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Molecular cloning of a new human G protein

Evidence for two $G_{i\alpha}$-like protein families

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The amino acid sequence of a novel G protein α subunit ($G_{x\alpha}$) has been deduced from the nucleotide sequence of a human cDNA clone isolated from a differentiated HL-60 cDNA library. The cDNA encodes a polypeptide of 354 amino acids (M_r 40 519) which is closely related to $G_{i\alpha}$ proteins. The amino acid sequence homology between $G_{x\alpha}$ and human myeloid $G_{i\alpha}$ is 86% with 15 nonconservative substitutions. $G_{x\alpha}$ also shares 86% homology with both rat brain and mouse macrophage $G_{i\alpha}$ but is more homologous (94%) to bovine brain $G_{i\alpha}$ with only 5 nonconservative amino acid differences. G proteins previously termed $G_{i\alpha}$ may fall into at least two distinct groups, with one including human myeloid $G_{i\alpha}$, rat brain $G_{i\alpha}$ and mouse macrophage $G_{i\alpha}$; and other $G_{x\alpha}$ and bovine brain $G_{i\alpha}$. One group probably contains true G_i and the other a new class of G protein whose function remains to be determined.

Guanine nucleotide regulatory protein; Receptor; Stimulus response

1. INTRODUCTION

Guanine nucleotide-binding (G) proteins play a critical role in transmembrane signalling. G proteins are composed of three subunits: α, β and γ. α-subunits show the greatest structural variability and are thought to determine specificity of G protein function. Distinct groups of G proteins have been functionally characterized and include G proteins that mediate the activation (G_s) and inhibition (G_i) of adenylate cyclase [1], transducin which couples rhodopsin to a cGMP phosphodiesterase [2], G_o, whose effector is unknown [3] but may be coupled to muscarinic receptors [4,5], and G proteins which couple receptors to phospholipase C, for which biochemical evidence exists [6–9] but which have not yet been structurally identified. Recent results have shown there to be two types of transducin, one that functions in retinal rods and the other in retinal cones [10,11], and multiple forms of $G_{s\alpha}$ subunits [12,13]. Gathering evidence now suggests that there may be multiple forms of $G_{i\alpha}$ [14,15], however definite proof is lacking. We report here the cloning of a cDNA encoding an α-subunit of a novel human G protein. The amino acid sequence is highly homologous but distinct from a previously identified human $G_{i\alpha}$ [26] and may represent a second member of a $G_{i\alpha}$-like gene family.

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2. MATERIALS AND METHODS

Total cellular RNA from HL-60 cells differentiated with dibutyryl cyclic AMP for 48 h was extracted as described [16] and poly(A) RNA for cDNA library construction was isolated by two

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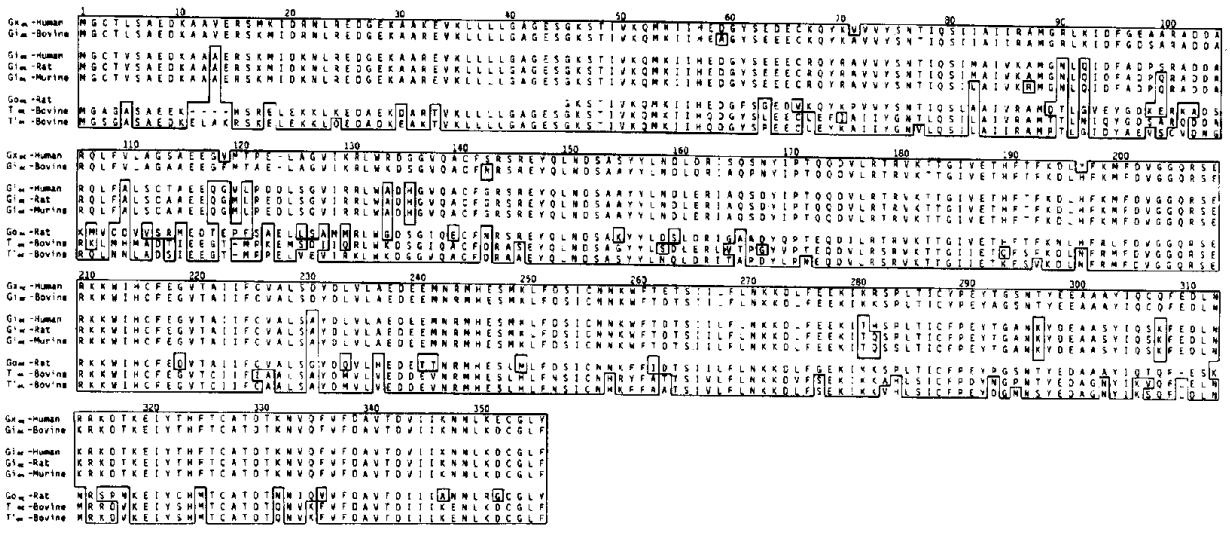

Fig.2. Comparison of amino acid sequences of G protein α-subunits. The amino acid sequences (single letter notation) of human $G_{s\alpha}$, human $G_{i\alpha}$ [26], bovine $G_{i\alpha}$ [14], rat $G_{i\alpha}$ [15], and $G_{o\alpha}$ [15], and bovine transducins T_{α} [10] and T'_{α} [11]. Boxed are sets of identical or conservative residues.

considerably less similar to the above $G_{i\alpha}$s and to each other (longest sequence of 80% or more homology being 14 bp). Characterizing these two classes of $G_{i\alpha}$-like proteins in terms of specificity of function is clearly necessary. It is likely that one of these classes is truly G_i and regulates adenylate cyclase. The other, or an as yet unidentified class of $G_{i\alpha}$-like protein will possibly be the one regulating polyphosphoinositide breakdown.

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