Volume 17 Number 5 1989 Nucleic Acids Research

Expression of functional *Xenopus* TFIIIA in *Escherichia coli*

J.Yun Tso, Christian Siebel and Laurence Jay Korn

Department of Genetics, Stanford University, Stanford, CA 94305, USA
Submitted November 16, 1988

The transcription factor TFIIIA binds to the internal control region of the 5S RNA gene to initiate the first step of transcription. A Xenopus TFIIIA cDNA clone, which codes for a 344 amino acid protein had been isolated (1). Here we report a bacteriophage T7 RNA polymerase/promoter system (2) to express the TFIIIA cDNA in E. coli. The expression vector used, pT7-7, is a derivative of pT7-1 (2) provided by Stanley Tabor (Department of Biological Chemistry, Harvard Medical School). It contains the T7 RNA polymerase promoter 10 and the translation start site for the T7 gene 10 protein. Since the translation initiation codon for the T7 gene 10 protein overlaps with the unique NdeI site in this vector, we used a synthetic adaptor, 5'TATGGGAGAGAAGGCGC , containing sequence from the initiation codon (Nde I ACCCTCTCTTCC5' site) to the first HaeII site of the TFIIIA cDNA to link the rest of the gene to the vector pT7-7. The resulting plasmid, pT7-TF, has the TFIIIA cDNA inserted between the NdeI site and the EcoRI site of pT7-7 in such a way that the initiation codon of TFIIIA cDNA precisely replaced that of the T7 gene 10 protein (Fig.1). A compatible plasmid, pGP1-2, was also used to provide T7 RNA polymerase (T7 gene 1) under the control of λpL promoter as well as the gene for heat labile λ repressor cI-857 (2). Upon heat induction at 42 C for 30 min in LB medium, E. coli K38 harboring pT7-TF and pGP1-2, expressed a 40 kd protein identified by Western blot analysis to be TFIIIA. This protein can be partially purified from the soluble fraction of the sonicated E. coli extract with 5 M urea in 50 mM Hepes, pH 7.5/5mM $MgCl_2$/1mM DTT/10 μM $ZnCl_2$/20% glycerol, and then fractionating on a Bio-Rex 70 column with 0.25 M, 0.5 M, and 0.75 M NaCl step gradients in the same buffer without urea (3). Partially purified TFIIIA eluted at 0.75 M NaCl and was about 30% pure based on SDS PAGE analysis, with overall yield of 500 μg TFIIIA/l of culture. At TFIIIA: gene ratio of 20:1 the partially purified protein bound to the 5S RNA gene and protected it from DNase I digestion (Fig. 2). It also promoted transcription of the 5S RNA gene in an egg extract deficient in TFIIIA. This system thus appears to be valuable in producing functional TFIIIA or its varient for structure-function study.

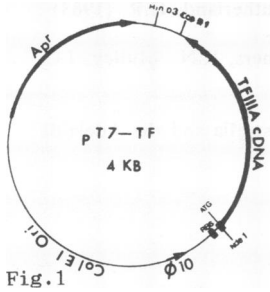

Fig.1

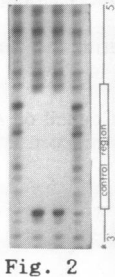

Fig. 2

Fig.1 Physical map of the plasmid pT7-TF. RBS indicates the ribosome binding site of T7 gene 10. Fig. 2 DNase I footprint assay for protection of 5S DNA by TFIIIA. The X. laevis somatic 5S RNA gene in plasmid pXLS11 was labeled on the coding strand at the 3' end and was preincubated either without TFIIIA (lanes 1 and 4) or with TFIIIA from oocytes (lane 2) or from E. coli K38/pT7-TF/pGP1-2 (lane 3). Conditions for preincubation and digestion were the same as those described by Smith et al (3)

Present address: Protein Design Labs, Palo Alto, CA 94304, USA

References: (1) Ginsberg, A.M., et al (1984) Cell 39, 479-489. (2) Tabor, S, and Richardson, C.C. (1985) Proc. Natl. Acad. Sci. 82, 1074-1078. (3) Smith, D.R., et al (1984) Cell 37, 645-652.