Volume 268, number 1, 27–31 FEBS 08626 July 1990

Molecular cloning and sequence determination of four different cDNA species coding for α-subunits of G proteins from *Xenopus laevis* oocytes

Juan Olate[1], Sixta Martinez[2], Patricia Purcell[1], Hugo Jorquera[1], Juan Codina[3], Lutz Birnbaumer[3] and Jorge E. Allende[1]

[1] *Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile, Casilla 70086, Santiago 7, Chile,* [2] *Departamento de Química, Universidad Nacional, Bogotá, Colombia* and [3] *Department of Cell Biology, Baylor College of Medicine, Texas Medical Center, Houston, TX 77030, USA*

Received 9 May 1990

A cDNA library preprared from *Xenopus laevis* oocytes in λgt10 was screened with a mixture of three oligonucleotide probes designed to detect sequences found in different mammalian genes coding for α-subunits of G-proteins. In addition to a clone coding for a Gαo-type subunit previously reported [(1989) FEBS Lett. 244, 188–192] four additional clones have been found coding for different Gα protein subunits. By comparison with mammalian α-subunits, these oocyte cDNAs correspond to two closely related Gαs-1a, to a Gαi-1 and to a Gαi-3 species. The derived amino acid sequences showed that both Gαs species contain 379 residues, corresponding to the short species without the serine residue and with a calculated M_r of 42720. The Gαi-1 gene encodes a 354 amino acid protein with an M_r of 39000 and the Gαi-3 encodes an incomplete open reading frame of 345 residues, lacking the first 9 amino acid residues at the NH_2 terminus. All these Gα-subunits showed high identity with their respective mammalian counterparts (75–80%), indicating a great degree of conservation through the evolution and the important cellular regulatory function that they play.

G-protein; cDNA cloning; Nucleotide sequence; *Xenopus laevis* oocyte

1. INTRODUCTION

The transduction of many external signals towards the interior of the cells involves trimeric proteins that bind guanine nucleotides and that are known as G-proteins [1]. There is a large family of these proteins since more than 16 different G-proteins have been isolated from different species and tissues [2–5]. Although the function of some of these G-proteins has been elucidated in particular signal transduction pathways, there are still many questions open as to the role that each one of these may play in different systems.

The *Xenopus laevis* oocyte has become a popular system for researchers studying the function of receptors and who have isolated mRNAs coding for these receptor proteins. The reason for this popularity is the fact that the oocyte microinjected with these mRNAs has shown itself to be capable of both translating these receptors and also of coupling the newly synthesized receptors to transducing systems. The microinjected oocyte thus acquires the capacity to respond physiologically to the agonist that binds to that particular receptor [6–9].

Correspondence address: J. Olate, Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile, Casilla 70086, Santiago 7, Chile

It has become important, therefore, to study the endogenous transducing systems of the oocyte in order to be able to determine the entities that participate in the mechanism of action of various signals and that couple to their respective effector systems. These considerations have induced us to clone the genes coding for different G-proteins that are expressed in this amphibian oocyte. In a previous communication [10], we reported the cloning of the cDNA coding for the oocyte Gαo-type subunit which showed a high degree of identity to the mammalian Gαo.

In this report, we present the cloning and sequencing of four other different cDNAs from *Xenopus laevis* oocytes coding for α-subunits highly analogous to Gαs, Gαi-1 and Gαi-3 of mammalian systems. These results indicate that this single cell type has at least 5 different types of G-proteins.

2. MATERIALS AND METHODS

2.1. *cDNA library*

A *Xenopus laevis* oocyte cDNA library constructed in the vector gt10 (kindly donated by Dr D.A. Melton of Harvard University) was utilized [11].

2.2. *Screening of the cDNA library*

Close to 2×10^5 recombinant plaques were screened by plaque hybridization [12] with three synthetic probes labeled at the 5′-end with ^{32}P. The probes used for this purpose and their respective se-

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00145793/90/$3.50

quences were the same utilized in the previous work [10]. Phages from five positive lytic plaques from the first screening round were plaque-purified through secondary and tertiary screening. Four of these clones were fully sequenced. Hybridizations were done overnight at 40°C in a solution containing 6 × SSC, pH 6.8, 100 μg of heat-denatured salmon sperm DNA per ml and 0.1% SDS. Filters were washed three times at 40°C in 6 × SSC and then three times at 45°C for 15 min. Films were exposed overnight at room temperature.

2.3. *cDNA sequence analysis*

Nucleotide sequences were determined by using the M13mp19 and the dideoxynucleotide chain-termination method [13] as described in the 'Sequenase' booklet provided by the US Biochemical Corp. (USB).

3. RESULTS

3.1. *Screening of the λgt10* Xenopus laevis *oocyte cDNA library*

Using the same strategy described previously by Olate et al. we screened about 2×10^5 lysis plaques and five positive clones were obtained. All these clones were subjected to a secondary and tertiary screening, their DNAs purified and finally analyzed by nucleotide sequencing of the cDNA inserts following the strategy shown in Fig. 1.

3.2. *Nucleotide sequences of the Gαi-1 cDNA clone*

Fig. 2 shows the nucleotide sequence of the cDNA encoding the Gαi-1 type α-subunit. The sequence is 2759 nucleotides long and predicts an open reading frame of a 354 amino acid protein (M_r 40200), with a 5′-untranslated region of 184 nucleotides and a 3′-untranslated region of 1510 nucleotides. The sequence contains two poly(A) addition sequences, AATAA, at positions 1979 and 2539 and it ends with a 14 residue poly(A) tail. The deduced amino acid sequence showed a high degree of identity (85%) with the human Gαi-1 [14].

3.3. *Nucleotide sequence of the Gαi-3 cDNA clone*

Fig. 3 shows the nucleotide sequence analysis of an insert with great similarity to human Gαi-3. The sequence is 2037 nucleotides long and it predicts a single open reading frame of 1035 nucleotides coding for a continuous sequence of a 345 amino acid protein. Since no ATG initiation codon was found, we assume that this sequence contains the partial sequence for a Gα-type protein missing a short stretch of the NH_2-terminus. At the protein level, comparison of corresponding residues between the oocyte and human Gαi

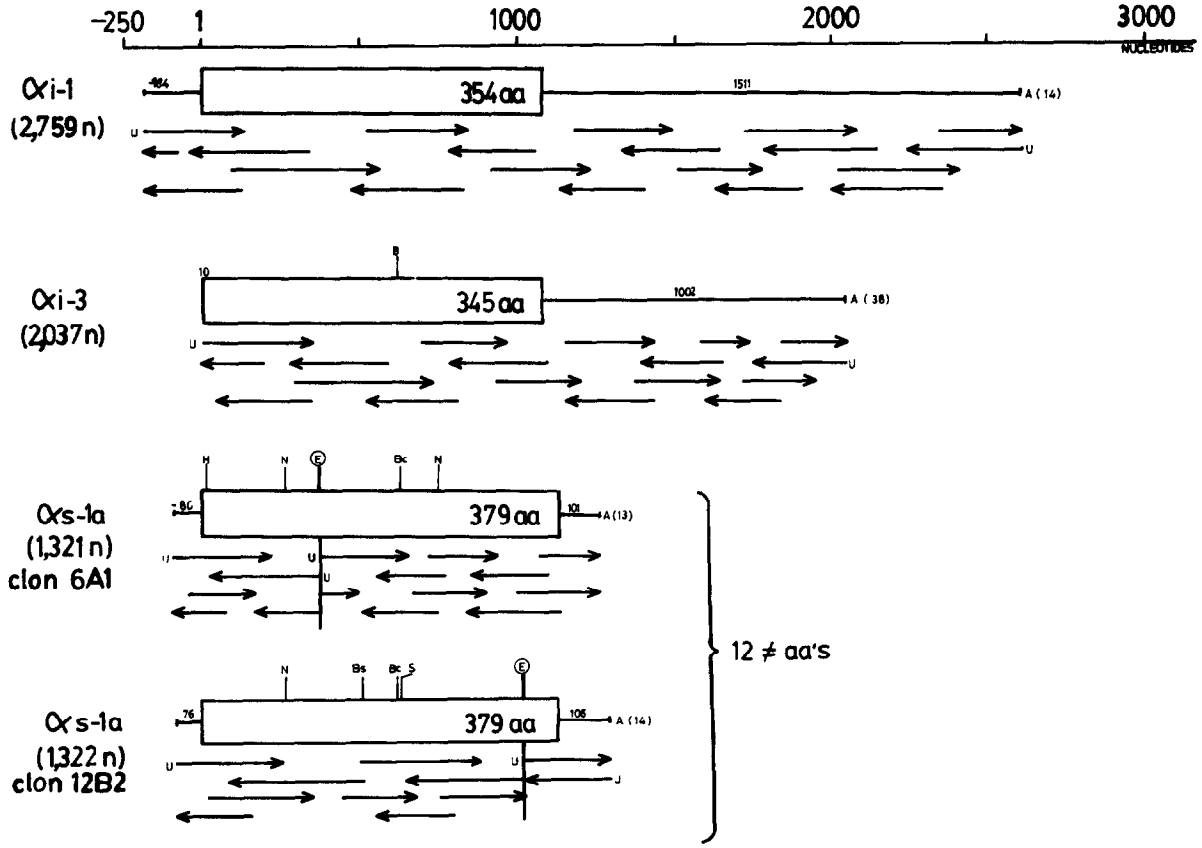

Fig. 1. Sequencing strategy and partial restriction endonuclease map for the *Xenopus laevis* Gα cDNAs. The top scale indicates cDNA length in nucleotides. Open boxes show the open reading frames (ORF) for the different proteins. The thin black lines show the 5′- and 3′-untranslated regions of the mRNA. The arrows indicate extent and direction of sequencing obtained with the oligonucleotide primers. All the cDNA inserts were sequenced in their complete length after subcloning them into the M13mp19 vector. The numbers in parentheses correspond to the length of each cDNA. The restriction endonuclease sites are denoted by one letter. B, *Bam*HI; H, *Hin*dIII; N, *Nc*II; E, *Eco*RI; Bc, *Bc*II; S, *Sph*I; Bs, *Bst*I.

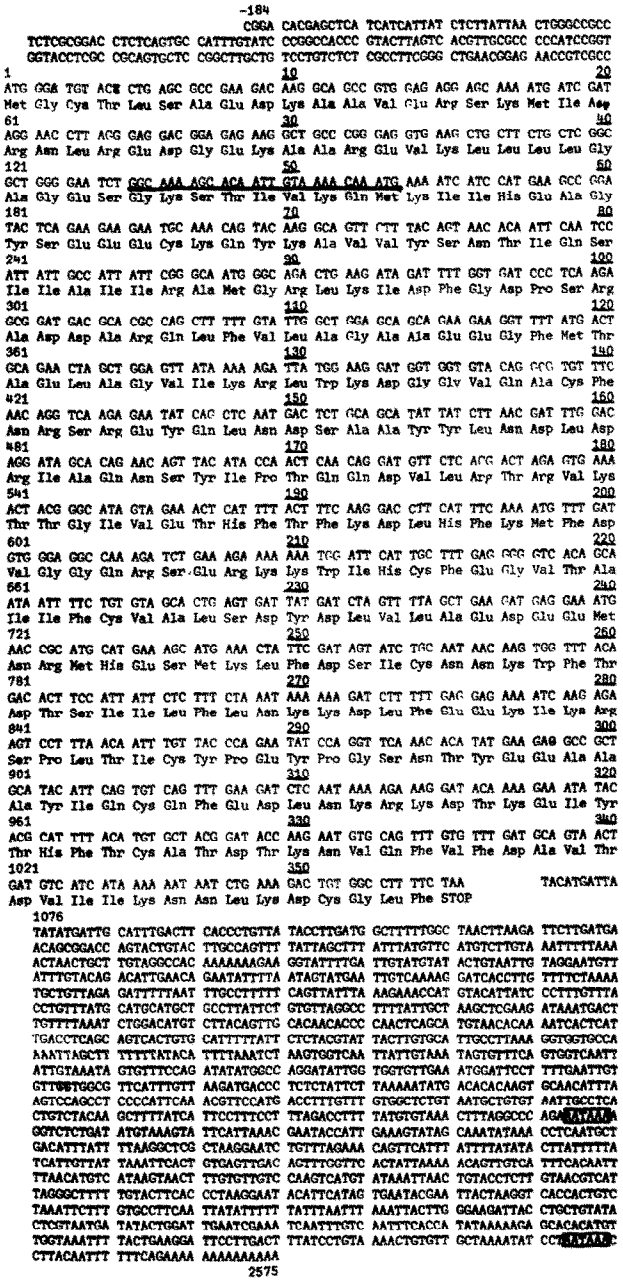

Fig. 2. Nucleotide and predicted amino acid sequence of the cDNA insert for Gαi-1. Numbers indicate the position of nucleotides or amino acid residues starting at the initiation codon. Sequences enclosed in open boxes correspond to the poly(A) addition sequences. Nucleotide sequence recognized by the common 27-base probe is underlined [10].

Fig. 3. Nucleotide and predicted amino acid sequence of the cDNA insert for Gαi-3. Numbers indicate the position of nucleotides or amino acid residues starting at the hypothetical initiation codon. Sequence enclosed in an open box correspond to the poly(A) addition sequence. Nucleotide sequence recognized by the common 27-base probe is underlined [10].

proteins indicated a high degree of identity (87%) with the human Gαi-3 [15], so this protein was classified as a Gαi-3 oocyte protein. The sequence has a 3′-untranslated region of 1002 nucleotides, it contains a poly(A) addition sequence at position 2010 followed by a stretch of 38 adenosines.

3.4. *Nucleotide sequence of a Gαs-type cDNA*

Fig. 4 shows the nucleotide and predicted amino acid sequence for one of the Gαs cDNA (clone 6A1). The sequence is 1321 nucleotides long and predicts an open reading frame of 379 amin acid residues (M_r 42720) and the protein presents more similarity (90%) to the short species of human Gαs-1a [16]. At the nucleotide level, the 5′-non-coding region contains 80 nucleotides and a small 3′-non-coding region of 101 residues containing a poly(A) addition sequence at position 1226 and a poly(A) tail of 13 adenosines. Clone Gαs 12B2 is very similar to clone 6A1 at the nucleotide level, showing only 42 differences which generate different restriction sites (see *Eco*RI site in Fig. 1) and 12 different amino acid residues (Fig. 5).

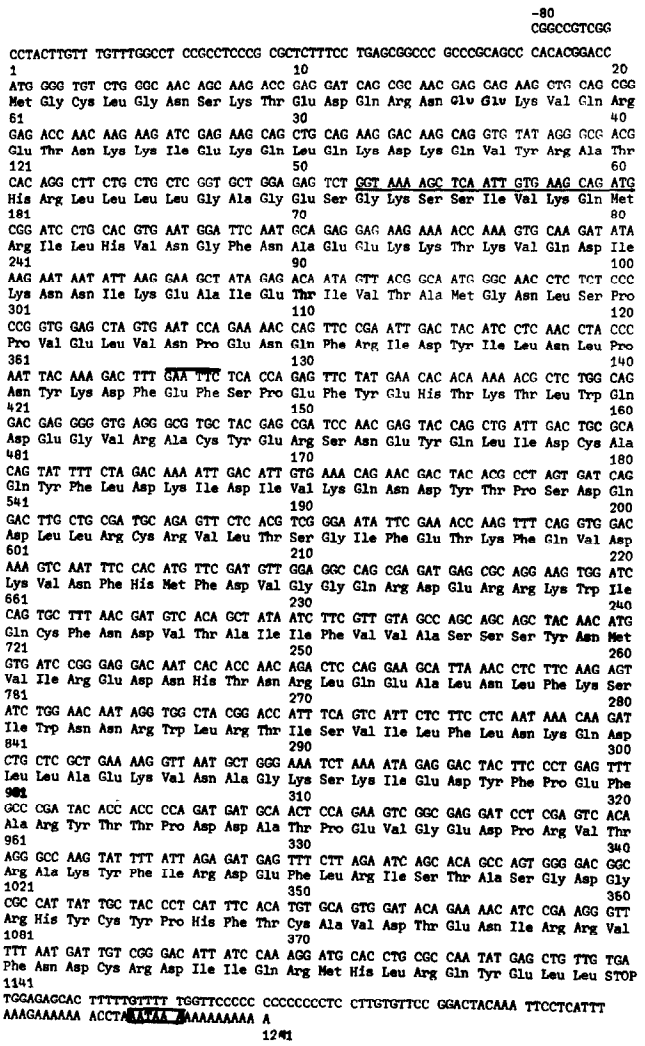

Fig. 4. Nucleotide and predicted amino acid sequence of the cDNA for Gαs (clone 6A1). Numbers indicate the position of nucleotides or amino acid residues starting at the initiation codon. Sequence enclosed by the open box corresponds to the poly(A) addition sequence. Nucleotide sequence recognized by the common 27-base probe is underlined [10] and the *Eco*RI site is indicated by a line over the sequence GAATTC.

3.5. *Comparison of the deduced amino acid sequences of the* Xenopus laevis *oocyte Gα subunits*

Fig. 5 shows the alignment of the predicted protein sequences of each of the cDNA clones reported here. The sequences share great similarity among themselves and with their mammalian counterparts. The regions of identity are enclosed in boxes. Also the figure shows the sequences labelled A, C, E and G which are supposedly involved in the binding of GTP.

4. DISCUSSION

The results presented in this communication and in the previous publication [10] demonstrate that one single cell type, the amphibian oocyte, contains at the mRNA level at least 5 different types of α G-proteins. Since our search has not been exhaustive, it is quite possible that there are more different α-subunits not yet detected.

The analysis of the nucleotide and deduced amino acid sequences allows us to classify the oocyte subunits according to their analogy to the structure of mammalian G-protein α-subunits. Thus it has been established that two of the isolated clones correspond to αs-type subunits, and the other two clones correspond to αi-1 and αi-3 subunits.

The two αs clones correspond to the 'short' version of the mammalian αs which arises through alternative splicing and elimination of exon 3, present in the 'long' αs [17]. Another variant found in mammals is that the short version can be found with or without a serine in position 72. The two oocyte clones are short versions without this serine. This is to our knowledge the first time that two different αs coding genes have been found in the same cell type and differ in other characteristics than those mentioned above. Since the 5′- and 3′-non-coding regions of the two clones are quite similar, it seems probable that they arose through a rather recent gene duplication. The small number of differences that account for 12 amino acid changes are all scattered through the polypeptide and therefore cannot be generated through alternative splicing. One of the changes alters an *Eco*RI site, a finding which may be useful for future analysis of the function of these proteins. One of the most interesting differences between the two αs clones is the conservative change of serine-178 present in the 6A1 clone for threonine in the clone 12B2. On the basis of the results obtained with mutations done in rats, the region encompassing residues 192–196 in long species of the αs subunits has been proposed to interact with the catalytic subunit of adenylyl cyclase and any mutation in this region could cause a change in its activity [18]. It is interesting to note that in this highly conserved region, all the α-subunits that are not stimulatory (non-αs-type subunits) contain a threonine in position 178 (short αs species) while all previously sequenced αs subunits have a serine in this position. The only other exception to this latter generality is the short αs-type found in rat olfactory neurons which contains a threonine in position 180 [19]. Interestingly, in the same tissue, olfactory neuroepithelium, a different long αs species from non-neuronal origin was found by the same group and this contained a serine in position 193 [20]. The presence of these two types of αs subunits in the oocyte suggests that they may have different functions in this cell.

Several G-protein regions share considerable similarity with the guanine nucleotide binding regions of elongation factor Tu and $p21^{ras}$ [21]. Consistent with this role, these regions (Fig. 5, labeled A, C, E and G) are all highly conserved within the four oocyte G-proteins. Other regions, as the one implicated in receptor, Gβγ, and effector protein interaction [22] are not

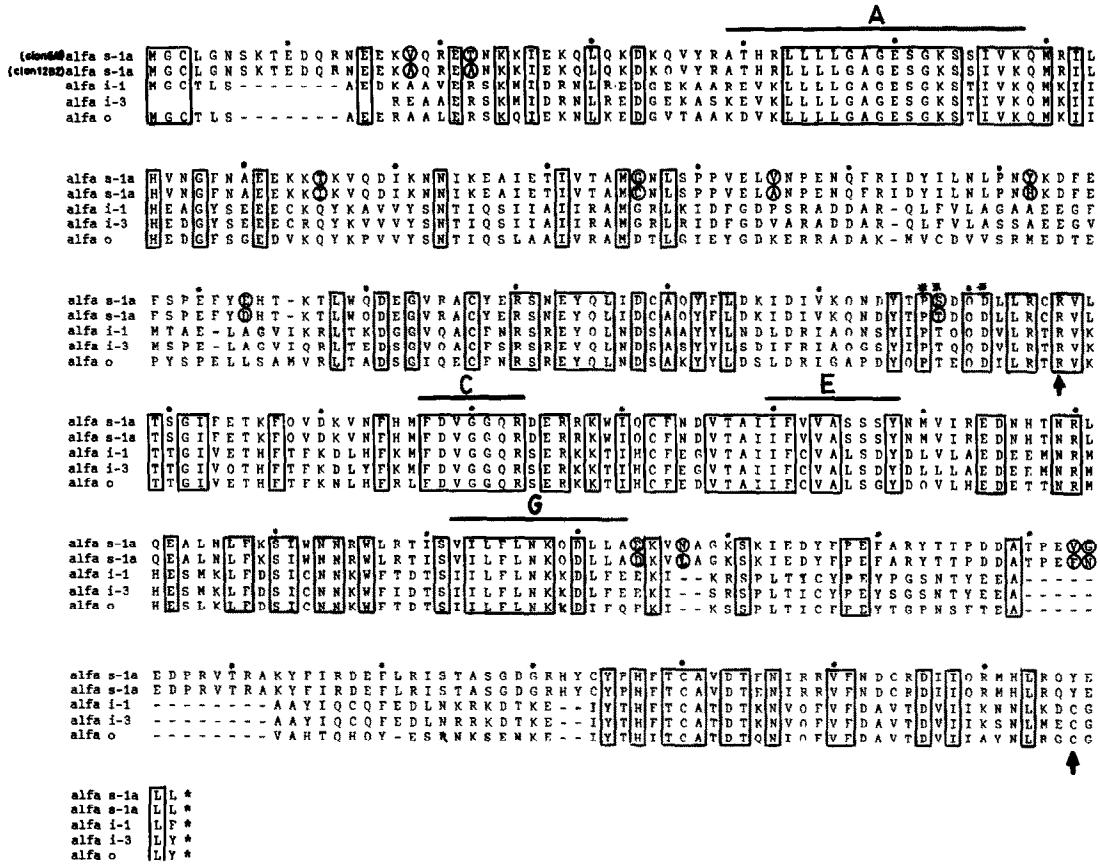

Fig. 5. Alignment of the amino acid sequences of the oocyte Gα subunits. Amino acid sequences are presented by the standard one-letter abbreviation code. The oocytes Gαs, Gαi-1, Gαi-3 and the already published Gαo [10] are shown. Amino acid regions that are identical are enclosed by open boxes. The arrows indicate the arginine and cysteine residues that are ADP-ribosylated by *Cholera* and *Pertussis* toxins, respectively. The 12 amino acid differences between clones 12B2 and 6A1 of Gαs are enclosed by circles. The amino acid regions that participate in the binding of GTP are overlined. The residues marked by asterisks correspond to the Gαs region proposed to be important for the adenylyl cyclase activation [18].

as conserved as the guanine nucleotide binding regions, reflecting their role in the independent modulation of different signal transduction pathways.

The proteins encoded by Gαi-1 and Gαi-3 cDNAs are potential substrates for pertussis toxin (PTX)-catalyzed ADP-ribosylation because they have a cysteine residue at the appropriate site near the carboxyl-terminus. Also the two Gαs clones contain the arginine that is modified by cholera toxin (Fig. 5, arrowheads).

Currently, we are expressing these proteins in *E. coli* and in an in vitro system in order to study some of their G-protein properties and functions.

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