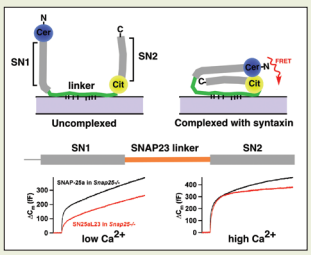
INCYTES from MBC
September, Vol. 19, No. 9



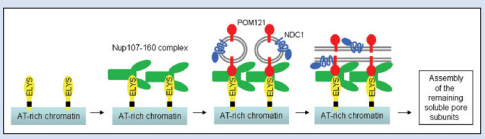
Structural and Functional Implications of Linked SNARE Motifs in SNAP25

Li Wang, Mary A. Bittner, Daniel Axelrod, and Ronald W. Holz

The SNAP-25 Linker as an Adaptation towards Fast Exocytosis

Gábor Nagy, Ira Milosevic, Ralf Mohrmann, Katrin Wiederhold, Alexander M. Walter, and Jakob B. Sørensen

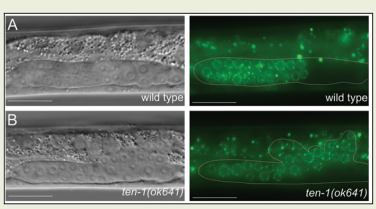
Membrane fusion is catalyzed by the assembly of tetrahelical coiled-coil SNARE complexes between the fusing compartments. Typically, the four SNARE domains are encoded by separate membrane-targeted proteins, but in the exocytic pathway one SNARE protein (exemplified by SNAP25) encodes two SNARE motifs, connected by a flexible linker. To probe the significance of the highly conserved two-SNARE-motif configuration of SNAP25, Wang *et al.* constructed membrane-bound, intramolecular and intermolecular fluorescence resonance energy transfer (FRET) probes that report the folding of the two SNARE motifs (SN1 and SN2) in a natural membrane setting in the absence and presence of other SNAREs. FRET was measured in vitro by spectroscopy and in living cells by total internal reflection fluorescence microscopy. Parallel experiments investigated the functionality of SNAP25 constructs with different folding characteristics by measuring their ability to rescue secretion in botulinum neurotoxin type E–expressing cells. Linking SN1 and SN2 together in the same protein has at least two important outcomes. First, it facilitates the formation of a complex with syntaxin that brings the N-termini of SN1 and SN2 close together. Second, the linkage enables fast secretion in chromaffin cells. Independently, Nagy, Milosevic, *et al.* used systematic mutagenesis and chimeric proteins between SNAP25 and the non-neuronal homologue SNAP-23 together with fast biophysical methods to study SNAP25-specific functions. They identified residues in the linker domain that are crucial for the ability of SNAP25 to rapidly couple membrane fusion to the intracellular calcium concentration—the distinguishing feature of neurosecretion. They thus suggest that the fusion of two SNARE domains has developed as an adaptation towards rapid calcium triggering.



Capture of AT-rich Chromatin by ELYS Recruits POM121 and NDC1 to Initiate Nuclear Pore Assembly

Beth A. Rasala, Corinne Ramos, Amnon Harel, and Douglass J. Forbes

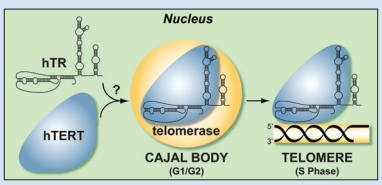
In vertebrates, the nuclear pore complexes that mediate nucleocytoplasmic transport are assembled from approximately a dozen soluble subunits and several membrane proteins. Recent studies have shown that a specific chromatin-binding nucleoporin, ELYS, initiates nuclear pore complex (NPC) assembly at the chromatin surface at the end of mitosis. Here, the authors demonstrate that ELYS contains multiple chromatin-binding domains. Through use of a *Xenopus* in vitro nuclear reconstitution assay, point mutation in ELYS, and AT- or GC-binding antibiotics, the authors show that the AT-hook motif of ELYS is critical for focusing NPC assembly at AT-rich chromatin. Strikingly, the AT-binding antibiotic distamycin blocks nuclear pore assembly, whereas the GC-binding chromomycin does not. The authors further find that chromatin-bound ELYS/Nup107-160 complex is required to attract the integral membrane pore proteins POM121 and NDC1 to the nuclear membrane. Results point to an order of NPC assembly consisting of: AT-rich chromatin, ELYS, the Nup107-160 complex, recruitment of POM121- and NDC1-containing vesicles, vesicle fusion, and assembly of the remaining soluble pore subunits.



Caenorhabditis elegans Teneurin, *ten-1,* Is Required for Gonadal and Pharyngeal Basement Membrane Integrity and Acts Redundantly with Integrin *ina-1* and Dystroglycan *dgn-1*

Agnieszka Trzebiatowska, Ulrike Topf, Ursula Sauder, Krzysztof Drabikowski, and Ruth Chiquet-Ehrismann

Basement membranes (BMs) are vital extracellular matrix sheets that both connect and separate different cells and tissues in all multicellular animals. BMs determine organ differentiation and homeostasis. The authors provide the first report linking teneurin, a phylogenetically conserved transmembrane receptor, to BM structure and function. They discovered that *Caenorhabditis elegans* teneurin, *ten-1,* is essential for the maintenance of gonad and pharyngeal BMs. In the absence of TEN-1, the BM surrounding the gonad formed properly at hatching but ruptured during larval development. Genetic interactions were found between *ten-1* and the BM components laminin and nidogen as well as the well-known BM receptors integrin and dystroglycan. Thus teneurin, integrin, and dystroglycan apparently have related and partly redundant functions. These studies in *C. elegans* have wider implications for the role of teneurins, integrins, and dystroglycans in vertebrates as well, since they reveal redundancy not only between several receptors of the same family but also between structurally distinct receptor families.



Telomerase Reverse Transcriptase Is Required for the Localization of Telomerase RNA to Cajal Bodies and Telomeres in Human Cancer Cells

Rebecca L. Tomlinson, Eladio B. Abreu, Tania Ziegler, Hinh Ly, Christopher M. Counter, Rebecca M. Terns, and Michael P. Terns

Telomerase, the enzyme that maintains telomeres at the ends of chromosomes, must be assembled from two essential components and must be trafficked to telomeres in order to function. In cancer cells, but not normal human cells, the RNA subunit of telomerase (TR) is found at telomeres and Cajal bodies. A number of factors could account for this cancer cell–specific localization pattern, but this study indicates that the primary factor is the presence of the essential protein subunit of telomerase–telomerase reverse transcriptase (TERT). Ectopic expression of TERT in normal cells induces TR localization to telomeres and Cajal bodies, and knockdown of TERT in cancer cells eliminates localization. These findings suggest that, like for other ribonucleoprotein enzymes, critical steps in the assembly and subcellular trafficking of telomerase are tightly linked. ■