Wiskott–Aldrich syndrome: a gene, a multifunctional protein and the beginnings of an explanation

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Patients with Wiskott–Aldrich syndrome show various defects in the normal function of platelets and lymphocytes. The recent identification of the gene responsible for this syndrome has led to a surge of studies aimed at solving the puzzle posed by the varied phenotype observed in this disease. It is now known that WASP, the protein product of this gene, can interact with a large number of other proteins known to be involved in the regulation of signal transduction and cytoskeletal organization. Thus, WASP appears to integrate these two basic and fundamental cellular mechanisms. Several groups are now focusing on understanding the function of WASP in detail, and translating this new knowledge into improved therapies.

THE story of Wiskott–Aldrich syndrome (Box 1) is a fascinating scientific odyssey[1]. The original observations in the 1930s of a syndrome in which patients had eczema and thrombocytopenia[2] were followed 30 years later by the recognition of immunological deficits[3]. Analysis showed that the immunological problems were primarily due to defective T cells[4–6], and discoveries of perturbations in the appearance of the cell surface[7,8] led to the notion that the cytoskeleton might be involved in the basic defect. However, the connection between T-cell activation and the cytoskeleton remained obscure[7,9]. Four years ago, the identification of the gene for Wiskott–Aldrich syndrome protein (WASP)[10,11] provided a route to understanding the disparate defects in Wiskott–Aldrich syndrome[12], and an explosion of research that has led to a new appreciation of the importance of the compartmentalization of signal transduction. The exciting new ideas resulting from the study of this disease seem almost certain to lead to new therapies, although the nature of these therapies remains to be determined.

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Interactions with the cytoskeleton

My own involvement in the study of WASP started shortly after the gene had been cloned. We noticed that, among the large number of domains within the molecule, there was a motif that is characteristic of molecules that bind to the small GTP-binding protein Cdc42 (Ref. 13). Hall and co-workers had previously shown that Cdc42 is involved in actin organization in mammalian cells[14], as was already

Box 1. Wiskott–Aldrich syndrome

Wiskott–Aldrich syndrome is a rare inherited X-linked disease that maps to the short arm of the X chromosome at Xp11.23 to Xp11.3. Female heterozygous carriers show non-random inactivation of the X chromosome in all blood cells, but not in cells of other lineages. All affected males display thrombocytopenia, but the severity of the immunological defect varies depending on the mutations in the *WAS* gene. Untreated children die within the first decade of life – often of a brain hemorrhage or of severe infections. At present, bone marrow transplantation is the main curative treatment, particularly for severe cases.

known to be the case in yeast. Three groups, including our own, used different methods to show that Cdc42 indeed interacts with WASP, indicating that WASP might be involved in regulating cytoskeleton structure or function[13,15,16]. Symons *et al.* bolstered this possibility by overexpressing WASP in mammalian cells and showing that the intact protein causes co-precipitation of actin and WASP into 'pebbles' within the cytoplasm (Fig. 1)[16]. Surprisingly, however, truncated WASP, lacking the C-terminal domain but still containing the Cdc42-binding site, did not have this effect on actin. Furthermore, yeast WASP lacks the Cdc42-binding site yet is clearly involved in organizing cortical actin in yeast cells[17,18].

The exact nature of the interaction between WASP and actin remains to be clarified. It is possible that mammalian WASP interacts with actin in two ways, only one of which involves Cdc42. It is also possible that the interaction with Cdc42 has nothing to do with the actions of WASP on actin, but is instead involved in the signal transduction functions that have recently emerged as an important aspect of WASP's functions[19–22]. If so, the discovery that WASP interacts with actin was pure serendipity – a reflection of the high level of connectivity between WASP and signal transduction pathways in the cell. This theme was later repeated in the accidental discovery of the brain homolog of WASP, N-WASP, which was found in a search for proteins that bind to Src homology 3 (SH3) domains[23]. Proteins that contain these domains are typically involved in protein–protein interactions that mediate signal transduction cascades.

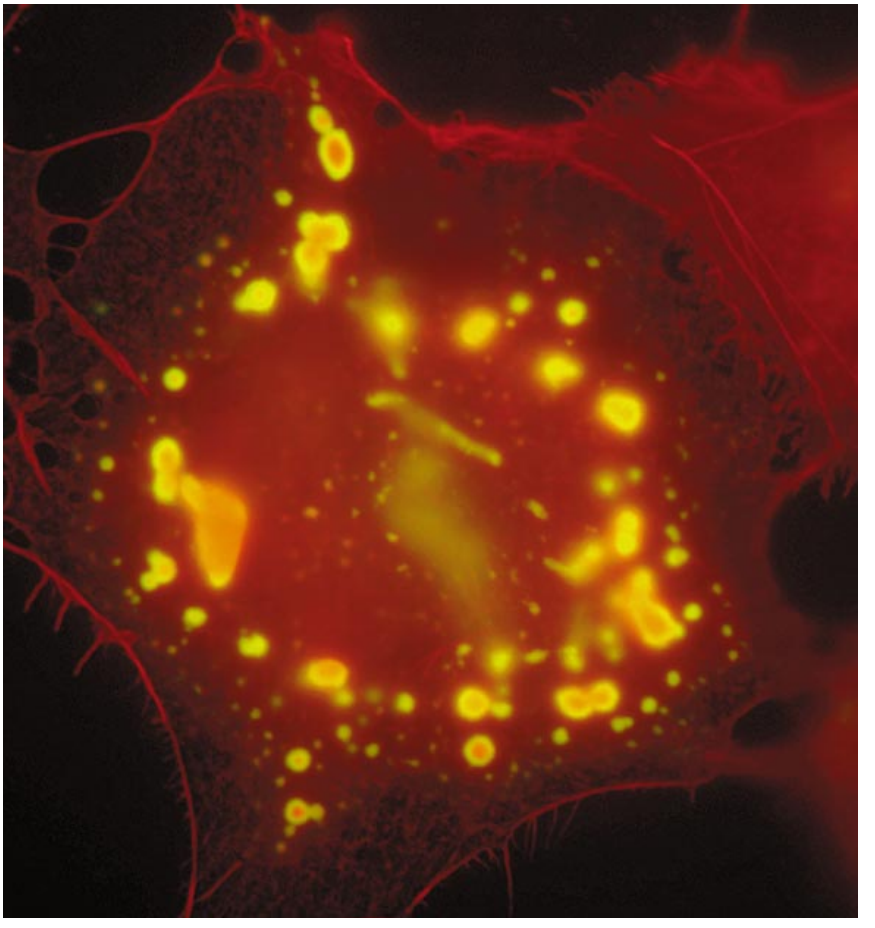

Figure 1. Immunofluorescence image of COS cells overexpressing WASP, stained with an antibody specific for WASP (green) and with phalloidin (red), which specifically labels actin filaments. The pebbles (yellow) corresponding to the extensive co-localization of WASP and actin were originally described by Symons *et al.*[16] Photograph courtesy of Dr M. Lopez.

Dissecting the domains of WASP

Soon after the initial discovery of the Wiskott–Aldrich syndrome gene, it became clear that WASP might have a wide range of protein partners. The protein has three distinct domains, each of which has a different function. The identification of these domains was made possible by the recognition of WASP homologs in brain (N-WASP, see above), *Saccharomyces cerevisiae* and *Caenorhabditis elegans*[16]. The availability of the complete *S. cerevisiae* genome sequence, which makes it trivial to discover whether homologs for a certain gene exist in yeast, first allowed the identification of a WASP homolog; the *C. elegans* database is not yet complete, but in this case yielded a second member of the gene family. Both the *C. elegans* and *S. cerevisiae* WASP gene homologs have high levels of sequence similarity to WASP at the N- and C-terminal domains (Wiskott homology domains WH1 and WH2), while the central domain is divergent (Fig. 2). The strong sequence similarity in the WH1 and WH2 domains in these very divergent species suggests that the functions of these two domains are highly conserved during evolution, and that the interacting partners will also be strongly conserved.

The WH1 domain binds phosphoinositides

The first clue to the function of the WH1 domain came from the observation that it has weak homology with pleckstrin homology (PH) domains, which interact with phosphorylated lipids in the phosphoinositide pathway[23]. Accordingly, a recombinant fragment of N-WASP containing the WH1 domain interacts with phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2][23], and a corresponding fragment of WASP has a similar binding activity (C. L. Carpenter, unpublished). More than half of the mutations identified in Wiskott–Aldrich syndrome patients map to the WH1 domain[24]. In patients that have mild immune deficiency and severe thrombocytopenia, almost all of the known mutations map to this region[24]. Whether the function of the lipid is to target WASP to membranes or to modulate its affinity for one or more of its partners, this function is clearly more important for the production of normal platelets than for lymphocyte activity. The WH1 domain also interacts with WIP (Wiskott–Aldrich interactor protein), a new partner that was discovered using WASP as a bait in a yeast two-hybrid screen[25]. Overexpression of WIP in transfected cells induces reorganization of the actin cytoskeleton[25].

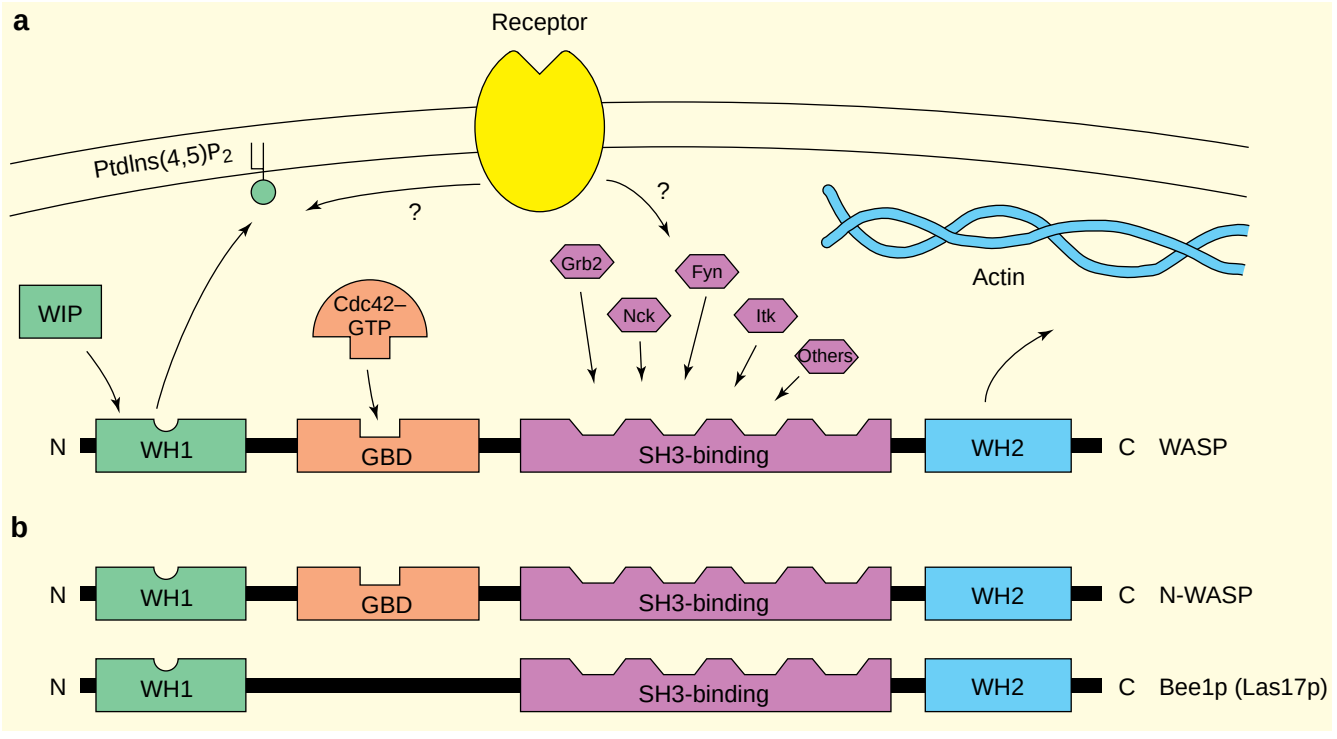

Figure 2. (**a**) Schematic representation of known protein–protein and protein–lipid interactions of WASP and its mammalian homolog N-WASP. WASP and N-WASP probably act as integrators of signal cascades that start at the cell surface, involve actin cytoskeletal rearrangements and can lead to proliferation and cell homing effects. The sequence of events and the position of WASP and N-WASP in these processes are not known. WH1 and WH2, Wiskott homology domains 1 and 2. GBD, GTPase-binding domain. WIP, Wiskott–Aldrich interactor protein. PtdIns(4,5)P_2, phosphatidylinositol (4,5)-bisphosphate. Grb2 and Nck are SH3-domain-containing 'adaptor' proteins. Fyn and Itk are kinases that contain SH3 domains. (**b**) Structure of WASP homologs: N-WASP (from mammalian cells) and Bee1p (Las17p) from *S. cerevisiae*.

The WH2 domain interacts with actin

The function of WH2 has been more elusive. A significant percentage of the known human mutations are found in this domain[24,26]. Patients who carry these mutations have very serious immune deficiency, unlike those who carry mutations in WH1 (Ref. 24). WH2 is obviously involved in actin binding, first because WASP lacking this domain does not co-precipitate actin (see above), and second because a recombinant fragment of N-WASP containing the WH2 domain binds to actin *in vitro* and prevents actin polymerization *in vitro*[23]. The effect of the overexpression of intact N-WASP on actin *in vivo* is to cause co-precipitation of N-WASP and actin in the cortical region of the cell[23], a phenomenon that is similar in general terms, though different in detail, to the effect of the overexpression of WASP, which co-precipitates with actin throughout the cytosol[16]. Taking these observations together, we can conclude that the interactions between WASP or N-WASP and actin are largely mediated through the WH2 domain, although it is not known whether this interaction is direct, or exactly where the sites of interaction might be.

The central domain binds SH3 domains

The characteristic feature of the central domain of WASP homologs is that it is remarkably proline-rich. At least seven SH3-binding sites have been identified in the central region of WASP, and a number of SH3-domain-containing proteins have been shown to interact with WASP either *in vitro* or *in vivo*. These proteins include kinases such as Fyn and Itk (Refs 19,22), which are involved in T-cell signaling, and adaptor proteins such as Grb2 (Ref. 20), which is involved in both the T-cell receptor signaling pathway and the signaling pathway downstream of tyrosine kinase receptors. Given these observations, it is hardly surprising that defects in WASP affect T-cell activation, although the relevance to platelet development and B-cell activation is less obvious. There are some indications that the epidermal growth factor (EGF) receptor, a receptor tyrosine kinase, physically associates with WASP or N-WASP in response to EGF activation[20,23] (probably via Grb2), indicating that the importance of WASP is not restricted to the T-cell receptor pathway. Mutations in the central domain of WASP produce severe Wiskott–Aldrich syndrome[24]. Interestingly, all of the known mutations in this area cause gross disruptions of the structure of the central domain. For example, frameshift mutations in this region are known, but no point mutations have been identified. This could mean that subtle mutations that affect only one or two SH3 interaction sites do not appear as a recognizable Wiskott–Aldrich syndrome phenotype, and thus there might be redundancy in the signaling pathways accessed via this domain.

The central domain is not at all conserved among the different homologs of WASP, indicating that each WASP homolog interacts with a different set of SH3-domain-containing proteins, and therefore presumably with a different set of signaling cascades. N-WASP is highly expressed in brain tissue (presumably neurons) and in lung and intestinal tissues (presumably endothelial cells). In both of these

cell types, actin remodeling is an important part of development: in the case of neurons, the formation and motion of a growth cone requires dramatic cytoskeletal reorganization, and endothelial cells have a remarkably active luminal surface that remodels in response to pathogens and circulating blood cells and actively performs pinocytosis. The SH3-containing proteins interacting with N-WASP might therefore be involved in signaling the actin remodeling that occurs in response to these varied stimuli.

The picture that emerges from the recent explosion of WASP research is that WASP is a nexus for several signaling pathways, integrating the response of the cytoskeleton to a variety of different inputs. The importance of the lipid-binding domain indicates that WASP must be in the correct cellular compartment to function normally. The study of WASP seems certain to teach us more about the control of cytoskeletal organization; there are intriguing hints that other proteins involved in cytoskeletal function might interact with WASP, and might soon be identified by means of this interaction[25,27]. WASP is also expressed in B cells, and indeed all hemopoietic cells, but the connection between mutations on the Wiskott–Aldrich syndrome gene and the B-cell defects and eczema seen in patients remains unclear. A second genetic factor is likely to be involved because the existence and severity of eczema in these patients vary among siblings.

The next steps

Before the new understanding of the molecular basis of Wiskott–Aldrich syndrome can be translated into progress in drug development, several crucial research tools need to be developed. First, we need a way to trace the effects of the different mutations, by studying their effects on the interactions of WASP with its various partners and the downstream signaling events that result from these interactions. At present, the only systems available are fresh or transformed T cells derived from patients. Fresh T cells are in extremely short supply, and the transformed T cells are extremely delicate and hard to re-transfect. Furthermore, their signaling pathways might be distorted by the effects of the herpesvirus Saimiri used to transform them. The study of the effects of WASP in platelets is hampered by the fact that its function appears to be largely developmental, thus WASP might largely be redundant by the time the platelets become accessible to study. A potentially useful alternative assay was recently reported, in which activation of a platelet precursor cell line resulted in vesicle formation and co-localization of actin and WASP to the cytoplasmic face of the vesicle[21]. This effect is inhibited by the introduction of antisense WASP DNA (Ref. 21). In principle, therefore, it might be possible to introduce mutant WASP genes and thus map the domains of the molecule that are required for vesicle formation.

The most obvious general way forward, however, is to construct a WASP or N-WASP knockout mouse. If a WASP-deleted mouse shows the alterations in T-cell and platelet signaling and development that would be expected from the human phenotype, then mutant mice could be made in which WASP proteins that lack the WH1, WH2 or central domains are expressed in the WASP-deleted background. Successful efforts in this direction are currently under way (S. Snaper, pers. commun.). My own expectation is that an N-WASP deletion will be lethal, because the profound defects in T-cell and platelet function that occur in WASP-deficient humans suggest that N-WASP-deleted neurons will be severely functionally impaired. The problem of N-WASP will probably be solved by biochemical approaches.

Glossary

Actin – One of the proteins that act as a dynamic scaffold in the cytoskeleton of eukaryotic cells.

Fyn – An SH3-domain-containing kinase found to bind to one of the SH3-binding sites of WASP.

Itk – An SH3-domain-containing kinase found to bind to one of the SH3-binding sites of WASP.

N-WASP – A ubiquitously expressed protein whose protein sequence is highly related to that of WASP.

Pleckstrin homology (PH) domain – A conserved protein structure that functions as a localization module for signaling proteins by binding to phosphoinositides on membranes.

SH3 domain – A conserved protein structure originally found in the Src oncoprotein, involved in protein–protein interactions.

Thrombocytopenia – Platelet count of $<10^7$ platelets ml^{-1} in peripheral blood, resulting in a significant shortage of platelets.

WASP – The protein product of the Wiskott–Aldrich syndrome (*WAS*) gene. It is a 502 amino acid protein composed of several domains. These domains can interact with lipids or with other proteins involved in the regulation of signal transduction cascades and remodeling of the cytoskeleton.

Wiskott–Aldrich homology domains (WH1 and WH2) – Regions of WASP whose sequences are found in other related proteins such as N-WASP in mammals and Las17p (also called Bee1p) in *Saccharomyces cerevisiae*.

Wiskott–Aldrich interactor protein (WIP) – A protein that binds to the WH1 domain of WASP and induces reorganization of the actin cytoskeleton when overexpressed.

Yeast two-hybrid system – A method used to identify proteins that bind to the protein of interest. One gene is expressed in yeast as a fusion protein with the DNA-binding site of the GAL4 transcription factor. The other gene is co-expressed as a fusion protein with the transcriptional activator domain of GAL4. Interaction of the two proteins brings the two GAL4 domains sufficiently close together to activate the transcription of a marker gene (such as *lacz*) downstream of the GAL4-binding promoter sequence.

Therapeutic possibilities

Wiskott–Aldrich syndrome is an X-linked disease, and mothers of boys with Wiskott–Aldrich syndrome show non-random X-chromosome inactivation in hemopoietic cells[28,29]. As cells that express wild-type WASP appear to survive better than cells expressing mutant WASP, one would expect that correcting the WASP mutation in stem cells by gene therapy would allow the corrected cells to replace most of the mutant cells, ameliorating or curing the disease. Although it is possible that the mutant WASP protein might be dominant, in general Wiskott–Aldrich syndrome patients with a severe manifestation of the disease have very low levels of WASP expression[13,24,30]. It therefore seems very likely that the majority of patients will be helped by addition of the wild-type WASP protein to hemopoietic stem cells.

The outstanding questions

- What are the partners of WASP and N-WASP: are they shared or are they different?
- Do all the WASP-binding or N-WASP-binding proteins interact with their target simultaneously or is there a temporal organization?
- What are the roles of WASP and N-WASP; are they localized to defined regions in the cell or do they move to different cell compartments depending on the development and activation status of the cell?
- Is N-WASP required for the survival of cells or animals?
- Can Wiskott–Aldrich syndrome be treated by gene replacement or by other therapies?

Until gene therapy becomes a reality, however, splenectomy is recommended for patients with mild Wiskott–Aldrich syndrome and thrombocytopenia; with severe Wiskott–Aldrich syndrome, bone marrow transplantation and cord blood stem cell infusions remain the treatments of choice. In cases where identical or haplo-identical bone marrow is available, bone marrow transplantation may represent a cure.

The tremendous progress in understanding the disease in the past 5 years, made possible by the cloning of the gene, the genome projects, and synergies with the recent explosion of understanding of signaling pathways in cell biology, would have been unimaginable 20 years ago. If this productive mingling of basic research and clinical research continues in this area, I predict that the molecular basis of Wiskott–Aldrich syndrome will be understood, and at least one potential cure will be under development in the early years of the 21st century. I wish I could tell you what it will be.

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