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INSIGHTS FROM MODEL SYSTEMS
Learning from the Slime Mold: *Dictyostelium* and Human Disease

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When readers of this Journal think about *Dictyostelium*—rarely, if at all, I suspect—they may conjure up dim memories either of college laboratory demonstrations involving cell aggregation and cAMP or of watching the cells develop into tiny fruiting bodies made of spores and stalks. Yeasts and several invertebrate or vertebrate model systems are widely known for their contributions to our understanding of human disease, but *Dictyostelium* is seldom included in this list. Quietly, over the last few years, however, research on this social amoeba has revealed some common cellular characteristics shared across diverse phyla. I wish to suggest that, despite the wide evolutionary distance that separates humans and the cellular slime molds, mutations in *Dictyostelium discoideum* can provide direct insight into human disease processes. Here, I focus in detail on the relationship between the Wiskott-Aldrich syndrome (WAS) and a new family of proteins, the SCARs (suppressor of cAMP receptor defects), identified first in *Dictyostelium* and subsequently in humans. This class of proteins appears to be of fundamental significance to the control of actin cytoskeleton dynamics, which is perturbed in WAS and other human genetic diseases.

Experimental Virtues of *Dictyostelium*

Dictyostelium offers numerous advantages as an experimental organism. Like other microbial genetic organisms, *Dictyostelium* is cultured easily and cheaply and can be frozen and stored indefinitely. Its genome is small (~34 Mb) and, where vertebrates may express a large number of similar genes with overlapping functions, *Dictyostelium* often carries only a single orthologous gene. In addition, expressed sequence tag and genomic sequencing projects are now well under way. Most importantly, for ease of genetic manipulation, *Dictyostelium* may be surpassed only by the yeasts, and yeast cells differ from both human and *Dictyostelium* cells in important respects—notably as regards motility. DNA-mediated transformation and homologous recombination make creating knockout and knock-in organisms relatively straightforward. Because multiple selectable markers are available, strains can be constructed with complex genotypes. The organism is haploid throughout its life cycle, so loss-of-function mutations usually cause phenotypes without the need for further manipulation. For the same reason, neither recombination nor complementation is possible by the usual means (mating) in this system, but mutants may be rescued by introducing the gene of interest directly, in either wild-type or mutant form, and with variable levels of expression.

Dictyostelium researchers now have available two means to identify second site suppressors and so to dissect genetic interactions. One recently described method involves the overexpression of cDNAs from a *Dictyostelium* (Robinson and Spudich 1998). As with the plasmid-rescue method in yeast, this approach permits the screening of libraries for unlinked genes that complement a specific defect. The other (and older) method is restriction enzyme–mediated integration (REMI; see sidebar). This insertional mutagenesis approach typically yields null alleles in interacting genes. Hence, when REMI is used to identify a second site suppressor gene, the suppressor may be predicted to serve as a negative regulator of the original gene of interest. Unmasking negative regulatory pathways is often difficult in other model genetic systems, but REMI makes it relatively easy in *Dictyostelium.* These two mutagenesis approaches would be expected to yield a different set of interacting genes, and both are well suited to facilitate the cloning of these genes.

Signaling and Motility in *Dictyostelium*

Dictyostelium is a free-living amoeba that grows and divides in the soil. When the food supply, usually bacteria, is exhausted, individual cells stop growing and collect together into a multicellular organism made up of as many as 10^5 cells. Complex signal transduction networks are activated at the beginning of this process and are used, throughout the remainder of development, to coordinate the morphogenetic and cellular differen-

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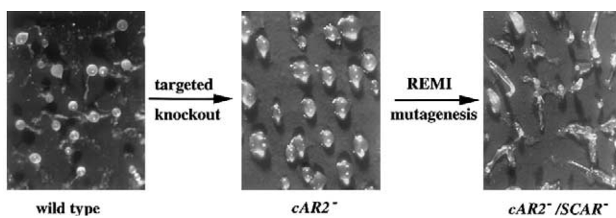
REMI-ing Out the *Dictyostelium* Genome

Restriction enzyme-mediated integration (REMI) is perhaps the most valuable tool in the *Dictyostelium* geneticist's kit. An insertional mutagenesis technique first described by Schiestl and Petes (1991) in yeast, REMI involves introducing, into *Dictyostelium* cells, linearized plasmid DNA along with limiting amounts of a restriction enzyme that will generate ends compatible with those of the plasmid. The restriction enzyme clips the genomic DNA at one or more sites, permitting the plasmid to integrate into and disrupt genes more or less at random. Cells in the resulting mutagenized population will usually carry only a single genetic lesion, and they can then be screened for a specific phenotype. Identification of the gene disrupted is generally straightforward because the plasmid itself marks the region of interest. Because of the ease of culturing *Dictyostelium* cells and the efficiency of DNA-mediated transformation, it is possible to start a mutagenesis screen with $>10^9$ cells, recover $>10^4$ tranformants, and end up with 10–50 mutants of interest. Saturation mutagenesis is at least conceivable.



The figure shows the use of molecular genetic techniques to generate first the cAR2 null strain and then the suppressor strain that identified SCAR. The left panel shows fruiting bodies that mark the terminal stage of wild-type *Dictyostelium* development. A *cAR2*-null strain was produced by targeted gene disruption, and, as seen in the middle panel, development is arrested at an earlier stage. The *cAR2*$^-$ strain was then subjected to REMI mutagenesis, and strains able to complete development were isolated. The *cAR2*$^-$/*SCAR*$^-$ strain shown in the right panel resulted from that screen. The identification of *SCAR,* which encodes an actin-binding protein, as a suppressor of the motility defects in the *cAR*$^-$ background provides important clues about the regulation of cytoskeletal remodeling in *Dictyostelium* cells and in our own.

tiation events that result in the terminal structure, the fruiting body. Over the years, significant effort has gone into understanding the signaling mechanisms involved in regulating *Dictyostelium* growth and development.

Signaling in *Dictyostelium* involves the use of extracellular cues to coordinate changes in cell behavior, cell fate, and cell-cell communication. For example, in vegetative, growing cells, extracellular folate stimulates a chemotactic response, which is thought to be used to hunt bacteria. This response is GTP-sensitive and is thought to be mediated through heterotrimeric G protein signaling (Parent and Devreotes 1996). Another series of G protein–mediated signals, initiated with cAMP as the extracellular cue, is used, during the aggregation process, to collect individual cells into the multicellular organism. Study of vegetative growth and early developmental signaling events has led to the identification of serpentine-type, G protein–coupled receptors, heterotrimeric and low–molecular weight G proteins, and effectors such as adenylyl cyclase, protein kinase A, and cAMP-dependent phosphodiesterases (reviewed in Parent and Devreotes 1996; Soderbom and Loomis 1998). Molecular genetic studies of these signaling pathways have also led to the identification of a number of other well-known signaling components, including MAP kinases, phosphatidylinositol-3 kinases, phospholipase C, protein kinase B, and STAT proteins (Drayer and Van Haastert 1992; Zhou et al. 1995; Aubry et al. 1997; Kawata et al. 1997; Meili et al. 1999). It appears that pathways familiar to mammalian cell biologists are conserved in *Dictyostelium*.

In addition to fruitful studies in the realm of signaling, *Dictyostelium* has proved to be a major contributor to our understanding of amoeboid cell movement. That *Dictyostelium* cells and leukocytes share certain characteristics (Devreotes and Zigmond 1988) has been appreciated for many years. Both use G protein–mediated signaling to regulate chemotaxis. The second messenger pathways activated seem to be similar between the two types of cells, and Rho family GTPases are important for controlling chemotactic responses.

Closely related to the questions of cell motility is the study of cytoskeletal reorganization. *Dictyostelium* cells have long been a favorite of researchers interested in the changing interactions of actin filaments and actin-associated proteins during cell movement and cytokinesis. The ability to generate null mutants in cytoskeletal protein genes in this system by means of homologous recombination has allowed cell biologists to evaluate the roles of myosin proteins and other actin-binding proteins (ABPs) in actin dynamics (Noegel and Luna 1995). For example, the *Dictyostelium* "gelation factor," ABP-120, is related to a class of human actin–cross-linking proteins, the filamins. A point mutant in *ABP-120* results in a very mild phenotype, but a targeted ablation of this gene disrupts actin filament networks, blocks pseudopod formation, and impairs cell motility dramatically. Interestingly, mutations in a human *ABP-120* relative, *Filamin-1* (also known as *"ABP-280"*), lead to periventricular heterotopia, a developmental abnormality in which cortical neurons fail to migrate (see Fox and Walsh 1999 [in this issue]).

The possibility that this migration defect and the motility defects seen in the *ABP-120* mutants are similar may make *Dictyostelium* a powerful system for studying the molecular basis of this disease. Because it is feasible to construct strains with mutations in genes for multiple ABPs, it is possible to uncover morphogenetic or motility phenotypes that might otherwise be obscured by functional redundancy among gene products. Thus, although

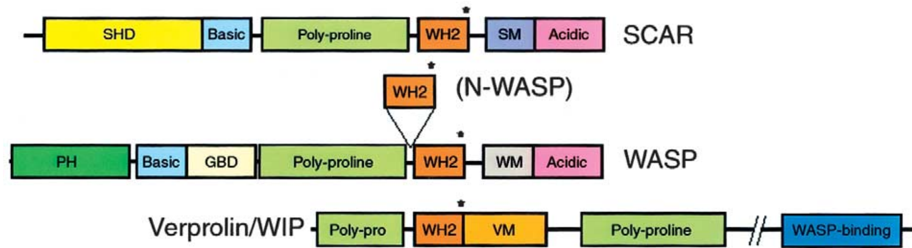


Figure 1 Structure of SCAR and its human homologues. Two human relatives of *Dictyostelium* SCAR, WASp, and N-WASp and the yeast protein verprolin are all known to interact with actin, as does human SCAR. The conserved domains are indicated, labeled as in Bear et al. 1998.

the ABP-120–deficient cells are abnormal in chemotaxis and cell movement, they can complete development, and mutants lacking another actin–cross-linking protein, α-actinin, have an even milder phenotype. The double mutant, however, is unable to complete development, and the synthetic phenotype suggests that the two proteins have nonoverlapping roles in coordinating actin networks.

Motility Defects in Human and *Dictyostelium* Cells

Signal transduction meets the actin cytoskeleton whenever cell motility is regulated, as in lamellipodial or filopodial extension by leukocytes, the development of microvilli on the surface of epithelial and immune cells, and the elaboration of neuronal processes.

One specific place where signaling and the actin cytoskeleton converge is revealed in patients with WAS. Patients with this rare X-linked disease typically have immune deficiencies, thrombocytopenia, and eczema, and, without an allogenic bone marrow transplant, they often die at an early age (reviewed in Brickell et al. 1998). This disorder is clinically quite variable, and some mutations in WASP lead not to full blown WAS but to a milder condition, X-linked thrombocytopenia (XLT). Platelets in patients with WAS are unusually small, and the thrombocytopenia is believed to be primarily due to increased platelet destruction. The autoimmune defect is believed to be, at least in part, associated with abnormally shaped T cells. These cells show a dramatic reduction in the number of actin-containing microvilli and a lack of actin filament assembly under the plasma membrane. Macrophages and monocytes also show abnormal cell motility and chemotaxis. All of this points toward WAS being a defect in some aspect of actin organization. The gene responsible for WAS was positionally cloned in 1994 (Derry et al.), and the product of this gene, WASP, was subsequently identified as a binding partner for the Rho family GTPase, CDC42 (Aspenstrom et al. 1996; Kolluri et al. 1996; Symons et al. 1996). More recently, WASP has been shown to bind both actin and the actin-nucleating protein complex, arp2/3 (Machesky and Insall 1998); the WASP-related protein, N-WASP, has been shown to facilitate the formation of actin filaments by coordinating the action of CDC42 and arp2/3 (Rohatgi et al. 1999).

How do *Dictyostelium* and SCAR fit into this picture? We identified the gene for SCAR last year in a second site suppressor screen involving one of the *Dictyostelium* cAMP receptors, cAR2 (Bear et al. 1998). Cells lacking cAR2 progress through the early hours of *Dictyostelium* development but then arrest about halfway through the process (Saxe III et al. 1993). Using REMI (see sidebar), we isolated strains of cells that bypassed the developmental block and completed development in the absence of cAR2. One of these strains identified the *SCAR* gene.

SCAR-null cells are unusually small (~25% the size of normal cells) and misshapen in suspension culture. They have reduced amounts of F-actin, particularly at the leading edge (Bear et al. 1998), and show striking defects in cell movement and chemotaxis (J. R. Steiner and C. L. Saxe, unpublished data). In several respects, the SCAR$^-$ cells seem similar to hemopoietic cells in WAS patients. The connection becomes more apparent when the SCAR protein is analyzed.

Structure of SCAR, WASP, and Their Homologues

Examination of the SCAR protein revealed a connection to WASP (fig. 1; Bi and Zigmond 1999). Both proteins have a central polyproline-containing region fitting the consensus of $X(P)_{4–9}$ (where X = G, A, L, M, or S). This type of repeat is present in actin-associated proteins and is thought to be important for interactions like those between actin and profilin (Purich and Southwick 1997). The regions C-terminal to the polyprolines most clearly connect SCAR with the WASP family of proteins. This region contains a so-called WASP homology 2 (WH2) or verprolin domain shown to be necessary for binding to monomeric actin (Machesky and Insall 1998). Fol-

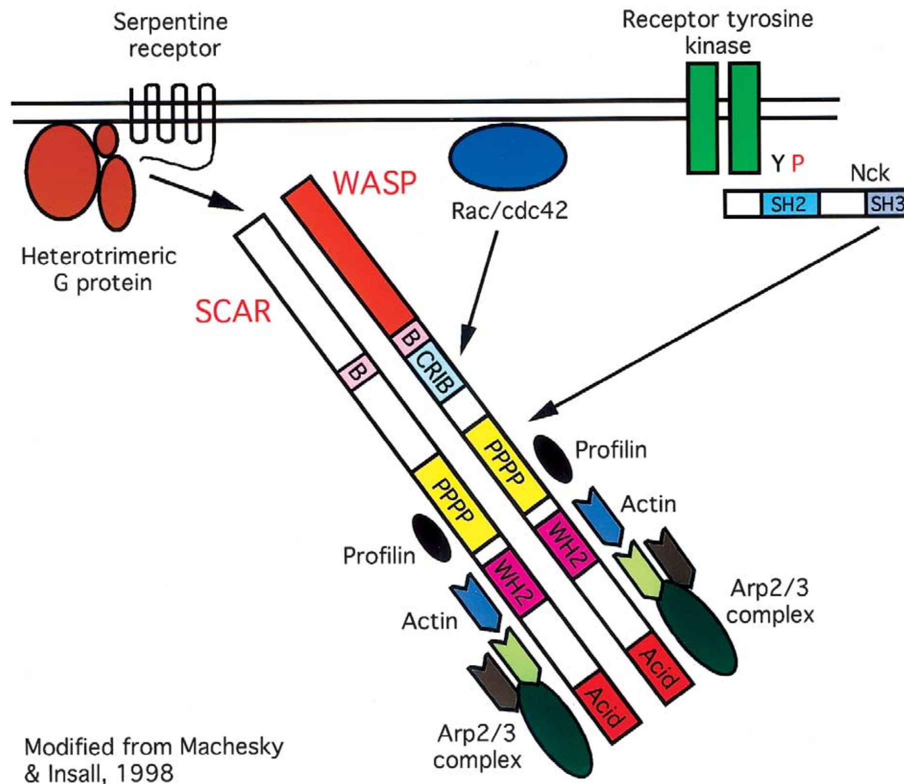


Figure 2 A model of SCAR and WASP function in actin polymerization. In this model, receptor-mediated events stimulate GTP exchange on Rho family GTPases. This signaling may be initiated through cytokines or growth factors binding receptor tyrosine kinases, or possibly through chemoattractants that act on serpentine receptors. As a result of this signal, WASP and SCAR become activated or localized in a specific region of the submembranous cytoplasm, presumably close to the activating receptor. SCAR and WASP may also be regulated through the polyproline-rich region by interactions with profilin and/or SH3-containing proteins such as Nck. Once activated, SCAR and WASP recruit or activate the arp2/3 complex, which binds to the pointed end of actin and nucleates new actin filaments in this cytoplasmic region. Monomeric actin and arp2/3 bind to distinct but adjacent regions of SCAR/WASP (the verprolin/WH2 region and acidic regions, respectively), which may facilitate their interaction. Ultimately, the assembly of these actin filaments drives the formation of filopodia or lamellipodia at the leading edge of motile cells.

lowing the verprolin/WH2 domain is a region related to the actin-depolymerizing protein cofilin. Finally, at their C termini, SCAR and WASP share an acidic domain containing a single tryptophan residue (Bi and Zigmond 1999). As discussed below, this region was recently shown to be critical for binding SCAR, WASP, and the WASP-related protein, N-WASP, to the arp2/3 complex and for facilitating the polymerization of actin filaments.

One region that distinguishes SCAR from WASP is the N terminus. In WASP, this region contains a CDC42/rac-binding domain (GBD/CRIB) and a region that binds the membrane phospholipid PIP_2. No GBD is apparent in SCAR, but preliminary data suggest that SCAR does bind the *Dictyostelium* racC protein (D. J. Seastone, J. A. Cardelli, W. R. Mahler, and C. L. Saxe, unpublished data). The N terminus also defines SCAR as a member of a family of proteins present in organisms as diverse as *Drosophila, Caenorhabditis elegans,* mice, and humans (Bear et al. 1998; Miki et al. 1998). Shortly after we identified the SCAR family, Machesky and Insall (1998) reported the protein we called “hSCAR1” as a binding partner for the p21 component of the arp2/3 complex. WASP was also found to bind p21. arp2/3 is a complex of seven proteins, comprising the actin-related proteins arp2 and arp3, as well as p41, p34, p21, p20, and p16 (Machesky et al. 1997; Welch et al. 1997). This complex binds to the pointed end of actin and nucleates barbed end growth and branching of new filaments.

The identification of WASP and a human homolog of SCAR as binding partners for the arp2/3 complex led Machesky and Insall (1998) to propose the currently accepted model of SCAR and WASP function. The model (fig. 2) predicts that the C-terminal domains of SCAR/WASP family proteins bind actin and arp2/3 and are required for the activation of the actin-nucleation function of the arp2/3 complex. This in turn leads to the

assembly of actin filaments either at the leading edge of cells or in filopodia-like structures (Machesky et al. 1999; Rohatgi et al. 1999). In vitro experiments have shown that SCAR, WASP, and N-WASP directly bind to actin via the WH2 region and to arp2/3 via the acidic domain (Machesky and Insall 1998; Rohatgi et al. 1999). SCAR and N-WASP have also been shown, in vitro, to enhance the ability of arp2/3 to stimulate actin polymerization (Machesky et al. 1999; Rohatgi et al. 1999).

Modeling WAS in *Dictyostelium*

On the basis of the molecular model shown in figure 2, one may begin to explain the anomalies associated with actin assembly in WAS patients. WASP is apparently needed to properly activate arp2/3 and to stimulate new actin-network formation. The absence of functional WASP may explain the lack of normal microvilli formation on T cells, the abnormal shape of platelets, and the motility defects in macrophages and monocytes. Without WASP, filopodia (structures dependent on CDC42 activity) and lamellipodia formation (stimulated by rac1) may be deranged. The fact that different WAS alleles show different severities of disease is consistent with different regions playing unique roles in WASP function.

Structure/function studies in *Dictyostelium* may play an important role in fleshing out the details of this model. There appears to be a single gene for SCAR in the *Dictyostelium* genome, as well as a bona fide *WASP* gene (Bear et al. 1998). In humans, there are at least three different SCARs and two WASPs. Use of genetics to understand the role(s) of individual players will be much easier in *Dictyostelium* because of this more limited redundancy. Introduction of site-directed, random, or chimeric mutants of SCAR or WASP into a null genetic background will be simple in *Dictyostelium.* Several of the most severe disease alleles found in WAS individuals occur in the C-terminal region of *WASP,* in sequences that are conserved between WASP and SCAR. These mutations can be introduced into the cloned *Dictyostelium* genes and expressed in $SCAR^{-}$ strains. The ability to rescue some parts of the SCAR phenotype but not others could help in our understanding of the different severities of the disease among WAS and XLT families. Recreation of different mutations and use of the *Dictyostelium* mutant phenotype as the readout should provide much information about the functional importance of parts of WASP and SCAR. Once the *Dictyostelium WASP* gene is knocked out, this strategy can be expanded to include studies in that null background. There may also be differences between the SCAR and WASP nulls, which would reveal unique functions for these homologous proteins. Most likely, human *WASP* and *SCAR* genes can be expressed successfully in *Dictyostelium* and studied directly in this system (author's unpublished data). *Dictyostelium*-suppressor genetics may also reveal new levels of regulation of this developmental pathway. Starting with a *SCAR*- or *WASP*-null strain, the phenotype can be suppressed (partially or completely) by overexpression or REMI rescue. The genes identified in such experiments should expand our understanding of the pathways involved in regulating SCAR/WASP and may provide the connections from the cell surface to the actin cytoskeleton.

Although the outlines of SCAR/WASP function are now becoming clear, there is still a great deal about the mechanisms of their action that is unknown. How do the small GTPases regulate SCAR/WASP? Are there other proteins that interact with them? Do SCAR and/or WASP function as dimers? How critical is localization for their function and how is it determined? How is this coupled to receptor-mediated signaling? *Dictyostelium* may not be the only organism in which all of these questions can be answered, but use of *Dictyostelium* genetics, the relative simplicity of the system, and its wealth of information regarding signaling and actin cytoskeletal organization may reveal many parts of the puzzle. The time is ripe to exploit the power of this system to answer fundamental questions about how cells use extracellular cues to make differential decisions. WAS may be among the first human diseases to be elucidated in this system, but it is unlikely to be the last.

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