The *nusG* gene of *Streptomyces griseus*: Cloning of the gene and analysis of the A-factor binding properties of the gene product

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Abstract: The *nusG* gene of *Streptomyces griseus* was cloned and the nucleotide sequence determined. It encodes a protein with an identity of 76% to the reported receptor (VbrA) for VB-C, an autoregulatory factor in *Streptomyces virginae*. NusG protein was expressed in *Escherichia coli*. However, no binding activity for A-factor, an butyrolactone autoregulator in *S. griseus* very similar to VB-C, could be detected. The *nusG* gene of *S. griseus* does not seem to encode the A-factor-binding protein.

Key words: A-factor; A-factor binding protein; *nusG* gene; *Streptomyces griseus*

Introduction

A-factor (2-isocapryloyl-3R-hydroxymethyl-γ-butyrolactone, Fig. 1) is an autoregulatory factor triggering streptomycin production and differentiation in *Streptomyces griseus* at a concentration as low as 1 nM [1]. Beppu and co-workers identified an A-factor-binding protein of approx. 26 kDa in the cytoplasmic protein fraction of *S. griseus* [1]. In the absence of A-factor, this receptor protein seems to act as a repressor of streptomycin synthesis and differentiation [2]. A-factor triggers streptomycin production in *S. griseus* by inducing the transcription of *strR*, coding for a transcriptional activator of at least three different promoters within the streptomycin biosynthesis gene cluster (L. Retzlaff, J. Distler and W. Piepersberg, unpublished results) [3–5]. The induction of *strR* transcription by A-factor is not a direct result of removing the inhibitory effect of the A-factor-binding protein, but results in an A-factor-dependent expression of a transcriptional factor binding to an upstream activation sequence in the *strR* promoter region [6]. Similar γ-butyrolactone autoregulators involved in the regulation of secondary metabolism have been identified in other *Streptomyces* species. The most extensively studied autoregulator is the virginia butanolide (VB-C, Fig. 1) responsible for virginiamicin production in *S. virginae* [7]. Recently the gene of the VB-C-binding protein (*vbrA*) was cloned and analysed. The VB-C receptor protein has a signif-
significant homology to the anti-termination protein NusG of *E. coli* [7]. Like the *nusG* gene of *E. coli* *ubrA* of *S. virginiiae* is also located in close neighbourhood of the *rplK* gene encoding the ribosomal protein L11 [7]. Because of the similarities in chemical structure and regulatory function of VB-C to A-factor, it is conceivable that in *S. griseus* the A-factor-binding protein is also similar to NusG. In this paper we describe the cloning and characterisation of the *nusG* gene of *S. griseus* and the A-factor binding properties of its gene product.

**Materials and Methods**

**Bacteria, plasmids and culture conditions**

*S. griseus* N2-3-11, *S. griseus* M881 (an A-factor non-producing mutant [3]), and *S. lividans* 66 TK23 [8] were grown on SMA agar [3] or in TSB media [8] at 28°C. *Escherichia coli* JM83 (*ara, rpsL, A(lac-prAB), lacZ* M15, *II80*) [9] used as a host for subcloning and *E. coli* NM539 (*supF, hsdR, trpR, lacY*) [10] for amplification of the recombinant phages λ EMBL3 [10,11] were grown as described [10]. *E. coli* BL21 [12] was the host for the expression vector pT7-6 [13]. Preparation, transformation and regeneration of *S. griseus* and *S. lividans* protoplasts were carried out according to published procedures [8]. Plasmids pWKD12 and pWKD3 were constructed by cloning the 2.9-kb *BamHI* fragment (Fig. 2) into pJ702 [14] or pUC18 [9], respectively. The 1.3-kb *BglII–BamHI* fragment (Fig. 2) was fused with pWKD3, partially digested with *BamHI* and cloned as an *EcoRI–HindIII* fragment into pJ486 [15] to give pWKD11. The 1.2-kb *BamHI–StuI* DNA fragment (Fig. 2) was cloned into vector pT7-6. In the resulting plasmid pKDW105, transcription of *nusG* was under the control of promoter *III10* which is dependent on the isopropyl-β-thiogalactoside (IPTG)-inducible T7 polymerase of *E. coli* BL21 [13].

**DNA manipulation, DNA sequencing and sequence analysis**

Conventional procedures were used for nuclease digestion, ligation, Southern blots, plaque lifts, plasmid and phage DNA preparation [8,16]. DNA sequence was determined by the dideoxynucleotide chain-termination method [17] using the AutoRead sequencing kit (Pharmacia, Freiburg, FRG) and an A.L.F. DNA sequencer (Pharmacia, Freiburg, FRG). The entire sequences of both
strands were determined from double-stranded plasmid DNAs prepared by the alkaline lysis method [16]. Nucleic acid and deduced protein sequences were analysed using the programs PC-GENE (Genofit, Heidelberg, FRG) and FASTA [18].

**Preparation of cell-free extracts and A-factor binding assay**

Cell-free extracts were prepared from *E. coli* or from late exponential *Streptomyces* cultures as described previously [1]. *E. coli* BL21 (pWKD105) grown in Luria broth at 30°C was induced with 0.4 mM IPTG in the mid-exponential growth phase [13]. 10 min after IPTG induction, 0.3 mg ml⁻¹ rifampicin was added to the culture and after an additional 50 min the cells were collected by centrifugation. A-factor-binding activity was analysed by measuring the binding of ³H-labelled A-factor (1.85 TBq mmol⁻¹) in the absence and presence of non-labelled A-factor (0.1 mM, 3500-fold excess) according to a published procedure [1]. Protein concentrations were determined with the Bio-Rad protein assay (Bio-Rad, München, FRG) according to the recommendation of the manufacturer.

**Protein gel electrophoresis**

NusG expression was analysed by one-dimensional polyacrylamide gel electrophoresis [19]. Induced and non-induced *E. coli* BL21 cells from 0.5 ml culture lysed by boiling in sample buffer [19] or cell-free extracts (25 μg protein) were separated in sodium dodecyl sulphate polyacrylamide (12.5%) gels.

**Synthesis of radioactive A-factor**

6-Oxohexanoic acid methylester was synthesized from 2-acetylcylopane (Aldrich, Steinheim, FRG) according to a published procedure [20]. It was converted via a Wittig reaction and acidic cleavage of the ester into 6-methyl-6-heptenoic acid [21] and subsequently into the corresponding tert-butyldimethylsilyl ester [22]. The condensation with 3-trimethylsilyloxyethyl-4-butanolide (synthesis described in [22]) and deprotection yielded 2-(6'-methylhept-6'-enoyl)-3S,R-hydroxymethyl-4-butanolide [23]. [³H]A-factor was obtained by the catalytic reduction of 60 mg 2-(6'-methylhept-6'-enoyl)-3S,R-hydroxymethyl-4-butanolide with tritium gas (740 GBq) and 5% Pd-C catalyst for 3 h at room temperature (Amersham, Braunseweig, FRG). The [³H]A-factor purified by silica gel chromatography [1] restored sporulation and streptomycin production of A-factor-deficient mutants of *S. griseus*.

**Results and Discussion**

**Isolation and sequencing of the nusG gene**

The *vbrA* gene similar to *nusG* encoding the VB-C receptor is located in the close vicinity of the gene for the ribosomal protein L11 (*rplK*) on the chromosome of *S. virginiae* [7]. Therefore, a *S. griseus* DNA library in λ EMBL3 [11] was screened with an oligonucleotide probe derived from a highly conserved amino acid (aa) sequence of the ribosomal protein L11 (Fig. 3). A total of five phage clones containing approx. 28 kb co-linear *S. griseus* DNA could be identified. A 3-kb *BamH*I DNA fragment hybridising to the oligonucleotide probe was cloned in pUC18 (pWKD3, Fig. 2) and partially sequenced. The sequence (1251 bp) which comprises the *nusG* and part of the *rplK* gene coding for the ribosomal protein L11 is presented in Fig. 3. The *nusG* gene putatively starts at a GTG codon at nucleotide 58 and ends at position 942. A potential ribosomal binding site is located 7 bp upstream of this GTG. The *nusG* gene encodes a 31.7-kDa polypeptide (294 aa) with an overall identity of 76% to the VB-C receptor (*VbrA*) of *S. virginiae* [7] and with 33% identity in its C-terminal part (aa 98–294) to the shorter NusG of *E. coli* (181 aa) involved in transcriptional antitermination and Rho-dependent termination [24,25]. The values of identity between *VbrA* of *S. virginiae* and NusG of *S. griseus* are 95% in the C-terminal part, but only 41% in the N-terminal parts. This pattern of similarity probably reflects the existence of two domains in *Streptomyces* NusG proteins: a conserved C-terminal domain interacts with components of the RNA polymerase–Nus factor complex which may be essential in all
bacteria. Therefore, a proposed function of the additional N-terminal domain not occurring in *E. coli* may be the binding of small effector molecules like VB-C or A-factor or of additional proteins participating in the transcriptional antitermination in *Streptomyces* species. An inverted repeat located downstream of the *nusG* gene could be a termination signal of transcription. A similar structure (65% identity) is also present downstream of the *vbrA* gene of *S. virginiae* [7]. The partial ORF starting at nt 1119 encodes a protein which has 95% and 74% identity to the N-terminal parts of the ribosomal proteins L11 of *S. virginiae* and *E. coli* [26], respectively. The *nusG* and *rplK* genes are located in the centre of the *r/f* gene cluster (*tufB-secEnusG-rplKAJL-rpoBC*) of *E. coli* which encodes components involved in transcription, translation and protein secretion. Preliminary sequence analysis of DNA fragments adjacent to *nusG* and *rplK* of *S. griseus* shows distinct homology to *secE, rplAJL* and *rpoB* of *E. coli* suggesting a similar organisation within the gene clusters of these two bacteria (C. Küster, W. Piepersberg and J. Distler unpublished results).

**Expression and analysis of A-factor binding activity of the NusG protein**

The A-factor-binding protein was detected in cell-free extracts from *S. griseus* N2-3-11 and mutant M881. Radioactivity eluted with the high-molecular mass fraction of a Sephadex G-25 column, when cell extracts from both *S. griseus* N2-3-11 and M881 were incubated with [3H]A-factor (Fig. 5). This radioactivity peak was caused by specific binding of [3H]A-factor since it was absent when [3H]A-factor was displaced by an excess amount of non-radioactive A-factor (Fig. 5). A second peak due to free [3H]A-factor which was unaffected by non-radioactive A-factor appeared in all runs. The cell-free protein extracts of the mutant M881 bound about two-fold more radioactive A-factor than that of *S. griseus* N2-3-11. A possible explanation for this observation is the presence of A-factor in these extracts which was synthesized by *S. griseus* N2-3-11. This was verified by the successful induction of streptomycin production of strain M881 with A-factor extracted from cell-free extracts of *S. griseus* N2-3-11. Therefore, quantification of eventual differences in the amount of A-factor receptor in both strains was not possible. The A-factor binding
activity of the cloned nusG gene of S. griseus was analysed in E. coli (pWKD105). One hour after the induction of the T7-polymerase expression with IPTG the accumulation of an additional protein of 30 kDa was observed (Fig. 4). The determined molecular mass of this protein was in good agreement with calculated mass of the putative NusG protein (31.7 kDa). There was no expression of an additional protein in non-induced cells (Fig. 4) or from the vector pT7-6 alone (data not shown). No A-factor-binding activity was detectable in cell-free extracts of E. coli (pWKD105) induced with IPTG (Fig. 5). In addition, no binding activity was found in either S. lividans (pWKD12), although nusG transcription was enabled by the melC promotor of pIJ702 [14], or in S. lividans (pWKD11) containing nusG

![Fig. 4. Expression of NusG in E. coli. Protein extracts of induced (+; lane 2, 4, 6) and non-induced (−; lane 3, 5, 7) E. coli (pWKD105) were analysed before (0; lane 2,3) and 30 min (30; lane 4, 5) or 60 min (60; lane 6, 7) after IPTG induction. The cell-free extracts of induced (+; lane 8) and non-induced (−; lane 9) E. coli (pWKD105) used in the A-factor binding assay were separated in SDS-polyacrylamide gels. Arrows indicate the expressed NusG protein. The molecular mass (kDa) of the marker proteins (lane 1, 10) are: a-lactalbumin, bovine (14.2); /β- lactalbumin, bovine (18.4); trypsinogen, bovine (24); carbonic anhydrase, bovine (29); glyceraldehyde-3-phosphate dehydrogenase, rabbit (36); ovalbumin, chicken (45); albumin, bovine (66); phosphorylase b, rabbit (97); galactosidase, E. coli (116); myosin, rabbit (205).](https://academic.oup.com/femsle/article-abstract/119/1-2/33/586844)

and a 1.3-kb upstream DNA fragment (data not shown). An increase of the nusG gene dosis in either S. griseus (pWKD11) or in S. griseus (pWKD12) had no effect on A-factor-binding activity, sporulation or streptomycin production. Therefore, NusG does not seem to be the A-factor-binding protein in S. griseus. Despite the great similarities between the autoregulators VB-C of S. virginiae and A-factor of S. griseus regarding the chemical structure and biological effects, A-factor has a different target site. The differences could be explained by the assumption that another component of the RNA polymerase–Nus
factor complex, probably interacting with NusG, is the A-factor receptor. The involvement of an antitermination complex containing Nus proteins in the regulation of secondary metabolism in *Streptomyces* species is supported by data published recently [27]. The sporulation and streptomycin production of *S. griseus* bald (bld) mutants were complemented by a *S. griseus* gene encoding a protein with similarity to NusA of *E. coli* [27]. Further work is required to determine the possible relationship of NusG and A-factor receptor in transcriptional antitermination and/or regulation of secondary metabolism in *S. griseus*.

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References


