renewal.

ISC differentiation is, however, not strictly unidirectional. Cre expression in absorptive or secretory cell types tags those cells selectively, but upon ablation of LGR5$^+$ CBCs, the label appears throughout (*9*). These observations imply that differentiated daughter cells have reverted into ISCs. Moreover, *Bmi1* expression was found to mark differentiated crypt endocrine cells (*10*), and putative +4 markers are expressed in many crypt cells including LGR5$^+$ CBCs. Accordingly, when Cre is expressed from these loci, the traced lineage might simply reflect CBC activity in resting animals and reverse differentiation of crypt cells after ISC ablation. But is dedifferentiation a rare and physiologically inconsequential event or the predominant mode of stem cell recovery? Dedifferentiation may obviate the need to invoke a dedicated reserve population, or it is possible that ISC recovery may reflect both dedifferentiation and contributions from a reserve stem cell population.

To investigate these issues, researchers activated a fluorescent label in LGR5$^+$ CBCs and waited for this label to pass into progeny cells before ablating CBCs (*11*). Thus, only the CBCs that recover by dedifferentiation should be labeled, and any cells arising from reserve ISCs should not. Nearly every restored crypt and CBC was fluorescent, with substantial contributions from both enterocytes and secretory cells (*11*). Cells captured early in the restorative process coexpressed mature-cell and ISC genes, which is compatible with recovery by dedifferentiation. Another study found that damaged ISCs are reconstituted wholly by the progeny of LGR5$^+$ CBCs (*8*). Thus, dedifferentiation would seem to be the principal mode of ISC regeneration, and prior conclusions about +4 ISCs likely reflect unselective Cre expression.

Stem cells in homeostasis and recovery

Physiologic cell turnover and recovery from injury occur from different cellular sources in diverse epithelia (intestine, upper airway, and prostate gland). Homeostatic turnover is driven by the stem cell pool, and tissue restoration from injury occurs through transient expansion and dedifferentiation of specified mature cells.



ISC, intestinal stem cell; KRT5, keratin 5; LGR5, leucine-rich repeat–containing G protein–coupled receptor 5.

GRAPHIC: KELLIE HOLSKI/*SCIENCE*

Different tissues might deploy distinct regenerative strategies, and recent studies in mouse airway, prostate, intestinal, and liver epithelia provide insightful lessons. After ablation of KRT5$^+$ airway stem cells, specified secretory and club cell precursors were found to undergo clonal multilineage expansion and accounted for up to 10% of restored KRT5$^+$ cells in vivo (*12*). Chemical or viral damage was subsequently reported to induce migration and dedifferentiation of submucosal gland myoepithelial cells into the basal layer to reconstitute the surface lining, including KRT5$^+$ stem cells (*13*). Thus, dedifferentiation into native stem cells occurs upon injury to both airway and intestinal linings in mice.

Single-cell RNA sequencing (scRNA-seq) analysis of mouse prostate glands recently revealed distinct gene expression profiles in ~3% of luminal cells, which are more clonogenic than others, express putative stem cell markers, and hence qualify as a pool enriched for native stem-like cells (*14*). After androgen reexposure following castration, however, the scale and distribution of cell replication and the location of restored clones were incompatible with an origin wholly within that small pool. Rather, the principal source of gland reconstitution in vivo, including new KRT5$^+$ basal cells, was the dominant population of differentiated luminal cells (*14*). These observations parallel those in the liver, where recovery of organ mass after tissue injury occurs by renewed proliferation of mature resting hepatocytes (*5*), abetted by expansion of bile duct cells that transdifferentiate into hepatocytes (*6*). Cell plasticity is thus widespread, whether tissues have or lack native stem cell compartments.

Reverse differentiation in the intestine, airways, and prostate gland was generally observed after near-total elimination of resident stem or luminal cells, an extreme and artificial condition. However, several observations suggest that this dedifferentiation reflects a physiologic process designed to maintain a proper cell census. Contact with a single KRT5$^+$ airway stem cell prevents secretory and club cell dedifferentiation in vitro (*12*), and tracheal submucosal glands exhibit limited stem cell activity even in the absence of injury (*13*). Live imaging of intestinal crypts reveals continuous and stochastic exit from and reentry into the ISC compartment (*15*), implying that barriers for differentiation or dedifferentiation are inherently low. However, the primary purpose of dedifferentiating airway, intestinal, liver, and prostate cells is not to enable tissue recovery. Therefore, they should be regarded as facultative stem cells; that is, they have other physiologic functions and realize a latent stem cell capacity only under duress.

This distinction from "reserve" stem cells is not merely semantic. Emphasis in regenerative therapy research belongs on any cell population with restorative potential; in vivo findings now direct attention away from putative reserve cells and toward dedifferentiation as a common means for tissue recovery. The absence of dedicated reserves and the inherent cellular ability to toggle between stem and differentiated states also inform cancer biology. Because mutations realize oncogenic potential only in long-lived cells, both frontline and reserve stem cells represent candidate sources of cancer, in contrast to differentiated cells, which are generally short-lived. However, oncogenic mutations that arise in differentiated cells could become fixed upon dedifferentiation, thus enabling tumor development.

Notably, stem cell properties and interconversion with their progeny are not stereotypic. ISCs divide daily into two identical daughters, whereas hematopoietic stem cell replication is infrequent and asymmetric. Severe loss of blood stem cells does not elicit substantial dedifferentiation and is rescued only by adoptive stem cell transfer. Immature secretory precursors dedifferentiate more readily than terminally mature airway cells (*12*), whereas fully differentiated cells fuel liver and prostate regeneration. Cell plasticity in each case is determined by local signals. Unknown factors from KRT5$^+$ tracheal stem cells, for example, suppress secretory cell dedifferentiation (*12*), and specific factors secreted from the prostate mesenchyme stimulate luminal cell dedifferentiation (*14*). The intestinal mesenchyme probably senses ISC attrition to trigger tissue recovery, but the spatial and molecular determinants remain unknown. Outstanding challenges are to identify the signaling pathways that ensure a stable cell census and to harness diverse regenerative responses to ameliorate acute tissue injuries or prevent organ failure. Knowing the cellular basis for stem cell recovery in different contexts brings us closer to those goals. ■

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10.1126/science.abb6848

CELL THERAPY

Emerging cell therapy for biliary diseases

Gallbladder organoids repair bile ducts in mouse and human liver

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Cell therapy has potential as an alternative to liver transplantation. The liver cell therapy field has been focused on diseases affecting hepatocytes, which provide most of the liver's metabolic and synthetic functions (*1*). Less attention has been paid to biliary diseases affecting cholangiocytes, the cells that form the biliary tree. This system consists of intrahepatic and extrahepatic bile ducts and the gallbladder, and drains bile into the intestine. Because bile is toxic when it accumulates in the liver, biliary diseases can lead to liver fibrosis and failure, necessitating liver transplantation (*2*). Despite progress in bioengineering bile ducts for treating extrahepatic biliary diseases (*3*), few studies have explored cell therapy for diseases affecting intrahepatic bile ducts. Still, evidence is accumulating that supports the feasibility of cell therapy for intrahepatic biliary diseases, and on page 839 of this issue, Sampaziotis *et al.* (*4*) provide the most definitive example to date.

Cell therapy for intrahepatic biliary diseases requires repopulating large stretches of injured bile ducts or forming new ones. As in any emerging field, distinct approaches have been taken to meet these requirements, varying in the source of transplanted cells, recipient characteristics, and route of cell delivery (see the table). Most studies have used primary cells, including expandable cells isolated from the extrahepatic part of the human biliary tree by two-dimensional or organoid culture (*4, 5*). In culture, these cells lose regional specification but maintain core cholangiocyte identity; after engraftment in the liver, they acquire intrahepatic cholangiocyte

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Tissue regeneration: Reserve or reverse?

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Science **371** (6531), 784-786.
DOI: 10.1126/science.abb6848