MICROBIOME

Plant microbiome blueprints

A plant defense hormone shapes the root microbiome

By **Cara H. Haney**[1,2]
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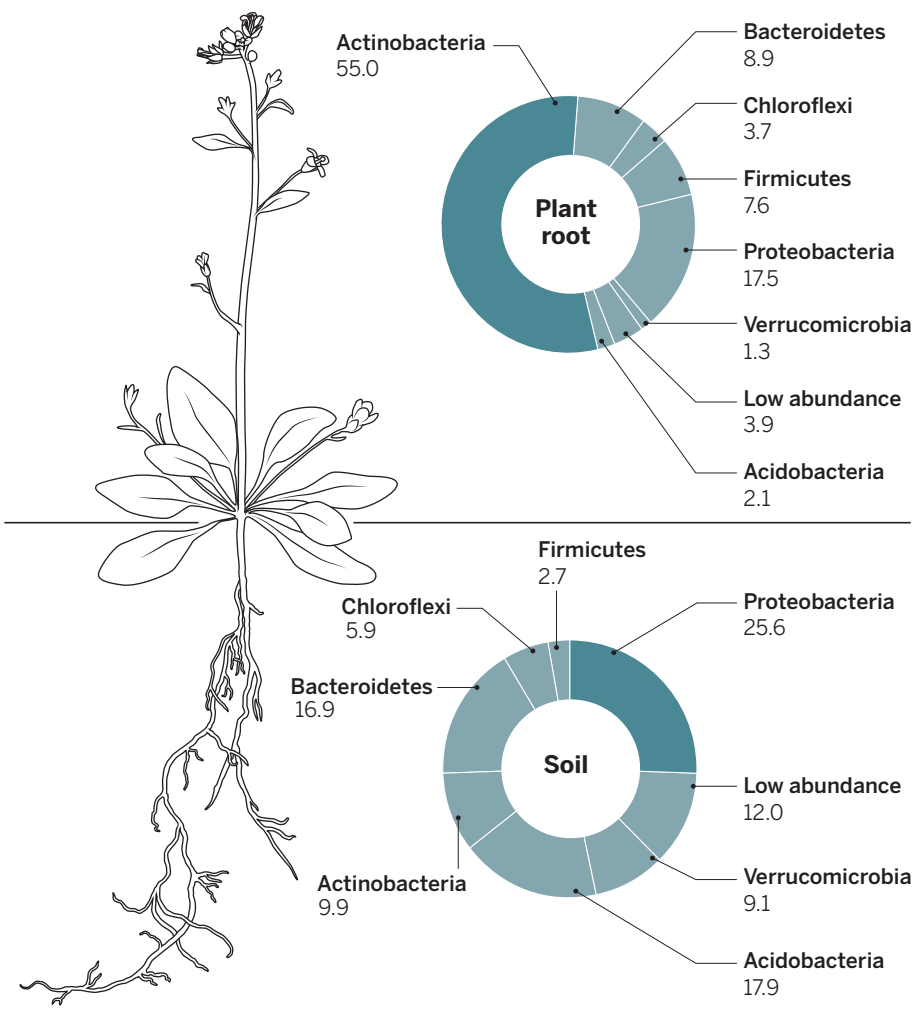
Just as the number of petals in a flower or the number of limbs on an animal follow predictable rules, host-associated microbial communities ("microbiomes") have predictable compositions. At the level of bacterial phylum, the structure of the host-associated microbiome is conserved across individuals of a species (*1*, *2*). The consistency and predictability of host-associated microbiomes—like many of the phenotypes of a particular multicellular organism—suggest that they too may, in part, be under the regulation of a genetic blueprint. Indeed, evidence in animals shows that through production of broad-spectrum antimicrobials, the innate immune system shapes the composition of the gut microbiome (*3*, *4*). On page 860 of this issue, Lebeis *et al.* (*5*) reveal a critical role of the plant hormone salicylic acid in determining the higher-order organization of the root-associated microbiome of the reference plant *Arabidopsis thaliana*.

The plant root system, where nutrients are taken up by the host and exposure to environmental microbes occurs, is functionally analogous to the animal gut. As such, the associated microbial communities perform analogous functions, including aiding with nutrient uptake and protecting the host from pathogens. Correspondingly, the plant innate immune system must simultaneously allow beneficial microbes to survive while limiting the growth of potential pathogens.

The plant hormones salicylic acid, jasmonic acid, and ethylene are key regulators of innate immunity in plant leaves (*6*). Mutants impaired in salicylic acid synthesis and signaling are hypersusceptible to a number of biotrophic pathogens (microbes that colonize the host plant, rather than kill host tissue, to obtain nutrients), whereas mutants impaired in jasmonic acid and ethylene synthesis and signaling are hypersusceptible to herbivorous insects and necrotrophic pathogens (microbes that kill host cells and extract nutrients). Most studies that demonstrate these features of plant hormones have been carried out in leaves with a single or limited number of pathogens. However, the challenge of modulating a community of diverse microbes in plant roots is distinct from that of clearing a relatively limited number of pathogens from inside a plant leaf. Consequently, it is not clear that the same immune mechanisms that control foliar microbes will be effective in regulating root microbiome composition.

Using 16*S* ribosomal RNA sequencing, Lebeis *et al.* profiled the root microbiomes of a panel of *Arabidopsis* hormone mutants impaired in synthesis or signaling of individual or combinations of plant hormones. Specifically, the authors looked at the microbial community in the rhizosphere (the soil adjacent to the root) and in the endophytic compartment of roots (see the figure). The latter refers to bacteria living within plant root tissue as endosymbionts. They found that when salicylic acid signaling was constitutive [in an *Arabidopsis* strain (*cpr5* mutant) that displays hyperimmunity] or disrupted [in a hypoimmune *Arabidopsis* strain (*pad4* mutant)], a reproducible shift in the relative abundance of bacterial phyla in the endophytic compartment occurred. These changes were consistent across many families within the affected phyla, indicating that salicylic acid signaling does not simply modulate the growth of particular bacterial taxa, but may be a key component of the blueprint that determines microbiome community structure.

To complicate matters, the classical plant defense hormones also function in plant growth, metabolism, and responses to abi-



Bacterial profiles. Plant roots consistently enrich for and deplete certain bacterial phyla relative to their abundance in bulk soil. This process depends on salicylic acid synthesis and signaling, indicating that plant hormones regulate the root microbiome composition. Shown are the relative abundance of bacterial taxa in the plant root (endophytic compartment) of *Arabidopsis thaliana* and bulk soil (*5*).

ILLUSTRATION: ADAPTED BY P. HUEY/*SCIENCE*

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otic stresses, obfuscating the precise mechanism by which salicylic acid regulates the root microbiome. By constructing a synthetic microbial community in an artificial soil, Lebeis *et al.* found that salicylic acid produced by the plant had either a direct negative or direct positive effect on the growth of a subset of bacteria in the root microbiome. In one case, salicylic acid directly inhibited the growth of some microbes, whereas in two other cases, the hormone served as a metabolite for bacteria. This finding is consistent with previous work showing that direct application of plant hormones to soil can affect microbial community composition (*7*). Thus, by using a synthetic microbial community, Lebeis *et al.* reveal that the role of salicylic acid in structuring the plant root microbiome might be intertwined with its function in plant physiology and as a microbial catabolite. Future work using similar reductionist approaches will hopefully elucidate the basic mechanisms that shape the rhizosphere and endophytic microbiome. Indeed, defined synthetic microbial communities have helped reveal mechanisms that shape the animal gut microbiome (*8*, *9*).

During plant domestication, humans have selected for traits related to plant improvement for food, fiber, and fuel. However, we have not consciously selected for plant associations with a beneficial microbiome. Lebeis *et al.* have shown that both hyper- and hypoimmune plants have altered root microbiome communities, indicating that selecting for plant disease resistance traits (or the accidental loss of resistance traits through inbreeding) might change the plant microbiome blueprint. The importance of microbial community structure on host health is largely unknown. However, even minor changes in abundance of certain bacteria can have a major effect on plant defenses and physiology, with only minimal effects on the overall structure of the microbiome (*10*, *11*). Understanding the factors that contribute to a healthful microbial community may allow for conscious selection of microbiomes for improvement of crop yields and increased crop resilience in the face of biotic and abiotic stresses. ■

ILLUSTRATION: V. ALTOUNIAN/*SCIENCE*

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10.1126/science.aad0092

CELL SIGNALING

Lipids link ion channels and cancer

Membrane voltage connects lipid organization to cell proliferation

By **Alessio Accardi**

How does membrane voltage control cellular proliferation? This is a key but poorly understood step in understanding how dysregulation of the electrical balance in a cell can lead to uncontrolled proliferation and, eventually, to tumor development. Although the phenomenon is well established (*1*–*3*), the underlying mechanisms have been unclear. On page 873 of this issue, Zhou *et al.* (*4*) show that persistent changes in the resting membrane potential (the voltage across the membrane of a cell), caused by the uncontrolled expression of ion channels, can cause negatively charged lipid in the inner membrane leaflet to cluster and attract the signaling protein K-Ras, enhancing its ability to promote cell proliferation.

Ion channels control the rapid movement of ions across cellular membranes (*5*). They are best known as the gatekeepers of excitatory cellular processes such as neuronal firing, muscle contraction, and heartbeat. They also control ion homeostasis and set the membrane potential of all resting or non-excitable cells. Less well understood is their role in regulating cell division (mitosis). It has been known for nearly 40 years that cells undergoing mitosis are more depolarized—less negatively charged on the inside—than their quiescent counterparts (*6*–*8*) and that, as cells transition through the different states of the cell division cycle, their membrane voltage changes (*9*) in concert with the expression of many different ion channels (*3*). Furthermore, overexpression of K^+, Na^+, Ca^{2+}, and Cl^- channels has been observed in numerous tumors (*2*), and their pharmacological inhibition can restore normal proliferative behavior (*2*, *10*).

In addition to their role in tumorigenesis, ion channels are also involved in other aspects of cancer biology such as cell adhesion, cell volume regulation, programmed cell death (apoptosis), and angiogenesis (*1*, *8*). It is therefore not surprising that ion channels are viewed as highly promising targets for cancer treatment, especially given the great variety of readily available compounds that specifically target various channel types and can serve as scaffolds for the development of anticancer drugs (*10*). However, efforts in these directions have been stymied by a poor understanding of the cellular signaling pathways that are affected by channel overexpression and by the ensuing alterations in the resting membrane potential.

Zhou *et al.* investigated whether changes in membrane potential might alter the lateral spatiotemporal distribution of charged lipids in the membrane, which in turn might affect the distribution of membrane-bound signaling proteins, such as the Ras family of guanosine triphosphatases (GTPases). The human genes encoding H-, N-, and K-Ras are among the most commonly occurring mutated oncogenes. Mutations that constitutively activate K-Ras are found in nearly 25% of all human tumors (*11*). Positively charged residues in the C termini of Ras proteins interact with negatively charged lipids that sequester these proteins into spatially localized assemblies called nanoclusters. Such aggregation is essential for K-Ras–induced activation of the RAF–mitogen-activated protein kinase (MAPK) cascade (*12*).

> ***"The study provides...a breakthrough that should pave the way for developing strategies that silence oncogenic pathways."***

Using an elegant combination of electron microscopy, electrophysiological recordings, and fluorescence imaging, Zhou *et al.* show that membrane depolarization specifically and reversibly promotes clustering of two types of negatively charged lipids, phosphatidylserine and phosphatidylinositol 4,5-bisphosphate (PIP_2). Upon depolarization, nanoclustering of phosphatidylserine and K-Ras increased with closely matching

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Science **349**, 788 (2015);
DOI: 10.1126/science.aad0092