A model for structural similarity between different SNARE complexes based on sequence relationships

In the June 1998 issue of *trends in* CELL BIOLOGY, Götte and Fischer von Mollard summarized recent results in an updated picture of the structure and function of the SNARE machinery that mediates most if not all cellular membrane-fusion events (see Ref. 1 and references therein). The structure of the synaptic SNARE machinery has been studied in most detail, and it is clear now that the core structure involves two SNARE proteins at the target membrane (the t-SNAREs syntaxin 1 and SNAP-25) and one SNARE at the vesicle membrane (the v-SNARE synaptobrevin/VAMP). These three proteins form a stable trimeric complex held together by coiled-coil interactions between two domains of SNAP-25 and one each of syntaxin and synaptobrevin/VAMP. However, the structure of SNARE complexes at organelles other than the plasma membrane has remained more of a mystery. No SNAP-25-like proteins have been identified in those complexes, and, instead, a multitude of small v-SNARE-like proteins interact with syntaxin homologues on intracellular organelles. In some cases, evidence suggests that more than one v-SNARE-like protein is present in one complex[1]. We would like to add to this discussion our recent finding that v- and t-SNAREs are evolutionarily related to each other, and suggest a hypothesis to explain the absence of SNAP-25 homologues and the presence of more than one v-SNARE in intracellular SNARE complexes.

There are several homologues of v- and t-SNAREs involved in fusion to different membrane compartments in the cell. It has been difficult to analyse sequence relationships between SNAREs because the only conserved regions show a propensity for heptad repeats, which occur in many unrelated proteins. Using profile-based sequence analysis, we demonstrated recently the evolutionary relationship of t-SNAREs of the syntaxin and SNAP-25 families[2]. Syntaxins contain one copy of a conserved 't-SNARE domain' of 60 amino acids, whereas SNAP-25 proteins have two copies. These domains are identical to the coiled-coil domains that mediate interactions between SNARE proteins. A classification of the small v-SNARE-like proteins has been more difficult despite the fact that they share common features.

We have now extended our sequence analysis of SNARE proteins using generalized profiles, which are derived from multiple alignments and contain information on which part of the sequence is most highly conserved and which regions of the sequence are likely to tolerate deletions or insertions[3]. Iteratively refined profile searches are a sensitive method of detecting distant sequence similarities, and, unlike protein threading methods, similarities found by profile searches typically reflect relationships based on divergent rather than convergent evolution. We created a profile using the sequence of the Golgi v-SNARE Bos1p and recently reported sequences of related proteins. Database searches with this profile revealed several new and uncharacterized, yet clearly related, yeast and nematode sequences, which were included into the profile. The resulting refined profile, consisting of the central conserved regions and the C-terminal membrane anchor, was used for further database searches. Surprisingly, the highest-scoring matches were members of the syntaxin and synaptobrevin/VAMP families, the best matching of which indicated a statistically significant relationship. This strongly suggests that the Bos1-, syntaxin- and synaptobrevin/VAMP-families are evolutionarily related to each other. Since we had previously already reported the relationship between the syntaxin and SNAP-25 families and Bet1p[2], we conclude that all currently known v- and t-SNAREs are likely to have evolved from a single common ancestral gene and belong to a common superfamily. Five distinct SNARE subfamilies can be identified by nearest-neighbour dendrogram analysis (see Box 1). Figure 1 shows an alignment of the homologous sequences of representative members of these subfamilies. Several features are striking: the conservation of the heptad repeats, a cluster of basic amino acids that separates the coiled-coil and transmembrane domains, and the conservation of a glutamine residue in the d-position of a heptad repeat in all but the synaptobrevin/VAMP family.

The evolutionary relationship of SNARE proteins leads us to propose the following hypothesis. There is reason to believe that members of the SNAP-25 family are exclusively involved in fusion at the plasma membrane. First, the completely sequenced genome of *Saccharomyces cerevisiae* contains only two SNAP-25 members[2], Sec9p and Spo20p (YMR017w), both of which function in fusion to the plasma membrane[4]. Second, in mammalian systems, only two family members have been characterized: SNAP-25 at the presynaptic plasma membrane and SNAP-23 at plasma membranes in other tissues[5]. Given the evolutionary relationship between the coiled-coil domains of all SNAREs, we suggest that the intracellular SNARE complexes are structurally similar to the synaptic SNARE complex in that the number of coiled-coil domains in each complex is fixed. One molecule of SNAP-25, which contributes two coiled-coil domains in the synaptic complex, could be substituted by two small SNAREs of the synaptobrevin/VAMP-, Bet1- and/or Bos1-families, which could therefore be regarded as 'half-SNAP-25s'. Several arrangements are possible, one of which is depicted in Figure 2b. It is possible that the binding of one or two given small SNARE(s) to a syntaxin isoform could alter the binding properties of the resulting dimer or trimer, thereby creating t-SNAREs with different specificities – which could explain how one single

BOX 1 – FURTHER INFORMATION

Supplementary material supporting this hypothesis is available on the World Wide Web at the following URL: `http://itsa.ucsf.edu/~mostov/supplementary/`

This site contains a more detailed sequence alignment and a nearest-neighbour dendrogram analysis of SNARE proteins.

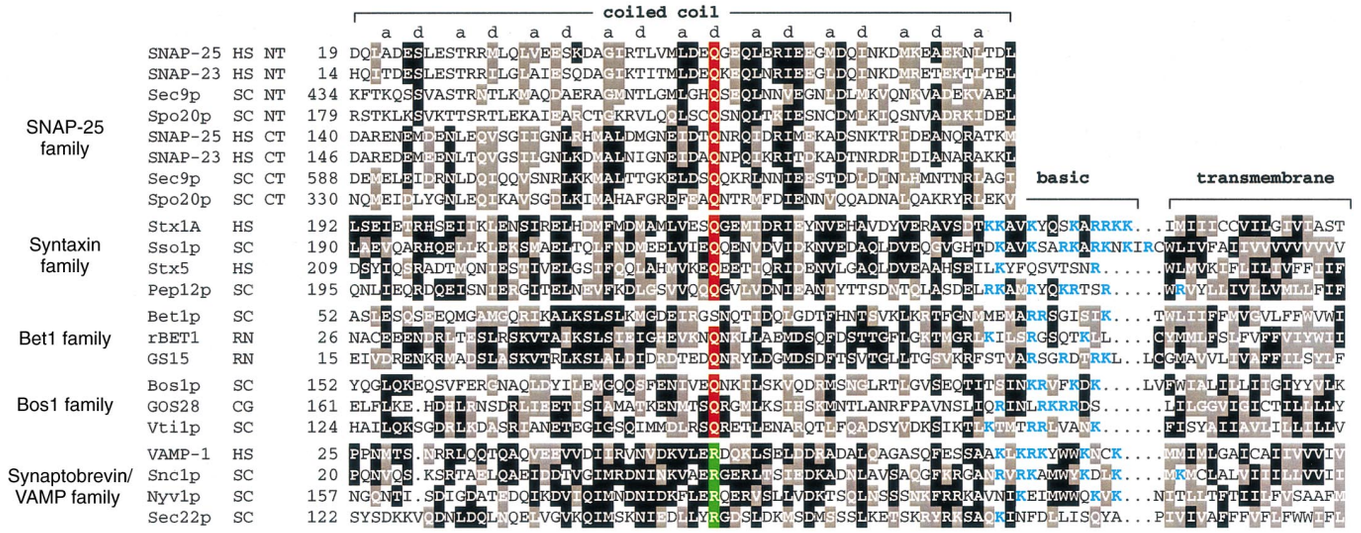

FIGURE 1

Alignment of the conserved domains of representative members of the SNARE subfamilies. Five SNARE families can be identified by their clustering in nearest-neighbour dendrogram analysis (see Box 1). The cytoplasmic regions with a high likelihood of coiled-coil structure are highlighted together with the register of the heptad repeat. NT and CT refer to the N-terminal and C-terminal coiled-coil domain of SNAP-25-related proteins, respectively. All subfamilies, except the SNAP-25 family, possess a C-terminal transmembrane domain, which is separated from the coiled-coil domain by a cluster of basic amino acids (cyan). Typically, 3–8 excess positive charges occur in a stretch of 10–16 residues. Note that the region connecting the two coiled-coil domains of members of the SNAP-25 family is also basic. Abbreviations: RN, *Rattus norvegicus*; HS, *Homo sapiens*; SC, *Saccharomyces cerevisiae*; CG, *Cricetulus griseus*. A glutamine residue (red) in the d-position of a heptad repeat (normally hydrophobic) is conserved in all subfamilies except the synaptobrevin/VAMP family, where an arginine residue (green) is invariant at this position. The conservation of a nonhydrophobic amino acid in the d-position of a heptad repeat suggests that this residue is of particular importance. Interestingly, there is also a conserved glutamine in a similar position in the middle of a coiled-coil domain in the cartilage oligomeric matrix protein (COMP), which has five identical subunits that form a pentameric coiled-coil tunnel with five glutamine residues extending into the interior[6]. It is tempting to speculate that the SNARE fusion complex also consists of a pentameric coiled-coil with the glutamine residues arranged in a similar manner.

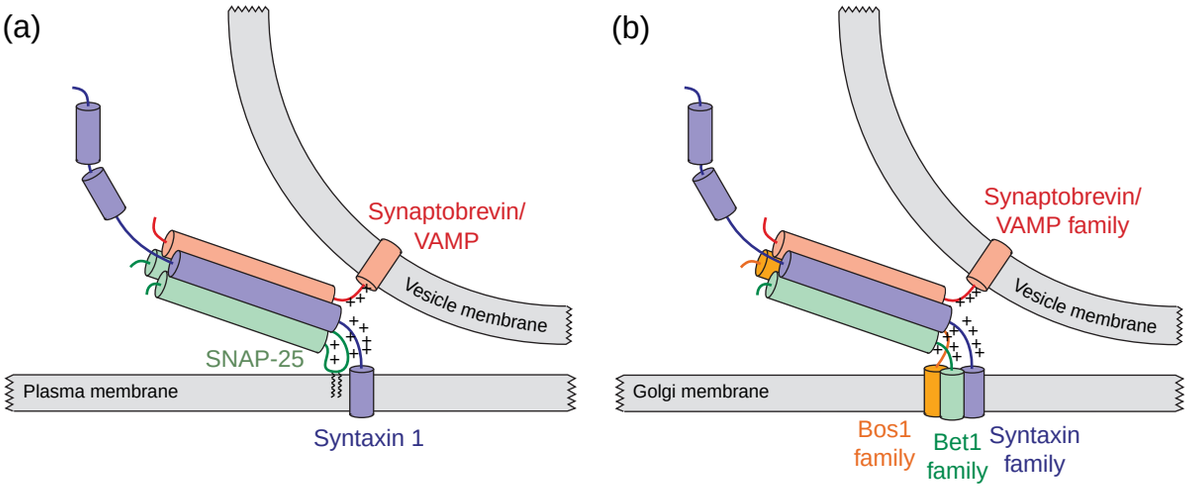

FIGURE 2

Schematic model of the structure of SNARE fusion complexes at the synapse and at intracellular membrane compartments. (a) A coiled-coil complex is assumed to form at the presynaptic plasma membrane between the membrane-proximal coiled-coil domain of syntaxin 1, the two coiled-coil domains of SNAP-25 and the coiled-coil domain of synaptobrevin/VAMP. Coiled-coil interactions between these three proteins have been reported, but from existing data it is not clear whether the resulting SNARE complex must be a tetramer of these proteins alone. The basic clusters (see Fig. 1) in the regions connecting the coiled-coil and transmembrane domains of the SNAREs are highlighted (+). The domains connecting the two coiled-coil domains of the SNAP-25 family members are also basic, and this might suggest that these hinge regions play a functional role in membrane fusion perhaps by interaction with acidic membrane lipids. (b) Despite the absence of SNAP-25 family members in intracellular fusion complexes, the SNARE complexes could still be structurally similar to the plasma membrane complexes if one molecule of SNAP-25 is replaced by two small v-SNARE-like proteins of the synaptobrevin/VAMP-, Bet1- and/or Bos1-families. The proposed complex in (b) most closely resembles the plasma membrane SNARE complex in that three coiled-coil domains are contributed by SNAREs located in the target membrane. It is conceivable, however, that these coiled-coil domains could also be contributed by small SNAREs located in the vesicle membrane, which would be more compatible with the observed presence of some small Golgi SNAREs on transport vesicles. Direct *in vitro* interactions between small SNAREs of the endoplasmic reticulum (ER)/Golgi (e.g. Bet1p and Bos1p) have been reported[1], supporting this model. Note that, in the way the model is drawn, the C-terminal coiled-coil domain of SNAP-25 in (a) would be antiparallel, whereas all coiled-coil domains in (b) would be parallel.

syntaxin can accomplish the specific fusion of different classes of transport vesicles with different Golgi subcompartments.

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A novel centrosomal lattice with a cutting edge

DICTENBERG, J. B. *et al*. (1998)
Pericentrin and γ-tubulin form a protein complex and are organized into a novel lattice at the centrosome
J. Cell Biol. 141, 163–174

HARTMAN, J. J. *et al*. (1998)
Katanin, a microtubule-severing protein, is a novel AAA ATPase that targets to the centrosome using a WD40-containing subunit
Cell 93, 277–287

At the centrosome, microtubules are nucleated by the 25S γ-tubulin ring complex (γ-TuRC). How the centrosome manages to organize spatially the nucleation of microtubules and how they are dynamically attached (necessary for spindle microtubule flux) is unknown.

Dictenberg *et al*. investigated the three-dimensional environment for microtubule nucleation. Using biochemical, deconvolution immunofluorescence microscopy and fluorescence resonance energy transfer methods, they showed that pericentrin and γ-tubulin form a large 38–48S centrosomal complex distinct from the γ-TuRC. The complex is present as large interconnected ring-like structures, aligned side by side with a circumference of 800–900 nm and a diameter of 250 nm. The backbone of the rings is made of pericentrin, which fits with the 218 nm linear coiled-coil polypeptide structure predicted from the primary amino acid sequence. The novel lattice was found in several distinct microtubule-organizing centres (MTOCs), indicating that this lattice may be a basic organizer of γ-TuRCs at all MTOCs. Moreover, cell-cycle-dependent changes in lattice structure were reported, suggesting that the lattice regulates the centrosomal microtubule nucleation capacity during the cell cycle.

Microtubule-severing proteins promote the disassembly of microtubules by generating internal breaks within a microtubule. The heterodimeric microtubule-severing factor katanin (from katana, the Japanese word for samurai sword) is localized to centrosomes. Hartman *et al*. cloned and characterized the 60-kDa and 80-kDa subunits of katanin. The 60-kDa subunit contains the ATP-binding site, the ATPase activity and the severing activity of katanin. The 80-kDa subunit contains a C-terminal domain required for interaction with the 60-kDa subunit. Moreover, the N-terminus contains six WD40 repeat motifs, sufficient to localize a green-fluorescent protein (GFP) to centrosomes. This suggests that the WD40 motifs of the 80-kDa subunit target katanin to the centrosome. Rotary-shadowing electron microscopy showed that katanin forms oligomeric/ring-like structures (as does the AAA protein *N*-ethylmaleimide-sensitive fusion factor, NSF), and this might be important for the ability to sever microtubules.

Future work will show whether microtubules emanating from the Dictenberg *et al*. lattice are severed by katanin and whether this contributes to spindle microtubule flux.

Sex and the single worm

SINGSON, A., MERCER, K. B. and L'HERNAULT, S. W. (1998)
The *C. elegans spe-9* gene encodes a sperm transmembrane protein that contains EGF-like repeats and is required for fertilization
Cell 19, 71–79

The meeting of egg and sperm is arguably the most important single event in the life of a multicellular organism. For this meeting to result in successful fertilization, the egg must be able to recognize and accept a single sperm of the same species. The winning sperm must be attractive to the egg, penetrate its outer surface, fuse with the plasma membrane, enter and activate the egg. Doubtlessly, there are many sperm surface molecules that participate in one or many of the steps to successful fertilization, but studies of mice and marine organisms have revealed only a handful of molecules crucial to this process. Singson *et al.* exploit the power of *Caenorhabditis elegans* genetics to identify *spe-9* as a candidate egg ligand.

C. elegans has an unusual reproductive cycle: most worms are self-fertilizing hermaphrodites, bearing both eggs and sperm. The rare male is capable of producing only sperm and of copulating with hermaphrodites. In this study, the authors found that hermaphrodite and male worms containing a temperature-sensitive mutation in the *spe-9* gene are sterile because their sperm are unable to fertilize their own or wild-type eggs. When grown at the restrictive temperature, *spe-9* mutant worms produce wild-type levels of morphologically normal spermatozoa. Hermaphrodite *spe-9* mutants are able to deposit the spermatozoa correctly into the spermatheca (internal

This month's *headlines* were contributed by Søren Andersen, Donald Gullberg, Arianne Heinrichs, Martin Latterich, Chung Lau, Wallace Marshall, Robin May, Tom Misteli and Kirsten Sadler.