

Hypothesis

Regulation of adenylate cyclase by hormones and G-proteins

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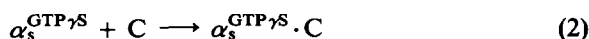
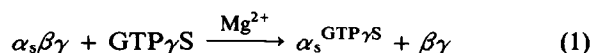
Over the past few years, it has become apparent that a large number of transmembrane signaling systems operate through heterotrimeric G-proteins ([1] Gilman, A.G. (1984) *Cell* 36, 577–579; [2] Baker, P.F. (1986) *Nature* 320, 395). Adenylate cyclase is regulated by stimulatory hormones through $G_s(\alpha_s\beta\gamma)$ and inhibitory hormones through $G_i(\alpha_i\beta\gamma)$ ([2]; Katada, T. et al. (1984) *J. Biol. Chem.* 259, 3586–3595), whereas the breakdown of phosphatidylinositol biphosphate (PIP_2) to inositol trisphosphate (IP_3) and diacylglycerol (DG) by phospholipase C is probably also mediated by a heterotrimeric G-protein (G_o or G_i) [1,2]. Similarly, the activation of cGMP phosphodiesterase by light-activated rhodopsin is mediated through the heterotrimeric G-protein transducin (Stryer, L. (1986) *Rev. Neurosci.* 9, 89–119). Other transmembrane signaling systems may also be found to involve G-proteins similar to those already recognized. Because of the emerging universality of G-proteins as transducers of receptor-triggered signals, it may be useful to evaluate the current models prevailing in the adenylate cyclase field, as these models seem to guide our way in evaluating the role of G-proteins in transmembrane signaling, in general.

G-protein; Adenylate cyclase; Receptor

1. INTRODUCTION

G-proteins are heterotrimeric proteins composed of three subunits: a GTP-binding subunit α -, β - and γ -subunits. These proteins function in transmembrane signaling of hormones, neurotransmitters and light (transducin) [1]. The molecular mechanism of G-protein action as a transducer between the receptor and its biochemical effector system is believed to be similar in all heterotrimeric G-proteins. Detailed studies on the hormonal regulation of adenylate cyclase through the stimulatory G-protein, G_s , and the inhibitory G-

protein, G_i (reviews [1,3]), provided a popular molecular model promoted mainly by Gilman and his colleagues [1,3]. The model (fig.1) is mainly based on the observation that the stimulatory G-protein, G_s , dissociates in the presence of the non-hydrolyzable analog, $GTP\gamma S$, to produce a $GTP\gamma S$ -bound $\alpha_s(G_s\alpha)$ -subunit which is sufficient to activate the purified catalytic unit of adenylate cyclase (C) [5]:



Similarly, the α -subunit of transducin, when bound with the non-hydrolyzable guanyl

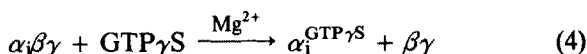
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nucleotide GppNHp, is sufficient to activate the rod outer-segment cGMP phosphodiesterase [6]:



GTP γ S has also been shown to activate the breakdown of phosphatidylinositol bisphosphate (PIP $_2$) to the second messengers diacylglycerol (DG) and inositol trisphosphate (IP $_3$) [7], which by analogy implies an interaction of a G α that is as yet undefined but may be G α [8] with a specific phospholipase C [9]. Support for the involvement of G-proteins in the activation of phospholipase C is abundant [9–12].

G $_i$, a heterotrimeric G-protein which mediates hormonal inhibition, can also [1,3,13–15] dissociate to its GTP γ S-bound α_i with the release of $\beta\gamma$ -subunits:



So far it has not been possible to demonstrate a G $_i$ or an α_i cyclase complex. Since the $\beta\gamma$ -subunit of G $_i$ seems to be identical to the $\beta\gamma$ -subunit of G $_s$ [16], the suggestion that G $_i$ confers inhibition on adenylate cyclase through the release of $\beta\gamma$ -subunits with no direct α_i -C interaction [1,3] is very attractive, as explained below.

2. THE G-DISSOCIATION MODEL FOR ADENYLATE CYCLASE REGULATION

The G-dissociation model for adenylate cyclase regulation by hormones suggests (fig.1) that:

(i) Stimulation of adenylate cyclase occurs as follows: The agonist-bound stimulatory receptor catalyzes the GDP–GTP exchange on G $_s$, and G $_s$ dissociates to α_s^{GTP} and $\beta\gamma$. α_s^{GTP} seeks the adenylate cyclase catalyst C and activates it. Upon GTP hydrolysis, α_s^{GDP} dissociates from C and reassociates to form the GDP-bound heterotrimer $\alpha_s^{GDP}\beta\gamma$. A new cycle of G $_s$ activation by the stimulatory hormone can now begin.

(ii) Inhibition of adenylate cyclase occurs as follows: The agonist bound at the inhibitory receptor catalyzes the GDP–GTP exchange, and G $_i$

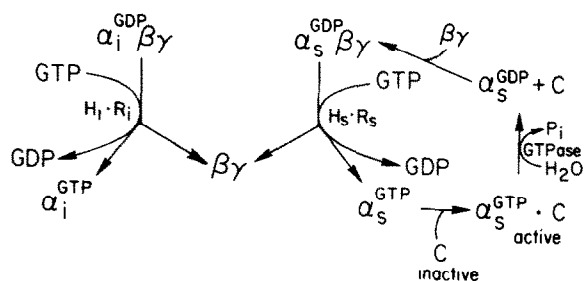


Fig.1. The G-dissociation model for adenylate cyclase regulation. Interaction of a stimulatory receptor R_s bound with an agonist H_s with G $_s$ in its resting GDP-bound state and in the presence of GTP leads to its dissociation. The active α_s^{GTP} released from the heterotrimer combines with the catalytic unit C and activates it to the cAMP-producing form. The $\beta\gamma$ -subunits compete with C for α_s . When GTP is hydrolyzed at the $\alpha_s^{GTP}\cdot C$ complex to form $\alpha_s^{GDP}\cdot C$, the complex is dissociated to α_s^{GDP} and C and the former recombines with $\beta\gamma$ to reform the inactive G $_s$. According to this model, the intramembraneous concentration of the $\beta\gamma$ -subunits determines the level of adenylate cyclase activity, since they compete with the catalyst C for α_s^{GTP} . When the inhibitory G-protein G $_i$ is activated by interacting with an inhibitory receptor R_i bound with an agonist H_i , it dissociates to α_i^{GTP} and $\beta\gamma$. This reaction leads to an increase in the intramembraneous concentration of $\beta\gamma$ and, therefore, to adenylate cyclase inhibition. The two basic features of the model are: (i) all 5 components, R_s , R_i , G $_s$, G $_i$ and C, are physically separate and interact with each other. This type of interaction leads to a complex kinetic pattern of activation, typical for the 'shuttle' models (see text). (ii) The native form of the enzyme C, while its active form is $\alpha_s^{GTP}\cdot C$.

dissociates to α_i^{GTP} and $\beta\gamma$. The $\beta\gamma$ -subunits released elevate their level within the bilayer, thus causing a more effective scavenging of α_s , with an effective cyclase inhibition.

The model as described is depicted in fig.1 and is essentially the model described by Katada et al. [3]. This molecular model has achieved prominent status, mainly because of two features: (i) it gives a functional role to the $\beta\gamma$ -subunits and, because of their identity in G $_i$ and G $_s$, enables a cross-talk between the two G-proteins; (ii) it accounts for adenylate cyclase inhibition by G $_i$ without the necessity of a direct G $_i$ -C interaction.

3. EVALUATION

A number of experimental observations do not fit with the dissociation model and therefore call for modifications or even alternative hypotheses:

(i) The model, as it stands, treats adenylate cyclase as a five-component system, where all the functional units, R_s , R_i , G_s , G_i and C , are physically separate but dynamically interact with each other. According to this model, α_s 'shuttles' between G_s and C which are two physically separate molecules. Such a mode of interaction will yield complex kinetics of activation, whether GTP or a non-hydrolyzable analog is used [17,18]. This is in contrast to the experimental observation that the kinetics of adenylate cyclase activation by hormones and guanyl nucleotides, both in native membranes [18–20] and in systems reconstituted from resolved components [21–24], are first order. Even if one assumes a moderate G_s – C dissociation, complex kinetics of activation of adenylate cyclase by hormones and guanyl nucleotides will result [17,20]. The linear dependence of the first-order rate constant of adenylate cyclase activation on the concentration of the activating receptor further supports the assertion that the functional entity of adenylate cyclase is a complex between G_s and C [17,20].

Biochemical studies have also shown that to separate G_s from C , a combination of detergent and high salt is required [25–27]. Furthermore, α_s or even G_s stay attached to C through a 240-fold purification as a complex when the turkey erythrocyte enzyme is purified in mild detergents in the presence of phospholipids. The complex is stable whether the adenylate cyclase is in its inactive, GDP-bound form or in its preactivated GppNHp-bound form [28]. It is feasible to modify the original [1,3] dissociation model and actually accommodate these latter findings, if one assumes that α_s is *always* associated with C and the $\beta\gamma$ -subunits dissociate from the $G_s \cdot C$ complex, leaving behind $\alpha_s \cdot C$ [29]. This modified [29] dissociation model (fig.2) does not conflict with the basic kinetic properties of the complete system and still retains its basic feature, namely, a central role for the $\beta\gamma$ -subunits as the regulators of adenylate cyclase activity.

Recent experiments on the light-catalyzed cGMP phosphodiesterase (PDE) in rod disk membranes

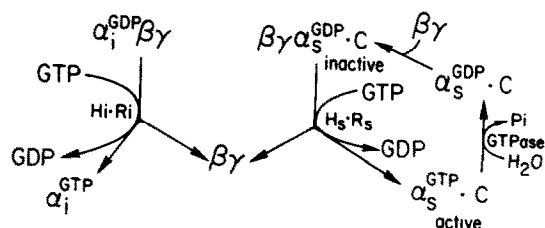


Fig.2. The modified G-dissociation model. In this model, it is assumed that the α_s -subunit is physically attached to the catalyst C at all times. The active form of the enzyme is $\alpha_s^{GTP} \cdot C$ and its inactive form $\beta\gamma \alpha_s^{GDP} \cdot C$. Like in the dissociation model (fig.1), the intramembraneous concentration of the $\beta\gamma$ -subunits determines the level of adenylate cyclase activity. The absence of a complete dissociation between α_s and C allows simple overall kinetics of activation, and therefore is the minimal modification required for the dissociation model in order to accommodate it with the kinetic results obtained for hormonally regulated adenylate cyclase in native membranes as well as hormone-sensitive adenylate cyclase reconstituted from purified components (see text).

also suggest that the G-protein is associated with the catalyst PDE during the entire cycle of its activation by light-excited rhodopsin [30].

(ii) In T-cell S49 lymphoma cell AC⁻ (cyc⁻) variant, normal hormonal inhibition is observed but hormonal stimulation is nullified because of the complete absence of G_s . The ability of somatostatin to inhibit adenylate cyclase through G_i in S40 cyc⁻ cells [31] argues for a direct G_i – C interaction. Kinetic studies performed on the S49AC⁻ membranes, into which increasing amounts of G_s have been inserted, also suggests that G_s and G_i interact at independent domains of C [32]. As indicated above, no $G_i \cdot C$ complex has thus far been reported. This, however, can result from a weak protein-protein interaction between G_i and C , as compared with the G_s – C interaction. Furthermore, the experimental conditions under which G_i – C interactions were tested [5] may not have been optimal.

(iii) As indicated above, the mode of action of GTP on G-proteins is frequently deduced from the mode of action of its non-hydrolyzable analogue GTP γ S. It should, however, be noted that: (i) GTP γ S permanently activates turkey erythrocyte

adenylate cyclase, even in intact membranes, and cannot be 'reversed' by GTP and β -adrenergic agonists [33], probably because of the irreversible nature of the G-protein dissociation in the presence of GTP γ S. (ii) In contrast to GTP γ S, the GTP analogue GppNHp-activated adenylate cyclase in turkey erythrocytes *can* be reversed by GTP and β -agonists or adenosine, due to a GppNHp-GTP exchange [34,35]. These results have been reproduced in reconstituted systems: preactivated G_s^{GppNHp} from duck erythrocytes was reconstituted with turkey erythrocyte β_1 -adrenoceptors, and the G_s^{GppNHp} could be reversed to G_s^{GTP} when the mixture was challenged with (-)-isoproterenol, GTP and excess of $\beta\gamma$ -subunits [23]. This result suggests that the purified G_s^{GppNHp} tends to lose its $\beta\gamma$ -subunits and that the guanyl nucleotide exchange can occur only when the α_s^{GppNHp} subunit combines with the $\beta\gamma$ -subunits. The GppNHp-GTP exchange reaction is most probably catalyzed by the β -agonist-receptor complex by a mechanism similar to the hormone-catalyzed GDP-GTP exchange during the activation process of adenylate cyclase. These observations strongly suggest that GppNHp-preactivated adenylate cyclase in *native* membrane possesses all three G_s subunits, α_s , β and γ , since reversal occurs with no addition of excess $\beta\gamma$ -subunits [35].

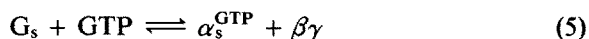
Direct measurements of subunit dissociation of GppNHp-preactivated G_s in detergent show that it occurs *subsequent* to G_s activation [36]. It seems that GppNHp, which is isoelectronic and probably isosteric to GTP, mimics GTP more closely than GTP γ S, excluding, of course, the GTPase 'turn-off' reaction which is absent in the GppNHp-activated G-proteins.

(iv) GTP, the natural guanyl nucleotide, is hydrolyzed to GDP and P_i on the GTP-binding subunit with a rate constant of $6\text{--}15\text{ min}^{-1}$ [18,33,37,38]. Thus, the residence time of GTP at its binding site as a triphosphate has a half-life of 2.8–7 s. It is unlikely, although possible, that during the 'on-off' cycle a complete α - $\beta\gamma$ separation takes place. It is more reasonable to assume a conformational transition which involves all three subunits but does not necessarily involve complete subunit separation.

Codina et al. [36] showed that G_s as well as G_i can exist in an active undissociated GppNHp-

bound form which is distinct from the inactive GDP-bound form. Furthermore, Codina et al. [36] make the point that NaF and Mg^{2+} activate G-proteins reversibly without subunit dissociation. Indeed, during the purification of G_s , NaF, Al^{3+} and Mg^{2+} , which activate G_s , are always present, and still the protein is obtained in its trimeric undissociated form [1].

(v) The concentration dependence of adenylate cyclase activity on guanyl nucleotide is not complex and can be described by classical Michaelian kinetics ([39] and references therein; [40]). Had the activation of adenylate cyclase depended on G_s dissociations, followed by a bimolecular interaction between α_s^{GTP} and the catalytic unit:



one would have expected a complex, non-Michaelian dependence of the rate of activation on GppNHp concentration [17,20]. In the case of cAMP-dependent protein kinase, where enzyme activation depends on a dissociation step occurring subsequently to cAMP binding, one indeed observes a characteristic kinetic pattern with a complex dependence on the concentration of the activating ligand cAMP [41].

(vi) A prediction of the dissociation model [1,3] or of its modified form [29] is that activation of G-proteins which mediate the action of other receptors should yield adenylate cyclase inhibition. This is so, because the $\beta\gamma$ -subunits of different G-proteins seem to be similar or identical, and their hypothesized release by the G-proteins should yield an elevation of their level within the bilayer. Only in one system [9] was this issue tackled directly, where it was shown that the activation of phospholipase C through a G-protein-mediated process by bradykinin results in a very small percentage of inhibition of adenylate cyclase.

4. ALTERNATIVE MODELS

In view of this discussion, it is apparent that the molecular model which assumes that regulation of adenylate cyclase depends exclusively on the dissociation of G_s and G_i suffers from certain severe weaknesses. It may, therefore, be necessary

to formulate a revised or perhaps a completely distinct hypothesis to account for the regulation of adenylate cyclase by G-proteins. Except for the modified G-dissociation model (fig.2) [19,29], there is room for completely different molecular models which account for the known experimental data. These models (fig.3) are based on the following assumptions:

- (i) The G_s -protein does not dissociate as part of its mode of action.
- (ii) The G_s -protein exhibits high affinity towards the catalyst which it regulates, and remains associated with it at all times.
- (iii) The complex $G_s \cdot C$ interacts with the stimulatory receptor R_s which, when *bound* with agonist catalyzes the GDP-GTP exchange on the $G_s \cdot C$ complex.
- (iv) One receptor can catalyze the activation of many $G_s \cdot C$ units.
- (v) G_i interacts either directly with C or with G_s and confers inhibition on adenylate cyclase in a non-competitive manner with respect to G_s .

Two variant models are shown in fig.3. Model A is very similar to the model originally proposed by Hildebrandt et al. [42] but differs from it in postulating that the G_i -C interactions are weaker than the G_s -C interactions. Model B has an important feature in common with the dissociation models, in that it postulates that G_i does not in-

teract with C but rather with G_s . Both models A and B can actually account for all the experimental findings quoted in this article. Experimental determination of the mode of physical interactions between G_s and G_i , and between G_i and C, would discriminate between the two models. It is likely that G_i -C or G_i - G_s interactions are much weaker than the G_s -C interaction, and have therefore been missed so far. Weak physical interaction is already known to occur between receptors and G-proteins ([43] and references therein). Furthermore, it has already been pointed out that even in native membranes, the β -adrenoceptor- G_s coupling can be perturbed by very low concentrations of detergent, such as Lubrol-PX, while the G_s -C association remains intact even at very high Lubrol-PX concentrations [44]. In reconstituted systems, where the β_1 -adrenoceptor, G_s and C have been co-reconstituted, it has also been found that the β -adrenoceptor- G_s interface is much more sensitive towards detergents than G_s -C coupling [24]. Hence, it seems essential to optimize experimental protocols such that the ability of G_i to interact with C and/or with G_s can be carefully examined.

In summary, future experiments should be aimed at determining whether the dissociation of G_s and G_i is part of their mode of action, and to explore in full whether G_i interacts with G_s or C.

REFERENCES

- [1] Gilman, A.G. (1984) *Cell* 36, 577-579.
- [2] Baker, P.F. (1986) *Nature* 320, 395.
- [3] Katada, T., Bokoch, G.M., Smigel, M.D., Ui, M. and Gilman, A.G. (1984) *J. Biol. Chem.* 259, 3586-3595.
- [4] Stryer, L. (1986) *Rev. Neurosci.* 9, 87-119.
- [5] Smigel, M.D. (1986) *J. Biol. Chem.* 261, 1976-1982.
- [6] Fung, B.K.-K. and Stryer, L. (1981) *Proc. Natl. Acad. Sci. USA* 77, 2500-2504.
- [7] Cockcroft, S. and Gomperts, B. (1985) *Nature* 314, 534-536.
- [8] Sternweis, P.C. and Robishaw, J.D. (1984) *J. Biol. Chem.* 259, 13806-13813.
- [9] Hipesida, H., Streaty, R.A., Klee, W. and Nirenberg, M. (1986) *Proc. Natl. Acad. Sci. USA* 83, 942-946.
- [10] Nakamura, T. and Ui, M. (1985) *J. Biol. Chem.* 260, 3584-3593.
- [11] Bradford, P.G. and Rubin, R.P. (1985) *FEBS Lett.* 183, 317-320.

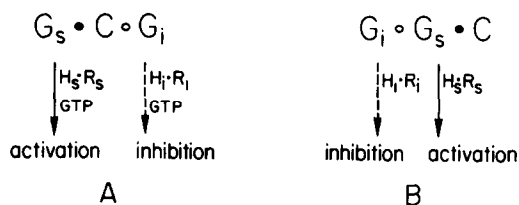


Fig.3. Alternative molecular models for G_s - G_i -C interrelations. Model A: This is essentially the original model of interaction between the two G-proteins and the catalyst. It differs, however, from the symmetric relationship between G_s and G_i vis-à-vis C, which was originally postulated [32]. According to the stated hypothesis, G_i makes a weaker (○) interaction with C than G_s (●). Model B: In this model, G_i does not interact with C but rather with G_s . Here, too, the G_i - G_s interaction is weaker than the G_s -C interaction. Both models A and B account for a non-competitive relationship between G_s and G_i at C, as observed experimentally [32].

- [12] Volpi, M., Nacache, P.H., Molski, T.F.P., Shefyck, J., Huang, C.-K., Marsh, M.L., Munoz, J., Becker, E.L. and Shaafi, R.I. (1985) *Proc. Natl. Acad. Sci. USA* 82, 2708–2712.
- [13] Ui, M. (1984) *Trends Pharmacol. Sci.* 5, 277–279.
- [14] Katada, T. and Ui, M. (1982) *Proc. Natl. Acad. Sci. USA* 79, 3129–3133.
- [15] Katada, T., Northup, J.P., Bokoch, G.M., Ui, M. and Gilman, A.G. (1984) *J. Biol. Chem.* 259, 3578–3585.
- [16] Hildebrandt, J.D., Codina, S., Rosenthal, W., Birnbaumer, L., Neer, E.J., Yamazaki, A. and Bitensky, M. (1985) *J. Biol. Chem.* 260, 14867–14872.
- [17] Tolkovsky, A.M. and Levitzki, A. (1981) *J. Cyclic Nucleotide Res.* 7, 139–150.
- [18] Tolkovsky, A.M. and Levitzki, A. (1978) *Biochemistry* 17, 3795–3810.
- [19] Levitzki, A. (1986) *Physiol. Rev.* 66, 819–842.
- [20] Tolkovsky, A.M., Braun, S. and Levitzki, A. (1982) *Proc. Natl. Acad. Sci. USA* 79, 213–217.
- [21] Citri, Y. and Schramm, M. (1980) *Nature* 287, 295–300.
- [22] Pedersen, S.E. and Ross, E.M. (1982) *Proc. Natl. Acad. Sci. USA* 79, 7228–7232.
- [23] Hekman, M., Feder, D., Keenan, A.K., Gal, A., Klein, H.W., Pfeuffer, T., Levitzki, A. and Helmreich, E.J.M. (1984) *EMBO J.* 3, 3339–3345.
- [24] Feder, D., Im, M.J., Hekman, M., Klein, H.W., Levitzki, A., Helmreich, E.J.M. and Pfeuffer, T. (1986) *EMBO J.* 5, 1509–1514.
- [25] Strittmatter, S. and Neer, E. (1980) *Proc. Natl. Acad. Sci. USA* 77, 6344–6348.
- [26] Ross, E.M. (1981) *J. Biol. Chem.* 256, 1949–1953.
- [27] Bender, J.L. and Neer, E. (1983) *J. Biol. Chem.* 258, 2432–2439.
- [28] Arad, H., Rosenbush, J. and Levitzki, A. (1984) *Proc. Natl. Acad. Sci. USA* 81, 6579–6583.
- [29] Levitzki, A. (1984) *J. Receptor Res.* 4, 399–409.
- [30] Stitaramayya, A., Harkness, J., Parkes, J.H., Gonzalez-Oliva, C. and Liebman, P. (1986) *Biochemistry* 25, 651–657.
- [31] Jakobs, K.H., Aktories, K. and Schultz, G. (1983) *Nature* 302, 706–709.
- [32] Hildebrandt, J.D., Codina, J. and Birnbaumer, L. (1984) *J. Biol. Chem.* 259, 13178–13185.
- [33] Cassel, D. and Selinger, Z. (1977) *Biochem. Biophys. Res. Commun.* 77, 868–873.
- [34] Sevilla, N. and Levitzki, A. (1977) *FEBS Lett.* 76, 129–134.
- [35] Arad, H., Rimón, G. and Levitzki, A. (1981) *J. Biol. Chem.* 256, 1593–1597.
- [36] Codina, J., Hildebrandt, J.P., Birnbaumer, L. and Sekura, R.D. (1984) *J. Biol. Chem.* 259, 11408–11418.
- [37] Cassel, D., Levkovitz, H. and Selinger, Z. (1977) *J. Cyclic Nucleotide Res.* 3, 393–406.
- [38] Arad, H. and Levitzki, A. (1979) *Mol. Pharmacol.* 16, 749–756.
- [39] Ross, E. and Gilman, A.G. (1980) *Annu. Rev. Biochem.* 49, 533–564.
- [40] Braun, S., Tolkovsky, A.M. and Levitzki, A. (1982) *J. Cyclic Nucleotide Res.* 8, 133–147.
- [41] Swillens, S. and Dumont, J.E. (1976) *J. Mol. Med.* 1, 273–288.
- [42] Hildebrandt, J.D., Codina, J., Risinger, R. and Birnbaumer, L. (1984) *J. Biol. Chem.* 259, 2039–2042.
- [43] Lefkowitz, R.J., Stadel, J.M. and Caron, M.G. (1983) *Annu. Rev. Biochem.* 52, 159–186.
- [44] Gal, A., Keenan, A.K. and Levitzki, A. (1982) *Biochem. Biophys. Res. Commun.* 105, 615–623.