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A karyopherin acts in localized protein synthesis

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Multiple mechanisms are in place to regulate adequate synthesis of proteins, ranging from ways to ensure sequence fidelity, polypeptide folding and protein modification, to control of amounts and subcellular localization of the molecules. Some of these mechanisms act at the level of mRNA export and mRNA targeting. mRNA nuclear export consists of three coupled consecutive steps: (1) the packaging into messenger ribonucleoprotein (mRNP); (2) the transport through the nuclear pore complexes (NPCs); and (3) the directional release into the cytoplasm (reviewed in refs. 1 and 2). The subsequent targeting of mRNA to particular subcellular locations is common in asymmetric cell division in many eukaryotes (reviewed in refs. 3–5) and ensures that proteins are produced at the desired place. Recent studies in *Saccharomyces cerevisiae* suggest that Karyopherin Kap104p plays a role not only in mRNA export but also in bud-localized protein synthesis.[6,7] In this report, we reflect on the possible mechanisms by which Kap104p links these events and hypothesize on a possible function of the localized protein synthesis.

Key words: *Saccharomyces cerevisiae*, Kap104, asymmetric division, translation, nuclear pore complex, mRNA targeting

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A Link between Karyopherin Kap104p and Localized Protein Synthesis

Transport through the nuclear pore complexes (NPCs) is mediated by soluble transport factors that bind specifically to a set of cargos that carry distinct nuclear localization signals (NLS) or nuclear export signals. Captured by a karyopherin the cargo molecules, including mRNAs, can transit the nuclear pores. The *Saccharomyces cerevisiae* transport factor Kap104p (transportin in vertebrates) recognizes the NLSs of the mRNA-binding proteins Nab2p and Nab4p/Hrp1p and mediates their reimport into the nucleus.[8-11] In a recent study,[6] it was observed that the nuclear accumulation levels of artificial cargo molecules of Kap104p, e.g., GFP fused to the NLS from Nab2p (rgNLS-GFP), change throughout the cell cycle; they were highest in G_1 and lowest during mitosis. The accumulation levels of this reporter are dependent on the rates of Kap104-mediated nuclear import and passive leakage from the nucleus as rgNLS-GFP is not significantly trapped by binding to nuclear structures. With constant cellular levels of rgNLS-GFP, the changes in accumulation thus seemed to be due to variations in availability of Kap104p.[12] Indeed, we did observe changes in cytoplasmic levels of Kap104p that correlated with the cell cycle-dependent accumulation of rgNLS-GFP and a redistribution of Kap104p pools to specific locations in the cell. Specifically, Kap104p-GFP accumulated ~10-fold compared to the cytoplasm at the distal-tip of the daughter cell during the early M-phase of the cell cycle and ~50-fold at the bud-neck during the late M-phase, i.e., at and after nuclear division (**Fig. 1A**). This redistribution approximately halved the Kap104p levels in the cytoplasm during M phase, resulting in an equivalent slowing of the import rate of rgNLS-GFP and thus nuclear accumulation levels. The pools of Kap104p at the bud tip and bud neck are apparently not available for binding and import of cytoplasmic rgNLS-GFP. Are they just parked

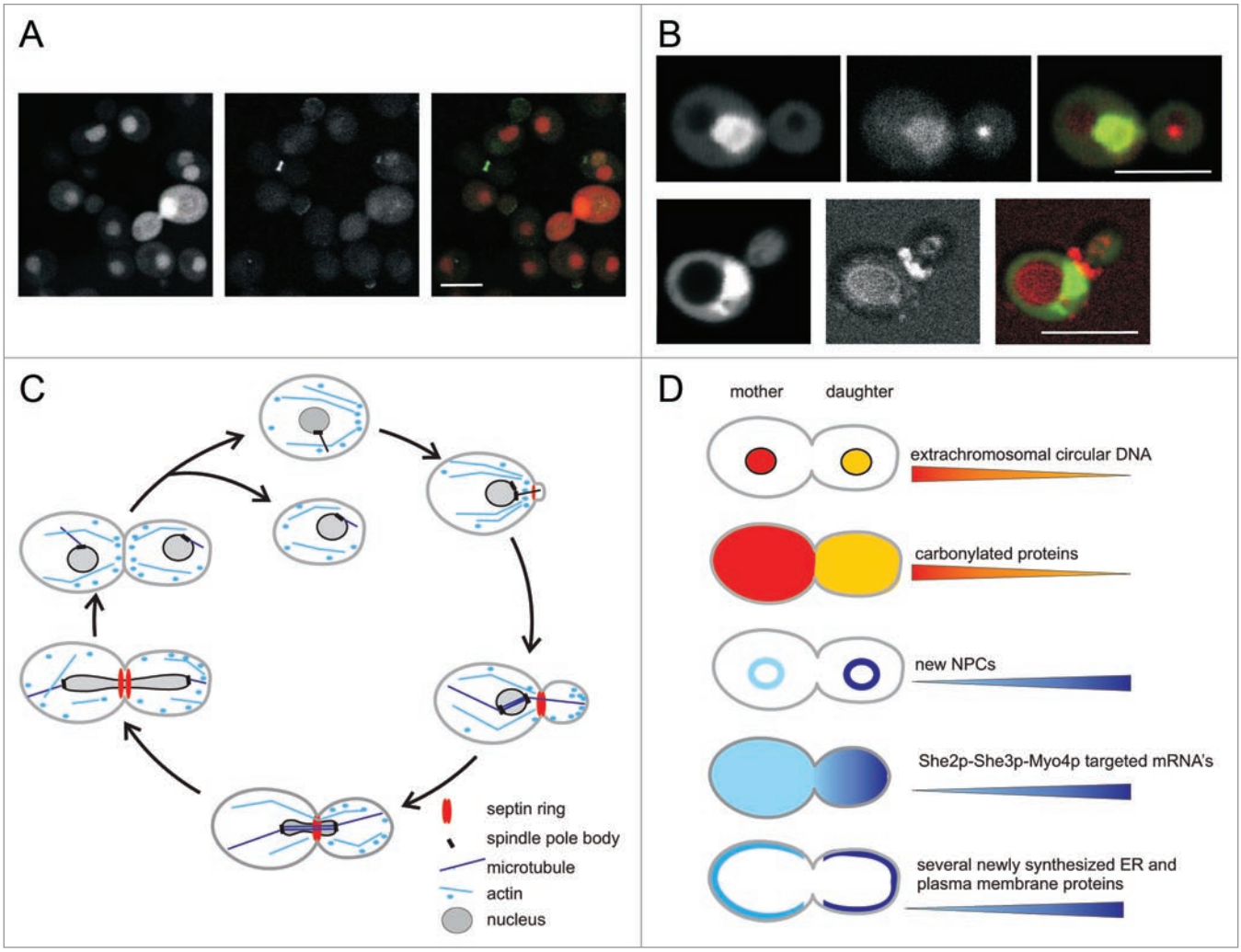

Figure 1. Growing young buds. (A) Confocal images of *Saccharomyces cerevisiae* cells expressing chromosomal GFP-tagged Kap104p (middle) and the rgNLS-mCherry (left). The merger of the two images is shown in the right panel (green: Kap104p-GFP; red: rgNLS-mCherry). In early mitosis Kap104p-GFP localized to the bud tip; in late mitosis it localized to the bud neck. Scale bars 5 µm. (B) Distribution of translation sites (red) in the bud of cycloheximide-treated cells (top three) and at the bud neck (bottom three). Left: rgNLS-GFP; middle: fluorescent staining to visualize the translation activity; right: merger of the two images. (C) *Saccharomyces cerevisiae* cells divide by formation of a bud, which is followed by the formation of a septum to separate mother and daughter. The divisions are asymmetric, resulting in daughter cells that are smaller than the mothers but with full replicative potential; (adapted from ref. 46). (D) A number of macromolecules have been described to divide asymmetrically between mother and daughter cells: extrachromosomal circular DNAs remain in the mother nucleus[39] and damaged proteins remain in the mother,[42] while new NPCs are assembled in the daughter nucleus,[45] specific mRNA's are targeted by the She2p-She3p-Myo4p pathway to the daughter (reviewed in ref. 5), and newly synthesized ER and plasma membrane proteins are compartmentalized to the daughter.[19,44] The here highlighted Kap104p-dependent translation activity[6] contributes to the asymmetry of yeast cell divisions by promoting protein synthesis in the bud. We speculate that this generates a gradient of old and new proteins and as such contributes to the rejuvenation of the daughter cell.

"off duty" or do they serve a different role at the bud tip and neck?

We proposed that the cell cycle-dependent localization of Kap104p may be related to the synthesis of proteins, which is required for outgrowth of the bud. To substantiate this hypothesis, we used a fluorescence-based assay to monitor the polypeptides as they are emerging from the ribosome and taking advantage of the increased ribosome packing of mRNA in cycloheximide-treated cells.[13] The assay allowed the visualization of increased translational activity in the bud,[6] i.e., at or near the site where Kap104p is accumulated (**Fig. 1B**). Are these two events, the cell cycle-dependent Kap104p localization and the increased translational activity, just incidentally co-occurring? The best evidence that Kap104p plays a more direct role in the increased translational activity came from the following experiments: In cells that expressed high levels of the Kap104p cargo (rgNLS-mCherry), Kap104p-GFP redistribution and increased translation activity were no longer observed. In this case, rgNLS-mCherry likely binds the majority of cytoplasmic Kap104p-GFP and thus competes with Kap104p interaction partner(s) in the tip and bud neck. Control experiments with similarly high levels of expression of cNLS-mCherry did not affect the Kap104p-GFP distributions

and locally increased translation stains. We thus concluded that the presence of high levels of Kap104p-GFP at the bud and the increased translational activity are interdependent events.

A Carrier Waiting for Cargo? Reflecting on Potential Mechanisms

Saccharomyces cerevisiae cells divide by a budding process (**Fig. 1C**) (reviewed in ref. 14). Cortical cues determine the site for bud formation, and an axis of polarity directed toward this site is formed by specifically localized signaling molecules. The initial bud then emerges after the formation of a cytoskeletal framework that targets secretion to the bud. Further cell growth is restricted to the bud as the cell orchestrates the duplication and segregation of its organelles. When the bud reaches nearly the same size as the mother, the cell undergoes mitosis and cytokinesis, and secretion is directed to the bud neck to lay down a septum, which then separates the mother and daughter.

A couple of dozen mRNAs have been shown to traffic to the bud many more have been predicted.[11,15-19] The best described example is that of the mRNA encoding the daughter-specific transcription factor Ash1p, which is transported to the bud in late anaphase. This targeting ensures that Ash1p only acts in the nucleus of the daughter so that mother and daughter cells have distinct mating types (reviewed in ref. 5). The molecular mechanism involves the She2p-She3p-Myo4p complex moving along the actin cytoskeleton in addition to a number of proteins that regulate *ASH1* mRNA transport and translation. We showed that increased translation at the bud is not dependent on Myo4p or She2p and thus must involve a mechanism that is different from the She2p-She3p-Myo4p pathway.

In our view the local high concentrations of Kap104p result in a mRNP remodeling step, including the release of Nab2p and Nab4p/Hrp1p from the mRNA. This freeing of the mRNA is required for translational activity. A high concentration of Kap104p would thus increase the protein synthesis activity locally. The exact molecular mechanism and the full complement of components involved remains to be resolved. Earlier data, using single stranded DNA, implied a direct role for Kap104p in releasing Nab2p and Nab4p/Hrp1p from oligonucleic acids.[20] However, other experiments using mRNPs did not confirm a direct role of Kap104p in releasing these proteins,[21] possibly because the affinities of Nab2p and Nab4p/Hrp1p for RNA are higher than for single-stranded DNA. In analogy with what happens at the NPC, that is, the release of Nab2p and Nab4p/Hrp1p from the mRNP by the joint action of Gle1p/IP6 and Dbp5p,[21-34] we speculate that the RNA-dependent DEAD box protein, Dbp5p, plays a crucial role in mRNP remodeling at the tip of the growing daughter cell. In fact, we could confirm that Dbp5p, like Kap104p, accumulates at the bud tip.[6] It seems possible that Kap104p and Dbp5p act together in the mRNP remodeling, whereby rapid binding of Nab2p and Nab4p/Hrp1p by Kap104p would shift the equilibrium towards translation competent mRNA.[7] Future experiments should clarify which are the binding partners for Kap104p at the bud neck and tip and what determines the cell cycle dependence of these interactions.

A role in remodeling of newly synthesized mRNA was recently also proposed for another member or the beta karyopherin family, human importin β.[35] In mammalian cells, the pioneer and steady-state translation initiation complexes are physically distinct. In the pioneer translation complex, the caps are bound by the cap-binding complex (CBP80-CBP20), while in the steady-state complex the cap is bound by eukaryotic translation initiation factor 4E (eIF4E). Sato and Maquat presented data which indicate that binding of the karyopherin importin β to CBP80-bound importin α promotes the replacement of the cap-binding complex by eIF4E. Indeed, from the structure of a complex of importin α with the cap-binding complex and biochemical data, it follows that importin β binding weakens the affinity of the cap binding complex for capped mRNA, promoting its release.[36] So, in analogy with yeast Kap104p, also this member of the β-karyopherin family is proposed to function in regulating protein synthesis.

Growing Young Buds: Reflecting on Potential Functions

Saccharomyces cerevisiae cells can divide approximately 30 times before they die (defined as replicative ageing) (reviewed in refs. 37 and 38). Replicative ageing is asymmetric and the mother cell ages. The age of the daughter cell is 'reset' such that daughter cells of older mothers retain replicative potential. Inheritance of toxic and deteriorated material, such as extra chromosomal rDNA circles (ERCs)[39] and oxidatively damaged (carbonylated) and aggregated proteins[40-42] by the mother and exclusion from the progeny is part of the mechanism (**Fig. 1D**).

The described Kap104p related increase in protein synthesis in the daughter cell[6] may contribute to increased efficiency of bud growth. It is tempting to speculate that the localized translation serves to provide the daughter cell with more than a fare share of newly synthesized proteins, whereas the mother cell gets a surplus of 'old' proteins. The question then arises how likely is it that the newly synthesized proteins are retained in the bud. The yeast cytosol is a crowded environment, but experimental determinations of lateral diffusion in situ indicate that proteins of the size of GFP should be able to migrate within seconds from the bud to the mother cell. On the other hand, for large supramolecular complexes assembled in the bud, the diffusion will be much slower. Thus, intermixing of newly synthesized soluble proteins and older proteins would happen in a timeframe of minutes, unless the proteins are rapidly incorporated in localized structures that are confined to the daughter cell. For membrane-associated protein complexes and certain integral membrane proteins there is compartmentalization between the mother and bud, and these proteins can thus retain their daughter-specific localization. For instance, the septin membrane diffusion barrier at the bud neck separates the mother and daughter plasma membrane.[43] Also, diffusion of endoplasmic reticulum membrane proteins from mother to daughter is restricted,[44] and outer nuclear membrane proteins, including nuclear pore complexes of anaphase nuclei, of mother and daughter are separated by a diffusion barrier.[43] Indeed some

of the known mRNAs that are targeted to the bud by the She2p-She3p-Myo4p complex encode proteins that are destined for these cellular structures. A careful evaluation of the rates of bud growth and replicative potential of the daughters, i.e., when interfering with Kap104p localization, may answer questions pertinent to retention and exclusion of proteins in different parts of the budding cell. Also, it will be important to obtain estimates of translational mobility of a variety of proteins and protein complexes in the mother and daughter cell.

Future Questions

The description of a new relationship between a cell cycle-regulated localization of karyopherin Kap104p at the bud of dividing yeast cells *and* bud localized translation has raised a number of questions. What does Kap104p actually do, and do differently at the various subcellular locations when the yeast progresses through its cell cycle? One may find answers to these questions if the following issues are addressed: Which mRNAs are localized to the sites of Kap104p accumulation? What are the different binding partners for Kap104p at the bud tip and neck and what determines the shift in subcellular localization? What are the remodeling steps in mRNP and is Dbp5p-Gle1p-IP6p involved? What is the function of the localized translation? Is it to ensure fast outgrowth of the bud, or does it affect the distribution of old and new proteins and as such secure the replicative potential of daughter cells? In this perspective, we have reflected on some of these questions and speculated on possible molecular mechanism and functions. Genuine answers will await thorough experimentation.

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