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SCAR/WAVE

A complex issue

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The SCAR/WAVE complex drives the actin polymerisation that underlies protrusion of the front of the cell and thus drives migration. However, it is not understood how the activity of SCAR/WAVE is regulated to generate the infinite range of cellular shape changes observed during cell motility. What are the relative roles of the subunits of the SCAR/WAVE complex? What signaling molecules do they interact with? And how does the complex integrate all this information in order to control the temporal and spatial polymerisation of actin during protrusion formation? Unfortunately, the interdependence of SCAR complex members has made genetic dissection hard. In our recent paper,¹ we describe stabilization of the *Dictyostelium* SCAR complex by a small fragment of Abi. Here we summarize the main findings and discuss how this approach can help reveal the inner workings of this impenetrable complex.

The SCAR/WAVE Complex and its inputs

The highly conserved SCAR complex (also called the “WAVE complex”) causes actin-based protrusion during cell migration in a diverse range of eukaryotes including the amoeba *Dictyostelium discoideum*, *Drosophila melanogaster*, and mammalian cells.²⁻⁴ SCAR/WAVE is a WASP family member that induces actin nucleation via recruitment and activation of the Arp2/3 complex.^{5,6} In vivo, SCAR activity is regulated by its inclusion within a ~400 kDa regulatory complex consisting of PIR121, Nap1, HSPC300, and Abi.⁷⁻⁹ This regulatory complex undoubtedly acts as a

signaling hub, where competing inputs are integrated and coupled to the activation of SCAR. However, we still lack even a basic knowledge of what the complex interacts with, never mind how such possible interactions are interpreted and translated into actin polymerisation, motility, and chemotaxis.¹⁰ Confusingly, WASP, which lacks the regulatory complex, can respond to many of the same inputs.²

The different roles of SCAR complex members

The recent resolution of the human SCAR complex crystal structure has greatly advanced our understanding of how these proteins interact with SCAR and one another.^{11,12} However, the relative contribution of the individual complex members to the activity of the whole remains poorly understood and still awaits elucidation.

Thus far, it has been established that the SCAR complex interacts with its best-known activator, Rac, via PIR121.^{11,13} As highlighted in **Figure 1**, Abi has been implicated in the recruitment of the SCAR complex to signaling complexes containing Rac.¹⁴ Furthermore, multiple, stimulus-responsive phosphorylation sites have also been identified across the different SCAR complex members.¹⁵

In vivo, SCAR is entirely dependent on its regulatory complex for stability, and in the absence of any one complex member, SCAR is rapidly degraded.^{3,16} Herein lies the problem that has confounded the study of the individual SCAR complex members: the inability to separate the function of the individual complex members from their requirement for complex stability.

Keywords: SCAR, WAVE, Abi, Arp 2/3, actin, pseudopod, chemotaxis, motility

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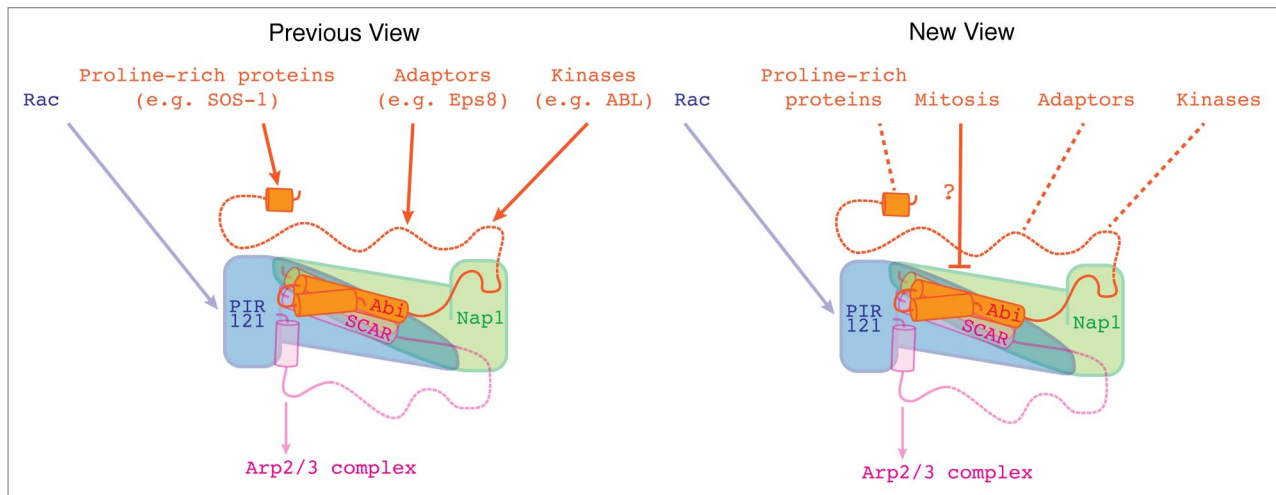


Figure 1. The role of Abi within the SCAR complex. The SCAR complex is composed of PIR121 (blue), Nap1 (green), HSPC300 (hidden in this image), SCAR (magenta), and Abi (orange). The SCAR complex promotes activation of the Arp2/3 complex (magenta arrow). Abi has long been considered a key regulator of the SCAR complex. In combination with the Rac/PIR121 interaction (blue arrow), Abi was thought to activate actin polymerization by coupling SCAR to various signaling and adaptor proteins (orange arrows) via its C-terminal SH3 domain (metazoans only) and polyproline tail (dashed ribbons). However, having deleted the majority of *Dictyostelium* Abi, it is now evident that Abi is not required for SCAR complex activation. Instead, Abi likely acts to tune the activity of the SCAR by integrating both positive and negative signals (dashed lines). One possible negative input could be acting through the N-terminal first α -helix to suppress SCAR activity during mitosis. However, the regulators that bind this domain have yet to be identified.

Numerous studies have endeavored to replace different SCAR complex members with mutant or truncated proteins in order to determine their function.^{17,18} However, all too often, the effect on complex stability has been ignored.

Most of Abi is dispensable

Previously, our lab has characterized the individual SCAR complex member gene disruptants in *Dictyostelium discoideum*.^{16,19-21} These mutants typically exhibit a *scrA* null phenotype due to the degradation of SCAR. We reasoned that if SCAR complex stability could be restored in the different SCAR complex member nulls, we could finally address the relative role of these proteins to the activity of the complex as a whole. In particular we sought to identify functional domains required for normal SCAR complex recruitment and activity. To achieve this, we set about generating a deletion series of the *Dictyostelium* PIR121, Nap1, and Abi with the objective of identifying minimal fragments of these proteins that could stabilize the complex. This would enable us to assign specific functions to the absent domains. Incremental truncation of both PIR121 and Nap1 failed to yield any fragments that could stabilize SCAR. Although not available at

the time, the SCAR complex crystal structure subsequently revealed the highly convoluted conformation of both PIR121 and Nap1 within the complex, which undoubtedly underlies the failure of this approach in these cases.

In contrast, in our recent paper we demonstrated that we could successfully delete 239 of the 332 amino acids comprising Abi and retain both SCAR and SCAR complex stability in the *Dictyostelium abiA* null.¹ Surprisingly, we found that none of this sequence was necessary for robust recruitment of the SCAR complex to the tips of pseudopodia. The suppressed rate of pseudopod formation in the *abiA* null was also restored, implying that the majority of Abi sequence is not required for SCAR complex activation either. Although the N-terminus of Abi was specifically involved in regulating the SCAR complex during cytokinesis, apparently negatively, we could find no phenotype associated with a loss of the C-terminal polyproline tail.

What is the true role of Abi within the SCAR complex?

As summarized in Figure 1, it was concluded that SCAR complex localization and activity do not depend on any signals that are transduced through Abi.¹ Instead,

we suggest that Abi serves to modulate the activity of SCAR, particularly during events such as cytokinesis. Despite low detailed amino acid identity, a C-terminal polyproline tail is a consistent feature of almost all Abi homologs, which implies that the polyproline domain does have a universal role in the regulation of the SCAR complex. We propose it acts as a non-essential signal integrator that tunes the activity of the SCAR complex after it has been activated by alternate pathways.

Such a role is consistent with the evolutionary recent acquisition of a C-terminal SH3 domain found in metazoan Abi homologs. Even though this domain has been implicated in the regulation of the mammalian SCAR complex, it is not required for SCAR complex recruitment during actin-based protrusion.^{14,22}

The attainment of multicellularity in metazoa has been accompanied with a huge increase in regulatory complexity, and cells within a tissue have very different requirements of the SCAR complex in comparison to free-living amoeba. For example, unlike amoebae that are constantly on the move, metazoan cells within a tissue presumably suppress SCAR complex activity until it is required during carefully choreographed events. We propose that Abi is the obvious candidate for

the application of these additional layers of control.

Future directions and conclusions

Although deletion series analysis allowed us to explore the role of Abi within the SCAR complex, this has proved too crude a method to similarly investigate the function of the other complex members such as PIR121 and Nap1. We believe a subtler approach will be

required to mutate these complex members while preserving complex stability. For instance, our lab has recently successfully used phosphomimetic mutation to study the activation of SCAR.²³ Few phosphosites have been identified within PIR121 and Nap1. PIR121 and Nap1 are also both so large that systematic alanine scanning mutagenesis is a daunting prospect. However, given the fragility of the SCAR complex, it may prove the only available option.

The intricacy of the SCAR complex continues to block investigation from every angle, and as a result we have barely scratched the surface of how these proteins collectively function. Despite the difficulty, we believe that genetic approaches could still offer the key to unlocking the secrets of the SCAR complex.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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