A novel 100 kDa protein, localized to receptor-enriched endosomes, is immunologically related to the signal-transducing guanine-nucleotide-binding proteins G_t and G_i

Linton M. TRAUB,* W. Howard EVANS† and Ronit SAGI-EISENBERG*‡

*Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel, and †National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

Antisera raised against the C-terminus decapeptide of the α -subunit of the retinal guanine-nucleotide-binding protein (G-protein) transducin (G_t) cross-reacted with the α -subunit of the inhibitory G-protein G_i . The same antisera also reacted with a 100 kDa protein (p100) found in rat liver homogenates. The immunoreactivity of both G_t and p100 was specifically inhibited by the immunizing peptide with similar dose-dependencies [concn. causing 50 % inhibition (IC_{50}) = 300 ng/ml]. This similarity in inhibition profiles implies that p100 contains within its structure the C-terminal sequence shared by both α_t and α_i . Tissue distribution studies demonstrated that p100 was particularly enriched in the liver and kidney, but was also present in other rat tissues, as well as in a number of cell lines tested. In the liver, p100 was found in both the soluble and membrane fractions. The membrane-associated form of p100 was specifically localized to an endosomal fraction (termed D-R), previously shown to be a ligand-free but receptor-enriched subfraction of liver endosomal vesicles. Two-dimensional gel electrophoresis revealed that both the cytosolic and membrane-bound forms of p100 occurred as a series of 100 kDa polypeptides with considerable charge heterogeneity (pI 6–7). Because the C-terminus domains of both α_t and α_i facilitate their association with their respective receptors, this region has been functionally assigned as the receptor binding site. Therefore the presence of an immunologically similar region within p100, together with its localization to the receptor-rich endocytic vesicles, suggests that p100 may be a receptor binding protein involved in receptor trafficking.

INTRODUCTION

Of the three subunits which comprise the signal-transducing guanine-nucleotide-binding proteins (G-proteins), the α -subunit is pivotal in receptor–effector coupling [1]. It is the α -subunit that interacts with the receptor system, exchanging bound GDP for GTP. The GTP-bound α -subunit then activates the relevant effector system and, finally, hydrolyses the bound GTP to GDP to terminate the cycle. The structural regions of the α -subunit involved in each of these different functional processes still remain uncertain. However, recent data have implicated the C-terminus domain of the α -subunit in its interaction with the receptor. A role for the C-terminal end of the α -subunit was first suggested by studies utilizing pertussis toxin [2]. Mono-ADP-ribosylation of susceptible α -subunits (the retinal G-protein G_t and the inhibitory G-protein G_i) by this toxin uncouples the receptor–G-protein interaction. The site of this ADP-ribosylation is a cysteine residue located within the terminal five amino acids of both α_t and α_i [3].

Antibodies directed against the C-region of the α_t -subunit have provided more direct evidence for its role in receptor coupling. A monoclonal antibody, mAb 4A, blocks the process of phototransduction by inhibiting the association of G_t with photolysed rhodopsin [4]. mAb 4A maps to amino acids 311–328 of α_t , within 50 amino acids of the C-terminus [5]. mAb 4A can also block the pertussis-toxin-mediated ADP-ribosylation of G_t , and synthetic peptides corresponding to that region of α_t which is recognized by mAb 4A effectively compete with G_t for interaction with photolysed rhodopsin [6]. Analogous findings have been made with similar antisera (AS/6 and AS/7), raised against a synthetic peptide corresponding to the last 10 amino acids of α_i [7]. Thus AS/7 inhibits the rhodopsin-stimulated

GTPase activity of G_t , again by blocking the association between rhodopsin and α_t [8]. These studies underscore the crucial importance of the C-terminus domain of α_t in the coupling process.

Because of the high degree of sequence homology evident within the G-protein family both mAb 4A and AS/7 cross-react immunologically with α_i [7,9]. Furthermore, the uncoupling effects of both of these antibodies are also observed in receptor– G_i interactions [9,10]. Taken together, these studies indicate that the C-terminus domain of the α -subunit endows the G-protein, in part, with the ability to interact with its cognate receptor [11]. This is true not only for rhodopsin– G_t [4,8] and opiate receptor– G_i [10] interactions, but for the β -adrenergic receptor– G_s interaction as well [12,13]. Significantly, the only other protein known to date to exhibit sequence similarity with the C-terminus decapeptide of α_t is arrestin [14]. Arrestin, a retinal protein, functions in the desensitization process of visual responses. It is believed to operate, in part, by competition with G_t for rhodopsin binding by virtue of this shared sequence [15]. Recently we have detected a protein which exhibits similarity to the C-terminal decapeptide sequence of α_t . Given the importance of this region, we have begun to examine this protein in detail. In this paper we describe the identification and distribution of this protein, and discuss its possible role in the light of its subcellular distribution and the importance of this common sequence.

MATERIALS AND METHODS

Materials

Bovine retinal rod outer segment (ROS) membranes and affinity-purified AS/6 and AS/7 antibodies were generously

Abbreviations used: G-protein, guanine-nucleotide-binding protein; ROS, retinal rod outer segment; mAb, monoclonal antibody; IC_{50} , concentration causing 50 % inhibition.

‡ To whom correspondence should be addressed.

provided by Dr. A. Spiegel (National Institutes of Health, Bethesda, MD, U.S.A.). Antisera KE/2 and KE/3 were raised as described for AS/6 [7]. The synthetic decapeptide corresponding to the C-terminus of α_i was synthesized by the Chemical Services Department of The Weizmann Institute of Science. A synthetic peptide of residues 364–374 of the δ -subunit of the acetylcholine receptor was kindly provided by Professor S. Fuchs (The Weizmann Institute). Iodinated second antibodies were either 125 I-labelled donkey anti-(rabbit IgG) from Amersham, or sheep anti-(rabbit IgG) from Bio-Makor, iodinated by the chloramine-T method. Pharmalytes were from Pharmacia. Nitrocellulose was from Schleicher and Scheull, and RP 2 X-ray film was from Agfa. All other reagents were analytical grade.

Preparation of subcellular fractions

Sprague-Dawley rats, approx. 3 months old, were used for all experiments. In all cases the animals were killed by cervical dislocation and the relevant tissues were rapidly removed and placed in ice-cold buffer A (20 mM-Tris/HCl, pH 7.5, 250 mM-sucrose, 2 mM-EDTA, 2 mM-EGTA, 10 mM-2-mercaptoethanol and 1 mM-phenylmethanesulphonyl fluoride), weighed and coarsely minced with scissors. All subsequent procedures were performed at 0–4 °C. The tissue was homogenized in a Dounce homogenizer with 8–10 strokes of a loose-fitting pestle. Homogenization was in 5 vol. of buffer A/g of tissue to yield a crude tissue homogenate.

To prepare the membrane and cytosolic fractions, the crude homogenate was centrifuged at 100 000 *g* for 60 min. The resultant supernatant represented the cytosol, while the pellet was resuspended in buffer A as the membrane fraction. Rat liver cytosol was further fractionated by the addition of various amounts of a saturated solution of $(\text{NH}_4)_2\text{SO}_4$ (pH 7.5). The additions were carried out over 30 min at 0–4 °C with continuous stirring. After an additional 30 min of stirring, the extract was centrifuged at 15 000 *g* for 20 min to collect the precipitated protein. The 45–60%-(NH_4)₂SO₄ fraction was resuspended in buffer B (20 mM-Tris/HCl, pH 7.5, 100 mM-KCl, 1 mM-EDTA and 10 mM-2-mercaptoethanol) and dialysed against buffer B overnight at 4 °C.

The basic subcellular fractionation of rat liver was carried out as described by Graham [16]. The fractionation of the various liver plasma membrane fractions, the Golgi fractions and the endosomal fractions was as described in detail previously [17,18]. The DN-2 'early' endosomes (density 1.090 g/ml) exhibit peak ligand incorporation at 2–3 min, and the DN-1 'late' endosomes (density 1.115 g/ml) show peak ligand incorporation at 10–15 min after ligand administration. The third endosome fraction, which is not associated with internalized ligands, but is rich in receptors, is termed the D-R 'receptor-rich' endosome fraction. Fao, RBL-2H3 and K562 cells were maintained as described previously [19]. Cytosol and membrane fractions from the sonicated cell lysates were prepared by centrifugation at 100 000 *g* as described above. The protein concentration of all of the various fractions was determined by the method of Bradford [20] using BSA as the standard.

Electrophoresis and immunoblotting

SDS/PAGE was performed as described by Laemmli [21]. Two-dimensional electrophoresis was performed as described by O'Farrell [22], as modified by Duncan & Hershey [23]. For the first dimension, 3.5% polyacrylamide rods, containing 2% pharmalytes 3–10 and 5–8 (1:1) were used, and focusing was for 12 000 V·h. The second dimension was run on standard SDS/10% polyacrylamide gels. Gels were either stained with Coomassie Blue R250 or transferred to nitrocellulose in 15.6 mM-Tris/120 mM-glycine at 200 mA for 3 h, as described by Gershoni

[24]. After transfer, the proteins and the molecular mass markers were visualized by brief staining of the blots in 0.5% Ponceau S, followed by complete destaining in 10 mM-Tris/HCl, pH 8.0, 150 mM-NaCl and 0.05% Tween 20 (TBST). The membranes were then incubated with 1% BSA in TBST for 4–5 h to block non-specific binding sites. The membranes were then incubated with pre-immune serum, antiserum or antiserum plus peptide at the indicated dilutions for 5–12 h at room temperature. All sera were diluted in 0.1% BSA in TBST, except when peptide was also present, when 1% BSA was used. The blots were then washed 4 times with TBST and incubated with 125 I-labelled anti-(rabbit IgG) (0.2 μ Ci/ml) for 1–2 h. The membranes were washed four times with TBST and twice with distilled water. The immunoreactive bands were visualized by autoradiography at –80 °C. For quantitative analysis, the regions of the blot corresponding to the labelled bands were excised and counted for radioactivity directly in a γ -scintillation counter. For the experiment presented in Fig. 3, KE/3 antiserum was affinity-purified on α_i immobilized on nitrocellulose as originally described by Olmsted [25], as modified by Burke *et al.* [26].

RESULTS AND DISCUSSION

Antiserum AS/6 was generated against a synthetic decapeptide (KENLKDCGLF) corresponding to the C-terminal end of α_i [7]. As expected, on Western blots the affinity-purified antibodies reacted with a 39 kDa protein present in ROS membranes (Fig. 1, lane h). This immunoreactivity aligned with the Coomassie Blue-stained α -subunit of G_i (lane a), confirming its identity as α_i . Because the C-terminus sequence of α_i has a high degree of sequence homology with α_{i1} and α_{i2} , AS/6 also detected α_i present in rat brain membranes (lane i) [7,27,28]. This antiserum does not recognize α_s , α_o or α_x , all of which show considerable divergence from the C-terminal sequence of α_i [27]. In brain the immunoreactivity was confined to the membrane fraction, and no proteins were detected in the cytosolic fraction (lane j). In liver the level of G_i is at least 5-fold lower than in brain [29], and the major form of G_i present, G_{i3} , is not recognized efficiently by AS/6 [27,28]. Therefore, in the crude liver homogenate and

Fig. 1. Detection of immunoreactive proteins with affinity-purified AS/6 antibodies

ROS membranes (5 μ g; lanes a and h) and 20 μ g each of rat brain membranes (b and i), rat brain supernatant (c and j), rat liver homogenate (d and k), liver membranes (e and l), liver supernatant (f and m) and a 45–60%-(NH_4)₂SO₄ cut of liver supernatant (g and n) were separated on a 10% gel and blotted as described in the Materials and methods section. Lanes a–g are from the Coomassie Blue-stained gel and lanes h–n are an autoradiograph of a replicate blot probed with affinity-purified AS/6 antibodies (10 μ g/ml). Molecular masses of standard proteins are indicated on the left.

membranes, α_i immunoreactivity was not discernible (lanes k and l). Purified liver plasma membranes, probed with AS/6, show higher levels of α_i reactivity (results not shown).

Affinity-purified AS/6 did detect two additional proteins present in the liver homogenate. The first, a protein of 42 kDa, was distinguished from G_i by its slower migration and its entirely soluble location (lane m). The second immunoreactive protein observed in the liver homogenate, a protein of 100 kDa (p100), was found on fractionation to be distributed between the membrane and cytosolic fractions (lanes l and m). A lower band of 95 kDa was also detected in the homogenate, but it appeared to be confined to the membrane fraction. However, this lower band has been observed variably in several different preparations, and we assume it to represent a degradation product of p100. The cytosolic form of p100 was selectively precipitated in a 45–60%-(NH₄)₂SO₄ fraction (lane n), resulting in a 3–5-fold enrichment of the p100 immunoreactivity.

The specificity of the immunoreactivity is shown in Fig. 2a. For these and the following experiments, we used a second series

of antisera, KE/2 and KE/3. These were raised by a protocol identical with that described for AS/6 using the same immunizing peptide. The reactivities of sera AS/6 and of KE/2 and KE/3 were comparable. Pre-immune KE/3 serum did not react with any of the proteins in ROS (lane c), but recognized two proteins present in the 45–60%-(NH₄)₂SO₄ fraction of liver cytosol (lane d). Immune serum from the same rabbit showed only a single new immunoreactive protein in each preparation. In ROS (lane e), a protein of 39 kDa, the α -subunit of G_i , was recognized. In liver (lane f), a 100 kDa protein, p100, was detected by the immune serum. Addition of the immunizing peptide inhibited binding in a dose-dependent manner (lanes g–n), and at 5 μ g/ml it almost abolished the immunoreactivity of both α_i (lane m) and p100 (lane n). The reactivity characteristic of the pre-immune serum was not affected by the inclusion of the peptide (lanes g–n). Inclusion of an irrelevant peptide (corresponding to amino acids 364–374 of the δ -subunit of the acetylcholine receptor) had little effect on the immunoreactivity of either α_i (lane o) or p100 (lane p). A quantitative analysis of these results showed that the two proteins had an almost identical dose-dependency for the inhibition of immunoreactivity by the synthetic peptide (Fig. 2b). The concentration causing 50% inhibition (IC₅₀) for both α_i and p100 was approx. 300 ng of peptide/ml. The findings that different antisera showed the same reactivity with both α_i and p100, and that the same degree of inhibition of this immunoreactivity by the immunizing peptide was observed for both proteins, confirmed the presence of a similar region in α_i and p100. The region of similarity corresponds to the C-terminus domain of both α_i and α_o , a region that plays a vital role in receptor–G protein coupling [4,8,10,12,13].

Because p100 was found to be distributed between both the membrane and cytosolic fractions, we analysed its subcellular distribution in greater detail. p100 immunoreactivity was absent in both 3000 g nuclear and 10000 g mitochondrial pellets (results not shown). The immunoreactivity was recovered in the 100000 g microsomal pellet and the 100000 g supernatant, consistent with our finding p100 in both membrane and cytosolic fractions in liver. In order to define more precisely the membranes with which p100 was associated, we extended our analysis of the subcellular distribution of p100. The additional samples analysed were purified plasma membranes, derived from the major functional regions of the hepatocyte, as well as Golgi and endocytic vesicle fractions. Affinity-purified KE/3 serum detected a protein of 40 kDa in the canalicular (Fig. 3, lane a), sinusoidal (lane b) and lateral (lanes c) plasma membrane fractions. As KE/3 was raised against α_i , and cross-reacts with α_o , this band represented the α -subunit of G_i present in these membranes. The α_i immunoreactivity was also noted in the DN-1 (lane d) and DN-2 (lane e) endosomal fractions and the 'heavy' Golgi fraction (lane i). This is in excellent agreement with the previous study on the subcellular distribution of G_i in liver [30].

Interestingly, the immunoreactivity of the membrane-associated form of p100 was restricted to the endosomal fractions. The endocytic compartment of the hepatocyte is a complex vesiculo-tubular structure [17,31,32]. In our previous study on rat liver [18] three endosomal subfractions were isolated. Two of these were designated 'early' and 'late', on the basis of the timing of their uptake from the circulation of a variety of radiolabelled ligands (insulin, transferrin and prolactin). An additional endosomal fraction was also isolated. This was designated a receptor-rich (D-R) fraction, as it also contained receptors (insulin and asialoglycoprotein), but did not appear to be associated with the radiolabelled ligands endocytosed by the liver. It was suggested that these vesicles were involved in returning the internalized receptors to the cell surface. Of these three endosomal fractions, p100 immunoreactivity was found in

Fig. 2. Peptide inhibition of α_i and p100 immunoreactivity

(a) ROS (2.5 μ g; lanes a, c, e, g, i, k, m and o) and 20 μ g of a 45–60%-(NH₄)₂SO₄ fraction of rat liver cytosol (lanes b, d, f, h, j, l, n and p) were separated on a 10% gel and transferred to nitrocellulose. Lanes a and b are from the Coomassie Blue-stained gel and lanes c–p are autoradiographs of replicate blots probed with a 1:250 dilution of pre-immune KE/3 serum (c and d) or a 1:250 dilution of immune KE/3 serum (e–p). Additionally, the blots contained concentrations of 100 ng/ml (g and h), 500 ng/ml (i and j), 1 μ g/ml (k and l) and 5 μ g/ml (m and n) of the immunizing peptide (KENLKDCGLF). Lanes o and p contained an irrelevant peptide (GYISKAQEYFN) (5 μ g/ml). Molecular masses of standard proteins are indicated on the left. (b) Quantitative analysis of the peptide inhibition. Immunoreactivity of α_i (○) and p100 (□) in the presence of different concentrations of the immunizing peptide. ● and ■ are the immunoreactivity of α_i and p100 in the presence of the irrelevant peptide.

Fig. 3. Distribution of p100 within purified plasma membranes, endosome and Golgi fractions

Samples of 50 μ g each of rat liver canalicular (lane a), sinusoidal (b) and lateral (c) plasma membranes (PM); DN-1 'late' (d), DN-2 'early' (e) and D-R receptor-enriched (f) endosomal fractions; and Golgi 'light' (g), 'intermediate' (h) and 'heavy' (i) fractions were resolved on a 10% gel and blotted on to nitrocellulose. An autoradiograph of the blot, probed with a 1:10 dilution of affinity-purified KE/3 antibodies, is presented. Molecular masses of standard proteins are indicated on the left.

the receptor-enriched (D-R) endosomes (Fig. 3, lane f). p100 was not observed in the 'late' (DN-1) endosome fraction (lane d) or in the 'early' (DN-2) endosome fraction (lane e). Very little is known about the non-receptor proteins that occur within endocytic membranes, and except for a 115 kDa calmodulin-binding protein [33] and a 60 kDa glycoprotein [34], both located in the DN-1 and DN-2 endosomal fractions, no other endocytic proteins have been identified. This is, therefore, the first

identification of a non-receptor protein that is specifically associated with the D-R endosomal fraction.

An analysis of the soluble and endosome-associated forms of p100, separated by two-dimensional gel electrophoresis and then probed with KE/3 antiserum, is shown in Fig. 4. The soluble form of p100, present in the 45–60%-(NH₄)₂SO₄ fraction, migrated as a series of four 100 kDa polypeptides with considerable charge heterogeneity (differing in pI by as much as one pH unit; Fig. 4b). The two other major immunoreactive bands seen in Fig. 4(b) were those components recognized by the pre-immune serum, as noted in Fig. 2(a). The Coomassie Blue-stained protein spots corresponding to the immunoreactivity are marked with arrows in Fig. 4(a). The stained gel showed the majority of the proteins to have focused into discrete single spots. Therefore the multiple-isoform property of p100 was not an artifact of the gel system. A similar analysis of the D-R endosome fraction again showed a series of immunoreactive charge isoforms (Fig. 4d). The electrophoretic mobility of the immunoreactive proteins in the D-R fraction was identical with that of the soluble form of p100. This confirmed the identity of p100 in the endosome fraction, and furthermore showed no differential localization of the various isoforms between the cytosol and the membrane fractions. The major isoform in both had a pI of approx. 7.0 (in 9 M-urea), with the remaining forms being successively more acidic. We do not yet know if this charge heterogeneity is due to post-translational modification (e.g. phosphorylation or ADP-ribosylation) or whether p100 is actually a family of immunologically related proteins, each the product of a distinct gene.

Serum KE/2 was used to assess the relative abundance of p100 immunoreactivity in several different rat tissues. p100 immunoreactivity was not detectable in all of the tissues examined (Fig. 5a). Homogenates of both liver (lane g) and kidney (lane h) showed an abundance of 100 kDa immunoreactivity which aligned with p100 present in the 45–60%-(NH₄)₂SO₄ fraction of

Fig. 4. Two-dimensional electrophoresis of p100-containing fractions

Samples of 50 μ g of a 45–60%-(NH₄)₂SO₄ fraction of liver supernatant (a and b) or 25 μ g of the purified D-R endocytic vesicles (c and d) were separated by two-dimensional electrophoresis and stained with Coomassie Blue (a and c) or transferred to nitrocellulose (b and d). Autoradiographs of the blots, probed with a 1:100 dilution of KE/3 serum (b and d), are shown. The gels are orientated with the basic end on the left. The arrows in (a) and (c) indicate the stained protein spots that correspond to the p100 immunoreactivity seen in (b) and (d). Molecular masses of the standard proteins are indicated on the left.

Fig. 5. Cellular distribution of p100

(a) Samples of 30 μ g of each of the 45–60% $(\text{NH}_4)_2\text{SO}_4$ fraction of liver cytosol (lane a) or brain (b), lung (c), thymus (d), heart (e), spleen (f), liver (g), kidney (h), testes (i) or skeletal muscle (j) homogenates were separated on a 10% gel and transferred to nitrocellulose. An autoradiograph of the blot, probed with a 1:200 dilution of KE/2 antiserum, is presented. Only the relevant region of the autoradiograph is shown. Molecular masses of the relevant standard proteins are indicated on the left. (b) Samples of 20 μ g each of membrane (a–c) and cytosolic (d–f) fractions of Fao rat hepatoma (a and d), K562 human erythroleukemia (b and e) and RBL rat basophilic leukaemia (c and f) cells were separated on a 10% gel and blotted. An autoradiograph of the blot, probed with affinity-purified AS/7 antibodies (10 μ g/ml), is presented. Only the relevant region of the autoradiograph is shown. Molecular masses of the relevant standard proteins are indicated on the left.

liver cytosol (lane a). The thymus also showed high levels of p100 (lane d), and brain (lane b), spleen (lane f) and testes (lane i) all exhibited p100 immunoreactivity, although to a lower extent. Lung, heart and skeletal muscle homogenates (lanes c, e and j) did not contain detectable levels of p100. p100 was also detected in a number of cell lines derived from different origins (Fig. 5b). These included the rat-derived Fao hepatoma (lanes a and d) and RBL-2H3 basophilic leukaemia (lanes c and f) and the human K562 erythroleukemia (lanes b and e) cell lines. As in liver, p100 was distributed between the membrane (lanes a–c) and cytosolic (lanes d–f) fractions. The 95 kDa immunoreactive protein present in Fao cells is probably the p100 degradation product noted above.

The cellular function of p100 is presently unknown. However, p100 differs from the heterotrimeric G-proteins in several respects. The data presented here show that, unlike G_i , p100 was completely absent from the purified plasma membrane fractions. Our two-dimensional electrophoretic analysis further revealed that p100 occurred as a series of charge isoforms, a property not observed for the heterotrimeric G-proteins [35]. Also, the presence of 9 M-urea in these experiments ruled out the possibility that p100 was a heterotrimeric G-protein not dissociated under the conditions that we used for one-dimensional electrophoresis. p100 is not likely to be an α -subunit precursor either, as all of the encoding cDNAs isolated to date code for proteins equivalent in size to the mature proteins. In addition, we have found that p100 is not recognized by antibodies raised against a synthetic peptide corresponding to a highly conserved region of the GTP-binding domain of the α -subunit (results not shown). Therefore, although p100 exhibits immunological identity with the C-terminus of both G_i and G_o , it does not appear to be a classical α -subunit.

Finally, we have recently obtained a partial amino acid sequence of the N-terminal end of p100. A search of a protein data base for related sequences failed to identify any protein with substantial sequence similarity to this N-terminus region of p100. This confirmed that p100 is a novel protein. Our specific localization of p100 to the receptor-rich D-R endosomal fraction raises the possibility that p100 plays a role in the process of endocytosis. Although no direct proof of this is presented here, the plausible presence of a putative receptor binding site within p100 favours the hypothesis that p100 may be involved in the process of sorting and receptor trafficking through the endosomal compartment of the cell.

We thank Dr. Yehiel Zick for most helpful discussions and critical reading of this manuscript, Dr. Allen Spiegel for providing the AS/6 and AS/7 antibodies, and Ms. Esther Gross for excellent secretarial assistance. This work was supported by the Israel Cancer Research Fund (ICRF), the Minerva Foundation and Yeda Fund. R.S.-E. is an incumbent of the Charles H. Revson Career Development Award.

REFERENCES

- Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56**, 615–649
- Van Dop, C., Yamanaka, G., Sternberg, F., Sekura, R., Manclark, C. R., Stryer, L. & Bourne, H. R. (1984) *J. Biol. Chem.* **259**, 23–26
- Milligan, G. (1988) *Biochem. J.* **255**, 1–13
- Hamm, H. E., Deretic, D., Hofmann, K. P., Schleicher, A. & Kohl, B. (1987) *J. Biol. Chem.* **262**, 10831–10838
- Deretic, D. & Hamm, H. F. (1987) *J. Biol. Chem.* **262**, 10839–10847
- Hamm, H. E., Deretic, D., Arendt, A., Hargrave, P. A., Koenig, B. & Hofmann, K. P. (1988) *Science* **241**, 832–835
- Goldsmith, P., Gierschik, P., Milligan, G., Unson, C. G., Vinitsky, R., Malech, H. L. & Spiegel, A. M. (1987) *J. Biol. Chem.* **262**, 14683–14688
- Cerione, R. A., Kroll, S., Rajaram, R., Unson, C., Goldsmith, P. & Spiegel, A. M. (1988) *J. Biol. Chem.* **263**, 9345–9352
- Hamm, H. E., Deretic, D., Mazzoni, M. R., Moore, C. A., Takahashi, J. S. & Rasenick, M. M. (1989) *J. Biol. Chem.* **264**, 11475–11482
- McKenzie, F. R., Kelly, E. C. H., Unson, C. G., Spiegel, A. M. & Milligan, G. (1988) *Biochem. J.* **249**, 653–659
- Masters, S. B., Stroud, R. M. & Bourne, H. R. (1986) *Protein Eng.* **1**, 47–54
- Simonds, W. F., Goldsmith, P. K., Woodard, C. J., Unson, C. G. & Spiegel, A. M. (1989) *FEBS Lett.* **249**, 189–194
- Palm, D., Munch, G., Malek, D., Dees, C. & Hekman, M. (1990) *FEBS Lett.* **261**, 294–298
- Wistow, G. J., Katial, A., Craft, C. & Shinohara, T. (1986) *FEBS Lett.* **196**, 23–28
- Wilden, U., Hall, S. W. & Kühn, H. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1174–1178
- Graham, J. (1984) in *Centrifugation, A Practical Approach* (Rickwood, D., ed.), pp. 168–171, IRL Press, Oxford
- Evans, W. H. (1987) in *Biological Membranes, A Practical Approach* (Findlay, J. B. & Evans, W. H., eds.), pp. 1–36, IRL Press, Oxford
- Evans, W. H. & Flint, N. (1985) *Biochem. J.* **232**, 25–32
- Sagi-Eisenberg, R. & Pecht, I. (1984) *Immunol. Lett.* **8**, 234–241
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021
- Duncan, R. & Hershey, J. W. B. (1984) *Anal. Biochem.* **138**, 144–155
- Gershoni, J. (1988) *Methods Biochem. Anal.* **33**, 1–58
- Olmsted, J. B. (1981) *J. Biol. Chem.* **256**, 11955–11957
- Burke, B., Griffiths, G., Reggio, H., Louvard, D. & Warren, G. (1982) *EMBO J.* **1**, 1621–1628
- Goldsmith, P., Rossiter, K., Carter, A., Simonds, W., Unson, C. G., Vinitsky, R. & Spiegel, A. M. (1988) *J. Biol. Chem.* **263**, 6476–6479
- Simonds, W. F., Goldsmith, P. K., Codina, J., Unson, C. G. & Spiegel, A. M. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7809–7813
- Jones, D. T. & Reed, R. R. (1987) *J. Biol. Chem.* **262**, 14241–14249

30. Ali, N., Milligan, G. & Evans, W. H. (1989) *Biochem. J.* **261**, 905–912
 31. Belcher, J. D., Hamilton, R. L., Brady, S. E., Hornick, C. A., Jaeckle, S., Schneider, W. J. & Havel, R. J. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6785–6789
 32. Mueller, S. C. & Hubbard, A. L. (1986) *J. Cell Biol.* **102**, 932–942
 33. Enrich, C., Bachs, O. & Evans, W. H. (1988) *Biochem. J.* **255**, 999–1005
 34. Wilson, J. M., Whitney, J. A. & Neutra, M. R. (1987) *J. Cell Biol.* **105**, 691–703
 35. Backlund, P. S., Aksamit, R. R., Unson, C. G., Goldsmith, P., Spiegel, A. M. & Milligan, G. (1988) *Biochemistry* **27**, 2040–2046
-

Received 3 July 1990/15 August 1990; accepted 20 August 1990