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The SEA complex – the beginning

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The presence of distinctive internal membrane compartments, dynamically connected via selective transport pathways, is a hallmark of eukaryotic cells. Many of the proteins required for formation and maintenance of these compartments share an evolutionary history. We have recently identified a new conserved protein complex – the SEA complex – that possesses proteins with structural characteristics similar to the membrane coating complexes such as the nuclear pore complex (NPC), the COPII vesicle coating complex and HOPS/CORVET tethering complexes. The SEA complex in yeast is dynamically associated to the vacuole. The data on the function of the SEA complex remain extremely limited. Here we will discuss a possible role of the SEA complex based on the data from genetic assays and a number of functional studies in both yeast and other eukaryotes.

Keywords: Saccharomyces cerevisiae, the SEA complex, vacuoles.

What is the SEA complex? The SEA complex is a ~1 MDa protein complex that was discovered in yeast *Saccharomy ces cerevisiae* during immunopurification of genomically tagged version of one of the components of the NPC, the nucleoporin Seh1 [1]. As a *bone fide* nucleoporin, Seh1 co-purifies with the Nup84 subcomplex, a key component of the NPC's membrane-coating scaffold. However, Seh1 also co-purifies with Npr2, Npr3, Sec13 and four high-molecular-weight proteins (Yjr138p (Iml1), Yol138p (Rtc1), Ydr128p (Mtc5), and Ybl104p). To reflect their association with Seh1, these proteins were given a common name, Sea (for Seh1-associated), and were renamed Sea1 through Sea4, respectively. Tagged versions of each of Sea1–Sea4 copurified with each other, and with Seh1, Sec13, Npr2, and Npr3. Sec13 is also a member of the NPC's Nup84 subcomplex. In addition, Sec13 interacts with Sec31 in ER-trafficking COPII vesicles; however, other NPC proteins or members of COPII vesicles were not found to be associated with the Sea proteins, and the Sea proteins did not localize to the NPC or ER, confirming that this group of proteins forms a novel and distinct complex, the SEA complex, entirely separate from the NPC or COPII.

How conserved is the SEA complex? The full SEA complex is specific to animals and fungi [1]. There is no evidence for any SEA complex gene in plants (with the exception of Seh1 and Sec13, which certainly fulfill other functions in this lineage). In other eukaryotic supergroups the SEA complex members are not always retained. The evolutionary profile of the SEA complex suggests that it might be dispensable in many biological contexts, having a specialized life-style specific role rather than a function central to eukaryotic fitness.

Where is the SEA complex localized? The expression level of the SEA complexes members is very low both in yeast and in human cells, which make the task of their localization challenging. Nevertheless the combination of subcellular fractionation and fluorescent microscopy detected yeast SEA complex as dynamically associated to the vacuole membrane [1]. The proteomic study of human placenta lysosomes also found homologues of Sea1, Sea2, Sea4, Seh1, and Sec13 to be associated to the lysosomal membrane [2]. However, because of the low level of expression of the SEA complex components it is possible that they can be found elsewhere in the cell.

What is the structural specificity of the SEA complex members? Sea proteins contain structural elements

present in intracellular structural trafficking complexes [1]. For example, the Sea2-Sea4 proteins are predicted to possess a -propeller/ -solenoid architecture characteristic of proteins that form coats around membranes and participate in membrane tethering. Notably, SEA complex contains five proteins with -propellers, a domain common in coating assemblies. Among those proteins with -propellers are Seh1 and Sec13 – two evolutionary conserved paraloges that are known members of other coating assemblies – NPC (both proteins) and COPII (Sec13 only). Seh1 forms a dimer with Sea4, which besides -propeller and -solenoid, contains a C-terminal RING domain. The structural organization of the Sea4 is identical to a number of proteins in HOPS and CORVET complexes, which have been implicated in tethering membranes together prior to their fusion. Similar to several proteins of HOPS/CORVET 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 ubiquitin ligase.

What did we learn about SEA complex function from yeast studies? Not too much so far. The majority of the data concerns Npr2 and Npr3. These two proteins in yeast act as upstream regulators of TORC1 kinase in response to nitrogen starvation [3]. Accordingly, the process of autophagy, which is induced in the response to nitrogen starvation, is impaired in the absence of Npr2 and to some extent of Npr3 [1, 4]. Interestingly, Npr2, Npr3 and Sea1 also required for a specific variation of autophagy, which can be induced in the absence of nitrogen starvation, but simply upon the change from rich to a minimal medium [5].

Otherwise SEA complex deletion strains exhibited relatively robust growth under a broad range of tests [1] (http://www.yeastgenome.org/). This presumes that the SEA complex functions alongside other related complexes and may be redundant under numerous growth conditions. However, complete redundancy is unlikely given evolutionary conservation of SEA complex subunits, especially in the animals and fungi. Recent papers on yeast synthetic genetic interactions [6] and chemical genetic profiles [7] shed a bit of a light on a possible role of the SEA complex. Analysis of these data suggests that SEA complex members are implicated in multiple genetic interactions with genes responsible for cell wall biogenesis and integrity, amino acid biogenesis and sorting, membrane trafficking, ubiquitination and autophagy.

One of the strongest genetic interactions that involve SEA complex members is the cooperation with the genes responsible for cell wall biogenesis and integrity. Thus, all five SEA complex genes appeared in Costanzo et al. [6] survey exhibit genetic interaction with MAP kinase Bck1; four SEA complex genes involved in genetic interaction with another MAP kinase – Slt2. In addition, Sea4 is synthetically lethal with Las21 – an integral plasma membrane protein involved in the synthesis of the glycosylphosphatidylinositol (GPI) core structure [8]. Similar to SEA complex genes Las21 is involved in negative genetic interactions with Pkc1 and Slt2 [6] and synthetically lethal with Bck1 [8].

A gene cohort involved in amino acid biosynthesis and sorting exhibits a large number of strong genetic interactions with SEA complex subunits. Notably, HOPS/CORVET belongs to this same interaction cluster [6], further underscoring the similarity between these complexes and the SEA complex. One module in the cluster is responsible for sorting of a general amino acid permease Gap1. Gap1 is a general amino acid permease that can transport all amino acids. GAP1 is transcriptionally regulated by the available nitrogen source via nitrogen catabolite repression (NCR). Interestingly, both Npr2 and Npr3 are probably involved in the NCR control [9]. Gap1 sorting is mediated by number of proteins and complexes, including EGO complex, Lst proteins and Rsp5-Bul1-Bul2 trimer, which ubiquitinate Gap1 itself. All of these genes display genetic interactions with the SEA complex members. In addition chemical genomic survey reports that Sea2, Sea4 and Sec13 show similar homozygous co-fitness with a number of genes involved in Gap1 sorting [7].

The most striking examples of SEA components genetic interaction is connection with genes involved in the biosynthesis of homoserine with almost entire set of the genes responsible for this pathway exhibiting very strong genetic interactions with 4 or 5 SEA complex members [6]. The majority of amino acids biosynthesis pathway genes, including those, involved in the genetic interactions with SEA complex genes are regulated by Gcn4 transcriptional activator [10]. Strikingly, Sea4 has multiple Gcn4 binding sites in its promoter [11], and therefore can be implicated in the control of amino acid biosyn-

thesis. Interestingly, a chemical genomic survey identified a small group of genes, required for resistance to diverse perturbations [7]. These genes referred to as multidrug resistance (MDR). Genes that involved in aromatic amino acid biosynthesis (and synthetic genetic interactions with SEA components), GCN4 and NPR2 together with NPR3 are all belong to the MDR group. By the way, NPR2 and NPR3 exhibit absolutely identical co-fitness of respective homozygous deletion strains in chemical genomic survey, which is a strong indication for these two proteins share similar biological process and molecular function [7]. All the observations described here suggest that the SEA complex plays a role in the regu- lation of amino acid biosynthesis and autophagy.

What do we know about SEA complex components in high eukaryotes? The fact that all SEA complex members were retained in higher eukaryotes underlines functional importance of this assembly. Nevertheless, information about SEA complex components in higher eukaryotes is even more limited than in yeast. So far no papers were published on Sea1, Sea2, Sea3 homologues. Sea4 homologue in *Drosophila* (*missing oocyte, mio*) is localized to the nucleus and required for the maintenance of the meiotic cycle and oocyte identity [12]. Importantly, Mio forms a complex with Seh1. Both proteins are dispensable for somatic development, but required for development of the mature egg. In addition Seh1 is required for Mio protein stability [13]. Human Seh1 also functions in chromosome alignment and segregation by regulating the centromeric localization of Aurora B and other chromosome passenger complex proteins [14]. Although Sea1-Sea4 and Npr2, Npr3 seems not to be present in plants, Seh1 in *Arabidopsis* is found in multiple locations, including nucleus, Golgi and prevacuolar compartment. AtSeh1 is suggested to play a role in membrane association of dynamin-related protein 2A, which is in turn required for protein trafficking from trans-Golgi network to the central vacuole [15]. Npr2 homologue in human – Nprl2 – has been characterized as a novel tumor suppressor [16]. Low expression of Nprl2 in different types of lung cancers and other tumors was correlated with resistance to cisplatin, one of the mainstays of chemotherapy for lung cancer [17]. Remarkably, the first information about cisplatin resistance of Npr2 deletion strains came from the studies in yeast [18]. Nprl2 interacts with Pdk1 kinase, a key regulator of cell proliferation and survival [19]. Interestingly, Pdk1, which plays a role in cellular transformation and tumor growth, is one of the well-defined upstream regulators of TORC1 pathway in mammalian cells. In addition Nprl2 forms a complex with Npr3 homologue Nprl3 [3], which suggests that Nprl2/Nprl3 might also signal to TORC1, similar to their yeast counterparts. Finally a recent study has demonstrated that Nprl3 (C16orf35) is a nucleocytoplasmic protein that interacts with transcriptional factor p73, a member of p53 family proteins involved in tumor suppression and embryonic development [20].

What are the future directions in the SEA complex study? The study of the SEA complex is in its primary steps and all directions are open and have to be explored. The importance of the SEA complex is underlined by the fact that all of its components have been retained in animals and fungi. The integrative approaches which can combine data on structure, function, regulation and coming from different model organisms should be the most informative. The results of these studies might be of a broad interest given that the Nprl2 in human has been characterized as a novel tumor suppressor [16] and the Nprl3 interacts with p73 [20] – a member of p53 family of transcription factors involved in tumor suppression.

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SEA-комплекс – початок

Резюме

Наявність різноманітних внутрішньоклітинних мембранних органел, динамічно пов'язаних за посередництвом селективного транспорту, є відмінною особливістю еукаріотичної клітини. Багато білків, необхідних для утворення та існування цих органел, мають подібну еволюційну історію. Недавно ми відкрили новий консервативний білковый комплекс – SEA-комплекс, якому притаманні структурні характеристики, схожі з такими мембран транспортных везикул (COPII) та їхніх допоміжних партнерів (HOPS/CORVET) і ядерної пори (NPC). Дріжджовий SEA-комплекс динамічно взаємодіє з вакуолями. Дані щодо функції SEA-комплексу є доволі малочисельними. В представленому міні-огляді обговорюється можлива функція SEA-комплексу з урахуванням результатів генетичних і функціональних досліджень, проведених на дріжджах та інших еукаріотах.

Ключові слова: дріжджі Saccharomyces cerevisiae, SEA-комплекс, вакуолі.

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SEA-комплекс – начало

Резюме

Наличие разнообразных внутриклеточных мембранных органелл, динамически связанных посредством селективного транспорта, является отличительной особенностью эукариотической клетки. Множество белков, необходимых для образования и существования этих органелл, имеют сходную эволюционную историю. Недавно мы открыли новый консервативный белковый комплекс – SEA-комплекс, обладающий структурными характеристиками, схожими с таковыми мембран транспортных везикул (COPII), а также их вспомогательных партнеров (HOPS/CORVET) и ядерной поры (NPC). Дрожжевой SEA-комплекс динамически взаимодействует с вакуолями. Данные о функции SEA-комплекса очень малочисленны. В представленном миниобзоре обсуждается возможная функция SEA-комплекса, основываясь на результатах генетических и функциональных исследований, проведенных на дрожжах и других эукариотах.

Ключевые слова: дрожжи Saccharomyces cerevisiae, SEA-комплекс, вакуоли.

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