1st large-scale map of a plant's protein network addresses evolution, disease process

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The eon-spanning clock of evolution – the millions of years that generally pass before organisms acquire new traits – belies a constant ferment in the chambers and channels of cells, as changes in genes and proteins have subtle ripple effects throughout an organism. In a study in the July 29 issue of Science, scientists at Dana-Farber Cancer Institute's Center for Cancer Systems Biology and an international team of colleagues capture the first evidence of the evolutionary process within networks of plant proteins.

In a companion article, the investigators use their new map of these networks to uncover how microbes like bacteria and fungi undermine plants' defenses against disease. The microbes accomplish this by disrupting a relatively small set of "virtuoso" proteins that play a variety of different roles within the cell.

Together, these findings promise not only to propel crop-improvement and anti-blight efforts, but also – because human diseases involve disturbances in protein networks as well – increase the understanding of a variety of human health disorders, including cancer, the authors state.

"Although these papers focus on the interactions of proteins in plants, they have implications for what occurs in animal – and human – cells as well," says Dana-Farber's Pascal Braun, PhD, who had a leading role in both studies. "The central role of protein interactions in all of life suggests that our findings hold important lessons for the study of health and disease in humans."

The model for the two studies was *Arabidopsis thaliana*, a small, gangly plant with prim white flowers that would likely be considered a weed if it appeared in someone's front yard, but which is a favorite subject of plant biology because it germinates rapidly and has a relatively simple genome of 27,000 genes. Despite decades of research involving the plant, however, scientists have yet to determine the roles of more than 60 percent of those genes, Braun remarks.

To gain the first inklings of those roles, investigators used genetic blueprints to produce about 8,000 of the proteins normally made by the plant's cells. They then mixed each of those proteins with each of the approximately 7,999 others to see which interact. (When two proteins interact, they bind together or modify each other to carry out a specific biological function, such as sending signals from the exterior of the cell to the interior, or escorting other proteins through the cell.)

The experiment yielded a map of 6,200 interactions between 2,700 proteins – far more than had been shown in any previous map of the plant's interactome (its collection of protein-protein interactions). Graphing the interactions in network form revealed that some proteins interact with a large number of other proteins, while most interact with only a few. (Interestingly, the World Wide Web and social networks such as Facebook show the same type of network structure, in which a relatively small number of pages attract a disproportionally large number of visitors.)

The investigators used a newly developed mathematical algorithm to scour the map for "communities," clusters of densely interconnected proteins that are likely to function together. They identified 26 such communities, many of which correspond to known processes but contain new proteins, while the function of other communities will need to be investigated. Knowing which genes work together offers clues to their roles within the cell.

Lastly, the investigators explored whether the interactome map could be used to answer a basic question in biology: Does natural selection – the evolutionary process by which certain traits "win out" over others by increasing an organism's likelihood of survival – operate at the level of protein networks?

Evolution is thought to begin when cells duplicate their DNA prior to dividing. Imperfections in the newly copied genes give rise to slightly altered proteins which, in combination with other proteins, gradually produce novel traits in an organism. If those traits – larger leaves, for example, or longer roots – give the organism an edge in the struggle for survival, its offspring are likely to inherit and retain those advantages. Because of the limited scope of most previous interactome maps, it has been difficult for scientists to trace the influence of gene duplication on protein-protein interactions.

"The proportion of near-duplicate genes is much higher in *Arabidopsis* than in many non-plant species, making it ideal for this kind of research," Braun remarks. "As novel proteins emerge as a result of gene alteration, those proteins produce a rewiring of the plant's protein networks," much as the arrival of a new tenant changes the lines of communication among residents of an apartment building.

When a novel protein first appears, the rewiring happens rapidly, as protein networks hasten to adapt to the new entrant. "Over time, these novel proteins assume new functions and become more critical for the plant's survival," Braun notes. "Evolutionary pressure tightens and the new proteins become fixed parts of the plant's operating machinery." The rewiring that occurred so quickly at first gradually tapers off as the interaction networks stabilize.

"In analyzing our interactome map of *Arabidopsis*, we found strong evidence of this rapid-then-slow process over the hundreds of millions of

years of the plant's evolution," says Braun. "It provides the first-ever empirical evidence of evolution acting on protein networks."

For the second paper, researchers used the interactome map to explore how certain bacteria and fungi cause disease in plants by subverting their immune system. Plants respond to infection by directing specialized proteins at the causative agent, be it viral, fungal, or bacterial. The disease agents, known as pathogens, respond by releasing virulence effector proteins that subdue the plant's defenses.

To bring the details of this process to light, investigators mixed effector proteins from a fungus and bacteria with thousands of proteins from *Arabidopsis* to see which would interact. Some of the *Arabidopsis* proteins are involved in the plant's immune system and some are not.

"We thought that mapping the interactions would enable us to identify the immune system proteins that are disabled by binding to the effector proteins," Braun recounts. "To our surprise, we found that the effectors bind to a small number of non-immune system proteins. These non-immune proteins tend to be highly connected; that is, they interact with a large number of other proteins and are therefore involved in a wide array of plant functions."

All of this points to the dexterity and sophistication of pathogens' attack on plants: instead of targeting proteins directly involved in the immune system, pathogens exploit other, highly linked proteins that control much of what happens within the cells of the organism.

"This suggests that the immune system is highly integrated with the rest of the plant cell – like having a guard at each point of activity within the cell," Braun remarks. "The system is much less compartmentalized than we'd thought.

"This work will help scientists derive general rules of how cells defend themselves from microbial infection, and how pathogens manipulate that system to their advantage. Ultimately, this may suggest new techniques for improving immune system functioning in plants."

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