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Abstract

The ribosomal proteins of the E. coli S4, S5 and S12 families that are part of the ribosome accuracy center control translation accuracy both in prokaryotes and eukaryotes. In *Podospora anserina*, genes coding for S4 and S5 have already been identified. Here, we identify the gene coding for the S12 protein homologue and show that it is identical to the genetically known *AS6* gene.

Podospora anserina AS6 gene encodes the cytosolic ribosomal protein of the _E. coli_ S12 family

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The ribosomal proteins of the *E. coli* S4, S5 and S12 families that are part of the ribosome accuracy center control translation accuracy both in prokaryotes and eukaryotes. In *Podospora anserina*, genes coding for S4 and S5 have already been identified. Here, we identify the gene coding for the S12 protein homologue and show that it is identical to the genetically known *AS6* gene.

Podospora anserina has been used in intensive search of translation accuracy mutants (Coppin-Raynal *et al.* 1988). Several factors involved in the maintenance of accuracy have been identified in this organism including the tRNA suppressors *su4-1* and *su8-1* (Debuchy *et al.* 1985), as well as elongation factor eEF1A coded by *AS4* (Silar *et al.* 1994), termination factors eRF1 and eRF3 coded by *su1* and *su2*/*AS2* respectively (Gagny *et al.* 1998), ribosomal proteins S12 coded by *AS1* (Dequard-Chablat *et al.* 1994), S7 coded by *su12* (Silar *et al.* 1997) and S1 coded by *su3* (Silar *et al.* 2003). S12, S7 and S1 refer to the *P. anserina* numbering for ribosomal proteins (Dequard-Chablat *et al.* 1986) since the *su12* and *su3* genes code for the ribosomal proteins homologues of the *E. coli* S4 and S5, respectively. These two proteins are part of an accuracy center that has been conserved for more than two billion years in both prokaryotes and eukaryotes (Alksne *et al.* 1993). The center contains a third protein corresponding to the *E. coli* S12 protein, which remains to be identified in *P. anserina*. This protein is highly conserved and essential in all prokaryotes and eukaryotes investigated to date (Alksne *et al.* 1993).

To identify the gene coding this protein in *P. anserina*, we took advantage of the availability of the complete genomic sequence of this fungus (available at http://podospora.igmors.u-psud.fr). We first located on the sequence map the gene encoding the *P. anserina* protein of the S12 family that would act in cytosolic translation. To do this, we searched the genome sequence by BLAST using the *Saccharomyces cerevisiae* S28 protein, which is a eukaryotic homolog of the *E. coli* S12 protein (Alksne *et al.* 1993). A single CDS, Pa_3_536, was obtained with a significant score (10^{-72}; Figure 1). It has 89% identity and 94% similarity with the *S. cerevisiae* S28 protein. At least one orthologue is present in the complete genomes of all organisms sequenced to date and the gene is highly conserved in fungi (figure 1). For example, the *P. anserina* and *Neurospora crassa* proteins are 99% identical .

Pa_3_5360 is located on the right arm of chromosome 3 in a region that would segregate with a 70% second division frequency, strongly suggesting that it could correspond to the CDS of the previously known *AS6* gene since (1) *AS6* is located at this position on the genetic map (Picard-Bennoun *et al.* 1980), (2) *AS6* controls translation accuracy (Picard-Bennoun 1981), (3) numerous mutations of this gene were recovered as suppressors of *su12* mutations and thus (Dequard-Chablat 1986) and (4) *AS6* encodes a ribosomal protein (named S19 in the *P. anserina* nomenclature; Dequard-Chablat *et al.* 1986).

To confirm this, we first sequenced the three alleles of Pa_3_5360 present in the *AS6-1*, *AS6-2* and *AS6-5* mutants. DNA fragments were amplified using oligonucleotides 5'- ttgcgagatcccatcaaag-3' and 5'-agcctcgcagactcctca-3' and the three mutant genomic DNAs as template. Complete sequence of the DNA fragments was then performed and compared with that of wild type. *AS6-1* contained a C to G transversion that changes arginine 73 to glycine, *AS6-2* a G to A transition that changes aspartate 90 to asparagine and *AS6-5* a C to T transition that changes alanine 113 to valine (Figure 1). Finally, we co-transformed the *AS6-1* mutant with plasmid pAS6 (plasmid GA0AA399BE01 from the *P. anserina* genome sequence project) containing a DNA fragment carrying the $AS6^{+}$ allele and the pBC-hygro vector carrying a hygromycin B resistance gene (Silar 1995). *AS6-1* has a strong sexual defect since it is unable to differentiate perithecia (Figure 2). No transformants with a restored wild-type phenotype was recovered among the 30 retrieved in the control experiment with the pBC-hygro vector alone, whereas, six transformants with a restored wild-type phenotype were recovered among 13 analyzed in the transformation with both pBC-hygro and pAS6 (Figure 2) further demonstrating that Pa_3_5360 is the CDS corresponding to *AS6*.

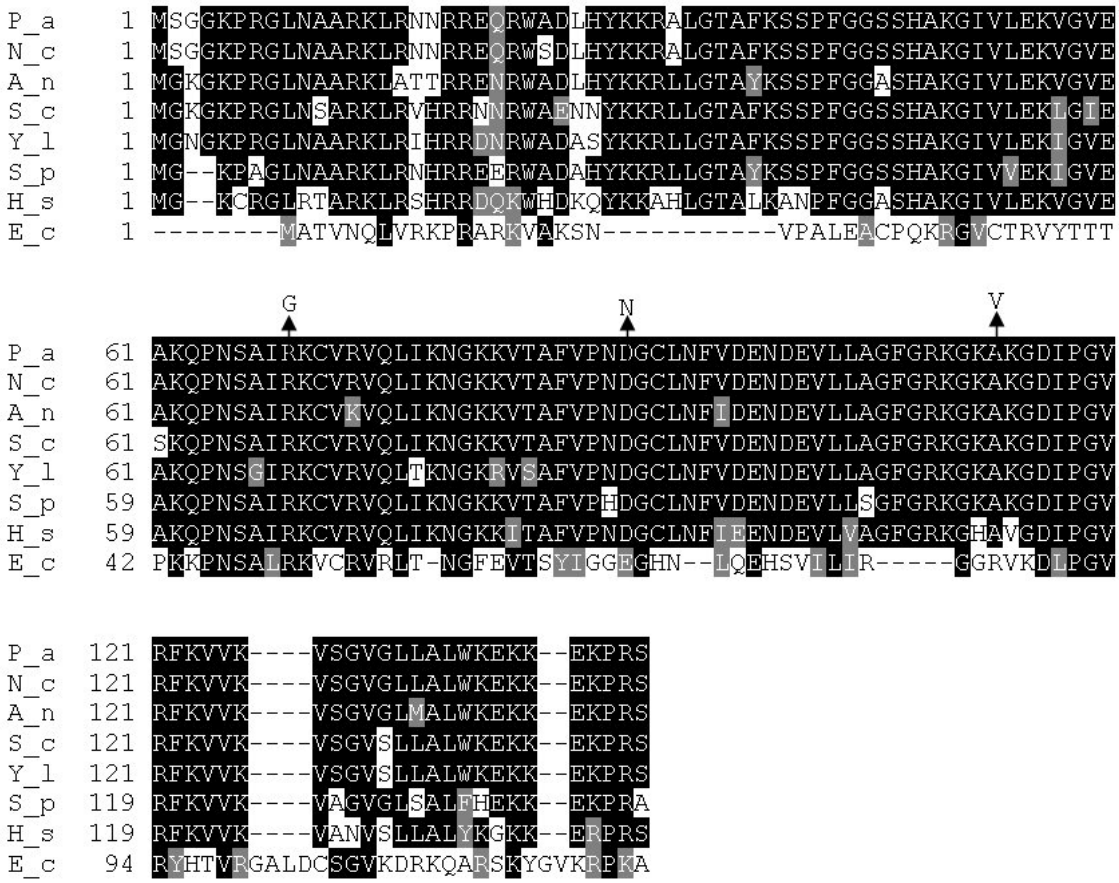

Figure 1. Comparison of the *P. anserina* S12 homologue (P_a) with orthologues in *Neurospora crassa* (N_c), *Aspergillus nidulans* (A_n), *Saccharomyces cerevisiae* (S_c), *Yarrowia lipolytica* (Y_l), *Schizosaccharomyces pombe* (S_p), *Homo sapiens* (H_s) and *Escherichia coli* (E_c). Arrows point the positions of the *AS6* mutations in the *P. anserina* protein.

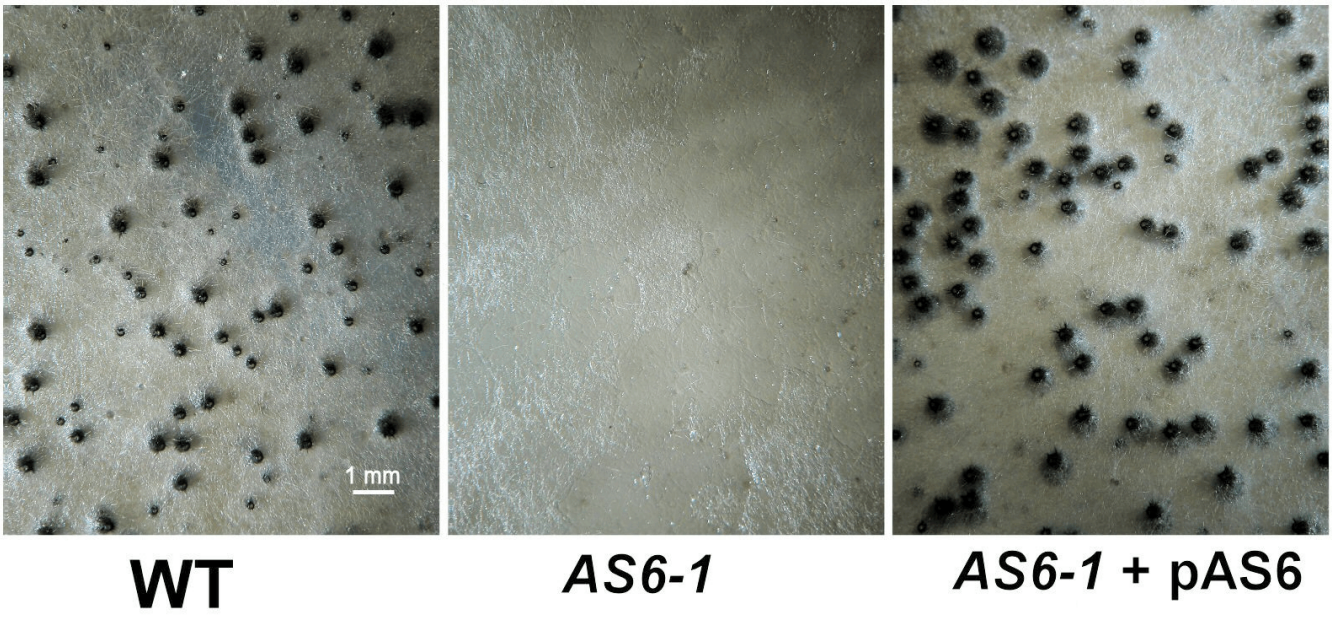

Figure 2. Perithecium production in the indicated strains.

In conclusion, the *AS6* gene codes the ribosomal protein of the *E. coli* S12 family acting in cytosolic translation. In *P. anserina*, mutations of this gene lead to an increased in translation accuracy (Picard-Bennoun 1981; Dequard-Chablat 1986; Dequard-Chablat *et al.* 1986), which in turn has many physiological effects. Indeed, it was demonstrated that increase translation accuracy in the *AS6-5* mutant triggers a specific suppressive effect on the accumulation of the circular senDNAα in the mitochondria during *P. anserina* senescence (Silar *et al.* 1997), entails a strong defect in sexual reproduction (Coppin-Raynal *et al.* 1988; Dequard *et al.* 1984) and permits the development of the epigenetic cell degeneration Crippled Growth (Haedens *et al.* 2005; Kicka *et al.* 2004; Silar *et al.* 1999). In all these cases, it was demonstrated that increase accuracy was responsible for the defects, indicating the translation errors are necessary for normal P. anserina physiology as postulated previously (Picard-Bennoun, 1982).

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