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Distribution of Sec24 isoforms to each ER exit site is dynamically regulated in *Saccharomyces cerevisiae*

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ABSTRACT

COPII vesicles are formed at specific subdomains of the ER, termed ER exit sites (ERESs). Depending on the cell type, ERESs number from a few to several hundred per cell. However, whether these ERESs are functionally and compositionally identical at the cellular level remains unclear. Our live cell-imaging analysis in *Saccharomyces cerevisiae* revealed that the isoforms of cargo-adaptor subunits are unequally distributed to each ERES at steady state, whereas this distribution is altered in response to UPR activation. These results suggest that in *S. cerevisiae* cargo loading to ERES is dynamically controlled in response to environmental changes.

1. Introduction

The endoplasmic reticulum (ER) is an essential organelle that is responsible for the synthesis, folding, and sorting of secretory and integral membrane proteins before their export to the Golgi [1]. Only successfully folded and assembled exocytic proteins are segregated from ER resident proteins and exported from the ER [2]. This process is mediated by a set of evolutionarily conserved cytoplasmic proteins, collectively known as the COPII coat, under the control of complex regulatory mechanisms. The COPII coat consists of an inner layer of the Sec23/24 heterodimeric complex surrounded by an outer layer of the Sec13/31 heterotetramer complex, and these components are sequentially assembled onto the ER membrane [3] through the action of a small GTPase Sar1 [4] to generate COPII-coated transport carriers. In short, assembly of the COPII coat is triggered by GDP-GTP exchange on Sar1 directed by the ER-resident guanine nucleotide exchange factor (GEF) Sec12 [5,6]. The binding of GTP induces a conformational change of Sar1 allowing association with the ER membrane. Membrane-associated Sar1-GTP recruits the Sec23/24 complex by binding to the Sec23 subunit, while the Sec24 subunit is involved in cargo selection via recognition of the cytoplasmically exposed ER export motif of the transmembrane cargo to form a prebudding complex [7–9]. Subsequently, the Sec23/24 of the prebudding complex recruits the Sec13/31 complex, which polymerizes adjacent prebudding complexes to drive membrane deformation and vesicle budding [10,11].

In all eukaryotic cells examined so far, COPII transport carrier formation appears to proceed at specialized subdomains of the ER termed ER exit sites (ERESs) [12]. Fluorescence microscopy imaging reveals that fluorescent proteins fused to COPII coat components assume a punctate pattern at the ER membrane. In addition to COPII coat components, recent data from our group and others suggest that the peripheral membrane protein, Sec16, localizes to ERES [13] and its GTPase inhibitory activity modulates the assembly of the COPII coat at ERES [14,15]. In mammalian and plant cells, hundreds of punctate ERESs are present along the cortical ER network under steady-state conditions [16–18]. In budding yeast species, the ERESs of *Saccharomyces cerevisiae* also appear as numerous punctate structures throughout the peripheral and perinuclear ER [19,20], whereas *Pichia pastoris* has only 2–5 ERESs next to the stacked Golgi complex [21]. While the size and number of ERESs differ between species and cell types in steady state, these parameters are also influenced by ER export competent cargo. For instance, in mammalian cells, acute and chronic increases in cargo load affect both the size and number of ERESs

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[22]. In plant cells, transient expression of membrane cargo causes an increase in the number of ERESs [23,24]. One emerging question is whether the ERESs found on the ER are all identical at the cellular level. The possibility that different ERESs could transport different secretory products has been implicated for mammalian and *Drosophila* cells [25,26]. In these cells, each ERES forms a unit with the Golgi stack, and ERES-Golgi units are scattered in different areas of the cell cytoplasm. These results raise the intriguing question of whether the ERESs of *S. cerevisiae* cells, which is a single-celled organism without the complexity of cellular differentiation, are all identical at the cellular level with respect to their composition and function. If so, could ERESs be reversibly differentiated in response to changing physiological and environmental conditions that alter the profiles of secretory proteins in the ER?

To address these crucial questions, we use *S. cerevisiae* and live-cell imaging analysis to examine whether Sec24 isoforms, the cargo-selection subunit of the COPII coat, are distributed uniformly or heterogeneously between different ERESs. Our results provide evidence for a non-uniform distribution of Sec24 isoforms to the ERESs at steady state. In addition, we showed that activation of the UPR causes a shift to a more homogeneous distribution of Sec24 isoforms. Our findings suggest that the ER of *S. cerevisiae* is able to undergo adaptive changes in COPII assembly at ERESs in response to environmental conditions.

2. Materials and methods

2.1. Strains, media, and growth conditions

The *S. cerevisiae* strain used in this work was the wild-type strain SEY6210 (*MATα leu2-3,112 ura3-52 his3-200 trp1-Δ901 lys2-801 suc2-Δ9*) [27]. Unless otherwise noted, the cultures were grown at 23 °C in MCD medium (0.67% yeast nitrogen base without amino acids, 0.5% casamino acids, and 2% dextrose) supplemented appropriately. For the induction of unfolded protein response, cells were grown to early log phase and treated with 8 mM dithiothreitol (DTT) for 2 h before microscopic observation.

2.2. Plasmid construction

The coding sequences of the *SEC24*, *LST1*, *ISS1*, and *SEC23* genes, together with upstream and downstream flanking regions, were amplified by PCR from *S. cerevisiae* genomic DNA and inserted into the *Sac*I and *Xho*I sites of pRS316 (*CEN*, *URA3*) or pRS314 (*CEN*, *TRP1*). For fusion of fluorescent proteins to the C-terminus, a *Sph*I site (for *SEC24*, *LST1*, and *ISS1*) or a *Bam*HI site (for *SEC23*) was created just before the stop codon of each gene, and the fragment encoding AcGFP, mCherry, or GFP(S65T), which was amplified by PCR from pAcGFP1, pmCherry (Clontech), or pFA6a-GFP(S65T)-kanMX6 [28], was inserted into these sites to yield pSEC24-AcGFP(316), pLST1-AcGFP(316), pSEC23-AcGFP(316), pLST1-mCherry(314), and pISS1-GFP(S65T)(316), respectively. A fragment encoding ISS1-GFP(S65T) was inserted into the *Sac*I and *Xho*I sites of pYO326 (*2μ*, *URA3*), yielding pISS1-GFP(S65T)(326). The generation of the plasmid expressing Sec23-mCherry has been described previously [29].

2.3. Fluorescence microscopy

Fluorescence microscopy observation was carried out using an Olympus IX71 microscope equipped with a CSU10 spinning-disk confocal scanner (Yokogawa Electric Corporation) and an electron multiplying charge coupled device camera (iXon, DU897, Andor Technology). In this setting, a 473 nm solid-state laser (Showa Optronics, J050BS) was used to excite GFP and mCherry at 561 nm (Jive, Cobolt). The acquired images were analyzed by using Andor iQ (Andor Technology) and ImageJ software (NIH). Each fluorescent spot (8 × 8 pixels) derived from ERES was quantified.

3. Results and discussion

In yeast and many other eukaryotes, gene duplication events have resulted in multiple isoforms for most of the COPII components. Several lines of evidence indicate that different isoforms of Sec24 recognize different sorting signals [30–33], expanding the variety of exported cargo [34]. In *S. cerevisiae*, there are two isoforms of Sec24 that have been identified to date: Lst1 and Iss1 [30–32]. The spectrum of cargo incorporated into COPII vesicles generated in vitro with Sec23/24 is markedly different from those formed with Sec23/Lst1 [9,35]. Therefore, to investigate steady-state organization of ERES in live cells, we used fluorescence confocal microscopy on *S. cerevisiae* cells expressing endogenous levels of Sec24-GFP paired with expression of one of the Sec24 isoforms tagged with mCherry. We measured the fluorescence intensities in a square centered on an ERES spot for the GFP and mCherry channels. The individual fluorescence intensities of the GFP and mCherry in each ERES were plotted, and the pairwise squared correlation coefficients (R^2) between the two were calculated. This approach allowed us to examine the relative spatial distribution of Sec24 isoforms with respect to each ERES in a single cell. For instance, if the distributions of Sec24 isoforms vary from one ERES to another, we would expect the R^2 pairwise correlation value between GFP and mCherry fluorescence intensity to be relatively low. Conversely, if the isoforms are distributed uniformly to each ERES, we would predict that the R^2 value of the fluorescence intensities between GFP and mCherry to be close to 1.0.

Initially, we examined the steady-state expression level of each of the GFP-tagged Sec24 isoforms driven by their own promoters on a single-copy plasmid (Fig. 1). A previous study demonstrated that Sec24, rather than Lst1, was highly expressed at steady state,

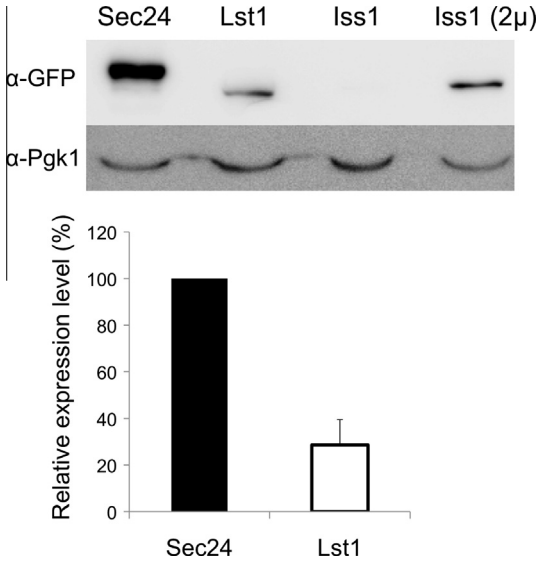

Fig. 1. Comparison of the steady-state expression levels of GFP-tagged Sec24, Lst1, and Iss1 driven by their own promoters. Equal amounts of proteins extracted from SEY6210 strain expressing the indicated GFP-tagged proteins were separated by SDS–PAGE followed by immunoblotting with anti-GFP antibody. One representative blot is shown (upper panel). 2μ indicates expression from a multicopy plasmid. Intensities of GFP-tagged protein bands were quantified and normalized relative to the total Pgk1 (loading control). Then these values were again normalized relative to Sec24-GFP. Data from three experiments were quantified (bottom panel). Values represent mean S.D.