Identification of an extranucleolar site of ribosome production in yeast

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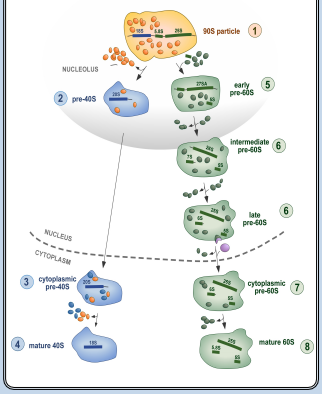
BACKGROUND

In *Saccharomyces cerevisiae* the nucleolus is a crescent-shaped structure that abuts the nuclear envelope and occupies up to one-third of the nucleus. The positioning of the nucleolus in the nuclear periphery is thought to be important for the genomic stability of the highly repetitive ribosomal DNA (rDNA) (1). Several inner nuclear membrane (INM) proteins and rDNA silencing factors have been implicated in tethering the rDNA to the nuclear envelope (1).

Many tRNA genes are clustered close to the nucleolus, suggesting that there is co-compartmentalization of tRNA and ribosome synthesis (2).

In mitosis, the nucleolus remains mostly intact and splits just when the rDNA is segregated, at the end of anaphase (3,4). Thus, the rDNA and the early ribosome synthesis factors are seen as part of a recognizable region inside the mother cell in metaphase and most of anaphase. They remain always in close proximity and stream into the bud at the end of anaphase to give rise to the daughter-cell nucleolus. It is therefore assumed that the mother-cell nucleolus is the major and sole place of ribosome production in early mitosis.

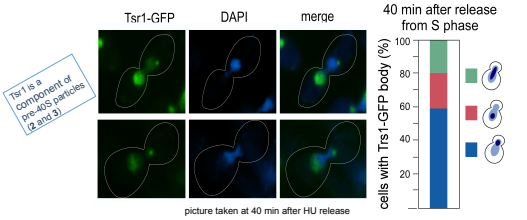
40S SUBUNIT AND 60S SUBUNIT PRE-RIBOSOMES



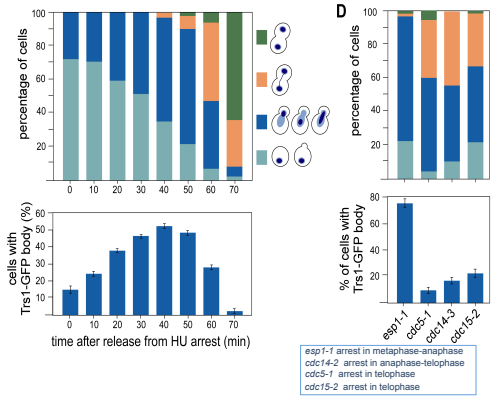
I. Presence of Tsr1 at a punctate body in the daughter cell during metaphase-anaphase

A. Localization of the pre-40S factor Tsr1 at a discrete site distant from the nucleolus

asynchronous culture → G1 arrest (α-factor) → S phase arrest (HU) → release from S phase arrest (microscopy 0-70 min)



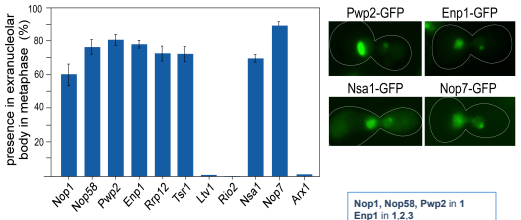
B. Formation of the extranucleolar Tsr1 body in wild type cells and in cell cycle mutants



esp1-1 arrest in metaphase-anaphase
cdc14-2 arrest in anaphase-telophase
cdc5-1 arrest in telophase
cdc15-2 arrest in telophase

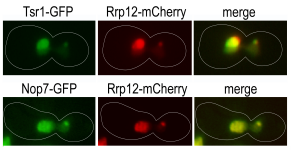
II. The Tsr1-containing body is a site of ribosome synthesis

A. Other nucleolar ribosome synthesis factors also localize at an extranucleolar site in metaphase-anaphase

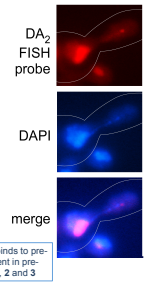


Nop1, Nop58, Pwp2 in **1**
Enp1 in **1,2,3**
Rrp12 in **1**, **2**
Tsr1 in **2,3**
Ltv1 and Rio2 mostly in **3**
Nop7 in **5**, **6** (mostly nucleolar)
Nsa1 in **6** (mostly nucleolar)
Arx1 in **6**, **7**

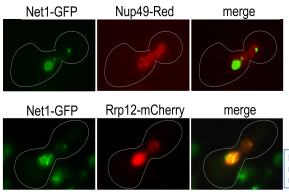
B. Co-localization of factors at extranucleolar site



C. Presence of pre-rRNA

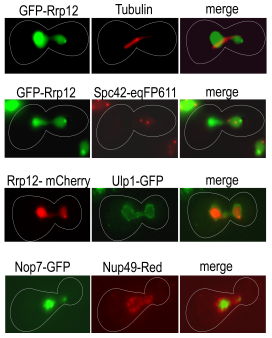


D. Presence of rDNA at extranucleolar site

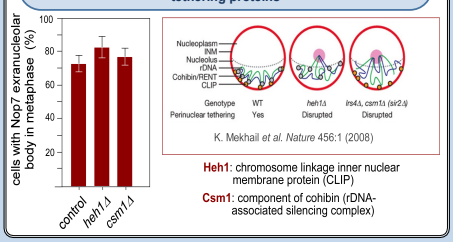


DA2 probe binds to pre-rRNAs present in pre-ribosomes **1**, **2** and **3**

III. The RiBi body is at the nuclear envelope and is not close to the SPB



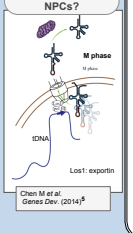
IV. Formation of the RiBi body does not depend on rDNA tethering proteins



Heh1: chromosome linkage inner nuclear membrane protein (CLIP)

Csm1: component of cohibin (rDNA-associated silencing complex)

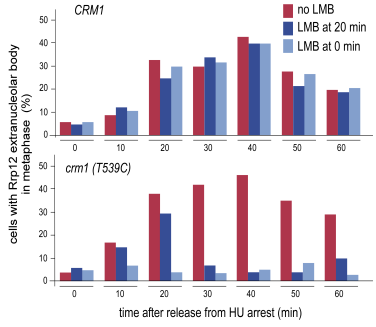
Similar to the M phase-specific coordination of tRNA transcription and export at NPCs?



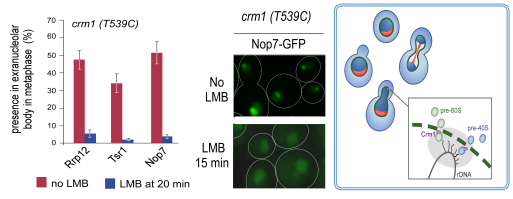
V. The Crm1 exportin is required for the focal accumulation of pre-ribosomes in metaphase-anaphase

A. Rrp12 does not accumulate at the extranucleolar body upon Crm1 inhibition

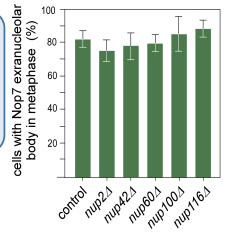
S phase arrest (HU)
LMB → 0 min
LMB → 20 min
release from S phase arrest (microscopy at 0-60 min)



B. Tsr1 and Nop7 localization upon Crm1 inhibition



VI. Several candidate nucleoporins are not required for the formation of the RiBi body



PROTEIN	RELEVANT INFORMATION	STRAIN
Nup2	Required to localize tDNAs at NPCs in mitosis[5]	*nup2.1* (viable)
Nup42	Interacts with Crm1[6] Involved in gene tethering at NPCs[7]	*nup42.1* (viable)
Nup60	Required to localize tDNAs at NPCs in mitosis[5]	*nup60.1* (viable)
Nup100	Interacts with Crm1 and Rrp12[6,8] Involved in gene tethering at NPCs[7]	*nup100.1* (viable)
Nup116	Interacts with Crm1 and Rrp12[8,9]	*nup116.1* (viable)

MAIN FINDINGS

1. During early mitosis in budding yeast, the initiation of ribosome synthesis not only takes place in the nucleolus, but also at a discrete region of the daughter-cell nucleus that is in close proximity to the nuclear envelope.
2. The extranucleolar ribosome-synthesis body is a site in which pre-ribosome maturation and export are highly efficient. This body contains rDNA and early pre-ribosomes, but has a low content of intermediate pre-export particles.
3. The exportin Crm1 is required for the focal accumulation of early pre-ribosomes at the extranucleolar body. This role is consistent with the function we have previously proposed for this exportin in coupling pre-40S particle maturation and export (10).
4. Initiating ribosome synthesis at a site that is in close proximity to the nuclear envelope might serve to expedite export of ribosomes and facilitate the growth of the bud during mitosis.

[1] AFFILIATION AND FUNDING

G.M., Centro de Investigación del Cáncer , CSIC/Universidad de Salamanca.
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This work is supported by grants from the spanish Ministerio de Economía y Competitividad (BFU2011-23668, BFU2014-52729, and RD12/0036/0002), the Samuel Solórzano Barruso Foundation (FS/17-2013) and the Castilla y León Autonomous Government (CSI039A12-1). G.M. and B.N. have been supported by graduate student contracts by the University of Salamanca and Santander Bank and, in the case of B.N., by the RD06/0020/0001 grant. Spanish funding is co-sponsored by the European Union FEDER Program.

REFERENCES

1. Mekhail, K., Seebacher, J., Gygi, S.P. and Moazed, D. (2008) Role for perinuclear chromosome tethering in maintenance of genome stability. *Nature*, **456**, 667-670.
2. Thompson, M., Haeusler, R.A., Good, P.D. and Engelke, D.R. (2003) Nucleolar clustering of dispersed tRNA genes. *Science*, **302**, 1399-1401.
3. Granot, D. and Snyder, M. (1991) Segregation of the nucleolus during mitosis in budding and fission yeast. *Cell Motil Cytoskeleton*, **20**, 47-54.
4. Bystricky, K., Laroche, T., van Houwe, G., Blaszczyk, M. and Gasser, S.M. (2005) Chromosome looping in yeast: telomere pairing and coordinated movement reflect anchoring efficiency and territorial organization. *The Journal of cell biology*, **168**, 375-387.
5. Chen, M. and Gartenberg, M.R. (2014) Coordination of tRNA transcription with export at nuclear pore complexes in budding yeast. *Genes & development*, **28**, 959-970.
6. Neville, M., Stutz, F., Lee, L., Davis, L.I. and Rosbash, M. (1997) The importin-beta family member Crm1p bridges the interaction between Rev and the nuclear pore complex during nuclear export. *Curr Biol*, **7**, 767-775.
7. Light, W.H., Brickner, D.G., Brand, V.R. and Brickner, J.H. (2010) Interaction of a DNA zip code with the nuclear pore complex promotes H2A.Z incorporation and INO1 transcriptional memory. *Molecular cell*, **40**, 112-125.
8. Oeffinger, M., Dlakic, M. and Tollervey, D. (2004) A pre-ribosome-associated HEAT-repeat protein is required for export of both ribosomal subunits. *Genes & development*, **18**, 196-209.
9. Yu, H., Braun, P., Yildirim, M.A., Lemmens, I., Venkatesan, K., Sahalie, J., Hirozane-Kishikawa, T., Gebreab, F., Li, N., Simonis, N. *et al.* (2008) High-quality binary protein interaction map of the yeast interactome network. *Science*, **322**, 104-110.
10. Moriggi, G., Nieto, B. and Dosil, M. (2014) Rrp12 and the Exportin Crm1 Participate in Late Assembly Events in the Nucleolus during 40S Ribosomal Subunit Biogenesis. *PLoS Genet*, **10**, e1004836