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THE HYG GENE PROMOTER FROM STREPTOMYCES HYGROSCOPICUS : A NOVEL FORM OF STREPTOMYCES PROMOTERS

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A 207 bp DNA fragment from the 5' region of the hyg gene of Streptomyces hygroscopicus was located preceeding a DNA sequence encoding the mature form of human interferon α2. This gene fusion, inserted in the Streptomyces vector pIJ702, expressed interferon activity in Streptomyces lividans indicating that the 207 bp sequence has promoter activity. The trancription initiation site was located. No significant homology with previously described Streptomyces promoters could be found. It appears therefore, it represents a novel class of Streptomyces promoters. © 1988 Academic Press, Inc.

The mycelial Gram-positive Streptomyces most often synthesize a variety of compounds, including antibiotics, during the secondary metabolism (1). In addition, these bacteria undergo a complex cycle of morphological differentiation (2). It is presumed that these characteristics involve the activation of transcription by specific promoters to direct the timely expression of the relevant genes. Indeed, different types of promoters have been identified in Streptomyces (reviewed by Hopwood et al (3)), which can be classified in two broad groups; those which have common features with the Escherichia coli consensus promoter sequence and others which share no homology with this element. The former group comprises only a small proportion of Streptomyces promoters which may be functional in E. coli (4,5,6,7). A holoenzyme form of the Streptomyces RNA polymerase seems to specifically recognize these "E.coli-like" promoters (8,9). In contrast, the great majority of Streptomyces promoters can be included in the second group and, when tested, do not detectably work in E. coli (4,6,10). Characteristically, these "Streptomyces-specific" promoters are in general much richer in [G+C]

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content than the *E. coli* ones and do not share a common consensus sequence between themselves. Two different types of "*Streptomyces*-specific" promoters have been described so far (3,11) suggesting that their distinct sequences may play an important role in regulation of gene expression.

In this work the 5′ region of the hygromycin B phosphotransferase (*hyg*) gene from *S. hygroscopicus* (12) has been isolated to examine its promoter activity.

MATERIALS AND METHODS

Streptomyces lividans strain 1326 (13) and plasmid pIJ702 (14) were obtained from D.A. Hopwood. Plasmid pFM4 was described previously (12). *S. lividans* was grown as described elsewhere (15). Strains carrying pIJ702 or its derivatives were grown in the presence of thiostrepton (10 and 25 μg/ml in either liquid or solid medium, respectively). *S. lividans* was transformed as described by Hopwood *et al.* (15). DNA cloning and transformation of *Streptomyces* was carried out as described by others (15).

S1 mapping was performed as described elsewhere (16). A 220 bp XbaI-SstI ^{32}P DNA fragment from pGIS21 (Fig. 1) end-labelled with at the XbaI site, was used as probe.

Preparation of cell extracts from *Streptomyces* clones, and assay of interferon activity were performed as described by Pulido *et al.* (17).

Restriction enzymes, T4 DNA ligase, Klenow fragment and calf intestinal alkaline phosphatase were obtained from Boehringer Mannheim, and used as recommended by the supplier. Other materials were obtained from Sigma and Merck. Radioactive nucleotides were obtained from Amersham (U.K.).

RESULTS AND DISCUSSION

The 207 bp NarI-AvaII DNA fragment (Fig 1) from the 5′ region of the *hyg* gene from plasmid pFM4 was fused, via *Escherichia coli* plasmid pUC19, to a gene construction (hIFNα2) containing the ribosome binding site of the *E. coli lpp* gene and a modified structural gene encoding the mature form of human interferon α2 (17). This construction had been shown to express biologically active human interferon in *S. lividans* if directed by the *aph* gene promoter from *Streptomyces fradiae* (17). The resulting gene fusion was inserted in the *Streptomyces* plasmid pIJ702 (14). Plasmids pGIS12 and pGIS21, differing in the orientation of the inserted DNA sequence, were obtained (Fig. 1). Cell-free extracts from *S. lividans* clones containing these plasmids had interferon activity (0.5×10^6 IU/l). In contrast, *S. lividans* clones containing these plasmids deprived of the 207 bp sequence totally lacked any antiviral activity (not shown). Moreover the constructed gene fusion expressed an Mr

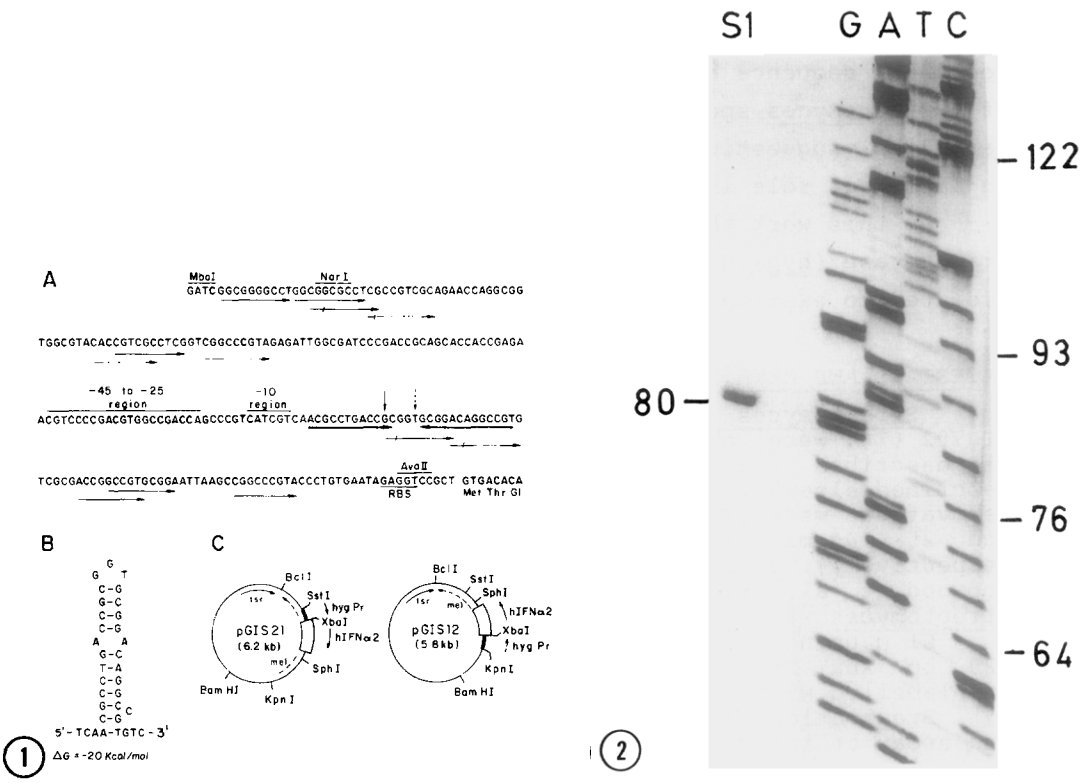

Figure 1. A) Nucleotide sequence of the 5′ region preceding the hyg structural gene. The restriction sites NarI and AvaII delimit the 207 bp fragment (16) which was fused to a DNA sequence encoding the mature form of hIFNα2 to assay promoter activity. Horizontal arrows indicate direct (thin) and inverted (thick) repeat sequences. The vertical arrows indicate the sites of transcription initiation from the hyg gene (16) (continuous arrow) or the hyg promoter-hIFNα2 gene construction (discontinuous arrow). Sites of mismatches between direct repeats are indicated by slashes. RBS indicates a possible ribosomal binding site. B) Putative secondary structure (stem-loop) of the inverted repeat sequence. The theoretical free energy (ΔG) from this structure was calculated as described by Tinoco et al (23). C)Restriction maps of the pIJ702-derivative recombinant plasmids pGIS12 and pGIS21 carrying the hyg promoter-hIFNα2 gene construction. The arrows indicate direction of transcription. Pr refers to promoter.

Figure 2. S1 mapping to determine the site for transcription initiation. The S1 mapping experiment was performed as indicated in Materials and Methods. Lanes labeled G, A, T and C contain standards from sequencing reactions. Numbers on the right indicate the number of bases of the standard and that on the left the size of the DNA fragment protected from the S1 nuclease action.

20000 polypeptide in an "in vitro" coupled transcription-translation system (18), which is the expected size of the mature form of HIFN α2 (17) (not shown).

A high resolution S1 mapping experiment (Fig 2) showed that transcription of hIFNα2 mRNA in plasmid pGIS21 starts at a T (Fig 1) four bases downstream from the transcription

initiation site from the hyg gene in the parental S. hygroscopicus strain (16). Taken all together, these results strongly suggest that the 207 bp NarI-AvaII DNA fragment contains the promoter of the hyg gene.

A variety of direct repeats and one inverted repeat sequence is apparent in the hyg promoter region (Fig 1). Similar structures were found in several promoter sequences from S. lividans which had homology to the promoter consensus sequence of E. coli (6) and in the 5' region of the genes encoding a streptomycin-phosphotransferase and an amidotransferase from S. griseus, although the promoter activity of these two regions was not reported (19). During the course of this work, it was found that these elements are also present in the promoter regions of the Streptomyces genes aph, vph, tsr (20) and pac (Lacalle et al, unpublished observations), indicating that they could be implicated in promoter activity.

The inverted repeat sequence of the hyg promoter is within nucleotides 147-174 (Fig 1A) immediately downstream from the -10 and -25/-45 regions. It could give rise to a stem-loop structure with a free energy of -20 Kcal/ml (Fig. 1B). This putative hair-pin sequence could act as an "attenuator", as it has been found for the trp operon from E. coli (21). Otherwise, it could bind some protein implicated in a transcription regulatory function in S. hygroscopicus. Indeed, putative-forming stem-loop sequences seems to be appropriate sites for DNA-protein interactions (22).

The sequence of the hyg promoter lacks any significant homology to the sequence of other Streptomyces promoters (3). Therefore, it seems to represent a new type of Streptomyces promoter.

At least three different types of Streptomyces promoters are known (3,11, this work) and additional ones may still appear. Further work is, therefore, necessary to clearly define the varieties and characteristics of Streptomyces promoters.

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