The Many Faces of WAS Protein

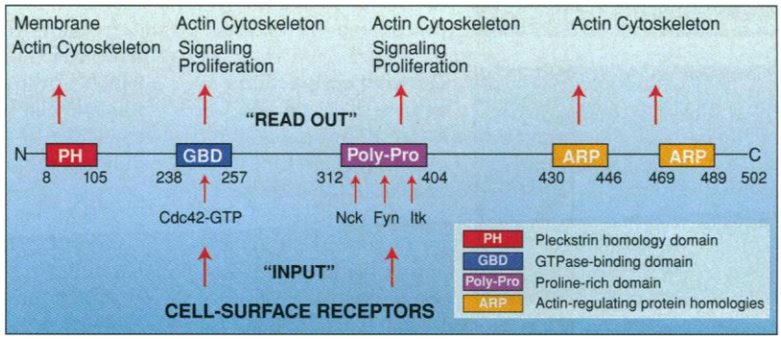
The protein made by the gene defective in Wiskott-Aldrich syndrome may have multiple functions, interacting with both the cell's internal skeleton and its growth-control pathways

How could a single errant gene cause everything from bleeding problems to eczema to cancer? That's the puzzle immunologists have faced in the rare hereditary disease known as Wiskott-Aldrich syndrome (WAS). Boys who inherit the most severe forms of this condition, which is transmitted by a gene carried on the X chromosome, have immune deficiencies that lead not only to eczema but also to frequent infections. They have few platelets—the tiny cells needed for normal blood clotting—and those few are abnormal. And WAS patients often develop immune-cell cancers—lymphomas and leukemias—that kill them by the age of 30. Now, a flurry of recent results from several labs has begun to connect these pieces of the puzzle.

The work is not only starting to reveal how a single defective gene can wreak such diverse havoc, but is also pointing to some intriguing new connections between the internal communication pathways of normal cells. It suggests that WASp, the protein made by the gene that is defective in WAS patients, is a multifaceted molecule that can interact with both the cell's internal skeleton and the signaling systems that control a cell's responses to its environment. "It has been a really important year," says Tomas Kirchhausen, whose group at Harvard Medical School is studying WASp. "For a long time, we've known that changes in the cytoskeleton are important aspects of the cell's response to its environment. Now we have a candidate molecule that seems to connect the cytoskeleton and signal transduction pathways."

On the one hand, WASp may help regulate the highly malleable, internal network of protein filaments known as the cytoskeleton. Those filaments that are made of the protein actin, for example, rapidly disassemble and reform to help immune cells move in response to external signals, such as the inflammatory molecules that draw them to infection sites, and also during the cell-to-cell interactions needed for triggering antibody production and other immune responses.

Exactly how receptor signals trigger this actin filament reorganization has been unclear. But the new work indicates that WASp may relay the signals by acting "like a bridge or a scaffold linking components on the plasma membrane with components on the cytoskeleton," says Kirchhausen. And on the other hand, WASp may provide a hub that connects receptors to pathways that regulate immune-cell proliferation, which is essential for normal immune responses. If the functioning of the WASp protein is impaired by mutation, the result could then be failure of both immune responses and the cell's normal growth-control systems.



Division of labor. The colored bars identify the different WASp domains that may contribute to its functions, with the numbers indicating the amino acid positions.

Buzzing about WASp

The current excitement about WASp had its origins in 1994 when Jonathan Derry and Uta Francke at Stanford University, in collaboration with Hans Ochs at the University of Washington, Seattle, cloned the gene that is mutated in WAS. Its sequence provided an early clue that the gene's protein product might have multiple functions. It showed, Ochs says, that "the gene is very complex, with well-defined regions that give the protein several faces." The first one recognized, however, turned out to be deceptive: a region containing many residues of the amino acid proline—a feature often seen in transcription factors, proteins that activate gene expression. If that were WASp's function, the protein should reside in the nucleus, where the genes are. But the work this year has instead helped pin down the protein to a location just beneath the cell surface.

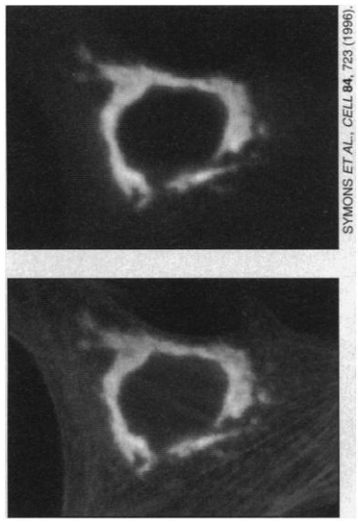
That would put WASp in the right position to relay incoming signals to the actin cytoskeleton, and there were already reasons to think that it might be doing just that: several features of blood cells from WAS patients. "Ages ago, [Harvard's] Fred Rosen thought that actin was not bundling properly [in T cells]," recalls Ochs. In work done more than 10 years ago, Rosen and his colleagues had noticed that WAS lymphocytes look strange, having too few of the cell-surface projections called microvilli that have actin at their core. The small, malformed appearance of the platelets in WAS patients also implied a problem with their cytoarchitecture. These malformed platelets are quickly destroyed by the spleen, leading to the patients' bleeding problems.

But researchers didn't begin considering the possibility that WASp might have direct effects on the cytoskeleton until the beginning of this year. The clue came when three independent teams, which were led by Alan Hall of University College London, Arie Abo of Onyx Pharmaceuticals in Richmond, California, and Harvard's Kirchhausen and Rosen, found that WASp binds to a protein called Cdc42, a member of a superfamily of small GTP-hydrolyzing proteins (GTPases) that act as on-off switches for many cellular activities. In Cdc42's case, the activities it controls involve actin remodeling, such as the formation of filopodia, fingerlike projections from the cell surface that help cells move in response to external stimuli, and the orientation of T cells toward antigen-presenting cells in the early stages of an immune reaction. Because WASp mutations were known to disrupt actin-containing structures, the finding raised speculations, says Hall, that Cdc42 might be working in conjunction with the normal WASp protein to regulate actin filament formation.

Since then, that idea has gathered support—and faced some contradictory evidence. The support came when the Hall and Abo groups found that Cdc42 binds WASp only when the GTPase is carrying GTP—the form that functions as the "on" switch for filopodia formation when Cdc42 is activated by an appropriate signal from a receptor. In addition, Abo's group provided evidence that WASp is localized in the cell with polymerized actin. When he and his colleagues genetically altered cells so that they ex-

pressed large amounts of WASp, they found that the protein clumped together with actin polymers in the cytoplasm in an interaction that is regulated by Cdc42. The WASp apparently binds to the actin through a region on its carboxyl end that is near regions with some structural similarities to certain known actin-regulating proteins. Taken together, these findings suggest that when Cdc42 is activated by an appropriate signal, it binds WASp, which in turn binds actin, bringing about the remodeling needed for filopodia formation and other cytoskeletal effects.

The latest results from Hall and his colleagues, published in the 1 November issue of *Cell*, indicate that this interpretation may not be correct, however. They found that fibroblasts, a kind of connective tissue cell that forms filopodia when injected with Cdc42, still produced them when they were injected with mutated Cdc42 proteins that could no longer bind WASp. "The simplest explanation," says Hall, "is that the interaction [of WASp and Cdc42] is not required to generate filopodia."



SYMONS *ET AL.*, *CELL* **84**, 723 (1996).

Together. Cells stained for WASp *(top)* and actin *(below)* show that the proteins colocalize.

Despite this apparent setback, researchers aren't giving up on the idea that the Cdc42-WASp interaction might be important. Cdc42 has other roles, such as transmitting stress signals to the genes in the nucleus, and WASp might be involved in that pathway in some way. And even if WASp doesn't cooperate with Cdc42 in regulating filopodia formation, there are other ways in which it could exert an effect on the actin cytoskeleton.

In addition to the actin-binding region that Abo's team found on the protein's carboxyl end, WASp's amino end contains a sequence known as the pleckstrin homology (PH) domain because it resembles a sequence originally found in the protein pleckstrin. The PH domain of WASp, like that in pleckstrin itself, binds to a phospholipid in the cell membrane, produced when many different types of receptors are activated, that regulates actin filament growth. "The homology [to the PH domain] is very hard to detect by looking at the sequence," says Kirchhausen, "but when you express [the domain from WASp] as a recombinant molecule, it binds tightly to [the phospholipid] PIP_2."

Perhaps the best evidence implicating WASp in actin remodeling comes from yeast biologist Rong Li and her colleagues at Harvard University, who have cloned a yeast counterpart of the WASp gene. Inactivating the yeast gene, says Li, disrupts the normal patchy distribution of actin just beneath the yeast cell surface and at sites where new yeast cells bud off during that organism's asexual reproduction. In yeast cells lacking a working WASp gene, "the [subsurface] actin was not in patches but more like cables that permeate the cytoplasm," Li says. The yeast WASp mutants are also defective in membrane growth and cell budding, both defects that could be explained by faulty actin organization. Abo's group, in collaboration with Mara Duncan and David Drubin at the University of California, Berkeley, has also observed an actin defect in yeast with an inactivated WASp gene.

Growing connections

Even as the case for WASp's actin connection builds, other work is linking the protein to the cell's growth-signaling pathways. In 1995, for example, Keith Robbins and colleagues at the National Institute of Dental Research in Bethesda, Maryland, noted that WASp binds to a protein called Nck, which is one of the first links in the chain of molecules that convey signals from certain growth-factor receptors into the cell. These receptors are called receptor tyrosine kinases because their intracellular portions are kinase enzymes that add phosphate groups to tyrosine residues in proteins, including the receptor proteins themselves, when the receptors are activated.

To do its job, Nck interacts with the kinase receptor through one of its domains, called SH2, but only when the receptor is in its activated, phosphorylated form. Through another of its domains, called SH3, Nck binds to WASp. Nck might therefore be a conduit for conducting signals between growth-factor receptors and WASp to influence the actin cytoskeleton when the cell mobilizes itself to begin proliferating.

And Nck might not be the only connection between WASp and the cell's growth pathways. In the last few months, a collaboration between several groups in London and another between Boston-based researchers reported that WASp, through its proline-rich domain, also binds to two nonreceptor tyrosine kinases that are thought to be links in intracellular signaling pathways. One of these, called Fyn, is related to the so-called Src kinase, which has been implicated in regulating both the actin cytoskeleton and cell division. Fyn itself may be involved in transmitting the T cell receptor signals that tell the immune cells to proliferate in response to antigen activation, although the evidence is controversial.

The other WASp-binding kinase, Itk, may help relay signals that trigger T cells to make interleukin 2, which sets in motion the T-cell proliferation and differentiation that are at the heart of a cellular immune response. "When the gene for Itk was inactivated in mice, T-cell development was defective," explains Leslie Berg of Harvard University, who is studying the interaction between WASp and Itk. "The mice just didn't produce the [normal] numbers [of T cells]."

WASp's connections may go even further. Berg's group has found in test-tube studies that WASp interacts through its proline-rich region with at least seven proteins that have SH3 domains. It is not yet certain which of these interactions might occur in the cell. But what is clear, Berg explains, is that "WASp is capable of binding several different [SH3-containing] proteins simultaneously." This multiple binding, which could enable WASp to coordinate the interactions of several signaling pathways, has led, says Kirchhausen, to the emerging view that WASp is "an integrator allowing regulatory elements to talk together."

The functional significance of this linkage between growth-factor signaling and the state of the actin cytoskeleton is not yet clear. Because cells usually need to be attached to their substrate before they can proliferate, one idea is that actin organization is important for cell attachment and therefore also for proliferation.

Researchers have a long way to go to work out just what WASp does and how its many roles might be related. But the answers may turn out to be important to the function of many cell types besides blood cells. In the October issue of the *EMBO Journal*, a group led by Tadaomi Takenawa at the University of Tokyo described a relative of the blood-cell WASp that is present in many tissues, reaching high concentrations in the brain. The functions of WASp in these other tissues are even more uncertain than they are in lymphocytes. But as Ochs says, "That we can see WASp in all kinds of cells is the most exciting thing. We still don't know all the answers, but one day soon undoubtedly someone will put the mosaic together."

–Carol Featherstone

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