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# Molecular cloning and sequence determination of a cDNA coding for the α-subunit of a $G_o$-type protein of *Xenopus laevis* oocytes

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*Xenopus laevis* oocytes are cells ideally suited to the study of signal transduction and of the G-proteins that are involved in this process. A *X. laevis* cDNA library in λgt10 has been screened with a mixture of three oligonucleotide probes designed to detect sequences found in various mammalian α-subunits of G-proteins. One of these clones has been purified through tertiary screening and the DNA insert has been sequenced. This clone was found to include the total sequence coding for a 354 amino acid protein that is 89% identical to the sequence of α-subunit of rat $G_o$. The differences with the mammalian protein were clustered in amino acids 290–315, which have been postulated to define the region interacting with the receptor and effector molecule. The homology with the α-subunits of other mammalian G-proteins is lower (65–70% to $G_i$ and 42% to $G_s$). On this basis, this clone can be classified as $G_o$-like.

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

## 1. INTRODUCTION

The transduction of many extracellular signals involves the participation of trimeric GTP-binding proteins, called G-proteins, that can interact both with the signal receptors and with effector molecules present in the cellular membrane (for reviews see [1–3]). Mammals are known to contain at least 7 different G-proteins, all of which have α-, β- and γ-subunits. The β- and γ-subunits are very similar or identical in all these G-proteins, but they contain different α-subunits. The α-subunits bind GTP and, when dissociated from β and γ, bind the effector molecules to stimulate or inhibit their activity. The mechanism for the hormonal regulation of adenylyl cyclase through stimulatory ($G_s$) and inhibitory ($G_i$) G-proteins has been established through the work of many laboratories [2]. Present attention in this field is centering on the G-proteins that regulate ion channels, phospholipases A and C, and exocytosis [4–7]. The recent cloning and sequencing of genes that code for the mammalian subunits of G-proteins have provided much insight as to their structure and function relationships [8–12].

There are several reasons for studying the G-proteins of *Xenopus laevis* oocytes. The adenylyl cyclase present in the membrane of these cells can be inhibited by progesterone in an unusual mechanism that is responsible for the induction of meiotic maturation [13–16]. This inhibition requires the participation of G-proteins but is not affected by ADP-ribosylation of membrane G-proteins by pertussis toxin which generally dampens the effect of $G_i$-mediated inhibition [17]. The mechanism is still unclear and requires a definite identification of the G-protein involved. The *X. laevis* oocyte has also become a popular system for the in vivo expression of hormonal and neuronal receptors. A number of laboratories have demonstrated that microinjection of mRNAs from different tissues leads to the synthesis of membrane-bound receptors. In such experiments the oocyte has shown an amazing versatility,

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because these cells not only express and process correctly the foreign proteins, but also can couple the newly acquired receptors to transducing systems that make them responsive to external signals [18–22]. These findings suggest that it would be possible to study in the oocyte the nature of the transducing components that are coupling to these receptors.

Since oocytes respond to acetylcholine stimulation by an increase in inositol 1,4,5-trisphosphate [23], these cells could also be used to study the G-proteins that are involved in the activation of the endogenous phospholipase C.

In this communication we report on the cloning and sequencing of cDNAs that code for the $\alpha$-subunit of a G-protein highly homologous to rat $G_o$.

## 2. MATERIALS AND METHODS

### 2.1. *cDNA library*

A cDNA library of *X. laevis* oocytes cloned in λgt10 (kindly donated by Dr D.A. Melton of Harvard University) [24] was used.

### 2.2. *Screening of the DNA library*

About $1.5 \times 10^5$ recombinant plaques were screened by plaque hybridization [25] with three synthetic probes labeled at the 5′-end with $^{32}P$. The probes used for this purpose were a 27-base oligonucleotide probe (probe A: 5′-CATTTGCTTCACAATGGTGCTTTTACC-3′) directed against a highly conserved G$\alpha$ mammalian region (Gly-Lys-Ser-Thr-Ile-Val-Lys-Glu-Met), a 57-base oligonucleotide probe (probe B: 5′-GAAGAGGCCACAGTCCTTCAGGTTGTTCTTGATGATGACGTCAGTGACGGCATCAAA-3′) directed against a sequence present in mouse macrophage G$\alpha_i$-type 2 (Phe-Asp-Ala-Val-Thr-Asp-Val-Ile-Ile-Lys-Asn-Asn-Leu-Lys-Asp-Cys-Gly-Leu-Phe) and a 48-base probe (probe C: 5′-CATCATGGCAGAAAGCAGTTCTGCAGAGAATGGTTCAGTGTCCTCCAT-3′) directed against a sequence present in rat heart G$\alpha_o$ (Met-Glu-Asp-Thr-Glu-Pro-Phe-Ser-Ala-Glu-Leu-Leu-Ser-Ala-Met-Met) [26]. Phages from three positive lytic plaques from the first screening round were plaque-purified through secondary and tertiary screening. One of these clones was fully sequenced. Hybridizations were done overnight at 45°C in a solution containing 6 × SSC (1 × SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7), 5 × Denhardt's solution (1 × = 0.02% BSA; 0.02% polyvinyl pyrrolidone; 0.02% Ficoll), 300 mM sodium phosphate, pH 6.8, 100 μg of heat-denatured salmon sperm DNA per ml and 0.1% SDS. Filters were washed three times at 45°C in 6 × SSC and then twice at 50°C in 6 × SSC for 15 min, before autoradiography. Films were exposed overnight at room temperature. Southern blots were done as described [27]. Nitrocellulose membranes were washed three times at 55°C for 15 min and exposed between 1 and 3 h at room temperature.

### 2.3. *DNA sequence analysis*

Nucleotide sequences were determined by using the phage M13mp19 and the dideoxynucleotide chain-termination method [28] as previously described [11].

## 3. RESULTS

### 3.1. *Screening of the* Xenopus laevis *oocyte cDNA library with a mixture of three synthetic probes*

A *Xenopus laevis* oocyte cDNA library cloned in λgt10 was screened using a mixture of three synthetic oligonucleotide probes complementary to conserved regions of different $\alpha$-subunits of G-proteins (see section 2). Approx. $1.5 \times 10^5$ plaques were screened yielding 25 positive clones. Three of these clones, XL$\alpha$310, XL$\alpha$811 and XL$\alpha$114 were chosen and carried through secondary and tertiary screening. DNA was prepared from these three clones, blotted onto nitrocellulose membranes and hybridized, separately in this case, to each of the labeled synthetic oligonucleotide probes. The 27-base probe A, which contains a sequence highly conserved in all $\alpha$-subunits of G-proteins, hybridized to all 3 cDNA clones. The 57-base probe B, which is present in mouse type 2-$\alpha_i$ subunit hybridized strongly with XL$\alpha$310 and XL$\alpha$811; the 48-base probe C directed against an $\alpha_o$ sequence, however, only hybridized strongly to the XL$\alpha$114 clone.

The clone XL$\alpha$114 was analyzed further by nucleotide sequencing of the cDNA insert following the strategy presented in fig.1.

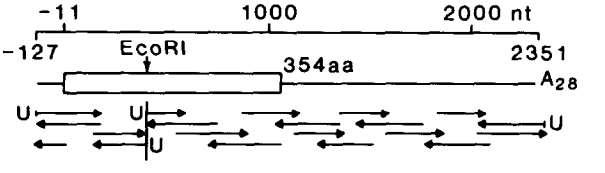


Fig.1. Restriction endonuclease map and sequencing strategy for the *X. laevis* G$\alpha_o$-like cDNA. The top scale indicates cDNA length in nucleotides. The open box shows the open reading frame (ORF) for the protein. The thin black lines show the 5′- and 3′-untranslated regions of the mRNA. The arrows indicate extent and direction of sequencing obtained with individual oligonucleotide primers. The cDNA insert from λXL$\alpha$114 was sequenced in its complete length, after subcloning its two *Eco*RI fragments in both directions into M13mp19. U, universal M13 primer used to begin the sequence walks [11].

### 3.2. *Nucleotide sequence of the Gα$_o$-type cDNA clone*

Fig.2 shows the nucleotide sequence of the cDNA insert of clone XLα114. This figure shows the translation of this sequence into an open reading frame that generates a 354 amino acid protein (40284 Da). The assignment of the initiation methionine codon is based on the existence of an in frame TAA stop codon in position −87 of the 5′-flanking sequence of the XLα114 cDNA insert. The sequence contains an internal *Eco*RI site at position 414 and predicts an mRNA with a 3′-untranslated region of 1269 nucleotides followed by a poly(A) tail.

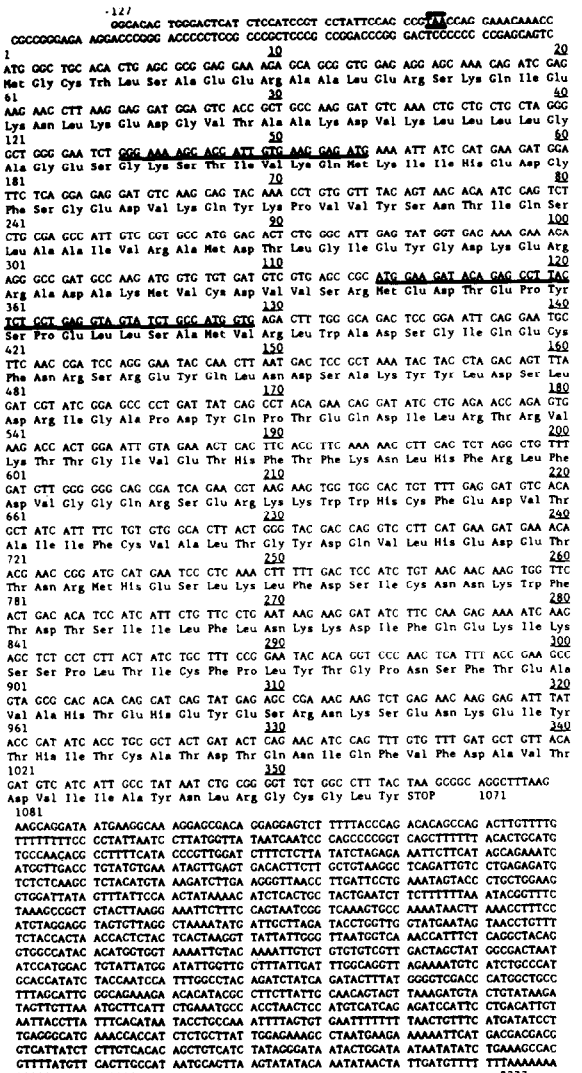

Fig.2. Nucleotide and predicted amino acid sequence of the cDNA insert from XLα114 (Gα$_o$). Numbers indicate the positions of nucleotides or amino acid residues starting at the initiation codon. Nucleotide and amino acid sequences recognized by the rat heart α$_o$ probe (48-base) and by the common 27-base probe are underlined.

The deduced amino acid sequence revealed a high degree of identity (89%) with that of a rat Gα$_o$ reported previously [29], as can be seen in fig.3. The differences from the mammalian sequences are scattered throughout the protein but are specially concentrated in the area of the 290–315 amino acids. Most of the changes, however, are conservative. On the other hand, there are many regions in which the identity between the two proteins is nearly perfect. The 5′- and 3′-non-coding sequences of the *X. laevis* clone are quite different from their mammalian counterparts [9,29].

## 4. DISCUSSION

The use of 3 mixed synthetic oligonucleotides has enabled us to detect 25 cDNA clones putatively coding for α-subunits of *X. laevis* G-proteins. This strategy uses the fact that the oligonucleotide mixture contains a 27-mer complementary to a sequence highly conserved in all known α-subunits of G-proteins (probe A) and two oligonucleotides designed to interact with the specific regions of Gα$_o$ (48-mer, probe C) and Gα$_i$ (57-mer, probe B).

The determination of a nucleotide sequence of one of the cDNAs cloned in this fashion has confirmed its identity as the α-subunit of G-protein and allows us to make comparisons with the sequence of the α-subunits of known mammalian G-proteins.

The first conclusion is that the degree of conservation of the sequences of the amphibian and mammalian α-subunits is very high indeed. This suggests that this amphibian protein is also fulfilling a very similar function in membrane signal transduction, as has been postulated for its mammalian counterparts. More detailed comparison with the various mammalian sequences allows a tentative identification of the clone obtained. The XLα114 clone has a sequence that is clearly closer to the mammalian Gα$_o$ (89% amino acid sequence identity) than to other α-subunits (65–70% identity to the three different Gα$_i$ subunits and only 42%

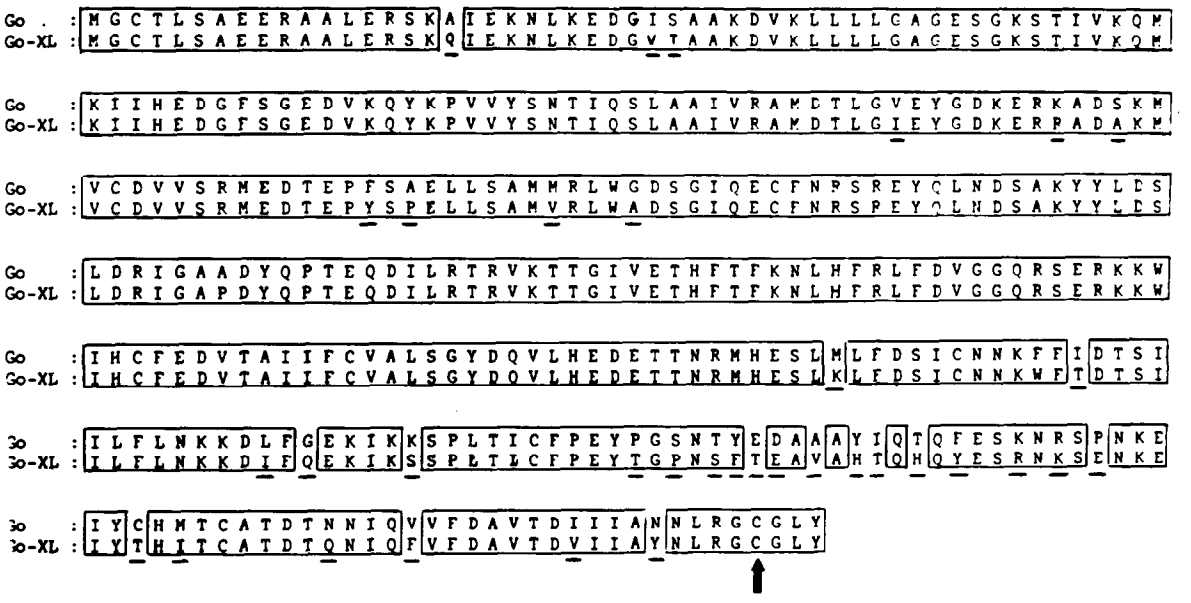

Fig.3. Alignment of the predicted amino acid sequence of *X. laevis* $G\alpha_o$ with those of rat $G\alpha_o$ [9,26]. Amino acid residues are presented by standard one-letter symbols. The residues in *X. laevis* $G\alpha_o$ that are not homologous to the corresponding residues at the same position in the rat $G\alpha_o$ are underlined. The arrow indicates the site of ADP-ribosylation by pertussis toxin.

identity to rat $G\alpha_s$). On this basis we can consider the XL$\alpha$114 sequence to be '$G\alpha_o$-like'. Unfortunately the function of $G_o$ proteins has not been clearly defined. It is interesting, however, that the differences between the *X. laevis* and rat sequences are clustered in a region (290–315 amino acids) close to the carboxyl-end, which contains the region that has been postulated to interact with receptors [30,31] as well as effectors [32]. These differences could indicate that the *X. laevis* sequences may represent a new class of $\alpha_9$ subunit. The common regions which contain the GTP-binding sites in other $\alpha$-subunits are also conserved in the amphibian sequences.

The *X. laevis* G-protein $\alpha$-subunit terminates at the carboxyl-end with the sequence CGLY in which the conserved cysteine is the ADP-ribose acceptor site in the reactions catalyzed by pertussis toxin [29,33]. This observation is pertinent because we have previously shown that the oocyte plasma membrane contains a substrate for pertussis toxin ADP-ribosylation [17]. Under special conditions of polyacrylamide gel electrophoresis of membrane proteins ADP-ribosylated by pertussis toxin, we have observed two closely migrating bands which may correspond to $G_i$ and $G_o$ $\alpha$-subunits (unpublished results). Endogenous substrates for pertussis toxin ADP-ribosylation have also been observed by Kaneko et al. [22]. In this regard it is interesting to note that ADP-ribosylation of oocyte membrane proteins by pertussis toxin does not abolish the inhibitory effect of progesterone on the oocyte adenylyl cyclase [17].

There has been some controversy about the presence of transducing proteins in *X. laevis* oocytes. Some groups claim that all the components of the transducing machinery are absent in the oocytes and are only present in the surrounding follicle cells [34–36]. This conclusion was based on the observation that oocytes denuded of follicle cells by collagenase treatment do not respond to some agonists. This claim was countered by a large number of experiments in which microinjection of purified mRNAs for different receptors resulted in functional signal transduction through the newly synthesized receptors, indicating that oocytes possessed all the other components required for transduction [21,37,38]. The finding described here that demonstrates the isolation of a clone of cDNA coding for a G-protein in a cDNA library constructed with an mRNA preparation obtained from denuded oocytes [24] should help to resolve

this controversy.

Preliminary sequencing experiments with some of the other isolated clones indicate the presence of two different $\alpha_i$-type subunits and one $\alpha_s$ subunit.

The cloning of the $\alpha$-subunits of the G-proteins of the very versatile oocyte system should be helpful in attempts to elucidate the different functions of the various components of G-protein family that are present in these cells.

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