Pietschmann, S.M., J. Eberle,

T. Sommer and V.E.A. Russo

On the direction of transcription of cloned genes of Neurospora crassa

Sometimes it may be important to know the in vivo direction of transcription of a cloned gene, for which neither the nucleotide sequence nor the sequence of the encoded protein is known. We tried different approaches to determine the transcriptional direction.

In the first approach we looked for the poly(A) tail in the corresponding cDNA-clone. This experiment requires a unique and asymmetrical restriction site in the cDNA, separation of the two resulting fragments by digestion, gel electrophoresis and gel elution. Finally, we made a dot blot of the two different fragments, using as probe radioactively labeled oligo(dT) (data not shown). The procedure is easy and fast. The only drawback was that one of the two cDNA-clones we used (bli-3) did not have a poly(A) tail.

The second approach was to clone the cDNA of two clones bli-3 and bli-4 in a Riboprobe Gemini system, pGEM vector (Promega, Biotec). The important feature of this vector is the two promotors for the SP6 and T7 RNA polymerases, which are located at the extremes of the cloned DNA. In the subsequent in vitro transcription using either the SP6 or the T7 RNA polymerase, radioactive transcripts from either strand of the cDNA may be obtained. These transcripts were used as probe for hybridization to Neurospora cellular RNA, bound on nylon membranes (GeneScreen, Du Pont). The expectation was that the transcript complementary to in vivo made mRNA would give a much stronger signal than the other. To prove the hybridization signals were not due to unspecific binding, we performed several controls. Some of the controls were based on our knowledge that the amount of mRNA of bli-3 and bli-4 is about 100 times more in a 60 min illuminated mycelium than in a corresponding dark grown mycelium of Neurospora crassa wt. Furthermore, we know that mycelia of the wc-1 (R100, ER53) and wc-2 (R100, ER33) mutants do not show this light induction. Therefore we immobilized several RNAs on nylon filters: total RNA, isolated from illuminated mycelia of wt, and total RNA from the corresponding dark mycelia (three independent experiments); a poly(A)+ RNA from illuminated and dark wt mycelia, total RNA from illuminated and dark mycelia from wc-1 (R110, ER53) and wc-2 (R100 ER33) mutants. To control the efficiency of hybridization we also bound DNA from the pGEM vector (negative control) and pGEM with cDNA insert (positive control). The hybridization procedure was performed according to Promega Biotec (procedure for Northern Blot #l) with the following modifications: prehybridization was done for 16 h at 60°C in a 10 ml volume, hybridization was started by addition of the probe (about 5 x 10^6 cpm) and continued at 60°C for 16 h, the filters were washed 3 times at 68°C for 30 min.

The whole procedure requires about three days of work once the cDNA is cloned. The data for the cloned genes (bli-3 and bli-4) are shown in Fig. 1.

Fig. 1 I) Scheme of dotted RNA and DNA. D = RNA, isolated from mycelia grown 24 hours in the dark, L = RNA, isolated from mycelia grown 24 hours in the dark and illuminated for 60 min (Chambers et al. 1985 EMBO J. 4:3649-3653), wt = total RNA from wt, poly(A)^+ = poly(A)^+ RNA from wt, wc-1 = total RNA from white collar 1 mutant, wc-2 = total RNA from white collar 2 mutant. The amount of total RNA was 5 ug/dot, of poly(A)^+ RNA was 0.5 ug/dot. The amount of DNA of pGEM or pGEM cDNA was 0.6 ng/dot.
II) Hybridization with RNA synthesized in vitro from bli-3 cDNA by T7 polymerese. The film was exposed for three days.
III) The same with RNA synthesized by SP6 polymerase.
IV) Like II, but for bli-4
V) Like III, but for bli-4

With the two methods we found the same direction for bli-4. This direction is further confirmed by sequence analysis of the promoter region of bli-4 where a consensus sequence for the start of translation and the beginning of an open reading frame was found. - - - Max Planck Institut für molekulare Genetik, D-1000 Berlin 33, Federal Republic of Germany