JOURNAL OF
MEDICINAL
CHEMISTRY

Volume 33, Number 5 May 1990

Perspective

Receptor Triggering and Receptor Regulation: Structure–Activity Relationships from the Receptor's Point of View†

Morley D. Hollenberg

Endocrine Research Group, Department of Pharmacology and Therapeutics and Department of Medicine, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada T2N 4N1. *Received September 27, 1989*

1.0 Introduction

1.1 Membrane Receptors and Cell Signalling. For an agonist-like acetylcholine to activate its target tissue, it must first be recognized in a highly specific manner by its receptor;[1] subsequently, the binding of acetylcholine to its receptor must be translated into an amplified signal that leads to a cellular response. The ligand binding and signal generation processes represent two distinct but closely related functions that a pharmacologic receptor must perform. It is now recognized that the receptor, and not the activating ligand, possesses in its structure the key elements that generate a cellular response. Nonetheless, traditionally structure–activity studies have dealt primarily with the stereochemical properties of the ligand that allow it to bind to the receptor either as an activator (agonist) or as an inhibitor (antagonist). Recently, however, attention is being focused on the structural properties of the receptor that allow it to bind its ligand and to trigger a cellular signal.

1.2 Mechanisms of Receptor-Mediated Cell Signalling. As summarized elsewhere,[2,3] the mechanisms whereby a pharmacologic receptor initiates a cellular signal may turn out to be few in number, as represented by the three basic paradigms portrayed in Figure 1: (1) the receptor (R_A in Figure 1) may form part of a ligand-gated oligomeric ion channel, like the nicotinic cholinergic receptor[4-7] or the receptor for gamma aminobutyric acid (GABA);[8-10] (2) the receptor (R_B in Figure 1) may be a transmembrane ligand-regulated enzyme (e.g. the tyrosine kinase receptors for insulin[11,12] and epidermal growth factor-urogastrone (EGF-URO)[13-16] or the guanylate cyclase linked receptor for atrial natriuretic factor[17]); alternatively, (3) the receptor (R_C in Figure 1) may interact in a ligand-regulated manner with membrane-associated guanine nucleotide binding proteins, or G-proteins, which

† Abbreviations used: EGF-URO, epidermal growth factor-urogastrone; IGF-I, insulin-like growth factor-I; neu/HER-2, cellular counterpart of the neu oncogene product homologous with the EGF-URO receptor; PDGF, platelet-derived growth factor; TGF-α, transforming growth factor-α.

(1) Langley, J. N. *Proc. R. Soc., Ser. B* **1906**, *78*, 170.
(2) Hollenberg, M. D.; Goren, H. J. *Mechanisms of Receptor Regulation*; Poste, G., Crooke, S. T., Eds.; Plenum Press: New York, 1985; p 323.
(3) Hollenberg, M. D. *Experientia* **1986**, *42*, 718.
(4) Conti-Tronconi, B. M.; Raftery, M. A. *Annu. Rev. Biochem.* **1982**, *51*, 491.
(5) Noda, M.; Takahashi, H.; Tanabe, T.; Toyosato, M.; Furutani, Y.; Hirose, T.; Asai, M.; Inayama, S.; Miyata, T.; Numa, S. *Nature* **1982**, *299*, 793.
(6) Noda, M.; Takahashi, H.; Tanabe, T.; Toyosato, M.; Kikyotani, S.; Hirose, T.; Asai, M.; Takashima, H.; Inayama, S.; Miyata, T.; Numa, S. *Nature* **1983**, *301*, 251.
(7) Noda, M.; Takahashi, H.; Tanabe, T.; Toyosato, M.; Kikyotani, S.; Furutani, Y.; Hirose, T.; Takashima, H.; Inayama, S.; Miyata, T.; Numa, S. *Nature* **1983**, *302*, 528.
(8) Schofield, P. R.; Darlison, M. G.; Fujita, N.; Burt, D. R.; Stephenson, F. A.; Rodriguez, H.; Rhee, L. M.; Ramachandran, J.; Reale, V.; Glencorse, T. A.; Seeburg, P. H.; Barnard, E. A. *Nature* **1987**, *328*, 221.
(9) Levitan, E. S.; Schofield, P. R.; Burt, D. R.; Rhee, L. M.; Wisden, W.; Kohler, M.; Fujita, N.; Rodriguez, H. F.; Stephenson, A.; Darlison, M. G.; Barnard, E. A.; Seeburg, P. H. *Nature* **1988**, *335*, 76.
(10) Pritchett, D. B.; Sontheimer, H.; Shivers, B. D.; Ymer, S.; Kettenmann, H.; Schofield, P. R.; Seeburg, P. H. *Nature* **1989**, *338*, 582.
(11) Goldfine, I. D. *Endocrine Rev.* **1987**, *8*, 235.
(12) Rosen, O. M. *Science* **1987**, *237*, 1452.
(13) Yarden, Y.; Ullrich, A. *Annu. Rev. Biochem.* **1988**, *57*, 443.
(14) Schlessinger, J. *Biochemistry* **1988**, *27*, 3119.
(15) Waterfield, M. D. *Br. Med. Bull.* **1989**, *45*, 541.
(16) Gill, G. N.; Bertics, P. J.; Santon, J. B. *Mol. Cell. Endocrinol.* **1987**, *51*, 169.
(17) Chinkers, M.; Garbers, D. L.; Chang, M.-S.; Lowe, D. G.; Chin, H.; Goeddel, D. V.; Schulz, S. *Nature* **1989**, *338*, 78.

0022-2623/90/1833-1275$02.50/0

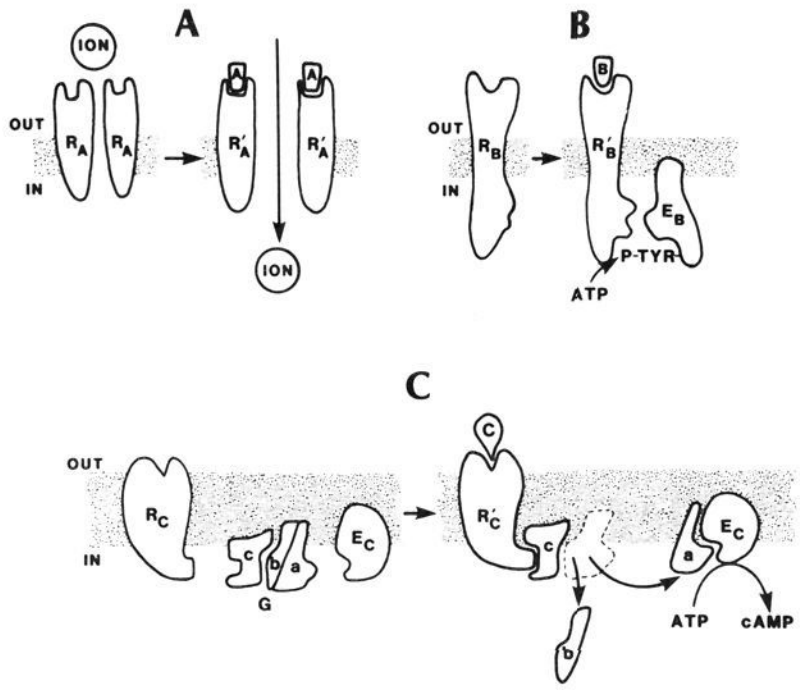

Figure 1. Receptor models. Three distinct mechanisms for receptor-mediated cell activation are portrayed: (A) a ligand-regulated channel, R_A, modulates ion flux; (B) a ligand-regulated transmembrane enzyme, R_B, acts on intracellular substrates; a tyrosine kinase receptor is depicted; (C) agonist binding promotes the interaction of the receptor with an oligomeric G-protein (G) leading to the dissociation of the oligomer into its α (a) and β/γ (b, c) substituents; the α subunit in turn regulates an enzyme-like adenylate cyclase (E_C) as shown. The fate of the β/γ subunits which are tightly associated is uncertain. The constituents of the system (receptor, G-protein, and cyclase) are *not* drawn to scale.

in turn modulate the activity of membrane-associated enzymes like adenylate cyclase.[18-20] As will be elaborated upon below, the amino acid sequences are now known for a number of the three types of receptors portrayed in Figure 1. One goal of molecular pharmacology is to analyze the receptor sequences in the context of the three signalling paradigms portrayed in Figure 1, so as to assign a function to a specific structural domain.

1.3 Receptor Mobility and Cell Signalling. Apart from receptors that are ligand-regulated ion channels (R_A, Figure 1), receptor mobility in the plane of the membrane (Figure 2) is a key feature related to receptor function, as outlined by the mobile receptor model of hormone action (summarized in ref 2). For a number of receptors like the ones for insulin or EGF-URO (portrayed as R_B in Figure 1), ligand binding triggers a series of mobile reactions, involving membrane-localized protein–protein interactions, that are thought to be involved in initiating a transmembrane signal (Figure 2). An important event in the activation of cells by a variety of receptors is the initial very rapid (i.e. less than 1 s) microclustering of the ligand-occupied receptor (dimers or more) followed by the less rapid (seconds to 10's of seconds) aggregation of receptors at sites of internalization (frequently, but not necessarily aloways, coated pit regions). Upon internalization, the receptor, localized to an endosomal organelle, can either migrate to a variety of intracellular locales (e.g. lysosome or nuclear membrane) or can recycle to the plasma membrane (Figure 2). It is during the course of these mobile reactions that a ligand-occupied receptor, according to the mobile receptor model of hormone action, can interact with the membrane effector moieties, so as to generate a transmembrane signal. The receptor domains that are responsible (1) for the binding of a ligand and (2) for the interaction of the receptor with other important membrane-associated constituents involved in cell signalling and receptor trafficking are largely unknown.

1.4 Amplication of Receptor-Triggered Signals. In terms of the cell triggering process per se, it is now believed that, for "enzyme receptors" like the ones for insulin or EGF-URO, signal amplification involves specific interactions with key phosphoprotein "effector" substrates (ref 21–29, represented by E_B in Figure 1). For receptors that interact with G-proteins, signal amplification is mediated via the receptor-induced dissociation of the G-protein oligomer into substituents (α and β/γ), which in turn regulate membrane enzymes like adenylate cyclase, so as to generate "second signal" molecules such as cyclic AMP. The amplification reactions triggered by the second signal molecules (e.g. phosphorylation–dephosphorylation cascades) can lead both to cell activation and to a feedback control of the receptor itself. The factors that govern the interaction between a ligand-occupied receptor and its G-protein signal mediator are partly (e.g. guanine nucleotide requirements) but not yet completely understood.[18-20] As yet, the factors that govern the interaction of specific receptor domains with feedback regulators (e.g. kinases or phosphatases) are only beginning to be documented.

In the light of the information summarized in the preceding sections, it is clear that receptor sequences will need to subserve a number of distinct, but related functions: (1) ligand binding, (2) membrane mobility, internalization and intracellular trafficking, (3) signal generation/amplification, and (4) feedback regulation. It is the goal of this perspective to outline some of the recent receptor structure–activity studies that are being done for a variety of receptors to identify those receptor domains that subserve each of these specific receptor functions.

2.0 Signalling and Domain Function

In terms of receptor function, the following kinds of questions relating to structure and activity may be asked: (1) What are the precise domain sequences that participate in ligand binding; and, are these the same domains involved in the ligand's ability to trigger the receptor? In this context, the terms "intrinsic activity" and "efficacy" may be seen in a new light. (2) What are the receptor domain sequences that are involved in the interaction of the receptor with "effector" moieties, like G-proteins in the plane of the membrane? (3) What are the receptor domains that predetermine its plasma membrane location and that regulate its mobility/internalization? and (4) For those receptors that are transmembrane enzymes (e.g. the

(18) Casey, P. J.; Gilman, A. G. *J. Biol. Chem.* **1988**, *263*, 2577.
(19) Neer, E. J.; Clapham, D. E. *Nature* **1988**, *333*, 129.
(20) Weiss, E. R.; Kelleher, D. J.; Woon, C. W.; Soparkar, S.; Osawa, S.; Heasley, L. E.; Johnson, G. L. *FASEB J.* **1988**, *2*, 2841.
(21) Bernier, M.; Laird, D. M.; Lane, M. D. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1844.
(22) Bernier, M.; Laird, D. M.; Lane, M. D. *Insulin Action and Diabetes*; Goren, H. J., Hollenberg, M. D., Roncari, D. A. K., Eds.; Raven Press: New York, 1988; p 117.
(23) Fava, R. A.; Cohen, S. *J. Biol. Chem.* **1984**, *259*, 2636.
(24) Valentine-Braun, K. A.; Northup, J. K.; Hollenberg, M. D. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 236.
(25) De, B. K.; Misono, K. S.; Lukas, T. J.; Mroczkowski, B.; Cohen, S. *J. Biol. Chem.* **1986**, *261*, 13784.
(26) Haigler, H. T.; Schlaepfer, D. D.; Burgess, W. H. *J. Biol. Chem.* **1987**, *262*, 6921.
(27) Wallner, B. P.; Mattaliano, R. J.; Hession, C.; Cate, R. L.; Tizard, R.; Sinclair, L. K.; Foeller, C.; Chow, E. P.; Browning, J. K.; Ramachandran, K. L.; Pepinsky, R. B. *Nature* **1986**, *320*, 77.
(28) Valentine-Braun, K. A.; Hollenberg, M. D.; Fraser, E.; Northup, J. K. *Arch. Biochem. Biophys.* **1987**, *259*, 262.
(29) Hollenberg, M. D.; Valentine-Braun, K. A.; Northup, J. K. *Trends Pharmacol. Sci.* **1988**, *9*, 63.

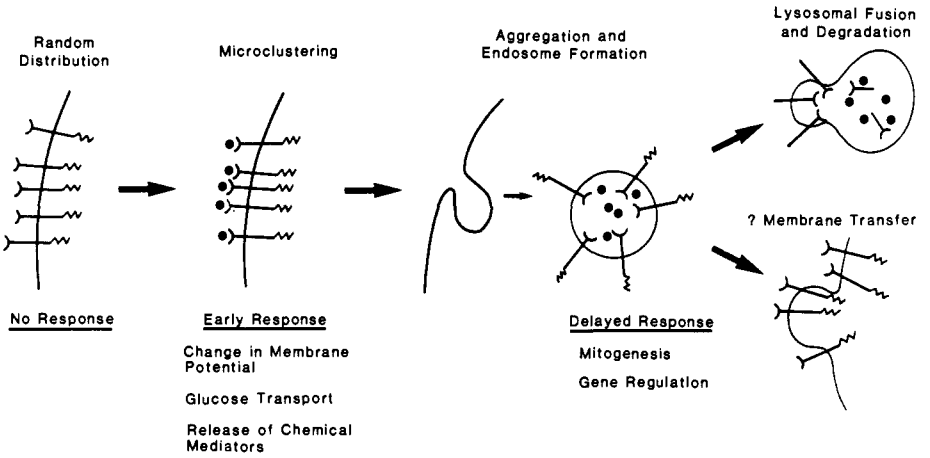

Figure 2. Receptor dynamics and agonist action. As outlined in the text, agonist binding triggers a number of mobile receptor reactions related to cell activation. The initial microclustering can be related to rapid cellular responses like changes in membrane potential or increases in metabolite transport. Subsequent receptor aggregation and internalization can be correlated with delayed responses like gene regulation and cell division.

insulin or EGF-URO receptor), what are the domain sequences that confer substrate specificity; and what are the receptor substrates that are involved in signalling? With the cloning, sequencing, and expression of a variety of receptors, along with the ability to site-specifically mutate and to express, in a cellular context, a variety of receptors, partial answers to some of the above questions are beginning to emerge. For illustrative purposes, this perspective will focus on work tht has been done with the receptors for insulin, epidermal growth factor-urogastrone (EGF-URO), β-adrenergic agents, and platelet-derived growth factor (PDGF). Thus, the perspective will not deal either with cytoplasmic receptors for agents like steroid hormones or with membrane-localized ion channels (like the one for calcium) or metabolite uptake sites (e.g. for amino acids) that can, in another context, be thought of as receptors.

In view of the several signalling paradigms and the four questions just posed, it is possible to single out specific receptor functions that one could expect to assign to defined sequence domains of an individual receptor protein: (1) a ligand binding domain, (2) oligosaccharide attachment sites, linked to the membrane-targetting properties of receptor glycosylation, (3) a transmembrane domain, responsible for anchoring the receptor effectively in the plasma membrane, (4) a catalytic domain (in the case of receptor–enzymes), (5) a domain involved in receptor microclustering, (6) a domain linked to the internalization process, (7) a substrate or G-protein binding domain, and (8) a phosphate-acceptor domain that may be a target for cellular protein kinases or for the receptor itself (autophosphorylation sites), so as to provide for enzymatic regulation of the receptor's activity. Given the expected multiple domain functions of a particular receptor, it is possible to approach structure–activity studies of a receptor on two levels. On the one hand, it may be possible to single out the function of an individual amino acid in the receptor sequence (for example, a serine, threonine, or tyrosine phosphate acceptor residue). On the other hand, it may be possible to assign more than one function to a discrete portion of the receptor comprising 10 to perhaps a few hundred amino acid residues. The specific amino acid sequences that confer a particular activity to a functional domain need not be contiguous, but may involve residues that are not adjacent when the receptor sequence is listed linearly; such residues may lie in close proximity only when the receptor is folded into its active conformation. For each domain so identified, it should be possible to single out a corresponding membrane-associated protein with which the receptor can interact. As will be elaborated upon below, work with a number of receptors is succeeding in delineating the kinds of domains discussed in the above paragraph and illustrated for the EGF-URO receptor in Figure 3.

3.0 The Ligand-Binding Domain

For the catecholamines, there is an extensive literature correlating the structural features of these monoamines with agonist activities. Similarly, for polypeptide growth factors, like EGF-URO and its close homologue, transforming growth factor α (TGF-α), there is an emerging literature, using site-directed mutagenesis and conventional peptide synthesis as bases for synthesizing peptide analogues, aimed at pinpointing the contribution of specific amino acids toward biological activity. Of particular interest with respect to the studies of peptide analogues is the ability to measure independently both agonist receptor affinity (by ligand-binding techniques) and biological potency (by tissue or cultured cell bioassay procedures). Such studies[30-33] provide a clear demonstration of the distinct nature of the receptor binding (K_D) and receptor triggering (ED_{50} and E_{max}) properties of agonist compounds, as encompassed by the "classical" concepts of "intrinsic" activity or "efficacy". Just as one can talk of distinct amino acid residues in the peptide agonist being responsible for (1) receptor binding and (2) receptor triggering, so one can talk of the complementary domains on the receptor that are involved in these two distinct processes. For instance, experiments with mutations introduced into the hamster β_2-adrenergic receptor point to the importance of the transmembrane domains of this receptor playing a key role in forming a ligand binding region close to the external domain of the plasma membrane.[34-36] Conversely, the

(30) Hollenberg, M. D.; Gregory, H. *Mol. Pharmacol.* **1980**, *17*, 314.
(31) Engler, D. A.; Matsunami, R. K.; Campion, S. R.; Stringer, C. D.; Stevens, A.; Niyogi, S. K. *J. Biol. Chem.* **1988**, *263*, 12384.
(32) Defeo-Jones, D.; Tai, J. Y.; Wegrzyn, R. J.; Vuocolo, G. A.; Baker, A. E.; Payne, L. S.; Garsky, V. M.; Oliff, A.; Riemen, M. W. *Mol. Cell. Biol.* **1988**, *8*, 2999.
(33) Lazar, E.; Vicenzi, E.; Van Obberghen-Schilling, E.; Wolff, B.; Dalton, S.; Watanabe, S.; Sporn, M. B. *Mol. Cell. Biol.* **1989**, *9*, 860.
(34) Dixon, R. A. F.; Sigal, I. S.; Rands, E.; Register, R. B.; Candelore, M. R.; Blake, A. D.; Strader, C. D. *Nature* **1987**, *326*, 73.
(35) Strader, C. D.; Sigal, I. S.; Candelore, M. R.; Rands, E.; Hill, W. S.; Dixon, R. A. F. *J. Biol. Chem.* **1988**, *263*, 10267.

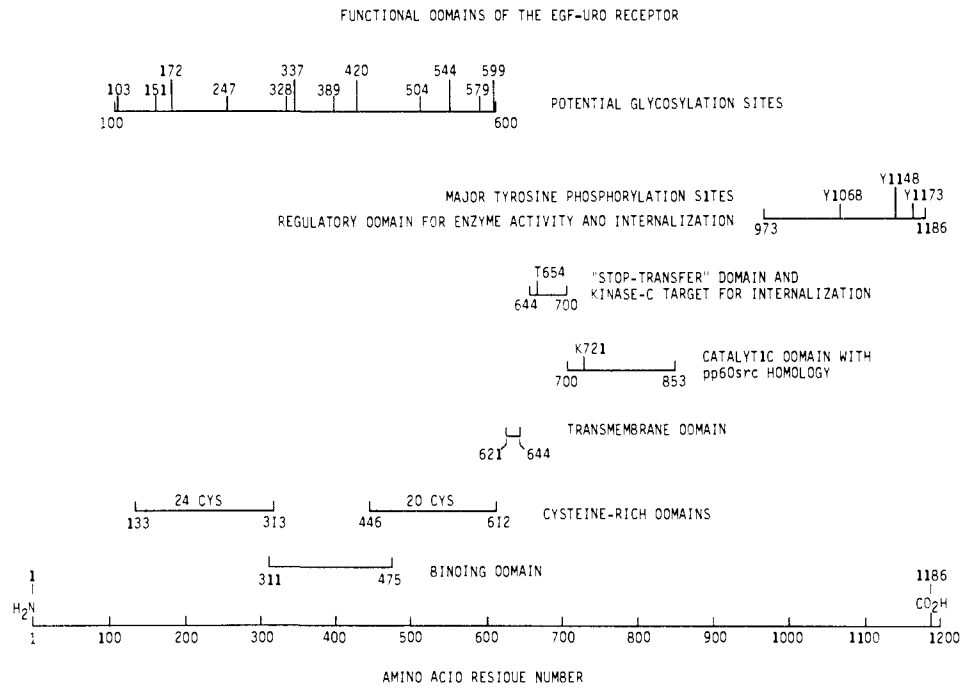

Figure 3. Functional domains of the EGF-URO receptor. As summarized in the text and elsewhere,[13-16] a number of specific receptor activities can be assigned to discrete portions of the receptor sequence.[58] In some instances, as discussed, the function of individual amino acid residues can be singled out (lysine (K) 721, threonine (T) 654, and tyrosines (Y) 1068, 1148, and 1173).

binding site for EGF-URO has been localized to a comparatively small stretch of sequence (residues about 320–500) in the extracellular domain.[37,38] It is of particular interest that the same receptor domain (domain III)[39] coming from two different species (human and chicken) can bind EGF-URO and TGF-α with reversed affinities (i.e. for the human receptor, EGF-URO binds with higher affinity than TGF-α, and vice versa for the chicken receptor, which binds TGF-α best). In a similar vein, the homologous receptors for (1) insulin and insluin-like growth factor-I (IGF-I) and (2) platelet-derived growth factor-AA and -BB possess binding domains that are able to distinguish between the closely related peptides insulin/IGF-I and PDGF-AA/-BB. As yet, the precise ligand-binding sequences in the α chains of the insulin and IGF-I receptors[40-42] and in the extracellular domains of the two PDGF receptors[43-46] have yet to be localized precisely.

Nonetheless, studies employing a mutational analysis and a comparative analysis of homologous receptors are beginning to define precisely the structural features of the receptors that are responsible for agonist binding. It is expected that the anatomy of the receptor sites involved in agonist triggering (i.e. intrinsic activity or efficacy) will also be similarly determined. It is important to note that although one principal extracellular receptor domain may be involved in ligand binding and ligand triggering, other cytoplasmically oriented receptor domains and transmembrane domains may also contribute to ligand-binding affinity, as will be discussed below for the adrenergic receptor. In certain respects the hydrophobic membrane-spanning receptor domain for receptors like the one for EGF-URO (Figure 3) does not appear to display characteristic sequence features in individual receptor families except for its content of hydrophobic amino acids. Nonetheless, more than simply anchoring the receptor, in the membrane, this domain can also play a role in cell triggering by a ligand-occupied receptor. For instance, switching the transmembrane domain in the PDGF receptor with the analogous hydrophobic domains of either the LDL receptor or the neu-oncogene tyrosine kinase (closely related to the EGF-URO receptor) resulted in a receptor that could not be triggered by PDGF even though PDGF could bind to the chimaeric receptor with high affinity and trigger receptor internalization.[46] In addition, a single amino acid mutation in the transmembrane domain of the neu/EGF-2 receptor appears to increase the constitutive tyrosine kinase activity of the unoccupied receptor.[13]

4.0 Domains Responsible for Receptor–Membrane Interactions

The cloning and sequencing of a number of receptors has delineated general domain features of receptors related

(36) Lefkowitz, R. J.; Caron, M. G. *J. Biol. Chem.* **1988**, *263*, 4993.
(37) Lax, I.; Burgess, W. H.; Bellot, F.; Ullrich, A.; Schlessinger, J.; Givol, D. *Mol. Cell. Biol.* **1988**, *8*, 1831.
(38) Lax, I.; Bellot, F.; Howk, R.; Ullrich, A.; Givol, D.; Schlessinger, J. *EMBO J.* **1989**, *8*, 421.
(39) Lax, I.; Johnson, A.; Howk, R.; Sap, J.; Bellot, F.; Winkler, M.; Ullrich, A.; Vennstrom, B.; Schlessinger, J.; Givol, D. *Mol. Cell. Biol.* **1988**, *8*, 1970.
(40) Ullrich, A.; Bell, J. R.; Chen, E. Y.; Herrera, R.; Petruzzelli, L. M.; Dull, T. J.; Gray, A.; Coussens, L.; Liao, Y.-C.; Tsubokawa, M.; Mason, A.; Seeburg, P. H.; Grunfeld, C.; Rosen, O. M.; Ramachandran, J. *Nature* **1985**, *313*, 756.
(41) Ullrich, A.; Gray, A.; Tam, A. W.; Yang-Feng, T.; Tsubokawa, M.; Collins, C.; Henzel, W.; Le Bon, T.; Kathuria, S.; Chen, E.; Jacobs, S.; Francke, U.; Ramachandran, J.; Fujita-Yamaguchi, Y. *EMBO J.* **1986**, *5*, 2503.
(42) Ebina, Y.; Ellis, L.; Jarnagin, K.; Edery, M.; Graf, L.; Clauser, E.; Ou, J.-H.; Masiarz, F.; Kan, Y. W.; Goldfine, I. D.; Roth, R. A.; Rutter, W. J. *Cell* **1985**, *40*, 747.
(43) Matsui, T.; Heidaran, M.; Miki, T.; Popescu, N.; La Rochelle, W.; Kraus, M.; Pierce, J.; Aaronson, S. *Science* **1989**, *243*, 800.
(44) Yarden, Y.; Escobedo, J. A.; Kuang, W.-J.; Yang-Feng, T. L.; Daniel, T. O.; Temble, P. M.; Chen, E. Y.; Ando, M. E.; Harkins, R. N.; Francke, U.; Fried, V. A.; Ullrich, A.; Williams, L. T. *Nature* **1986**, *323*, 226.
(45) Gronwald, R. G. K.; Grant, F. J.; Haldeman, B. A.; Hart, C. E.; O'Hara, P. J.; Hagen, F. S.; Ross, R.; Bowen-Pope, D. F.; Murray, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3435.
(46) Williams, L. T. *Science* **1989**, *243*, 1564.

to their localization in the plasma membrane. For instance, the hydrophobic transmembrane domains, suited to anchoring the receptor in the membrane, can be flanked, as for the EGF-URO receptor (Figure 3), by extracellular sites of glycosylation and intracellular "stop-transfer" sequences. Previous work has demonstrated the importance of receptor glycosylation both in terms of targetting the receptor to the plasma membrane and in terms of receptor function.[47-51]

As mentioned above, the transmembrane domain in some receptors (PDGF, neu/HER-2) may play a role in cell triggering. On the other hand, transmembrane domain exchanges in the EGF-URO receptor do not appear to affect its overall function.[13-16] In contrast with the tyrosine kinase receptors, in the adrenergic receptor family,[36] a number of the receptor sequences in the proposed seven transmembrane helices represent conserved sequences and are known to participate in ligand binding, as mentioned in section 3.0. A similar situation is thought to hold for the muscarinic receptor family.[52] Thus, some functions of receptor transmembrane domains, apart from receptor anchoring, are being elucidated by structure–activity studies. However, the exact sequences that are involved in the specific functions of this type of domain have not yet been identified precisely.

After a ligand triggers its receptor, the processes of receptor microclustering (dimers to 10's of receptors) aggregation (100's to 1000's of receptors) and ligand-induced internalization (so-called down-regulation) (see Figure 2) presumably also involve discrete receptor domains that are distinct from the ligand-binding site. To date, two kinds of domains involved in receptor internalization have been identified in the receptor for EGF-URO (Figure 3). In one domain, a specific threonine residue (no. 654) is the target for kinase C mediated phosphorylation.[53] A receptor lacking this phosphorylation site is no longer subject to kinase C mediated down-regulation, but the binding of EGF-URO is still able to accelerate internalization of the threonine-deficient receptor.[54] In addition, the intrinsic kinase activity of the insulin and EGF-URO receptors appears to play a role in ligand-triggered receptor internalization.[13,14,16,54] The putative phosphoprotein substrates involved in the ligand-directed "down-regulation" process have yet to be identified. Nonetheless, kinase-deficient receptors for EGF-URO are evidently still able to undergo spontaneous internalization and ligand-triggered microclustering (but not aggregation).[54] In a similar vein, a number of serine and threonine residues in the cytoplasmic portion of β-adrenergic receptor are targets for cellular protein kinases.[36] In particular, a cluster of potential serine and threonine phosphorylation sites can be identified in the C-terminal sequence between residues 365 and 413.[55] It is of interest that β-receptor phosphorylation by a number of protein kinases can be altered by agonist binding, so as to suggest a role for such phosphorylation sites in agonist-mediated receptor desensitization/down-regulation. In particular, one type of receptor phosphorylation appears to occur only in the presence of agonist (the so-called BARK-kinase).[36,56] The importance of the C-terminal phosphate acceptor domain has been evaluated in terms of (1) agonist-promoted phosphorylation and (2) agonist-triggered desensitization/down-regulation. Both a deletional (truncation of the C-terminal phosphate acceptor domain from residues 366 to 413) and a mutational (changes of serines and threonines to alanines or glycines) approach have established this domain as the target for agonist-enhanced phosphorylation, and the results also point to a role for this phosphate acceptor sequence in the process of ligand-induced desensitization/internalization.[36,55] In summary, the studies of domain function in the receptors for insulin, EGF-URO and β-adrenergic agents have brought to light sequences that are not only involved in the process of receptor internalization but are also regulatory targets for the same protein kinase-mediated phosphorylation reactions that participate in the signal amplification process. That is, the potential sites of receptor feedback regulation that may modulate receptor–membrane interactions can now be identified in the sequences of a variety of receptors.

5.0 Domains Involved in Interactions with Effectors

5.1 Tyrosine Kinase Receptors. Stemming from the work of Cohen and colleagues,[57] it has become evident that a number of receptors, including the ones for EGF-URO, insulin, IGF-I, and PDGF possess intrinsic tyrosine kinase activity.[13] There is now intense interest in pinpointing the key initial substrates with which these ligand-triggered receptors interact, so as to identify the "effectors" responsible for cell activation. In addition, it is of interest to identify the receptor domains responsible for substrate (i.e. effector) phosphorylation.

Largely from the work of Gill, Schlessinger, Ullrich, Waterfield, and their colleagues, a great deal is now known about the catalytic domains of the mammalian (human) and avian (chicken) receptors for EGF-URO which are both about 1200 amino acid residues long.[13-16] Most striking was the early discovery of the close relationship between the catalytic domain of the receptor, comprising its tyrosine kinase activity (approximately residues 690–940), and the catalytic domain of the sarcoma virus-coded tyrosine kinase pp60src.[13,58] This homology is now known to hold for the insulin and PDGF receptor families as well.[13] On the basis of this homology, lysine residue no. 721 in the EGF-URO receptor was readily identified as being critical for the binding of ATP, such that a mutated receptor lacking a lysine was devoid of catalytic activity. It has also become apparent that, despite considerable homology in this domain between receptors of the tyrosine kinase class,[13] there is considerable receptor substrate specificity. For example, the EGF-URO receptor readily phosphorylates calpactin II in a membrane reconstitution assay[24,28] but does not phosphorylate the related pp60src kinase substrate, calpactin I, under similar conditions.[28] The C-terminal EGF-URO receptor sequence (approximately residues 1060–1180), containing three principal autophosphorylation sites (Tyr 1068, 1148, and 1173), appears to play some role in regulating the K_m of peptide

(47) Mayes, E. L. V.; Waterfield, M. D. *EMBO J.* **1984**, *3*, 531.
(48) Soderquist, A. M.; Carpenter, G. *J. Biol. Chem.* **1984**, *259*, 12586.
(49) Slieker, L. J.; Lane, M. D. *J. Biol. Chem.* **1985**, *260*, 687.
(50) Cuatrecasas, P.; Illiano, G. *J. Biol. Chem.* **1971**, *246*, 4938.
(51) Hayes, G. R.; Lockwood, D. H. *J. Biol. Chem.* **1986**, *261*, 2791.
(52) Schimerlik, M. I. *Annu. Rev. Physiol.* **1989**, *51*, 217.
(53) Hunter, T.; Ling, N.; Cooper, J. A. *Nature* **1984**, *311*, 480.
(54) Lin, C. R.; Chen, W. S.; Lazar, C. S.; Carpenter, C. D.; Gill, G. N.; Evans, R. M.; Rosenfeld, M. G. *Cell* **1986**, *44*, 839.
(55) Bouvier, M.; Hausdorff, W. P.; De Blasi, A.; O'Dowd, B. F.; Kobilka, B. K.; Caron, M. G.; Lefkowitz, R. J. *Nature* **1988**, *333*, 370.
(56) Benovic, J. L.; Strasser, R. H.; Caron, M. G.; Lefkowitz, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 2797.
(57) Carpenter, G.; Cohen, S. *Annu. Rev. Biochem.* **1979**, *48*, 193.
(58) Ullrich, A.; Coussens, L.; Hayflick, J. S.; Dull, T. J.; Gray, A.; Tam, A. W.; Lee, J.; Yarden, Y.; Libermann, T. A.; Schlessinger, J.; Downward, J.; Mayes, E. L. V.; Whittle, N.; Waterfield, M. D.; Seeburg, P. H. *Nature* **1984**, *309*, 418.

substrates for the receptor kinase.[16,59,60] Interestingly, this C-terminal cytoplasmic domain also contributes to the high-affinity binding of EGF-URO.[61] It is now clear that most of the functions of the receptor (ranging from cell triggering to receptor internalization but exclusive of ligand binding and dimerization) rely entirely on the existence of an enzymatically active tyrosine kinase domain.

The receptors for insulin and IGF-I are seen as distinct from the single-chain EGF-URO receptor in that they are comprised of disulfide-linked heterotetramers of composition α_2/β_2, wherein the extracellular α subunits contain the ligand binding domains and the transmembrane/intracellular β subunits contain the tyrosine kinase domains which, like the kinase domain of EGF-URO receptor, are represented by a contiguous amino acid sequence[13,40-42] wherein lysine residue 1030 represents the ATP binding site, akin to lysine 721 in the EGF-URO receptor.[62] Autophosphorylation of the C-terminal portion of the insulin receptor is thought to facilitate the phosphorylation of other substrates.[12] In contrast, as reviewed recently,[13,46] both of the PDGF receptor subtypes, which are single-chain receptors like the one for EGF-URO, have tyrosine kinase domains that are split into two subdomains, one of which contains the ATP binding site, separated by 80–100 residues from a C-terminal subdomain that contains a tyrosine phosphorylation sites homologous with a site in the pp60src tyrosine kinase. Because of the intervening proline-rich insertion between the two tyrosine kinase subdomains, the PDGF receptor has been placed in a separate class of tyrosine kinase receptors, along with the receptor for colony stimulating factor-1, but distinct from the insulin/IGF-I and EGF-URO receptor classes.[13] In a sense, the members of the three distinct receptor classes, because of their sequence homologies, can be thought of as "isoreceptors" in the same manner as enzymes are designated "isoenzymes".

In terms of this perspective, focussed on receptor domain function, the above-mentioned isoreceptors are of interest not only because of their ability to be triggered in a distinct manner by closely related agonists (i.e. insulin and IGF-I for one receptor class, and PDGF-AA and -BB for the PDGF receptor subtypes) but also because of the substrate specificity built into the tyrosine kinase domains. Just as has been pointed out above for the EGF-URO receptor that can phosphorylate calpactin II but not the pp60src kinase substrate, calpactin I, in a membrane-reconstitution assay, it is clear that all of the tyrosine kinase receptors will display characteristic substrate specificities, as pointed out elsewhere.[63,64] Most instructive with respect to receptor domain function is the work of Williams and colleagues with the type-B PDGF receptor,[46] in which alterations were made in the domain separating the two tyrosine kinase subdomains. The interkinase domain deletion mutant of the PDGF receptor (designated δ-ki by Williams and colleagues) is of particular interest since this variant is able to stimulate phosphatidylinositol (PI) hydrolysis and increase intracellular calcium, but is deficient in the ability to trigger mitogenesis.[65] In addition, this receptor mutant appears to be unable to interact with phosphatidylinositol kinase, an enzyme that may be involved in the synthesis of novel phosphatidylinositol "signal messenger" precursors. Nonetheless, the ability of the δ-ki receptor mutant to trigger PI hydrolysis points to an interaction with a phospholipase C, a property that has been established for the EGF-URO receptor.[66] Thus, the data obtained with mutants of the PDGF receptor imply a selectivity of different receptor domains for interactions with a number of membrane-associated enzymes. It will be of substantial interest in the future to determine the precise structural features of the three classes of tyrosine kinase receptors that are responsible for substrate selectivity.

5.2 Domains Interacting with G-Proteins. The β-adrenergic receptor represents one of the most extensively studied receptors of the types that are coupled to guanine nucleotide regulatory proteins. As recently summarized,[36] all of the mammalian adrenergic receptor subtypes (β_1, β_2, α_1, and α_2) have now been purified to homogeneity, and mutational analysis has already begun for three of the subtypes (β_1, β_2, and α_2) for which the genes and/or cDNA's have been isolated and sequenced. The model that has been suggested[36] on the basis of the amino acid sequence data for the single-chain (about 410 residues long) adrenergic receptor (a member of the rhodopsin superfamily of G-protein-linked receptors) envisions a structure anchored by seven transmembrane helices (referred to as M-I through M-VII), with an extracellular N-terminal portion and an intracellular C-terminal sequence. In the suggested model, the looping back and forth of the seven proposed transmembrane helices results in the formation of three extracellular loops (designated E-I to E-III) and three cytoplasmic loops (C-I to C-III). As mentioned above and summarized elsewhere,[36] mutational analysis has emphasized the importance of the membrane-spanning regions (M-I etc.) for ligand binding, and deletion analysis has revealed that the largest extracellular loop, domain E-II, as well a the N-terminal portion, contributes only in a modest way to ligand binding.[34] Most interestingly, deletion of one of the putative intracellular loop domains (C-III) not only affects ligand binding (this receptor loses the "low affinity" site usually associated with GTP binding) but also results in a receptor that fails to couple to adenylate cyclase.[34] Thus, the C-III domain, comprising residues 240–270 of the β_2 receptor, would appear to be critically involved in the interaction of the receptor with the G-protein oligomer. Further work using a reconstitution approach with receptor variants and pure G-proteins should be able to corroborate the functional nature of domain C-III. Structural homologies have been observed for an enlarging number of G-protein-linked receptors including those for acetylcholine (muscarinic: M_1–M_4),[52,67-71] serotonin (5HT1a and 5HT1c),[72,73] sub-

(59) Honegger, A.; Dull, T. J.; Szapary, D.; Komoriya, A.; Kris, R.; Ullrich, A.; Schlessinger, J. *EMBO J.* **1988**, *7*, 3053.
(60) Honegger, A.; Dull, T. J.; Bellot, F.; Van Obberghen, E.; Szapry, D.; Scmidt, A.; Ullrich, A.; Schlessinger, J. *EMBO J.* **1988**, *7*, 3045.
(61) Livneh, E.; Prywes, R.; Kashles, O.; Reiss, N.; Sasson, I.; Mory, Y.; Ullrich, A.; Schlessinger, J. *J. Biol. Chem.* **1986**, *261*, 12490.
(62) Ebina, Y.; Araki, E.; Taira, M.; Shimada, F.; Mori, M.; Craik, C. S.; Siddle, K.; Pierce, S. B.; Roth, R. A.; Rutter, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 704.
(63) Hollenberg, M. D. *Clin. Invest. Med.* **1987**, *10*, 475.
(64) Klein, H. H.; Freidenberg, G. R.; Cordera, R.; Olefsky, J. *Biochem. Biophys. Res. Commun.* **1985**, *127*, 254.
(65) Escobedo, J. A.; Williams, L. T. *Nature* **1988**, *335*, 85.
(66) Wahl, M. I.; Nishibe, S.; Suh, P.-G.; Rhee, S. G.; Carpenter, G. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1568.
(67) Kubo, T.; Fukuda, K.; Mikami, A.; Maeda, A.; Takahashi, H.; et al. *Nature* **1986**, *323*, 411.
(68) Kubo, T.; Maeda, A.; Sugimoto, K.; Akiba, I.; Mikami, A.; et al. *FEBS Lett.* **1986**, *209*, 367.
(69) Bonner, T. I.; Buckley, N. J.; Young, A. C.; Brann, M. R. *Science* **1987**, *237*, 527.
(70) Peralta, E. G.; Ashkenazi, A.; Winslow, J. W.; Smith, D. H.; Ramachandran, J.; et al. *EMBO J.* **1987**, *6*, 3923.
(71) Peralta, E. G.; Winslow, J. W.; Peterson, G. L.; Smith, D. H.; Ashkenazi, A.; et al. *Science* **1987**, *236*, 600.

stance K,[74] angiotensin,[75] and the light receptor, rhodopsin,[76] which interacts with the G-protein homologue transducin. As mentioned above, structurally, rhodopsin may be taken as a prototype for the G-protein-linked receptor superfamily. In view of the studies pointing to the importance of the β-adenergic receptor domain C-III for G-protein interactions, it will be of great interest to examine the consequences of altering the homologous domain in the other receptors. In addition, since these receptors are thought to interact with distinct G-proteins,[18-20] it will be important to search for distinct sequences in the C-III domain region that confer G-protein selectivity. Reciprocally, structure–activity studies of the several G-proteins themselves should reveal the G-protein sequences that lead to a selective interaction of these proteins with their activating receptors.

6.0 Summary and Implications for Future Work

As outlined in sections 3.0–5.0, there are now a number of good examples of studies identifying specific receptor sequences involved in the domain functions discussed in section 2.0, and a number of the questions outlined in section 2.0 are, in part, being answered. The implications of these studies are at least 2-fold in terms of future work that can be done using the approaches outlined in this section. First, using site-directed and deletional mutational analysis of receptor sequences, it should be possible to determine with precision the functional role of specific amino acid residues, for instance as has been done for the ATP-binding function of lysine 721 of the EGF-URO receptor and for lysine 1030 of the insulin receptor. Such studies will very likely be complemented in the future by a crystallographic examination of receptor structure, as has been done for a variety of enzymes. Thus, a satisfying picture of the molecular basis of receptor function should emerge. A second implication of the studies relates to the substituents in or near the plasma membrane, with which specific receptors interact. For, once the domains on the receptor and on the interacting protein have been identified (e.g. domain C-III of the adrenergic receptor and a complementary G-protein domain), it may prove possible to design specific reagents related to these sequences that can modulate receptor–effector interactions. In terms of analogous enzyme–peptide substrate interactions of medical significance, one can point to the development of angiotensin converting enzyme inhibitors that are proving of enormous use in the treatment of hypertension. Thus, it may not be overly optimistic to hope for the development of specific compounds that may be able to regulate the interactions of specific cellular substrates with receptor tyrosine kinases. Such compounds might prove of use in controlling the oncogenic process that appears to result from the aberrant overproduction of tyrosine kinase receptor domains (e.g. the erythroleukemia virus erb-B counterpart of the EGF-URO receptor[13-16,58]). In a similar vein, studies of receptor domain function may lead to a better understanding of the interaction of peptide agonists (insulin, EGF-URO, etc.) with their receptors, so as to provide a novel basis for the design of new peptide antagonists and agonists. Overall, one can look forward with excitement to new developments in the area of receptor structure–activity studies, for which it can be said that a new era is just beginning.

Acknowledgment. Work in the author's laboratory is supported principally by operating funds from the Medical Research Council of Canada and by fellowship support from the Alberta Heritage Foundation for Medical Research.

(72) Fargin, A.; Raymond, J. R.; Lohse, M. J.; Kobilka, B. K.; Caron, M. G.; Lefkowitz, R. J. *Nature* **1988**, *335*, 358.
(73) Julius, D.; MacDermott, A. B.; Axel, R.; Jessell, T. M. *Science* **1988**, *241*, 558.
(74) Masu, Y.; Nakayama, K.; Tamaki, H.; Harada, Y.; Kuno, M.; Nakanishi, S. *Nature* **1987**, *329*, 836.
(75) Jackson, T. R.; Blair, L. A. C.; Marshall, J.; Goedert, M.; Hanley, M. R. *Nature* **1988**, *335*, 437.
(76) Dratz, E. A.; Hargrave, P. A. *Trends Biochem. Sci.* **1983**, *8*, 128.

Communications to the Editor

Cyclobut-A and Cyclobut-G: Broad-Spectrum Antiviral Agents with Potential Utility for the Therapy of AIDS

Sir:

In addition to the human immunodeficiency virus (HIV), virtually all adults with the acquired immunodeficiency syndrome (AIDS)[1] have been infected with one or more herpesviruses.[2] Cytomegalovirus (CMV) may threaten up to 25% of AIDS patients with blindness or death,[3] and at autopsy, evidence of active CMV infections is found with frequencies as high as 90%.[4] Although relatively benign in immunocompetent individuals, herpes simplex viruses (HSV-1 and HSV-2) can cause chronic ulcerative lesions in the immunocompromised.[3] Reactivation of varicella-zoster virus (VZV) afflicts many AIDS patients with painful vesicular eruptions.[3] Epstein-Barr virus (EBV) has been associated with AIDS related hairy leukoplakia and non-Hodgkin's lymphomas.[5]

Besides these direct contributions to morbidity and mortality, herpesviruses may play a more insidious role in the pathogenesis of AIDS by enhancing the replication and

(1) *AIDS: Modern Concepts and Therapeutic Challenges*; Broder, S., Ed.; Marcel Dekker: New York, 1987.
(2) (a) Quinnan, G. V.; Masur, H.; Rook, A. H.; Armstrong, G.; Frederick, W. R.; Epstein, J.; Manischewitz, J. F.; Macher, A. M.; Jackson, L.; Ames, J.; Smith, H. A.; Parker, M.; Pearson, G. R.; Parillo, J.; Mitchell, C.; Straus, S. E. *J. Am. Med. Assoc.* **1984**, *252*, 72–77. (b) Quinn, T. C.; Piot, P.; McCormick, J. B.; Feinsod, F. M.; Taelman, H.; Kapita, B.; Stevens, W.; Fauci, A. S. *J. Am. Med. Assoc.* **1987**, *257*, 2617–2621.
(3) Drew, W. L.; Buhles, W.; Erlich, K. S. In *The Medical Management of AIDS*; Sande, M. A., Volberding, P. A., Eds.; W. B. Saunders: Philadelphia 1988; Chapter 22.
(4) Macher, A. M.; Reichert, C. M.; Straus, S. E.; Longo, D. L.; Parrillo, J.; Lane, H. C.; Fauci, A. S.; Rook, A. H.; Manischewitz, J. F.; Quinnan, G. V. *N. Engl. J. Med.* **1983**, *309*, 1454.
(5) Ernberg, I.; Altiok, E. *APMIS* **1989**, *Suppl. 8*, 58–61 and references therein.

0022-2623/90/1833-1281$02.50/0