

Research Article

In vivo labelling of functional ribosomes reveals spatial regulation during starvation in *Podospora anserina*

Hervé Lalucque and Philippe Silar

Address: Institut de Génétique et Microbiologie, Université de Paris-Sud, France.

E-mail: Hervé Lalucque - lalucque@igmors.u-psud.fr Philippe Silar - silar@igmors.u-psud.fr

Published: 16 November 2000

Received: 30 August 2000

BMC Genetics 2000, 1:3

Accepted: 16 November 2000

This article is available from: <http://www.biomedcentral.com/1471-2156/1/3/>

Abstract

Background: To date, in eukaryotes, ribosomal protein expression is known to be regulated at the transcriptional and/or translational levels. But other forms of regulation may be possible.

Results: Here, we report the successful tagging of functional ribosomal particles with a S7-GFP chimaeric protein, making it possible to observe *in vivo* ribosome dynamics in the filamentous fungus *Podospora anserina*. Microscopic observations revealed a novel kind of ribosomal protein regulation during the passage between cell growth and stationary phases, with a transient accumulation of ribosomal proteins and/or ribosome subunits in the nucleus, possibly the nucleolus, being observed at the beginning of stationary phase.

Conclusion: Nuclear sequestration can be another level of ribosomal protein regulation in eukaryotic cells. This may contribute to the regulation of cell growth and division.

Background

Because translation utilizes a large proportion of the cell energy, its components are tightly regulated, especially ribosomes. Indeed, as cells are depleted of nutrients, a reduction in ribosome function occurs. In *E. coli*, ribosomes are converted by dimerization through the action of the RMF protein into 100S particles that are unable to perform translation [1]. In eukaryotes, ribosomal proteins are down regulated. This occurs through transcriptional regulation in yeast [2] and mostly through translational regulation in mammals and *Dictyostelium* [3]. To date, *in vivo* observation of a tagged and functional ribosomal particle during various cellular growth phases has not been reported (despite the fact that the large ribosomal subunit has recently been successfully tagged with GFP [4]), so there has been no evidence of whether spatial regulation can also be involved in the regulation of ribosomal proteins. Here, we report a spatial regulation of a small subunit ribosomal protein upon entrance into stationary phase.

Results and discussion

In order to observe the ribosomes *in vivo*, the S7 small subunit ribosomal protein of *P. anserina* was tagged with GFP. S7 belongs to the *E. coli* S4 family and is encoded by the *su12* gene [5]. To this end, the GFP coding sequence was inserted in frame in the place of the *su12* stop codon to yield the *su12-GFP* gene. Expression of this gene is thus driven from the *su12* promoter and should yield a chimaeric S7GFP protein composed of S7 at the N terminus of the protein and GFP at the C terminus. The *su12-GFP* gene was introduced by transformation in the *su12-1C1* mutant strain, which carries two mutations in the *su12* gene, and thus displays a strong decrease of paromomycin resistance [6]; this antibiotic binds to ribosomes and increases the decoding error rate [7, 8]. The transformants obtained were fluorescent and had the same level of paromomycin resistance as wild type (Fig. 1) indicating that the S7GFP protein is incorporated into the ribosome and is fully able to replace the *su12-1C1* mutant protein during protein synthesis. Genetic analysis for two transformants showed a complete co-segregation

of the restoration of paromomycin resistance and of the fluorescence.

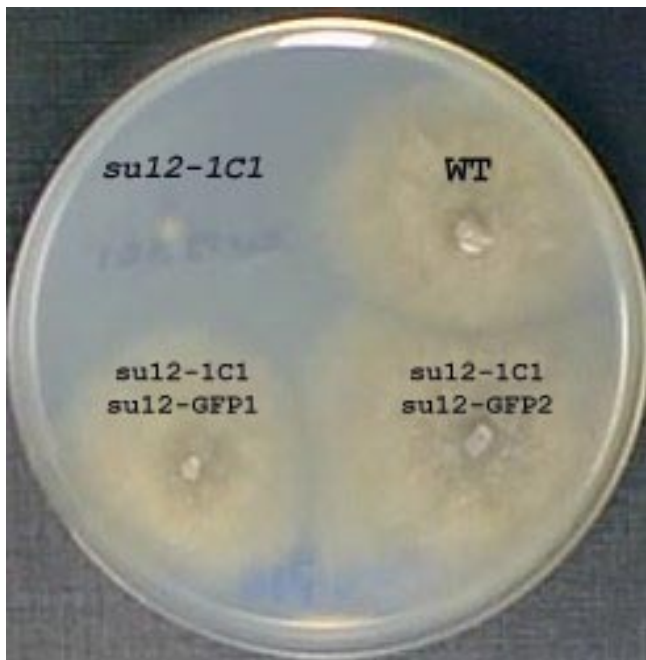


Figure 1
Functional complementation the *su12-1C1* mutation by *su12-GFP*. Growth after four days on medium containing 750 mg/l paromomycin of two independent *su12-1C1* transformants expressing the S7GFP protein, wild type and untransformed *su12-1C1* strain.

As seen in Fig. 2A and 2B, examination of hyphae taken from the growing edge of a culture revealed an intense fluorescence throughout the cytoplasm of most hyphae. The staining heterogeneity was due to the fact that organelles and vacuoles remained unstained. By contrast, examination of hyphae taken about 1 centimetre away from the growing edge, where cells have stopped their division for about 24 hours, revealed in many (but not all) of them a less intense fluorescence that was homogenous throughout the cytoplasm but for a few intense foci (Fig. 2C). These foci co-localised with nuclei (Fig. 2D). They were not observed in the control experiment where GFP was expressed alone from a GPD promoter (Fig. 2G and 2H). When cells had stopped their division for a longer time (i.e. for about a week), the fluorescence decreased further and no foci were observed (Fig. 2E and 2F). These *in vivo* data show that the ribosomes are diminished in the cytoplasm upon entrance into stationary phase but also provide evidence that the S7 protein is present during a transient period at a higher level within a define region of the nucleus. The staining is possibly localised in the nucleolus, since in 42 out of 50 nuclei observed, the foci were located at the periphery of the

nucleus in a region that appeared less stained with DAPI (Fig. 2I, 2 and 2). In the remaining 8 cases, the nuclei exhibited the shape of a pear and the foci were localised at the tip of the pear. In these cases, it was impossible to ascertain that the foci were located within the nucleolus.

Conclusions

We report here for the first time a nuclear sequestration of a ribosomal protein during a transient period at the onset of stationary phase. A plausible explanation is that this kind of regulation permits a rapid production of ribosomes if nutrients are encountered before a more pronounced stationary phase is entered. However, recent data [9, 10] show that release of *cde14*, sequestered in the nucleolus, is involved in the proper exit from mitosis. Because ribosomes might regulate cell cycle progression [11], it is possible that sequestering ribosome in the nucleus is an additional level of regulation involved in ensuring a correct cell cycle arrest.

Materials and Methods

The strains used for this study derive from the S strain [12]. Culture and methods used for *Podospora anserina* are described in [13].

DNA manipulation were made according to [14]. To construct the *su12-GFP* gene, the *su12* coding sequence was amplified from plasmid *psu12-S3* [5] by PCR with oligos 3382 (5'-ACTATAGGGCGAATTGG-3') and *su12-3'* (5'-CGGGATCCCCGAAAACATACCTGATCACGCAGAG-3'). This yielded a PCR product, in which the *su12* stop codon is replaced by a *Bam*HI site allowing for the fusion with the GFP coding sequence. To this end, the PCR product was digested by *Eco*RI and *Bam*HI and cloned into pEGFP-1 (Clontech) at the corresponding sites. The sequence of the complete *su12* coding sequence along with the junction with the *GFP* coding sequence revealed that no mutation had occur in the *su12* coding sequence during plasmid construction. *Podospora S* strain was co-transformed using the method of [15] by this plasmid and pBC-hygro vector [16]. Transformants resistant to hygromycin were selected and examined for GFP fluorescence and resistance to paromomycin. Several such transformants were obtained. Two were subjected to genetic analysis through a cross with wild type.

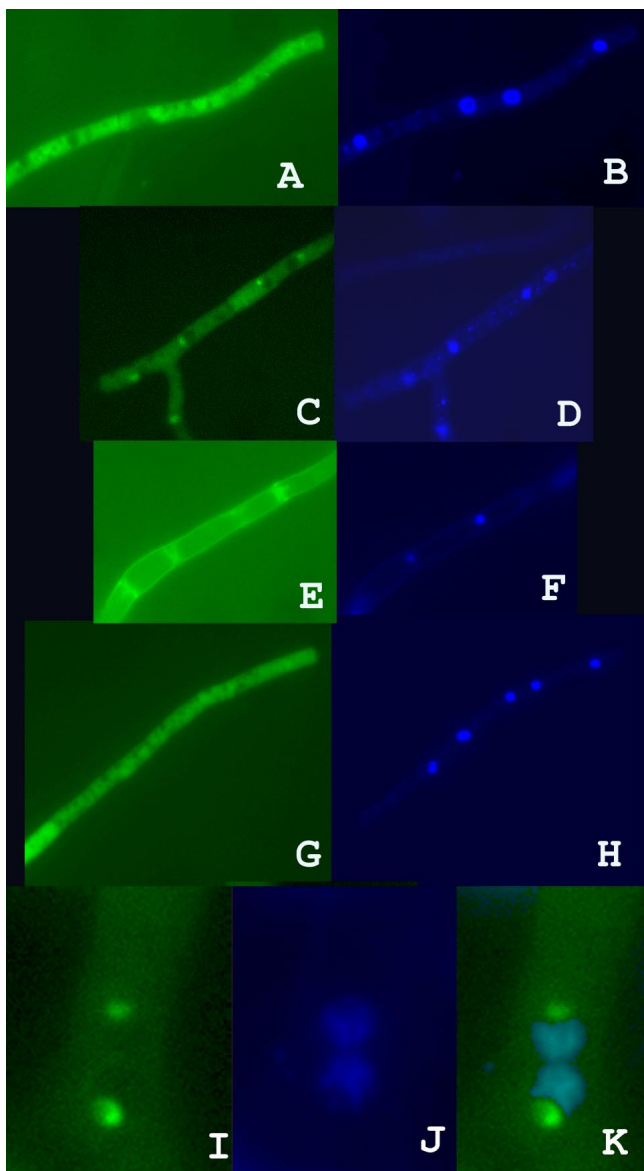


Figure 2
Microscopic observation of hyphae expressing the S7GFP protein. A-F, are experimental observations with strains carrying the *su12-GFP* chimaeric gene. A, C and E visualise GFP fluorescence and B, D and F visualise DAPI staining. The hyphae in A and B is from the growing edge; The hyphae in C and D is taken 1 centimetre away from the growing edge corresponding to about 1 day of stationary phase. The picture in E was taken with a pose time twice as long as the other pictures. G (GFP fluorescence) and H (DAPI staining) are control observations of GFP alone expressed from the GPD promoter; the hyphae is taken 1 centimetre away from the growing edge as in C and D. I (GFP fluorescence), J (DAPI staining) and K (superposition of I and J) show the sub-nuclear localisation of the foci in two adjacent nuclei. Clearly, the GFP foci are located in a depression of the DAPI staining, where the nucleolus is supposed to be located.

Acknowledgments

We would like to thank all members of the laboratory for useful discussion and Denise Zickler and Françoise James for helping with the microscope. This work was supported by ARC grant n° 5388.

References

1. Wada A: **Growth phase coupled modulation of Escherichia coli ribosomes.** *Genes to Cells* 1998, **3**:203-208
2. Warner JR: **The economics of ribosome biosynthesis in yeast.** *Trends Biochem Sci* 1999, **24**:437-440
3. Meyuhas O, Avni D, Shama S: **Translational control of ribosomal protein mRNA in Eukaryotes.** In *Translational control* Edited by Hersey JWB, Mathews MB, Sonenberg N Cold Spring harbor Laboratory Press 1996, 363-388
4. Hurt E, Hannus S, Schmelzl B, Lau D, Tollervey D, Simos G: **A novel in vivo assay reveals inhibition of ribosomal nuclear export in Ran-cycle and nucleoporin mutants.** *J Cell Biol* 1999, **144**:389-401
5. Silar P, Koll F, Rossignol M: **Cytosolic ribosomal mutations that abolish accumulation of circular intron in the mitochondria without preventing Senescence of Podospora anserina.** *Genetics* 1997, **145**:697-705
6. Dequard-Chablat M: **Different alterations of the ribosomal protein S7 lead to opposite effects on translational fidelity in the fungus Podospora anserina.** *J Biol Chem* 1986, **261**:4117-4121
7. Blanchard SC, Fourmy D, Eason RG, Puglisi JD: **rRNA chemical groups required for aminoglycosid binding.** *Biochemistry* 1998, **37**:7716-7724
8. Palmer E, Wilhem JM: **Mistranslation in a eukaryotic organism.** *Cell* 1978, **13**:329-334
9. Shou W, Seol JH, Shevchenko A, Baskerville C, Moazed D, Cheng ZWS, Jang J, Shevchenko A, Charbonneau H, Deshaies RJ: **Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from Nucleolar RENT complex.** *Cell* 1999, **97**:233-244
10. Visitin R, Hwang ES, Amon A: **Cfi1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus.** *Nature* 1999, **398**:818-823
11. Thomas G: **An encore for ribosome biogenesis in the control of cell proliferation.** *Nature Cell Biol* 2000, **2**:E71-E72
12. Rizet G: **Les phénomènes de barrages chez Podospora anserina. I Analyse génétique des barrages entre souches S et s.** *Rev Cytol Biol Veget* 1952, **13**:51-92
13. Esser K: **Podospora anserina.** In *Handbook of Genetics* Edited by King RC, vol. 1 New York: Plenum 1974, 531-551
14. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Strul (eds): **K: Current Protocols in Molecular Biology** 1987,
15. Brygoo Y, Debuchy R: **Transformation by integration in Podospora anserina. I. Methodology and phenomenology.** *Mol Gen Genet* 1985, **200**:128-131
16. Silar P: **Two new easy to use vectors for transformations.** *Fung Genet Newsltt* 1995, **42**:73-

Publish with **BioMedcentral** and every scientist can read your work free of charge

"BioMedcentral will be the most significant development for disseminating the results of biomedical research in our lifetime."

Paul Nurse, Director-General, Imperial Cancer Research Fund

Publish with **BMC** and your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours - you keep the copyright



Submit your manuscript here:

<http://www.biomedcentral.com/manuscript/>

editorial@biomedcentral.com