SH3 domain-containing proteins and the actin cytoskeleton in yeast

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Abstract

SH3 (Src homology-3) domains are involved in protein–protein interactions through proline-rich domains. Many SH3-containing proteins are implicated in actin cytoskeleton organization. The aim of our ongoing work is to study the functions of the SH3-containing proteins in actin cytoskeleton regulation. The yeast Saccharomyces cerevisiae proteome includes 29 SH3 domains distributed in 25 proteins. We have examined the direct involvement of these SH3 domains in actin polymerization using an in vitro polymerization assay on GST (glutathione S-transferase)–SH3-coated beads. As expected, not all SH3 domains show polymerization activity, and many recruit distinct partners as assessed by microscopy and pull-down experiments. One such partner, Las17p, the yeast homologue of WASP (Wiskott–Aldrich syndrome protein), was assayed because it stimulates actin nucleation via the Arp2/3 (actin-related protein 2/3) complex. Ultimately, proteins involved in specific biological processes, such as membrane trafficking, may also be recruited by some of these SH3 domains, shedding light on the SH3-containing proteins and actin cytoskeleton functions in these processes.

Introduction

The actin cytoskeleton is involved in diverse cellular processes including cell motility, cytokinesis, endocytosis and exocytosis. The WASP (Wiskott–Aldrich syndrome protein)/SCAR (suppressor of cAMP receptor) family proteins promote actin filament assembly by activating the Arp2/3 (actin-related protein 2/3) actin nucleation complex. SH3 (Src homology-3) domains are involved in protein–protein interactions, often through proline-rich motifs. In Saccharomyces cerevisiae, the homologue of WASP, Las17p, is involved in multiple SH3 interactions through its proline-rich regions [1–3]. Furthermore, several studies in different organisms highlight the roles of SH3 domain–containing proteins in the activation of WASP proteins and the Arp2/3 complex [4]. These proteins have to be precisely regulated to assemble specialized actin structures required for distinct cellular processes, such as the internalization step of endocytosis [5,6]. We are interested in determining the role of these SH3 domains in actin cytoskeleton functions and, in particular, in actin polymerization.

SH3 domain proteins in S. cerevisiae

The S. cerevisiae proteome includes 29 SH3 domains distributed in 25 proteins involved in multiple cellular functions. Some of these proteins are known to be part of or to interact with the actin cytoskeleton such as Myo3p, Myo5p, Abp1p, Bzz1p, Bbc1p, Rvs167p, Lsb1p–Lsb4p and Sla1p. Others are implicated in intracellular signalling (Sho1p, Nbp2p, Cdc25p and Sdc25p), in morphogenesis for bud growth (Bem1p, Bud14p, Boi1p and Boi2p), for shmoo formation (Fus1p), for cytokinesis (Hof1p, Cyk3p) or for snoRNP (small nucleotide ribonucleoprotein) assembly (Naf1p). Yet others such as Pex13p and Hse1p are membrane proteins of organelles. However, the function of several SH3 domain-containing proteins remains poorly understood [1]. Since most of the SH3-containing proteins are implicated in or connected to the actin cytoskeleton polymerization, we were interested in determining the possible roles of all yeast SH3 domains in actin polymerization.

SH3 domains and actin polymerization

Las17p is the key component of a multi-subunit complex that contains Vrp1p/End5p, Myo3p and Myo5p and several other SH3 domain-containing proteins. Furthermore, the type I myosins, Myo3p and Myo5p, that interact with a number of SH3-containing proteins are important for organization and polymerization of actin. The precise role of Vrp1 in actin polymerization is unknown, but this proline-rich protein is important for actin cytoskeleton polarization and for targeting other proteins to actin patches. Las17p has been shown to recruit, through its proline-rich region, SH3 domain-containing proteins such as Bzz1p, Bbc1p, Rvs167p and the type I myosins, Myo3p and Myo5p [1,2,7,8]. As many actin-associated proteins contain SH3 domains, this highlights the importance of SH3 domains in protein–protein interactions with the cytoskeleton. The aim of the ongoing work is to study, in yeast, the interactions between the SH3-containing proteins and the actin cytoskeleton. The SH3 domain function is analysed by in vitro actin polymerization assays followed by fluorescence microscopy analysis. Briefly, SH3 domains were expressed in Escherichia coli as GST (glutathione S-transferase)–SH3 fusions [1], purified

Key words: actin polymerization, Bbc1p/Abp1, Src homology-3 (SH3), type-I myosin, Wiskott–Aldrich syndrome protein/Las17 (WASP/Las17), yeast.

Abbreviations used: Arp2/3, actin-related protein 2/3; GFP, green fluorescent protein; GST, glutathione S-transferase; SH3, Src homology-3; TRITC, tetramethylrhodamine–thioisoucyanate; WASP, Wiskott–Aldrich syndrome protein.

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**Figure 1 | In vitro actin polymerization assay scheme**

GST–SH3 domain fusions were expressed in *E. coli* and purified on glutathione–Sepharose 4B beads (A). A total protein extract prepared from the DDY2736 strain expressing a Las17–GFP fusion (C) was added with an ATP regenerating buffer and fluorescent actin–Rhodamine on the GST–SH3-coated beads (B). After 20 min of incubation, beads were monitored for Las17–GFP binding and actin polymerization by fluorescence microscopy.

**Figure 2 | Effects of Myo5p and Bbc1p SH3 domains on actin polymerization**

Samples were visualized with a Nikon Optiphot-2 microscope and images were taken with a CCD (charge-coupled-device) camera (Photonics Science Coolview 10). The same field of beads was viewed by fluorescence (FITC filter, Las17–GFP panels, or TRITC filter, actin-Rhodamine panels) and by Nomarski optics. Beads coated with GST alone used as a negative control show neither actin polymerization nor Las17–GFP recruitment. The SH3 domain of Myo5p recruits Las17–GFP and shows actin polymerization. The Bbc1p SH3 domain recruits Las17p, but it does not trigger actin polymerization.
Our results show that not all of the 29 yeast SH3 domains interact with Las17p and/or induce actin polymerization. Here, we show that while both Myo5p and Bbc1p SH3 domains are able to recruit Las17p, only the Myo5p SH3 domain triggers actin polymerization (Figure 2).

**Conclusion**
The type I myosin Myo5p SH3 domain recruits Las17p and thus gives rise to actin polymerization. The actin polymerization machinery was shown to be recruited by a Myo5p C-terminal construct (containing the SH3 domain and the Tail homology domain 2) [9]. Here, we show that the SH3 domain alone is sufficient to trigger actin polymerization in our assay. This is in agreement with genetic data showing that myo3Δmyo5Δ mutant cells expressing an SH3 deleted Myo5p construct displayed the same actin defects as the mutant [9]. Thus the SH3 domain of type I myosin is critical for actin organization. We have also found that Bbc1p SH3 domain recruits Las17p, but does not induce actin polymerization. The SH3 domains of Myo3/5p myosins are known to bind to the proline-rich region of Bbc1p [1] and the bbc1Δ deletion suppresses the temperature sensitivity and endocytosis defects of a vrp1 mutant [10]. Furthermore, the SH3 domain of Bbc1p inhibits Las17p activity *in vitro* [11]. These results together show that the negative function of Bbc1p in actin polymerization is mediated via its SH3 domain.

In conclusion, this technique allows us to discriminate between SH3 domains recruiting Las17p and having a positive role in actin polymerization and SH3 domains not inducing or inhibiting actin polymerization even though they are associated with Las17p. Moreover, this technique can also be used with actin effectors other than Las17p to analyse their association with SH3 domains.

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**References**


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