male chicken embryos leads to feminization that involves activation of *Foxl2* expression (Smith et al., 2009).

It appears that two independent antitestis pathways are at work in the ovary: R-spondin1/Wnt4 via β-catenin and Foxl2 in the embryo, and at least Foxl2 in the adult. Is *Foxl2* the only gene required to constitutively repress the male pathway in the adult ovary? Given the importance of R-spondin1 and Wnt4 acting via β-catenin in establishing the embryonic ovary, it would be of interest to conditionally delete this pathway in adult ovaries to see whether the gonads also show ovary to testis transdifferentiation. In other words, is this effect solely mediated by *Foxl2*, or are other factors of equal importance also involved?

Many disorders of sex development in humans remain unexplained. Similarly, the molecular mechanisms underlying premature ovarian failure in women are not fully understood. If the same phenomenon observed in this study applies to humans, then it may at least partly explain the etiology of these conditions.

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A Tethering Complex Recruits SNAREs and Grabs Vesicles

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Protein tethers can bridge gaps between membranes. Ren et al. (2009) now provide evidence that the yeast Dsl1 complex tethers vesicles to the endoplasmic reticulum (ER) by binding ER SNARE proteins at its base and capturing vesicles using a loop region that extends 20 nm from the ER membrane.

In eukaryotic cells, membranes are often interconnected by tethers. For instance, tethering of the endoplasmic reticulum to mitochondria, vacuoles/lysosomes, or the plasma membrane is involved in calcium homeostasis or lipid exchange (Levine and Loewen, 2006). Tethers that connect the stacks of the Golgi apparatus at a predefined distance from each other provide another example (Gillingham and Munro, 2003). In comparison, tethers involved in membrane fusion have a more challenging task. They first need to bring the two membranes in close apposition and then need to step aside so that SNAREs can interact and mediate their merger. One group of tethers is formed by large multiprotein complexes, and it remains largely enigmatic how these bulky complexes complete these carefully orchestrated steps. To understand these events, it is essential to ascertain the architecture of the tethers and the relative positioning of their interacting partners, in particular the SNARE proteins and the membranes destined to fuse.

In this issue of *Cell*, Ren et al. (2009) describe a complete structural model of Dsl1, a multisubunit vesicle tethering complex in yeast. The Dsl1 complex as well as its mammalian counterpart, the syntaxin 18 complex (Hirose et al., 2004), mediate the fusion of COPI-coated vesicles with the endoplasmic reticulum (ER) as part of retrograde trafficking between the Golgi and ER. The Dsl1 structure is particularly interesting in comparison to the previously reported structure of the tethering complex TRAPP I (trafficking protein particle complex I) (Kim et al., 2006; Cai et al., 2008), which facilitates the fusion of COPII-coated vesicles involved in ER-Golgi anterograde transport. The mod-

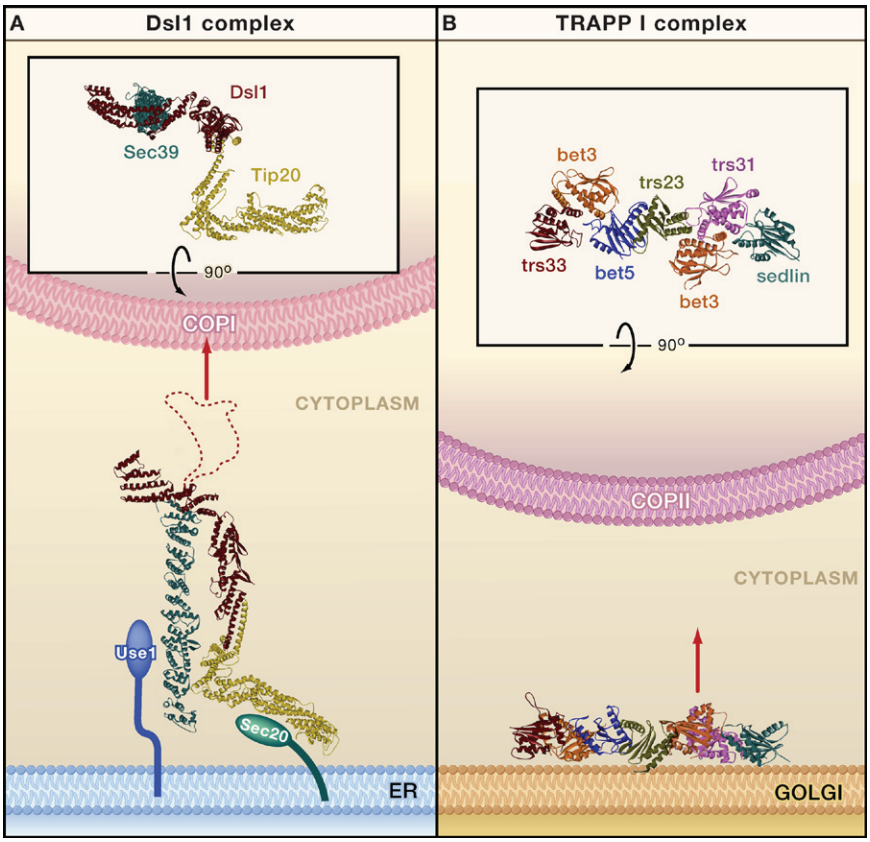

Figure 1. Tether-Coat Interactions in Membrane Traffic between ER and Golgi

(A) The Dsl1 complex. (Inset) View of the Dsl1 complex without interacting partners from above the membrane (Tip20, yellow; Dsl1, brown; Sec39, green). Side view of the Dsl1 complex anchored by the SNAREs Use1 (blue) and Sec20 (green) in the endoplasmic reticulum (ER) membrane. The complex may tether COPI-coated vesicles to the ER by exposing an unstructured central loop emanating from Dsl1 (Ren et al., 2009).

(B) Model of the mammalian TRAPP I complex that has been fit into the volume of the yeast complex (Kim et al., 2006) and how it may be positioned between a COPII vesicle and the Golgi membrane in yeast to initiate heterotypic membrane fusion. (Inset) The side of the TRAPP I complex facing forward represents the Ypt1-interacting surface (Cai et al., 2008). Note that the two Bet3 subunits are exposed in a way that they can interact with two COPII complexes during homotypic fusion of COPII vesicles in mammalian cells (Cai et al., 2008). This homotypic fusion event is thought to initiate the formation of the ER-Golgi intermediate compartment in mammalian cells.

els for both complexes are based on the crystal structures of subcomplexes that were then fitted into low-resolution structures determined by negative stain electron microscopy.

The two structures are strikingly different from one another. The six essential subunits of TRAPP I are globular proteins whose sizes range from 18 to 33 kDa in yeast, and their mammalian counterparts are even smaller (Kim et al., 2006; Sacher et al., 2008). These subunits form a chain in a staggered configuration with the Bet3 subunit present in two copies (Figure 1). In mammalian cells TRAPP I is thought to mediate homotypic fusion of COPII vesicles thereby initiating the formation of a vesicular tubular cluster (VTC) also known as ERGIC (ER-Golgi intermediate compartment), which is involved in sorting of anterograde and retrograde cargo (Cai et al., 2007). TRAPP I recruitment to membranes may encompass different mechanisms including direct interaction with the membrane via basic residues on the flat surfaces of the Bet3 and Trs31 subunits or binding to the COPII coat subunit Sec23. The significance of the Bet3 palmitoylation and the corresponding lipid-binding pocket in Bet3 for membrane binding is presently unclear. How this complex then engages the SNAREs for the subsequent fusion step is also unknown.

In contrast, the Dsl1 complex consists of three unique subunits, all between 80 and 90 kDa in size, that are long rods composed of stacks of α helices (Figure 1). Two of the subunits, Dsl1 and Tip20, resemble subunits of yet another tethering complex, the exocyst, which functions in the fusion of Golgi-derived vesicles with the plasma membrane (Cai et al., 2007). The third subunit, Sec39, also has a long rod-like structure that is different from the exocyst fold. Unlike the other complexes, the Dsl1 complex is firmly bound to the three ER SNAREs. Thus, for the first time it is possible to understand how tethering complexes structurally connect to the fusion machinery. As depicted in Figure 1, two of the three proteins, Sec39 and Tip20, are in contact with the N-terminal regulatory domains of two ER SNAREs. Findings by Ren et al. suggest a role for the tether in recruiting SNAREs. Essentially, Dsl1 lies on top of these two pillars. It is noteworthy to mention that the sites of protein interactions in the Dsl1 complex from yeast perfectly match with the sites of interactions mapped for the mammalian Dsl1 homolog Zw10 (Famulski et al., 2008). The complex exposes two different parts of Dsl1 at its top. The first comprises the terminal 100 amino acids, the only part of Dsl1-like proteins that exhibits extensive sequence conservation, and truncations within this region have effects that are different from the complete loss of Dsl1 (Zink et al., 2009). A second domain located in the center of Dsl1 is also exposed at the top of the complex. This disordered domain is known to interact with various subunits of the COPI coat complex. For the first time, a coherent picture emerges as to how such a tether might function: First, it collects the SNAREs and then it grabs the vesicle by its coat, perhaps even facilitating uncoating, before bringing membranes together for final fusion.

A similar interaction between a multisubunit tethering complex and a component of a vesicle coat has also been observed in the case of TRAPP I and the COPII coat (Cai et al., 2007). Here, Bet3 (TRAPP I) binds to the COPII subunit Sec23, and the importance of this interaction has largely been established in vitro using recombinant proteins. However, long-distance tethering of COPII

vesicles may also involve long coiled-coil proteins (Kim et al., 2006). It is clear that the Dsl1-COPI interaction is important for tethering as it has recently been shown that defects in the Dsl1 complex result in a massive accumulation of "stranded" COPI-coated vesicles (Zink et al., 2009). Thus, vesicle coats or their remnants on the vesicles are clearly involved in tethering.

How can the Dsl1 complex, sandwiched between the ER and COPI-coated vesicle, clear the way for fusion? Its side that faces the vesicle may assist in removing the COPI coat, thus rendering the vesicular SNARE accessible for its partners in the ER membrane (Zink et al., 2009). However, what happens at the other end, which faces the ER membrane, is likely more complicated. The Dsl1 complex is associated with the membrane-anchored ER SNAREs in a very tight and stoichiometric complex (Ren et al., 2009). However, Ren et al. suggest that there is substantial flexibility built into the Dsl1 complex. Such flexibility may allow the complex to bend away from the SNAREs (on both vesicle and ER) to clear the intermembrane space in a way that allows the SNAREs to interact in *trans* to induce fusion. Such a scenario is plausible given that the Dsl1 subunits interact with the regulatory domains of the SNAREs and not the SNARE motifs that drive fusion by the formation of helical SNARE complexes.

The findings by Ren et al. shed new light on the function of a very diverse group of protein complexes. Multisubunit tethering complexes exhibit many different interactions and biochemical activities (Cai et al., 2007). Many are effectors of Rab GTPases, whereas some possess Rab guanine nucleotide exchange activity; furthermore, two multisubunit tethering complexes, exocyst and HOPS (homotypic fusion and vacuole protein-sorting complex), may promote SNARE activity by interacting with or containing a member of the Sec1/Munc18 protein family; others interact with SNAREs directly. Interactions with SNAREs can be very stable, as with the Dsl1 complex, although they can also be more transient as is the case for the complexes involved in traffic between the endosome and the Golgi (GARP/VFT) or within the Golgi (COG). Finally, some multisubunit tethering complexes interact with coat complexes. In the light of this multitude of interactions and diverse activities for tethering complexes, structural information, as provided in work of Ren and colleagues, is undoubtedly the best way to unravel their molecular mechanisms of action.

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