New crystal structure sheds light on the function of Sro7/tomosyn/Lgl proteins in cell polarity

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The yeast proteins Sro7/Sro77 and their metazoan relatives Lgl and tomosyn interact with plasma membrane SNARE proteins and control cell polarity. Recently, the crystal structure of yeast Sro7 has been solved, yielding fascinating insights and even more unanswered questions concerning the molecular mechanism of these proteins.

The budding yeast is an attractive model organism to study cell polarity. At the onset of asymmetric cell division, vesicles are transported along cytoskeletal tracks to a predefined site of the plasma membrane. Here, they undergo exocytosis, causing the formation of a bud that enlarges to form the daughter cell. A large octameric protein complex, termed the exocyst, plays a key role in directing transport and defining the attachment site of the vesicles [1-3]. Fusion is mediated by the SNARE proteins Snc1/2p, Sso1/2p, and Sec9p. Two homologous proteins, Sro7p and Sro77p, bind both to a component of the exocyst (Exo84) [4] and a SNARE (Sec9) [5]. Deletion of these proteins leads to a cold-sensitive defect in vesicle exocytosis, suggesting a functional link between the exocyst and the SNAREs. Recently, Hattendorf et al. [6] have presented the crystal structure of the major portion of Sro7, shedding first light on the molecular mechanism by which this protein might control SNARE function.

SNAREs are evolutionary conserved membrane proteins involved in all fusion events of the secretory pathway, which are characterized by a conserved coiled coil segment termed the SNARE motif. During fusion, the SNARE motifs of interacting SNAREs assemble into a parallel four-helix bundle, starting from the N-terminal tips towards the C-terminal membrane anchors. As result, the membranes are pulled into close apposition and fusion is initiated. Based on a highly conserved set of amino acid side chains, SNARE motifs are classified into Qa-, Qb-, Qc-, and R-SNAREs, and every functional SNARE complex contains one SNARE motif of each subfamily (reviewed in [7,8]). Sso1/2 (Qa) and Snc1/2 (R) each contain a single SNARE motif that is located adjacent to a C-terminal transmembrane domain. In contrast, Sec9 contains two SNARE motifs (Qb and Qc) connected by a linker region and, in addition, it contains an extended N-terminal domain.

Sro proteins are large soluble proteins with close relatives in all eukaryotes. They contain multiple WD40 repeats of ≈ 40 amino acids, typically containing a Trp-Asp pair at the carboxyl end. WD40 repeats are known to form blade-like structures that circularly assemble into β-propellers. A metazoa relative of Sro proteins, the tumor suppressor lethal giant larvae (Lgl), plays a key role in establishing epithelial cell polarity during embryogenesis in *Drosophila* [9-11]. The salt-sensitivity of a yeast Sro double mutant to NaCl is rescued by ectopic expression of the *Drosophila lgl* gene [12]. A mammalian orthologue of Lgl was shown to interact with syntaxin 4 [14],

a ubiquitous Qa-SNARE of the plasma membrane. The closest relative of Sro in animals is tomosyn, originally isolated as a binding partner of the Qa-SNARE syntaxin 1 [13]. Syntaxin 1 mediates neuronal exocytosis together with its SNARE partners synaptobrevin 2 (R), and SNAP-25 (Qbc). Thus, it appears that Sro, Lgl, and tomosyn carry out similar functions by means of interacting with plasma membrane specific Q-SNAREs.

The new crystal structure of Sro7 shows that the 14 WD40 repeats fold into two consecutive seven-bladed β-propeller domains (Figure 1). The N-terminal portion of the chain associates with the C-terminal β-propeller, thus forming a molecular clasp, yielding an overall topology similar to the actin-interacting protein Aip1/Unc78 [15,16]. The arrangment of the two β-propellers into an open "clamshell"-shaped structure provides multiple protein binding surfaces oriented at specific angles with respect to one another. One of these surfaces is occupied by the 60-residue C-terminal tail of Sro7 (aa 892-951), which tightly packs in an extended conformation to the convex surface of the N-terminal β-propeller. A comparable arrangement has been found for the seven-bladed β-propeller proteins coronin-1 and Nup214/CAN. In coronin-1, the tight binding of a 130-residue C-terminal tail stabilizes the β-propeller [17] whereas in Nup214/CAN the interaction is not needed for maintaining the integrity of the β-propeller structure [18]. In the latter case, and probably also in Sro7, this interaction appears to be dynamic, suggesting that the C-terminal tail confers a regulatory function.

Using the new structure as a guide, Hattendorf et al. have investigated how Sec9 binds to Sro7, and how this interaction is related to the function of Sro7 in yeast exocytosis. Binding is mediated by a part of the N-terminal domain of Sec9 that associates with the convex surface of the C-terminal β-propeller. However, the binding region within the N-terminal domain is poorly conserved in other Sec9 proteins. Moreover, only fungal Qbc-SNARE proteins contain extended N-terminal domains, and in yeast the N-terminal domain of Sec9 was shown to be expendable [19].

To explore the observed discrepancy between the binding data and the functional complementation assays, the authors investigated whether the SNARE motifs of Sec9 alone are capable of interacting with Sro7. Weak binding was observed (with an approximate affinity of 50 μM), but only when the tail was deleted. In addition, deletion of the tail helix moderately increased the affinity of full-length Sec9 (from 3 to 0.8 μM), suggesting that the tail regulates the ability of Sro7 to interact with Sec9. This gave rise to the hypothesis that the two SNARE motifs of Sec9 compete with the tail of Sro for binding to the N-terminal β-propeller. In this case, binding may control the ability of Sec9 to enter SNARE complexes. Further support for this notion will require competition experiments between the tail and the SNARE motifs of not only

Sec9 but also its Qa-SNARE partner Sso, which might prove difficult considering the low affinities.

Intriguingly, the tail region of Sro is followed by a stretch of C-terminal heptad-repeats, which are not part of the new crystal structure. This stretch was predicted to form part of a degenerate R-SNARE motif [20] which is, in fact, less degenerate in the Sro7 orthologues of other fungi. Tomosyn (but not the Lgl proteins) contains a full R-SNARE motif at the C-terminal end [21,22]. The tomosyn R-SNARE motif is capable of substituting for synaptobrevin in forming a typical four helix-bundle with syntaxin 1 and SNAP-25 [20]. Thus, it appears that Sro7 and Sro77 are orthologues of tomosyn, with the two yeast isoforms probably arising from a recent gene duplication event. Lgl, however, is restricted to metazoa and probably plays a role in cell differentiation in multicellular organisms. Apparently, an evolutionary split occurred between the two factors [11], possibly resulting from an early duplication of the tomosyn gene in animals.

Considering that the R-SNARE motif of tomosyn can associate with the plasma membrane Q-SNAREs [20], the question arises whether the degenerate SNARE motif in the yeast orthologue Sro7 can bind to the R-SNARE site in the yeast exocytotic SNARE complex. In fact, the complex of Sro7 and Sec9 has originally been isolated together with Sso and Snc. Hattendorf et al., however, did not observe a significant change in binding affinity when the degenerate SNARE motif was deleted, albeit Sec9 binding was not investigated in the presence of Sso. Furthermore, in complementation assays, removal of 42 residues from the C-terminus (Δ42) of the degenerate SNARE motif of Sro7 was still able to rescue the Sro7/Sro77 deletion strain, whereas deletion of the entire tail region (Δ142) was not tolerated. Thus, neither of the two structurally defined interactions between Sro7 and its orthologue tomosyn and Q-SNAREs suffices to explain the function of these proteins at the molecular level. However, the new structure provides a sound basis for future experiments, and it is likely that the association of the tail peptide with the N-terminal propeller domain will indeed prove to be a critical element in the function of these fascinating proteins.

Figure 1 - Schematic overview of the Sro7 and the tomosyn SNARE complex structure.
a, View of the convex surface of the Sro7 structure. **b**, Side-view of the structure of Sro7 [6]. **c**, Schematic depiction of the domain structure of Sro7. The N-terminal β-propeller region is light green, the C-terminal β-propeller is violet, a putative regulatory loop and helix within blade 8 is yellow, the tail region is dark blue, and the C-terminal coiled coil region is orange. A short stretch between the blades 10 and 11 is red. This region contains phosphorylation sites in animal tomosyn and Lgl and is alternatively spliced in animal tomosyns, thus probably providing distinct binding surfaces for different regulating factors.
d, Sequence alignment of the C-terminal region of tomosyns and synaptobrevin showing that tomosyns generally contain an R-SNARE motif, which is partially degenerated in Sacchoromycota fungi. Shown are synaptobrevin 2, *Mus musculus* (MuMu_Syb2, gi 207626); and tomosyn homologues from *Rattus norvegicus* (gi 13540648, RaNo_Tom1); *Caenorhabditis elegans* (gi 17508275, CaEl_Tom); *Drosophila melanogaster* (gi 28571171, DrMe_Tom); *Arabidopsis thalania* (gi 15239173, ArTh_Tom1); *Magnaporthe grisea* (gi 39973855, MaGr_Tom); *Neurospora crassa* (gi 85090786, NeCr_Tom); *Aspergillus nidulans* (gi 40741778, AsNi_Tom); and *Schizosaccharomyces pombe* (gi 19113918, ScPo_Tom); and *Saccharomyces cerevisia* (gi 6325289, SaCe_Sro7p; gi 6319362, SaCe_Sro77). The transmembrane region of synaptobrevin 2 is indicated white on black background. The coiled coil layer residues are shown in black on a grey background. The numbers of the layers are indicated on top. The length of the deleted fragment (Δ42) is indicated [6]. Also note that some hydrophilic residues are present in the C-terminal layer residues of tomosyns from other species.
e, Structure of the SNARE complex of the C-terminal R-SNARE motif of tomosyn with the Q-SNAREs syntaxin 1 and SNAP-25 [20]. The structure representations were prepared with Pymol.

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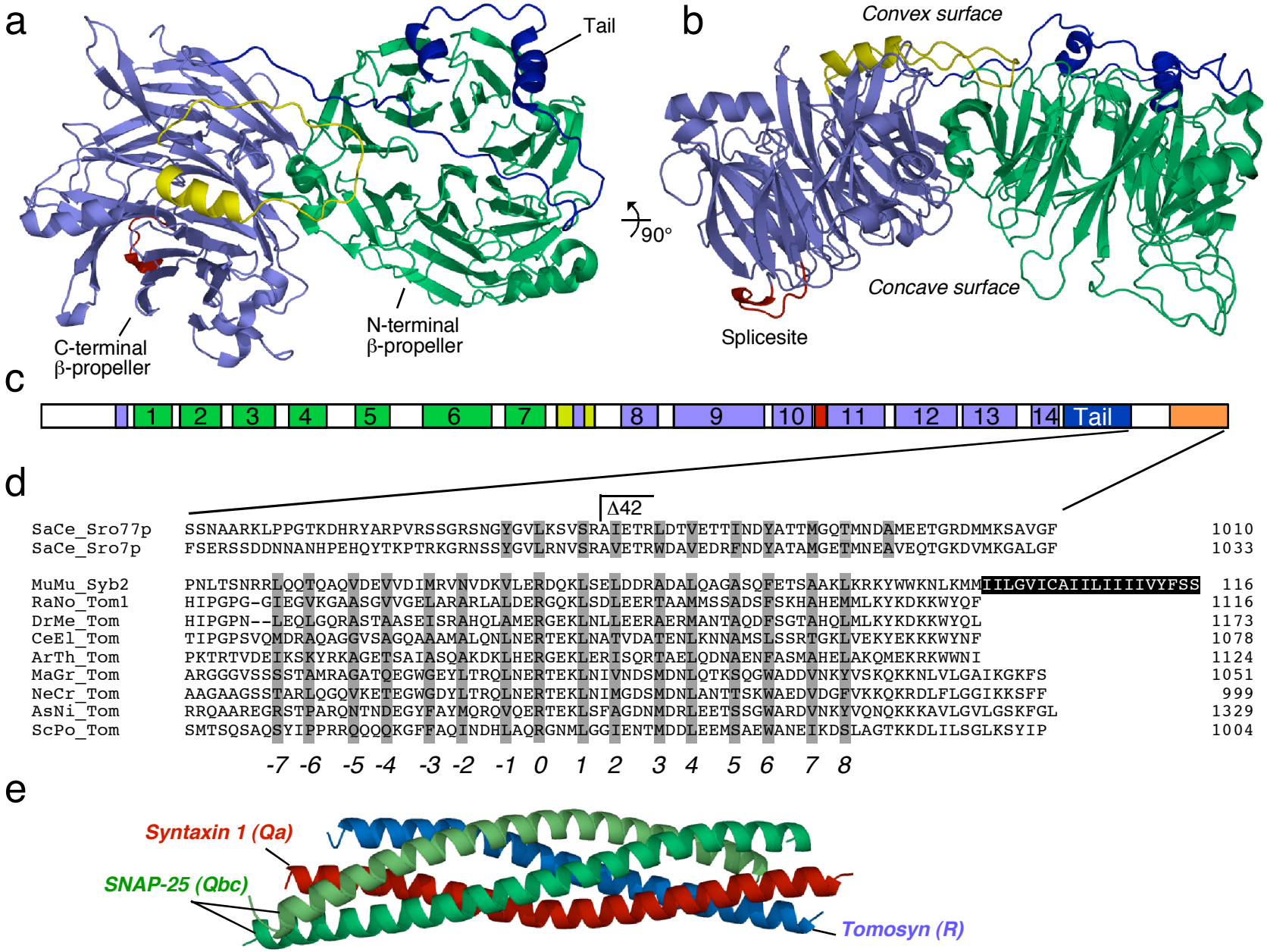
a
Tail
C-terminal
β-propeller
N-terminal
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Δ42
SaCe_Sro77p SSNAARKLPPGTKDHRYARPVRSSGRSNGYGVLKSVSRAIETRLDTVETTINDYATTMGQTMNDAMEETGRDMMKSAVGF 1010
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RaNo_Tom1 HIPGPG-GIEGVKGAASGVVGELARARLALDERGQKLSDLEERTAAMMSSADSFSKHAHEMMLKYKDKKWYQF 1116
DrMe_Tom HIPGPN--LEQLGQRASTAASEISRAHQLAMERGEKLNLLEERAERMANTAQDFSGTAHQLMLKYKDKKWYQL 1173
CeEl_Tom TIPGPSVQMDRAQAGGVSAGQAAAMALQNLNERTEKLNATVDATENLKNNAMSLSSRTGKLVEKYEKKKWYNF 1078
ArTh_Tom PKTRTVDEIKSKYRKAGETSAIASQAKDKLHERGEKLERISQRTAELQDNAENFASMAHELAKQMEKRKWWNI 1124
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AsNi_Tom RRQAAREGRSTPARQNTNDEGYFAYMQRQVQERTEKLSFAGDNMDRLEETSSGWARDVNKYVQNQKKKAVLGVLGSKFGL 1329
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Syntaxin 1 (Qa)
SNAP-25 (Qbc)
Tomosyn (R)