Functional prokaryotic gene control signals within a eukaryotic rainbow trout protamine promoter

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Following the construction of a series of pSV2-cat derived plasmids containing the chloramphenicol acetyltransferase (CAT) gene under the control of a eukaryotic trout protamine promoter, it was noted that Escherichia coli, transformed with these plasmids, developed resistance to chloramphenicol (CM). This result suggested that the eukaryotic trout protamine promoter possessed significant prokaryotic promoter activity. Modification of the trout protamine promoter region by removing the region containing the eukaryotic Goldberg-Hogness box in the plasmid p525-cat increased the expression of the CAT gene almost to the wild-type level and conferred strong CM resistance. Sequence comparisons of the plasmid series indicate that prokaryotic promoter elements are present in the trout protamine promoter and that their similarity to the prokaryotic promoter consensus sequences and the distance between the two elements is more favourable in p525-cat, the plasmid which confers the greatest CM resistance.

The genetic code appears to be universal for Prokaryota and Eukaryota, although there is an alternative use of certain codons in mitochondrial genes (5). It should be possible, therefore, to express prokaryotic proteins in eukaryotic cells, if appropriate eukaryotic promoter signals are juxtaposed to such genes. A good, recent example which confirms this prediction is the expression of the bacterial enzyme chloramphenicol acetyltransferase (CAT) in monkey CV-1 cells (6). However, the DNA sequence signals that control the synthesis of RNA in Eukaryota, and which involve three distinct RNA polymerases, appear to be quite different and easily distinguishable from those in Prokaryota, where only a single RNA polymerase is involved. Nevertheless, if present-day Eukaryota evolved from ancestral organisms that also gave rise to present-day Prokaryota, it should not be surprising that traces of these ancestral control signals might be found in Eukaryota. For example, the presence of a bacterial promoter within the eukaryotic genome of the SV40 virus has been reported by Zain et al. (20), Dhar et al. (4) and Rosenberg et al. (15).
In this paper, we report the presence of functional *Escherichia coli* gene control signals within the eukaryotic promoter of the rainbow trout protamine gene previously cloned and characterized by States et al. (17) and Jankowski and Dixon (9) which allow the transcription of the CAT gene in a bacterial host.

**Materials and Methods**

**Preparation of plasmid DNAs**

*E. coli* strains HB101 or DH1 were used as host bacteria. Plasmid DNAs were prepared by lysozyme-Triton X-100 lysis (11) and cesium chloride - ethidium bromide equilibrium gradient centrifugation (14).

**Enzymes**

Restriction enzymes were obtained from New England BioLabs, Bethesda Research Laboratories or Pharmacia - PL Biochemicals; T4 DNA ligase, kinase and Bal 31 exonuclease from New England BioLabs.

**Preparation of DNA fragments**

Restriction endonuclease digestions were carried out as recommended by the suppliers. Cohesive ends were made flush-ended by incubation with Bal 31 exonuclease (7), ligated with a Hind III synthetic linker (New England BioLabs) and then digested with Hind III endonuclease. DNA fragments were separated by agarose gel electrophoresis and visualized by ethidium bromide staining; selected fragments were purified by the low melting agarose method (13).

**Bacterial transformations**

Fragments were ligated by T4 DNA ligase overnight at 0°C. Transformations of *E. coli* were performed using the calcium chloride method (2). Colonies were selected on plates containing ampicillin (100 µg/ml).

**Assay of CAT activity**

One ml of an overnight bacterial culture was diluted to 100 ml of LB medium (Gibco) and grown at 37°C (on a rotary shaker). Cells were collected when the A600 reached 0.6 and were washed with 10 mM Tris/HCl, pH 8.0, and 1 mM EDTA. The pellet was resuspended in 4 times its weight of 0.1 M phosphate buffer pH 7.2. The cells were disrupted by sonication and CAT activity was determined by the colorimetric method according to Shaw (16).

**Results and Discussion**

**Construction of plasmids pP5-cat and p525-cat with the chloramphenicol acetyltransferase (CAT) gene under the control of the trout protamine promoter**

As outlined in Fig. 1 the entire trout protamine promoter fragment whose sequence is given in Fig. 2 was obtained by digestion of the pBR322-derived plasmid pP5, containing the entire protamine gene promoter fragment Bgl II-Ava II (constructed previously and described