

Molecular cloning of a novel ras-like protein from chicken

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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 Sp1, 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; Sp1; 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 palmitoylation 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.

2. MATERIALS AND METHODS

A λ gt11 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 μ g 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 fortuitously led to the isolation of cDNA clone Ju93. Sequencing studies indicated that this clone did not code for a collagenous

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1  GAATTCGGGAAAGACCCAGCATGGCCGCCAAGGAGAGCCCAAGTGCAGTTTAAAGCTT  60
1  M A A Q G E P Q V Q F K L  13
61  GTCTGTGTTGGCGATGGTGGCACTGGTAAACACATTGTAAACGTGCTTTGAGTGGT  120
14  V L V G D G G T G K T T F V K R H L T G  33
121 GAATTTGAGAAGAAATACGTAGCAACATTGGGTGTTGAAGTTTATCCACTGGTATTCAT  180
34  E F E K K Y V A T L G V E V H P L V F H  53
181 ACTAATAGAGGCCCTATTAAATTTAATGTATGGGACACAGCTGGCCAGGAGAAGTTGGT  240
54  T H R G P I K F N V W D T A G Q E K F G  73
241 GGTCTGGGAGATGGCTATTACATCCAAAGCTCAGTGTGCCATTATAATGTTTATGTAACA  300
74  G L R D G Y Y I Q A Q C A I I M F D V T  93
301 TCAGAGCTTACTTACAGAATGTACCTAACTGGCATAGAGACCTGGTACGGTATGTGAA  360
94  S R V T Y K N V P H H I R D L V R V C E  113
361 AACATCCCTATAGTGTGGTGGCAACAAAGTGGATATTAGGACAGAAAAGTCAAGGCA  420
114  N I P I V L C G N K V D I K D R K V K A  133
421 AAATCCATTGTCTCCACAGGAAGAAGATCTCCAGTATTATGACATTTCAGCCAAGAGT  480
134  K S I V F H R K K N L Q Y Y D I S A K S  153
481 AACTACAACCTTTGAGAAGCGCTTCTCTGGCTTGTAGGAAGCTAATTGGAGATCTCAAC  540
154  N Y N F E K P F L W L A R K L I G D F M  173
541 TTGGAATTTGTGTCCTGCTGCTCTTGCACACCTGAAGTTGTTTGGACCCGACACTG  600
174  L E F V A M F A L A P P E V V M D P A L  193
601 GCAGCAGATATGAGCAAGACTTACAGATTGCTCAAACTGCTGACCTGCCAGATGAAGAT  660
194  A A Q Y E Q D L Q : A Q T T A L P D E D  213
661 GATGACCTGTGAGGGATGAAGCTGGAGCCCGAGCGTCAGAAGTCTAGTTTATAGGCAACT  720
214  D D L *  216
721 GTCTGTGATGTCACTGGTGCAGCGTGTGTCACCTTTATTATCTAGCTGAGCAGAACAT  780
781 GTGCTTAATCTTTGGGATGCTGAAGAGATGAATGGGCTTCGGAGTGAATGTGGCAGTTC  840
841 AAAACGMAAAACAAACAACTTCATAATTTTGGACCTCCATATTAGCTGTTTITGG  900
901 ACTGCATTACTTCCCGTTTGAGTTTTCMAATATAAGACTGCTGCAGTCACATCAAAAGTGT  960
961 TATGTGGTAATCTTGTGTTCTGTCAATCCCGGAATTC  997

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

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 translation [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].

				G1		G2		
Ju93	1	MAAQGEFQVQFKLVLV	GDGGTGKT	TFVYKRLH	TGEFEKKY	VVATL	LGVEVHPL	50
								
H-ras	1	MTEYKLVVV	GAGGVGKS	SALTIQLI	QNHVDEY	YDPTI	EDSYRKQ	43
								
				G3				
Ju93	51	VFHTNRGPIKFNVM	DTAGQEK	FGGLRDGY	YIQAQCA	IMFDVTS	SRVTKYN	100
								
H-ras	44	VVIDGETCL	LDILDTAGQEK	EYSAMRDQ	YMRTEG	EFLCVFA	INNTKSPED	92
								
				G4				
Ju93	101	VPNWHRDVLRV	..CENIPIVLC	GNKVD	IRDAKVKAK	SIVFHR	AKNLQYY	147
								
H-ras	93	THQYREQIKRVK	SDSDVPMVLV	GNKCD	LPARTVET	RQAQDL	ARSYGIPYI	142
								
				G5				
Ju93	148	DISAK	SNYNFEK	PFLWLARK	LIGDPN	LEFVAMP	ALAPFEV	VMDPALAAQY 197
								
H-ras	143	ETSAK	TRQGV	EDAFYTLV	REIRQHK		LRKLN	PPDES
								186
Ju93	198	EQDLQIAQT	TALPDEDDDL					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.

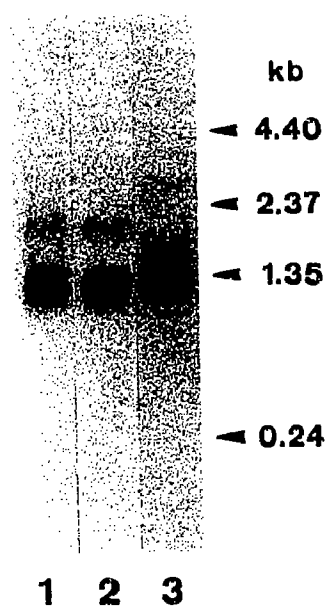


Fig. 2. Northern blot analysis of cDNA clone Ju93 with total RNA from chicken embryos (lane 1), chicken fibroblasts (lane 2) and human VA13 cells (lane 3). The migration positions of standard RNA molecules are indicated in the right margin.

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-

YEAST	1	MA-QPQNVPTFKLVLVGDGGTGKTTFVKRHLTGFEFEKKYIATLGVEVHPLHFHTNFGEIC	59
HUMAN	1	MAAQGEFQVQFKLVLVGDGGTGKTTFVKRHLTGFEFEKKYVATLGVEVHPLVFHTNRRGPIK	60
CHICK	1	MAAQGEFQVQFKLVLVGDGGTGKTTFVKRHLTGFEFEKKYVATLGVEVHPLVFHTNRRGPIK	60
		*** * ***** *	
YEAST	60	FNVWDTAGQEKLGGLRDGYIYIQGCQGIIMFDVTSRITYKKNVPHNWRDLVRVCENIPVLC	119
HUMAN	61	FNVWDTAGQEKFGGLRDGYIYQAQCAIIMFDVTSRVTYKNVPHNWRDLVRVCENIPVLC	120
CHICK	61	FNVWDTAGQEKFGGLRDGYIYQAQCAIIMFDVTSRVTYKNVPHNWRDLVRVCENIPVLC	120
		***** , ***** , * ***** *	
YEAST	120	GKNKVDYKERRVKAKAITFHRKKNLQYYDISAKSNYNFEKPFWLWARKLVGNPNLEFVASP	179
HUMAN	121	GKNKVDIKDRKVKAKSIVFHRKKNLQYYDISAKSNYNFEKPFWLWARKLIGDNLFEVAMP	180
CHICK	121	GKNKVDIKDRKVKAKSIVFHRKKNLQYYDISAKSNYNFEKPFWLWARKLIGDNLFEVAMP	180
		***** , ***** , ***** *	
YEAST	180	ALAPPEVQVDQQLLAQYQOEMNEAAAMPDPDEDDADL	216
HUMAN	181	ALAPPEVVMDPALAAQYENDLEVAQTALPDEDD-DL	216
CHICK	181	ALAPPEVVMDPALAAQYEQDLQIAQTALPDEDD-DL	216
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