Volume 306, number 2,3, 181–184 FEBS 11301 July 1992

Molecular cloning of a novel ras-like protein from chicken

Judith Trueb and Beat Trueb

Laboratorium für Biochemie I, Eidgenössische Technische Hochschule, CH-8092 Zürich, Switzerland

Received May 20 1992

We have isolated a cDNA clone from a chicken DNA expression library which codes for a ras-like polypeptide of 216 amino acid residues. This polypeptide is closely related to the human protein TC4 and to the yeast protein Spi1, two novel proteins that may be involved in the coordination of the cell cycle. In the amino-terminal region, the three polypeptides possess a P-loop motif characteristic of GTP-binding proteins. At the carboxy-terminal end, however, they lack the typical CAAX-box which is usually responsible for membrane anchorage of ras-like proteins. It is therefore likely that the three polypeptides define a new subclass of GTP-binding proteins within the ras-like superfamily.

Ras-like; Protooncogene; GTPase; GTP-binding; Spi1; Chicken

1. INTRODUCTION

Ras-like proteins constitute a superfamily of low molecular weight proteins that share 30–55% sequence identity among each other [1–4]. So far, more than forty different members of this superfamily have been identified which can be grouped into five distinct subfamilies, the ras-, ral-, rap-, rho-and rab-proteins. All ras-like proteins bind GTP with high affinity and catalyze its hydrolysis to GDP. In this way, the proteins act as molecular switches which are biologically active in the GTP-bound form, but inactive in the GDP-bound form. It is believed that ras proteins function as transducers of mitogenic signals from growth factor receptors to downstream targets. Rho proteins may play a role in controlling the architecture of the cytoskeleton, whereas rab proteins appear to be involved in the regulation of the secretory pathway.

All ras-like proteins exhibit a similar domain structure. The amino-terminal region (residues 1–165 in H-ras) represents the catalytic domain which binds and hydrolyses GTP. The following 20 residues form a heterogeneous region that differs largely among distinct ras-like proteins. The four carboxy-terminal residues, called the CAAX-box (where C is cysteine, A any aliphatic amino acid and X any uncharged amino acid), are responsible for membrane attachment which is essential for biological activity. This attachment is accomplished by several post-translational modifications including farnesylation and palmitylation of carboxy-terminal cysteine residues.

Ras genes have attracted considerable attention over the past ten years because they acquire transforming properties by point mutations that affect their GTP-binding region (primarily codons 12–13 and 59–61). In fact, activated ras oncogenes have been identified in many bladder carcinomas and in about 30% of all other human tumors.

We have been interested for some time in collagenous proteins of the extracellular matrix [5,6]. Screening of an expression library for such a protein fortuitously led to the isolation of a cDNA clone which encoded a novel ras-like protein. This protein revealed an extraordinary degree of sequence conservation between man, chicken and yeast and might therefore play a crucial role in normal cell growth.

Correspondence: B. Trueb, Biochemie I, ETH-Zentrum, CH-8092 Zürich, Switzerland. Fax (41) (1) 261 5677.

2. MATERIALS AND METHODS

A λgt11 expression library was screened with antibodies against type VI collagen as previously described [5,6]. Immunoreactive clones were amplified, subcloned into the sequencing vectors M13mp18 or M13mp19 and sequenced on both strands by the dideoxy chain termination method [7]. The sequences were analyzed with the software computer package of the Genetics Computer Group (University of Wisconsin, Madison WI).

Total RNA was prepared from 15-day-old chicken embryos and from several cell lines [8] by the guanidinium–isothiocyanate method [9]. For Northern blots, 15 μg of total RNA was resolved on a formaldehyde containing 1% agarose gel and transferred to nitrocellulose [10]. The nitrocellulose sheet was hybridized under standard conditions to the cDNA probe which had been labeled with [α-^{32}P]dCTP by the random primed oligolabeling method [11].

3. RESULTS AND DISCUSSION

3.1. *Isolation and characterization of cDNA clone Ju93*

Screening of a cDNA expression library with antibodies for a collagenous glycoprotein furtuitously led to the isolation of cDNA clone Ju93. Sequencing studies indicated that this clone did not code for a collagenous

```
1    GAATTCCGGGAAAGACCCAGCATGGCCGCCCAAGGAGAGCCCCAAGTGCAGTTTAAGCTT   60
1                        M  A  A  Q  G  E  P  Q  V  Q  F  K  L      13

61   GTCCTGGTTGGCGATGGTGGCACTGGTAAAACAACATTTGTAAAACGTCATTTGACTGGT  120
14   V  L  V  G  D  G  G  T  G  K  T  T  F  V  K  R  H  L  T  G    33

121  GAATTTGAGAAGAAATACGTAGCAACATTGGGTGTTGAAGTTCATCCACTGGTATTCCAT  180
34   E  F  E  K  K  Y  V  A  T  L  G  V  E  V  H  P  L  V  F  H   53

181  ACTAATAGAGGCCCTATTAAATTTAATGTATGGGACACAGCTGGCCAGGAGAAGTTTGGT  240
54   T  N  R  G  P  I  K  F  N  V  W  D  T  A  G  Q  E  K  F  G   73

241  GGTCTGCGAGATGGCTATTACATCCAAGCTCAGTGTGCCATTATAATGTTTGATGTAACA  300
74   G  L  R  D  G  Y  Y  I  Q  A  Q  C  A  I  I  M  F  D  V  T   93

301  TCAAGAGTTACTTACAAGAATGTACCTAACTGGCATAGAGACCTGGTACGGGTATGTGAA  360
94   S  R  V  T  Y  K  N  V  P  N  W  H  R  D  L  V  R  V  C  E  113

361  AACATCCCTATAGTGTTGTGTGGCAACAAAGTGGATATTAAGGACAGAAAAGTCAAGGCA  420
114  N  I  P  I  V  L  C  G  N  K  V  D  I  K  D  R  K  V  K  A  133

421  AAATCCATTGTCTTCCACAGGAAGAAGAATCTCCAGTATTATGACATTTCAGCCAAGAGT  480
134  K  S  I  V  F  H  R  K  K  N  L  Q  Y  Y  D  I  S  A  K  S  153

481  AACTACAACTTTGAGAAGCCGTTCCTCTGGCTTGCTAGGAAGCTAATTGGAGATCCTAAC  540
154  N  Y  N  F  E  K  P  F  L  W  L  A  R  K  L  I  G  D  P  N  173

541  TTGGAATTTGTTGCCATGCCTGCTCTTGCACCACCTGAAGTTGTTATGGACCCAGCACTG  600
174  L  E  F  V  A  M  P  A  L  A  P  P  E  V  V  M  D  P  A  L  193

601  GCAGCACAGTATGAGCAAGACTTACAGATTGCTCAAACCACTGCACTGCCAGATGAAGAT  660
194  A  A  Q  Y  E  Q  D  L  Q  I  A  Q  T  T  A  L  P  D  E  D  213

661  GATGACCTGTGAGGGATGAAGCTGGAGCCCAGCGTCAGAAGTCTAGTTTTATAGGCAACT  720
214  D  D  L  *                                                   216

721  GTCCTGTGATGTCAGTGGTGCAGCGTGTTTGCCACTTTATTATCTAGCTGAGCAGAACAT  780

781  GTGCTTAATCTTTGGGATGCTGAAAGAGATGAATGGGCTTCGCAGTGAATGTGGCAGTTC  840

841  AAAACGAAAAAAACAACAAAACTTCATAATTTTGGACCTCCATATTTAGCTGTTTTTTGG  900

901  ACTGCAATTACTTCCCGTTTGAGTTTCAAATATAAGACTGCTGCAGTCACATCAAAGTGT  960

961  TATGTGGTAAATCTTGTTTCTGTCATTCCCGGAATTC  997
```

Fig. 1. Nucleotide and derived amino acid sequence of cDNA clone Ju93.

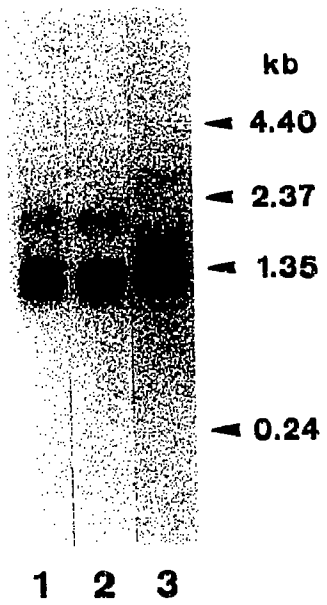

Fig. 2. Northern blot analysis of cDNA clone Ju93 with total RNA from chicken embryos (lane 1), chicken fibroblasts (lane 2) and human VA13 cells (lane 3). The migration positions of standard RNA molecules are indicated in the right margin.

polypeptide (Fig. 1). The nucleotide sequence contained an ATG codon (positions 22–24) in a surrounding that was consistent with a typical start site of transiation [12]. This initiation codon was followed by an open reading frame of 648 bp. The amino acid sequence derived from the nucleotide sequence predicted a protein of 216 residues with a molecular mass of 24,427 Da. At position 17–24, this sequence contained a P-loop motif (GDGGTGKT) which is characteristic of GTP-binding proteins [13].

On Northern blots with RNA preparations from chicken embryos and chicken fibroblasts, our cDNA clone detected a major band of 1,300 nucleotides and a minor band of 2,100 nucleotides (Fig. 2). Similar bands were obtained with RNA preparations from human VA13 cells, suggesting that both, avian and mammalian cells express the Ju93 gene.

3.2. *Similarity of Ju93 to ras-like proteins*

A detailed comparison of the Ju93 polypeptide sequence with all entries of the Swissprot databank revealed substantial similarities with members of the ras-like superfamily (Fig. 3). With H-ras, N-ras, ral-A, rap-

```
                    G1                                  G2
Ju93     1  MAAQGEPQVQFKLVLVGDGGTGKTTFVKRHLTGEFEKKYVATLGVEVHPL  50
H-ras    1        MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQ  43

                    G3
Ju93    51  VFHTNRGPIKFNVWDTAGQEKFGGLRDGYYIQAQCAIIMFDVTSRVTYKN  100
H-ras   44  VVIDGETCL.LDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFED   92

                                G4
Ju93   101  VPNWHRDLVRV..CENIPIVLCGNKVDIKDRKVKAK.SIVFHRKKNLQYY  147
H-ras   93  IHQYREQIKRVKDSDDVPMVLVGNKCDLPARTVETRQAQDLARSYGIPYI  142

            G5
Ju93   148  DISAKSNYNFEKPFLWLARKLIGDPNLEFVAMPALAPPEVVMDPALAAQY  197
H-ras  143  ETSAKTRQGVEDAFYTLVREIRQHK......LRKLNPPDESGPGCMNCKC  186

Ju93   198  EQDLQIAQTTALPDEDDDL  216
H-ras  187  VIS                 189
```

Fig. 3. Alignment of the amino acid sequences of the chicken proteins Ju93 and H-ras. The five conserved regions involved in GTP metabolism are boxed.

```
YEAST     1  MA-QPQNVPTFKLVLVGDGGTGKTTFVKRHLTGEFEKKYIATLGVEVHPLHFHTNFGEIC  59
HUMAN     1  MAAQGEPQVQFKLVLVGDGGTGKTTFVKRHLTGEFEKKYVATLGVEVHPLVFHTNRGPIK  60
CHICK     1  MAAQGEPQVQFKLVLVGDGGTGKTTFVKRHLTGEFEKKYVATLGVEVHPLVFHTNRGPIK  60
             ** * .    ********************************.********** **** * *

YEAST    60  FNVWDTAGQEKLGGLRDGYYIQGQCGIIMFDVTSRITYKNVPHWHRDLVRVCENIPIVLC  119
HUMAN    61  FNVWDTAGQEKFGGLRDGYYIQAQCAIIMFDVTSRVTYKNVPNWHRDLVRVCENIPIVLC  120
CHICK    61  FNVWDTAGQEKFGGLRDGYYIQAQCAIIMFDVTSRVTYKNVPNWHRDLVRVCENIPIVLC  120
             ***********.**********.**.*********.******.* ***************

YEAST   120  GNKVDVKERKVKAKAITFHRKKNLQYYDISAKSNYNFEKPFLWLARKLVGNPNLEFVASP  179
HUMAN   121  GNKVDIKDRKVKAKSIVFHRKKNLQYYDISAKSNYNFEKPFLWLARKLIGDPNLEFVAMP  180
CHICK   121  GNKVDIKDRKVKAKSIVFHRKKNLQYYDISAKSNYNFEKPFLWLARKLIGDPNLEFVAMP  180
             *****.*.******.*.*********************************.*.******* *

YEAST   180  ALAPPEVQVDQQLLAQYQQEMNEAAAMPLPDEDDADL  216
HUMAN   181  ALAPPEVVMDPALAAQYEHDLEVAQTTALPDEDD-DL  216
CHICK   181  ALAPPEVVMDPALAAQYEQDLQIAQTTALPDEDD-DL  216
             ******* .*..* ***..... *.. .****** **
```

Fig. 4. Alignment of the amino acid sequences of the chicken protein Ju93, the human protein TC4 and the yeast protein Spi1. Identical positions are marked by asterisks, conservative replacements by dots.

1B, rab, Rho-A and YPT1, the Ju93 protein shared 25–30% sequence identity. If conservative amino acid substitutions were included, these similarities increased to 49–53%.

As is the case for most ras-like proteins [2], the Ju93 sequence contained five particularly well conserved regions (G-1 to G-5) which are likely to be involved in GTP metabolism (Fig. 3). In contrast to other ras-like proteins, however, the typical CAAX-box was missing at the carboxy-terminus of the polypeptide sequence. Since this sequence motif is responsible for membrane attachment, the Ju93 protein might exist in a soluble, not membrane-anchored form.

3.3. *Ju93, TC4 and Spi1 define a new subclass within the ras-like superfamily*

A strikingly high homology was observed between the Ju93 sequence and two recent databank entries, human TC4 [14] and yeast Spi1 [15]. At the nucleotide level, the chicken sequence shared 68% identity with the yeast sequence and 89% identity with the human sequence. Under the assumption that the human sequence contained a minor sequencing error at codon 179, all three cDNA sequences could be translated into polypeptides of 216 amino acid residues. Compared to the (corrected) human amino acid sequence, the chicken polypeptide showed three amino acid substitutions (Fig. 4). All these substitutions occurred within the carboxy-terminal domain corresponding to the heterogeneous region of ras-like proteins [1–4]. Compared to the yeast protein, the chicken polypeptide displayed 82% sequence identity or 89% similarity, if conservative amino acid replacements were included. Since all three proteins lacked the characteristic CAAX box at their carboxy termini, they may define a new subfamily of GTPase signaling proteins within the ras-like superfamily.

The extraordinary degree of conservation throughout metazoan evolution strongly implies that the three novel proteins serve a crucial function in normal cell growth. What this function is, can only be speculated at present. Little is known about TC4 except that it was isolated from a teratocarcinoma cDNA library with a mixed oligonucleotide probe specific for ras-like sequences [14]. More information, however, is available on Spi1 from fission yeast [15]. Overexpression of Spi1 was found to rescue pim1 cells which undergo premature initiation of mitosis before completion of DNA replication. Thus, Spi1 appears to be involved in the maintenance of a coordinated cell cycle. The loss of a single copy of the Spi1 gene caused a dramatic instability of the genetic material and eventually led to mitotic haploidization. Since a wide variety of tumor cells display an abnormal karyotype, it seems conceivable that the functional loss of the Spi1 gene may contribute to the malignant transformation of a cell by affecting its genetic stability. It would therefore be interesting to examine the effects of overexpressing the Ju93 gene in normal cells or of introducing small mutations into the Ju93 sequence which may affect its GTP metabolism.

Acknowledgements: This work was supported by Grants 31–30881.91 and 31–9065.87 from the Swiss National Science Foundation and by a grant from the ETH Zürich.

REFERENCES

[1] Barbacid, M. (1987) Annu. Rev. Biochem. 56, 779–827.
[2] Bourne, H.R., Sanders, D.A. and McCormick, F. (1991) Nature 349, 117–127.
[3] Wittinghofer, A. and Pai, E.F. (1991) Trends Biochem. Sci. 16, 382–387.
[4] Downward, J. (1990) Trends Biochem. Sci. 15, 469–472.
[5] Trueb, B., Schaeren-Wiemers, N., Schreier, T. and Winterhalter, K.H. (1989) J. Biol. Chem. 264, 136–140.
[6] Koller, E., Winterhalter, K.H. and Trueb, B. (1989) EMBO J. 8, 1073–1077.
[7] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.

[8] Schreier, T., Friis, R.R., Winterhalter, K.H. and Trueb, B. (1988) Biochem. J. 253, 381–386.
[9] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[10] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[11] Feinberg, A. and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13.
[12] Kozak, M. (1987) Nucleic Acids Res. 15, 8125–8148.
[13] Saraste, M., Sibbald, P.R. and Wittinghofer, A. (1990) Trends Biochem. Sci. 15, 430–434.
[14] Drivas, G.T., Shih, A., Coutavas, E., Rush, M.G. and D'Eustachio, P. (1990) Mol. Cell. Biol. 10, 1793–1798.
[15] Matsumoto, T. and Beach, D. (1991) Cell 66, 347–360.