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A novel coatomer-related SEA complex dynamically associates with the vacuole in yeast and is implicated in the response to nitrogen starvation

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We now seem to live in a small world, in which everyone is highly interconnected. Cells, too, often also display tremendous interconnectivities in their component systems. As a recent case in point, we have identified a conserved protein complex—the SEA complex—that links the nuclear pore complex (NPC), the COPII vesicle coating complex, vacuoles and autophagy. In this punctum we will discuss the properties of this novel complex.

Keywords: vacuole, nuclear pore complex, coating assemblies, autophagy, Npr2/Npr3 complex, SEA complex

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The SEA complex, found in yeast, contains the nucleoporins Seh1 and Sec13, plus four previously uncharacterized proteins (Sea1–Sea4). This complex also contains Npr2 and Npr3, which Neklesa and Davis recently found during a genome-wide screen for regulators of TORC1 in response to nitrogen starvation; Npr2 and Npr3 specifically inactivate TORC1 when amino acids are scarce. Signals indicating an abundance of nutrients activate TORC1 (and deactivate autophagy), whereas signals of starvation or other stressors inhibit TORC1 (and activate autophagy). Accordingly we reasoned that, in the absence of Npr2 and Npr3, TORC1 is hyperactive, and that therefore autophagy should be impaired. Indeed, after nitrogen starvation, the vacuolar autophagy marker GFP-Atg8 was blocked in the cytoplasm in the *npr2*Δ strain and distributed equally between the vacuole and the cytoplasm in the *npr3*Δ strain, indicating impairment in autophagic processing. Therefore Npr2 and Npr3 can be considered as novel regulators of autophagy. Despite this important role, very little information is available about these proteins; hence, we would like to highlight here several findings that we made during the initial characterization of the SEA complex.

The SEA complex was discovered during immunopurification of a tagged version of one of the components of the NPC, the nucleoporin Seh1. As a bona fide nucleoporin, Seh1 co-purifies with the Nup84 subcomplex, a key component of the NPC's membrane-hugging scaffold. However, Seh1 also co-purifies with Npr2, Npr3, Sec13 and four high-molecular-weight proteins [Yjr138w (Iml1), Yol138c (Rtc1), Ydr128w (Mtc5) and Ybl104c)]. To reflect their association with Seh1, these proteins were given a common name, Sea (for *Se*h1-*a*ssociated), and were renamed Sea1 through Sea4, respectively. Tagged versions of each of Sea1–Sea4 co-purified with each other, and with Seh1, Sec13, Npr2 and Npr3. Sec13 is also a member of the NPC's Nup84 complex (above). In addition, Sec13 interacts with Sec31 in ER-trafficking COPII vesicles; however, we do not find other NPC proteins or members of COPII vesicles associated with the SEA proteins, and the SEA proteins do not localize to the NPC or ER, confirming that this group of proteins forms a novel and distinct complex, entirely separate from the NPC or COPII.

Previously, Seh1 and Sec13 had only been shown to function as part of membrane coating assemblies—NPCs and coated vesicles. Coated vesicles are membranous transport intermediates, encapsulated by distinctive proteinaceous coats such as those formed by the COPI, COPII or clathrin complexes. NPCs are

embedded within the nuclear envelope and are the sole mediators of macromolecular nucleocytoplasmic exchange. The structural scaffold of the NPC, comprising ~50% of the total NPC mass, is formed almost entirely from proteins consisting of only two folds, α-solenoid-like and β-propellers, including proteins with a particular arrangement of an amino-terminal β-propeller followed by a carboxy-terminal α-solenoid. The same structural modules dominate the major coated vesicle complexes, including COPI, COPII and clathrin. We previously proposed the "protocoatomer hypothesis," suggesting that various coated vesicles and the scaffold of the NPCs originated from a common evolutionary ancestor. Given that the SEA complex contains protein also found in the NPC and coated vesicles, we explored whether the SEA proteins also resemble proteins found in coating complexes by analyzing the fold compositions of their constituent protein domains. Strikingly, key Sea2-Sea4 proteins are predicted to possess the "protocoatomer" β-propeller/α-solenoid architecture. The SEA complex contains five proteins with β-propellers, a domain common in coating assemblies where it provides a molecular scaffold for protein interactions. Sea4 contains an N-terminal β-propeller, an α-solenoid and a C-terminal RING domain. Three SEA complex subunits, Sea2-Sea4, have a C-terminal RING domain. The high frequency of RING domains in the SEA complex suggests that the complex may act as an E3 ligase. Sea1 is a multidomain protein carrying an N-terminal Cdc48-like domain found in several AAA-ATPases, such as Sec18/NSF, immediately followed by a vWA-like domain, present in many membrane interacting proteins, including Sec23 of COPII vesicles. Sea1 also carries a DEP domain, which mediates interactions with membrane-bound receptors. Unfortunately, our analyses were unable to assign folds for Npr2 and Npr3. However both proteins seem to contain disordered regions and uncharacterized folds, the latter suggesting that suitable templates do not yet exist in the PDB.

We noticed that the predicted fold composition of several SEA proteins resemble those of proteins found in the HOPS and CORVET complexes, which have been implicated in tethering membranes together prior to their fusion. One of the major common defining features of NPC, coat and tethering proteins is their association with membranes. We found that the SEA complex proteins seem to be organized into large assemblies that dynamically associate with the vacuolar membrane surface. Despite this, deletion of one or several SEA members has only a minor effect on the examined vacuole functions, suggesting that the SEA complex functions alongside other related complexes and may be redundant under numerous growth conditions. We further tested whether SEA mutants are sensitive to rapamycin treatment and nitrogen deprivation. Growth of single deletion strains of SEA2–SEA4 is not affected by rapamycin treatment. However, double-deletion strains exhibit increased sensitivity to rapamycin and reduced survival upon nitrogen starvation. Consistent with all these observations, SEA complex synthetic genetic interactions implicate its members in multiple interactions with genes responsible for amino acid biogenesis and sorting, membrane trafficking, autophagy and ubiquitination.

Taken together, the SEA complex demonstrates remarkable relatedness at the structural and compositional levels to characterized vesicle coating complexes, and appears structurally most closely related to the HOPS/CORVET tethering complexes. Untangling the web of interactions and redundant functionalities of this newly identified complex will be the next challenge.

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