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needs to be overcome if a water molecule is to be placed inside the fullerene. Additionally, the water molecule needs to enter the closed fullerene cage.

To encapsulate the water molecule, Kurotobi and Murata began with an intact  $C_{60}$  molecule. Through a series of chemical reactions, they opened a hole in its surface without removing any of the 60 carbon atoms. This strategy had been used before for the insertion of molecular hydrogen into  $C_{60}$  to form  $H_2@C_{60}$  (5). However, in order to accommodate the greater size of  $H_2O$  relative to  $H_2$ , a larger hole needed to be cut into the fullerene. Kurotobi and Murata generated an opening that was ringed with oxygen atoms, whose presence allowed strong hydrogen bonding. Thus, the opening in the fullerene created an environment that could attract water molecules. Nevertheless, elevated pressure and temperature were needed to force a water molecule into the inside of the opened  $C_{60}$  container. Once the water molecule entered the cavity, a new set of chemical transformations was used to close the hole. An intact  $C_{60}$  cage emerged with the water molecule mechanically trapped inside.

The resulting molecule,  $H_2O@C_{60}$ , is a remarkable combination of a polar molecule encapsulated into a highly symmetric and nonpolar cage. Generally, the polarity of a molecule is associated with its external shape. The bent water molecule is polar, whereas the linear carbon disulfide and the highly symmetric  $C_{60}$  are not. Calculations by Kurotobi and Murata suggest that the polarity of  $H_2O@C_{60}$  is nearly the same as that of  $H_2O$ . Earlier computations at lesser levels of sophistication suggested that the polarity of  $H_2O@C_{60}$  would be lower than that of water (6–8). An experimental determination of the dipole moment of  $H_2O@C_{60}$  would clarify this issue, but the different chromatographic behavior of  $C_{60}$  and  $H_2O@C_{60}$  certainly points to a difference in their polarity.

Trapping a guest molecule inside a suitable host molecular container like a fullerene is a fascinating area of supramolecular chemistry. Other notable successes in this area using nonfullerene hosts include the stabilization of otherwise unobservable guest molecules such as the elusive cyclobutadiene,  $C_4H_4$  (9), and lowering the reactivity of a chemically energetic molecule like  $P_4$  (10). Fullerenes themselves can act either as guests or as hosts. As guests, fullerene cages may be found on the inside of carbon nanotubes where nano-peapods are formed (11). However, fullerenes are best known as hosts, which can encapsulate a variety of different entities to form endohedral fullerenes such as  $H_2O@C_{60}$ .

These endohedral fullerenes may be conveniently divided into two groups: those with neutral molecules or atoms inside and those with electropositive metals inside. In the latter category, there is appreciable transfer of electrons from the metal to the carbon cage, which becomes anionic (12). In these cases, the metal ions strongly interact with the carbon cages, determine the size and shape of the cage that traps the metal, and alter the chemical reactivity of the external surface of the molecule (13). In contrast, when neutral molecules or atoms are present inside fullerene cages, the interactions between guest and host are rather modest. Thus, spectroscopic studies by nuclear magnetic resonance show that both  $H_2$  and  $H_2O$  are free to move about inside the  $C_{60}$  cage. Future work will determine the extent of the effects of the internal water molecule on the reactivity of the carbon cage of  $H_2O@C_{60}$ , but interesting developments are anticipated.

The formation of  $H_2O@C_{60}$  represents an extreme in ongoing efforts that are examining the properties of small clusters of water molecules. Suitable molecular containers have been prepared that can contain assemblies of 4 to 45 water molecules (14, 15). Related studies have constructed a series of “molecular apple peels” from oligomers of aromatic amides that fold helically and can encapsulate one or two water molecules (16). How-

ever, these containers provide sites where the enclosed water molecules can undergo hydrogen bonding, a feature denied the water molecule in  $H_2O@C_{60}$ . Thus,  $H_2O@C_{60}$  is a remarkable molecule, a molecular container compound in which water cannot undergo conventional hydrogen bonding, but where it conveys an apparently sizable dipole moment onto a nearly isotropic fullerene cage.

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#### CELL BIOLOGY

## A Cellular Roadmap for the Plant Kingdom

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Mapping the *Arabidopsis* protein interactome helps show that plant pathogens target highly connected proteins.

One can learn a lot from an urban subway network just by looking at its map. It illustrates crossroads that must be under high surveillance, for example, because a disruption at these central stations can affect the entire system. The map also reflects the city's history, with old lines running through the urban center and recent ones along the periphery. In recent years, researchers have worked to draw analogous maps for cellular networks. These maps link

proteins that physically interact and reflect how cells organize biochemical and biophysical processes and convey molecular signals. Attempts to develop entire protein interaction maps (interactomes), however, have been limited to bacteria, unicellular fungi, and a few animals. Now, two studies in this issue, by the *Arabidopsis* Interactome Mapping Consortium (1) on page 601 and Mukhtar *et al.* (2) on page 596, add a flowering plant to the list. They report on the interactome of *Arabidopsis thaliana* and show how pathogens may exploit protein interactions to manipulate a plant's cellular machinery.

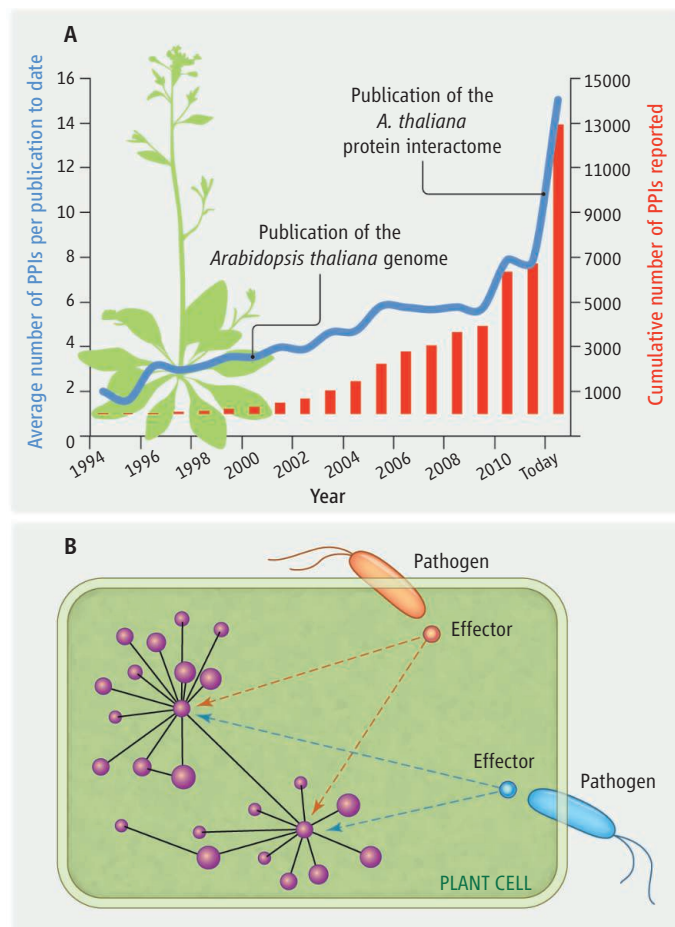
*A. thaliana* is an annual that is a major model organism for basic and applied

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research in genetics, development, and ecology. It has a relatively small genome and was the first plant to have its genome sequenced (3). Eleven years after the genome's publication, however, the function of a large fraction of *Arabidopsis* genes remains undiscovered. To fill this gap, investigators need large-scale methods for systematically associating proteins to functions, even in the absence of visible phenotypes. One powerful tool for completing this task is an interactome that maps the positions of proteins with unknown functions within a cellular network (4). Researchers have already systematically explored specific subnetworks of the *A. thaliana* protein interactome (5–7). The *Arabidopsis* Interactome Mapping Consortium expands these investigations to a proteome-wide scale. Using a collection of 8000 *A. thaliana* genes—about 30% of the total—they tested all possible pairings of protein-protein interactions (PPIs) (8). They identified 6200 PPIs among 2700 proteins; 40% involved plant-specific proteins. These results represent a major technical leap forward and establish a cellular roadmap for the plant kingdom. They also demonstrate that PPIs are a rich source of information for plant molecular and cell biologists who cannot rely on animal or fungal interactomes as references.

Proteins are not randomly connected; most interact with just a few others, whereas a limited number (“hubs”) interact with many others. Some investigators have suggested that this architecture provides a robust defense against random alterations, but highly connected proteins—which tend to play essential roles in organisms (9)—can also make networks brittle and vulnerable to attacks that target highly connected nodes, just as central stations make subways vulnerable to disruption (10). On the basis of these observations, one would predict that parasites and pathogens would target highly connected nodes in order to take control of host cells.

Mukhtar *et al.* elegantly test this prediction by mapping the physical interactions between *A. thaliana* proteins and virulence proteins produced by two pathogens with very different evolutionary histories. Plant pathogens often occupy extracellular niches,



**Interactome insights.** (A) The accumulation of data on the *Arabidopsis* protein interactome has accelerated recently, with researchers documenting new protein-protein interactions [data from (13)]. (B) The expanding interactome has helped researchers show that pathogen effectors target highly connected plant proteins (purple circles), most likely to control the host's cellular machinery.

but they also deploy proteins called effectors that manipulate the plant's immune system and lead to enhanced infection. Individual plant pathogens encode 20 to 30 such effectors (11). Mukhtar *et al.* found that, as predicted, effectors tend to interact with highly connected plant proteins and that, in this case, effectors from the two different pathogens targeted the same hub proteins. They also confirmed that these highly connected proteins are involved in immune defense by exposing mutant plants with impaired protein-coding genes to pathogens. The results illustrate how investigators can use PPIs to associate proteins to particular biological processes. In addition, they support a previous observation that one of the best predictors of the function of a protein is the function of the proteins with which it interacts (12).

With plant, animal, and fungal interactomes in hand, we can now ask whether more complex forms of life gave rise to specific network properties and architectures. We

can also ask whether eukaryotes share a central set of PPIs that could represent an ancestral eukaryotic interactome. In addition, we can explore how interactomes have been shaped by the requirements of multicellular life—such as the need for intercellular communication, synchronization of cell division, cell specialization, and a transcriptional program that regulates cell development and differentiation. Plants and animals evolved multicellularity independently, and comparing the organization of their interactomes provides a unique opportunity to gain insight. For instance, genes that contributed to the evolution of multicellularity may be different in the two groups but could have given rise to similar interactomes. The *Arabidopsis* Interactome Mapping Consortium briefly touches on this issue, reporting that the overall topology of the *Arabidopsis* network is qualitatively similar to those of yeast, worm, and human. The number of interactions per protein is also similar to numbers found in other species studied with the same technique.

Some answers may come from studying new dimensions of protein interactomes, such as how interactions respond to environmental changes and how complex transcriptional programs rearrange interactions within particular cell types. Developing interactome tools, in species spanning the range of biological diversity, will be key to the future of proteome annotation.

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