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Short communication

Cloning and sequencing of the genes encoding the hamster ribosomal transcription factors UBF1 and UBF2[1]

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Abstract

The RNA polymerase I transcription factor, UBF, belongs to a family of high-mobility-group DNA-binding proteins. Here, a human autoantibody reactive with the nucleolus organizer regions (NOR) was used to select cDNA clones encoding the hamster transcription factors, UBF1 and UBF2. Comparison at the nucleotide level showed a high degree of homology with other mammalian upstream binding factors (UBF) already identified. The deduced amino acid sequences are identical for both UBF1 and UBF2, except for a 37 amino acid insertion found in UBF1. This insertion is completely conserved among mammalian UBF1 which indicates a putative role of this region on the function of this transcript.

Keywords: Upstream binding factor; *Cricetulus griseus*; Nucleolus organizer regions

Nucleolus organizer regions (NOR) are the initiation sites for nucleologenesis where prenucleolar bodies fuse to form interphase nucleoli (Ochs et al., 1985). It has been shown that, in addition to RNA polymerase I, other auxiliary proteins are required for the initiation of transcription of ribosomal RNA (rRNA) genes (rDNA). Among them, UBF factors bind specifically to the rDNA promoter and form a stable preinitiation complex (Schnapp and Grummt, 1991). Human autoantibodies are useful tools to gain insight into the structural and molecular organization of intracellular organelles. Several of such antibodies recognize different nucleolar antigens and have been used in previous studies to provide answers to basic questions concerning the molecular components and the mechanisms involved in ribosome synthesis. We and others have previously described human anti-NOR autoantibodies specific to the NOR region of animal and plant cells (Rodríguez-Sánchez et al., 1987; Chan et al., 1991; Rendón et al., 1992; Goenechea et al., 1992). A CHO lambda ZAP expression library was screened with human autoantibodies to NOR. cDNA clones were isolated that encode sequences of hamster ribosomal transcription factors, UBF1 and UBF2. Sequence analysis showed that several clones codifying for partial hamster UBF were firstly identified. A 5′ RACE approach was used subsequently on hamster reverse transcribed mRNA as a template, to obtain full-length cDNA clones for UBF1 and UBF2. Those were sequenced completely on both strands. To confirm that those deduced nucleotide cDNAs were truly derived from mRNA, we amplified by PCR reaction full-length UBF1 and UBF2 cDNAs from CHO mRNA reverse transcribed. DNA sequence analysis of the PCR products (2.3 and 2.15 kb) confirm the UBF1 and UBF2 nt sequences initially obtained. As indicated in Fig. 1 both UBF are identical at the nt level, except for an in-frame 111 nt deletion in the coding region of hamster UBF2 that results in the deletion of 37 amino acids from the predicted protein sequence. This result, is in agreement with those found for human (Jantzen et al., 1990) and rat (O'Mahony and Rothblum, 1991) UBF. The deduced amino acid sequences of hamster UBF1 and UBF2 are highly homologous to those of other mammalian UBF previously described (Jantzen et al., 1990; O'Mahony and Rothblum, 1991; Maeda et al., 1992).

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[1] On request, the authors will supply detailed experimental evidence for the conclusions reached in this Short communication.

Abbreviations: bp, base pair(s); HMG, high mobility group; kb, kilobase(s) or 1000 bp; NOR, nucleolus organizer region(s); nt, nucleotide(s); Pol I, RNA polymerase I; rRNA, ribosomal RNA; rDNA, ribosomal RNA genes; UBF, upstream binding factor(s).

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TGTAGGATGAACGGAGAAGCAGACTGCCCCACAGACCTGGAAATGGCCGCCCCCAAAGGCCAAGACCGCTGGTCCCAGGAAGATATGCTAACTTTGCTGGAATGCATGAAGAACAACCTT   120
       M  N  G  E  A  D  C  P  T  D  L  E  M  A  A  P  K  G  Q  D  R  W  S  E  D  M  L  T  L  L  E  C  M  K  N  N  L  Q     38

CCTTCCAATGACAGCTCCAAGTTCAAAACCACAGAATCACATATGGACTGGGAAAAAGTTGCATTTAAGGACTTTTTAGGAGATATGTGCAAGCTCAAATGGGTGGAGATTTCTAATGAG   240
 P  S  N  D  S  S  K  F  K  T  T  E  S  H  M  D  W  E  K  V  A  F  K  D  F  L  G  D  M  C  K  L  K  W  V  E  I  S  N  E     78

GTGAGGAAGTTTCGTACGTTGACAGAATTGATCCTTGATGCTCAGGAACATGTTAAAAATCCTTACAAAGGCAAAAAACTCAAGAAACACCCAGATTTCCCAAAGAAGCCTCTCACCCCT   360
 V  R  K  F  R  T  L  T  E  L  I  L  D  A  Q  E  H  V  K  N  P  Y  K  G  K  K  L  K  K  H  P  D  F  P  K  K  P  L  T  P    118

TATCTCCGCTTCTTATGGAGAAGCGGGCCAAGTACGCGAAACTCCATCCTGAGATGAGCAACCTGGACCTAACCAAGATTCTGTCCAAGCAAGTATAAGGAGCTTCCAGAGAAGAAGAAG   480
 Y  L  R  F  F  M  E  K  R  A  K  Y  A  K  L  H  P  E  M  S  N  L  D  L  T  K  I  L  S  K  K  Y  K  E  L  P  E  K  K  K    158

ATGAAATATATTCAGGACTTCCAGAGGGAGAAACAGGAGTTCGAGCGAAACCTGGCCCGATTCAGGGAGGATCACCCTGACCTTATCCAGAATGCCAAGAAGTCGGACATCCCTGAGAAG   600
 M  K  Y  I  Q  D  F  Q  R  E  K  Q  E  F  E  R  N  L  A  R  F  R  D  E  H  P  D  L  I  Q  N  A  Q  Q  S  D  I  P  E  K    198

CCCAAAACTCCCCAGCAACTGTGGTATACCCATGAGAAGAAGGTGTATCTCAAAGTGCGGCCGGATGCCACTACGAAGGAGGTGAAGGACTCCCTGGGGAAGCAGTGGTCTCAGCTCTCG   720
 P  K  T  P  Q  Q  L  W  Y  T  H  E  K  K  V  Y  L  K  V  R  P  D  A  T  T  K  E  V  K  D  S  L  G  K  Q  W  S  Q  L  S    238

GACAAAAAGAGGCTGAAATGGATTCATAAGGCCCTGGAGCAGCGGAAGGAGTACGAGGAGATTATGCGAGATTATATCCAGAAACATCCAGAACTAAACATCAGCGAGGAAGGCATCACC   840
 D  K  K  R  L  K  W  I  H  K  A  L  E  Q  R  K  E  Y  E  E  I  M  R  D  Y  I  Q  K  H  P  E  L  N  I  S  E  E  G  I  T    278

AAATCTACCCTCACCAAGGCCGAACGCCAGCTCAAGGACAAGTTTGATGGCCGACCTACCAAGCCACCTCCGAACAGCTACTCATTGTACTGTGCAGAGCTCATGGCCAACATGAAGGAC   960
 K  S  T  L  T  K  A  E  R  Q  L  K  D  K  F  D  G  R  P  T  K  P  P  P  N  S  Y  S  L  Y  C  A  E  L  M  A  N  M  K  D    318

GTGCCGAGCACAGAGCGCATGGTGCTATGTAGCCAGCAGTGGAAGCTGCTGTCCCAGAAGGAAAAGGACGCCTACCACAAGAAGTGTGACCAGAAAAAGAAAGATTATGAGGTAGAACTG  1080
 V  P  S  T  E  R  M  V  L  C  S  Q  Q  V  K  L  L  S  Q  K  E  K  D  A  Y  H  K  K  C  D  Q  K  K  K  D  Y  E  V  E  L    358

CTGCGGTTCCTCGAGAGCTTGCCCGAGGAGGAGCAGCAGCGGGTCCTGGGCGAGGAAAAGATGTTGAACATCAATAAGAAACAGACCACCAGTCCAGCCTCCAAGAAGCCTTCTCAGGAA  1200
 L  R  F  L  E  S  L  P  E  E  E  Q  Q  R  V  L  G  E  E  K  M  L  N  I  N  K  K  Q  T  T  S  P  A  S  K  K  P  S  Q  E    398

GGTGGCAAGGGCGGCTCTGAAAAGCCCAAGCGGCCTGTGTCTGCCATGTTCATCTTCTCAGAGGAGAAGCGAAGGCAGCTGCAGGAGCAGCGGCCTGAGCTCTCAGAGAGCGAGCTGACC  1320
 G  G  K  G  G  S  E  K  P  K  R  P  V  S  A  M  F  I  F  S  E  E  K  R  R  Q  L  Q  E  Q  R  P  E  L  S  E  S  E  L  T    438

CGCCTGCTGGCCCGCATGTGGAACGACTTGTCAGAGAAGAAGAAGGCTAAATATAAGGCTCGGGAGGCTGCTCTGAAAGCACAGTCTGAGAGGATGCCAGGTGGGGAGCGTGAAGAGCGA  1440
 R  L  L  A  R  M  W  N  D  L  S  E  K  K  K  A  K  Y  K  A  R  E  A  A  L  K  A  Q  S  E  R  M  P  G  G  E  R  E  E  R    478

GGCAAGCTGCCTGAGTCGCCCAAGAGAGCTGAGGAAATATGGCAGCAGAGTGTCATTGGAGACTATCTGGCTCGCTTCAAGAATGACCGAGTCAAAGCCTTAAAAGCTATGGAGATGACT  1560
 G  K  L  P  E  S  P  K  R  A  E  E  I  W  Q  Q  S  V  I  G  D  Y  L  A  R  F  K  N  D  R  V  K  A  L  K  A  M  E  M  T    518

TGGAACAACATGGAGAAGAAGGAGAAGCTGATGTGGATTAAGAAGGCTGCCGAAGACCAAAAACGATATGAGAGAGAGTTAAGTGAGATGCGGGCCCCTCCAGCTGCTACGAACTCTTCC  1680
 W  N  N  M  E  K  K  E  K  L  M  W  I  K  K  A  A  E  D  Q  K  R  Y  E  R  E  L  S  E  M  R  A  P  P  A  A  T  N  S  S    558

AAGAAGATGAAGTTCCAGGTGGAGCCCAAAAAACCTCCCATGAACGGTTACCAGAAGTTCTCCCAGGAGCTGCTGTCCAATGGGGAGCTGAATCACCTGCCGCTGAAGGAGCGCATGGTA  1800
 K  K  M  K  F  Q  V  E  P  K  K  P  P  M  N  G  Y  Q  K  F  S  Q  E  L  L  S  N  G  E  L  N  H  L  P  L  K  E  R  M  V    598

GAGATTGGCAGCCGCTGGCAGCGCATCTCCCAGAGCCAGAAGGAGCACTATAAGAAGCTGGCGGAGGAGCAGCAGAAGCAGTATAAGGTGCACTTGGACCTCTGGGTCAAGAGCCTGTCT  1920
 E  I  G  S  R  W  Q  R  I  S  Q  S  Q  K  E  H  Y  K  K  L  A  E  E  Q  Q  K  Q  Y  K  V  H  L  D  L  W  V  K  S  L  S    638

CCCCAGGACCGTGCAGCATACAAAGAATACATCTCCAATAAACGTAAGAATATGACTAAGCTTCGAGGCCCAAATCCTAAGTCTAGCCGAACCACCCTGCAGTCTAAGTCGGAGTCTGAG  2040
 P  Q  D  R  A  A  Y  K  E  Y  I  S  N  K  R  K  N  M  T  K  L  R  G  P  N  P  K  S  S  R  T  T  L  Q  S  K  S  E  S  E    678

GAGGAGGAGGATGAGGAAGATGAGGATGATGATGAAGAGGAAGAGGAGGAAGATGATGAGAATGGGGACTCCTCTGAGGATGGCGGGGACTCTTCAGAGTCTAGCAGCGAAGATGAGAGC  2160
 E  E  E  D  E  E  D  E  D  D  D  E  E  E  E  E  E  D  D  E  N  G  D  S  S  E  D  G  G  D  S  S  E  S  S  S  E  D  E  S    718

GAAGATGGGGATGAGAATGAGGACGACGATGACGACGACGATGACGATGAGGATGACGATGAGGATGAAGACAATGAGTCTGAGGGCAGCAGCTCCAGCTCTTCCTCTTCAGGGGACTCC  2280
 E  D  G  D  E  N  E  D  D  D  D  D  D  D  D  E  D  D  D  E  D  E  D  N  E  S  E  G  S  S  S  S  S  S  S  S  G  D  S    758

TCAGACTCTGACTCCAACTGAGCTCAGCCCCCACCCTGGGCAGGCAGCCAAGGAGAGCCACTGCAGAGCTCCCCTCCCCACCTGACCACCTTTGTCTCTCCCTATGTTCTGTCCCCTCAG  2400
 S  D  S  D  S  N  *                                                                                                          764

CTTCCCCCACTTTCTT                                                                                                          2416
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Fig. 1. Nucleotide sequence of hamster UBF. The complete deduced aa sequence of hamster UBF1 is shown. Identical nt sequence for UBF2 was found except for the doubly overlined sequence that represents the delete region in the UBF2 polypeptide. TGA (asterisk) is the stop codon. Sequences for hamster UBF1 and UBF2 have been deposited in the EMBL/GenBank data base (accession Nos. L42570 and L42571).

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