

Isolation of a DNA fragment that encodes part of an ATP dependent RNA helicase in *Neurospora crassa*.

Ian E. Hunt and Barry J. Bowman- Sinsheimer Laboratories, Department of Biology, University of California, Santa Cruz, CA 95064.

A DNA fragment encoding part of an ATP dependent RNA Helicase was isolated as part of a search for an unrelated gene. The sequence is reported here and homology to other related genes is described.

DNA and RNA molecules frequently undergo a variety of dynamic conformational changes. Consequently a variety of proteins have evolved to control and regulate these changes. One such group of proteins is the RNA helicase family. This group of conserved proteins utilize ATP as an energy source for unwinding RNA. Members of this family are found in many different organisms ranging from *E.coli* to humans and have been implicated in a variety of physiological functions such as translation initiation, mitochondrial mRNA splicing, ribosomal assembly and germinal-line cell differentiation.

While searching for an unrelated gene we isolated a DNA fragment from *Neurospora crassa* that encodes part of a protein homologous to several eucaryotic RNA helicases. RT-PCR using degenerate oligonucleotide primers designed to amplify the gene encoding the 14-kDa subunit of the vacuolar H⁺-ATPase produced a band of 350 bp. It was subcloned into pT7blue plasmid (Novagen) and sequenced (Figure 1). Database searches revealed the 350 bp fragment to encode part of a protein that shared a high degree of sequence similarity with several ATP dependent DEAD-box RNA helicases, listed in Table 1. Even though RT-PCR usually amplifies mRNA, the region of the DNA fragment from nt #237 to #291 appears to correspond to an intron. This region contains typical 3' and 5' intron splice sites and occurs precisely at a point in which the derived amino acid sequence would deviate from the sequence of the homologous helicases. This sequence has been deposited in GenBank, Accession Number 1658275 and the plasmid is available on request at the above address.

Table 1. Comparison of an RNA helicase from *N. crassa* with homologous proteins from other organisms.

RNA helicase from:	<u>Sequence identity with <i>N.crassa</i> fragment</u>		Mol.Wt
	% Identity	% Similarity	(kDa)
<i>S. pombe</i>	70	86	50
<i>S. cerevisiae</i>	72	81	50
Human (DEAD box)	65	81	50
<i>D. melanogaster</i>	61	78	50

C. elegans 60 78 50

10	20	30	40	50	60	70
*	*	*	*	*	*	*
TTGTTATCNGGTGACGAGGACACCAAATTGACCCTTCACGGTCTTCAGCAATACTACATTCTCTTGAGG						
L L S G D E D T K L T L H G L Q Q Y Y I P L E						
80	90	100	110	120	130	140
*	*	*	*	*	*	*
AGCGCGAGAAGAACCGCAAGCTCAACGAGCTCTTCGACGAGCTGCAGTTCAACCAGGTCATCATCTTCGT						
E R E K N R K L N E L F D E L Q F N Q V I I F V						
150	160	170	180	190	200	210
*	*	*	*	*	*	*
CAAGAGCACTCTCCGTGCTACCGAGCTGGACAAGCTTTTGCGCGAGTGTAACCTCCCTTCGATCGCTGTC						
K S T L R A T E L D K L L R E C N F P S I A V						
220	230	240	250	260	270	280
*	*	*	*	*	*	*
CACTCCGGTGTGAGCCAGGAGGAGCGgtacgtacacctgtcccgacatcattgtgtgggcgcctactgac						
H S G V S Q E E R						
290	300	310	320	330	340	350
*	*	*	*	*	*	*
tctccttgtagTATCCGTCGTTACAAGGAATTCAAGGAATTCAACAAGCGTGTCTCGTCACCGATGACA						
I R R Y K E F K E F N K R V L V T D D						

Figure 1. Nucleotide sequence and derived amino acid sequence of a DNA fragment which appears to encode part of an ATP-dependent RNA helicase. The region sequenced corresponds to residues #267-#365 in the RNA helicase from *S. cerevisiae*. The putative intron within the fragment is shown in lower case.

[Return to the FGN 44 Table of Contents](#)

[Return to the FGSC home page](#)