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## COMMENTARY

# GENOMIC PHARMACOLOGY: MORE INTRACELLULAR SITES FOR DRUG ACTION

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Many drugs, if not the majority, interact at the cell membrane with receptors and ion channels. As a rule, drugs elicit short-term effects which are measured in acute animal models. However, with antidepressant drugs which are also tested acutely in animal models, several weeks of treatment are needed before patients improve. This discrepancy is quite frustrating for pharmacologists who are aware of the inadequacy of acute pharmacological tests, in particular when they are looking at novel antidepressant drugs. In the future, pharmacology will have to evolve towards long-term approaches rather than acute models, which are often inappropriate. This is particularly so to find more effective drugs for the treatment of degenerative and proliferative diseases—today, the major problems in human health—and also of affective disorders. Unfortunately, pathological animal models are often lacking, although this may change in the future with the development of transgenic mice.

Drugs of the future will be designed so that they lead to changes in gene expression; this implies that they will be tested chronically in animal models. Although the drugs acting on membrane receptors may also elicit changes in the gene expression (cf. Ref. 1), nuclear receptors, "third messengers" and transcription factors will become more important targets for drug action in the near future. Such a genomic approach is not new; it has been the framework for developing hormone therapy, in particular that involving steroids, which bind to nuclear receptors as agonists or antagonists [2, 3]. The aim of the present commentary is to try to define the concept of genomic pharmacology. This term is extended here to include nuclear receptors and intracellular proteins other than those belonging to the superfamily of nuclear hormone receptors. Indeed, recent evidence suggests the existence of a nuclear translocation process for membrane receptors. The intracellular proteins represent potential new targets for drug action. Furthermore, a more speculative hypothesis will be presented according to which the intracellular segments of membrane receptors could represent a new potential target for drug action. We will not discuss here all the data from the literature concerning the changes in gene expression induced by drugs after acute or chronic treatment. Instead, the reader is referred to a recent review [1].

*How to define genomic pharmacology?*

The term "genomic pharmacology" is restricted to drugs that affect gene expression in leading to long-term biological responses in terms of cell growth and division, differentiation, cell survival and plasticity. Drugs inducing only rapid or transient changes as for instance, through early genes, without other late consequences should not be included in the present definition. The biological responses may occur in different ways but essentially through drug binding to membrane-bound or nuclear receptors and to other intracellular proteins. A good example of genomic pharmacology is the steroids which exert their biological effects at the transcription level via nuclear receptors [2, 3]. Drugs acting on membrane receptors may also induce mRNA changes. However, little is known about the modifications in gene expression caused by chronic treatment with such drugs. The increasing number of probes commercially available to analyze mRNA by Northern blot and *in situ* hybridization techniques will allow this new pharmacological approach to be further developed.

As a rule, proteins, rather than DNA itself represent the main target for pharmacological agents. Anticancer drugs alter gene expression by interacting with DNA [4], but lack specificity since they affect normal cells as well as tumor cells. DNA does not appear to be a good target for drugs namely because of its repetitive helical structure and the fact that it consists of only two base pairs, AT and GC. Obviously, synthetic compounds capable of interacting with DNA sequences of one gene would be much too large to reach their target according to the principles of drug action. Of course, DNA remains the main target of antisense strategy [5], which certainly can contribute to solving numerous biological problems. However, antisense strategy is pharmacologically nonsense. Pharmacologists know from experience that mimicking nature is rarely a good way to generate effective drugs. The design of small synthetic compounds remains the most successful strategy as shown recently with the development of new classes of drug in the field of neuropeptides. Indeed, non-peptide antagonists have been found for cholecystokinin [6], substance P [7, 8] and angiotensin [9]. Besides membrane receptors, other intracellular proteins are expected to act as transcription factors and, thus, may become

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Table 1. Putative ligand-activated nuclear receptors

| Receptor family | Ligand | Reference |
|---|---|---|
| Nuclear hormone receptor superfamily | Steroid (estrogen, progesterone, androgen, glucocorticoid, mineralocorticoid) | [cf. 2, 3, 10, 12–14] |
| | Retinoic acid | |
| | Thyroxine | |
| | Vitamin $D_3$ | |
| Peroxisome proliferator-activated receptor | Peroxisome proliferator including hypolipidemic drugs and plasticizer | [15] |
| Cytokine receptor | Interleukin 1 (IL-1) | [17–23] |
| | Interleukin 2 (IL-2) | [27–31] |
| Growth factor receptor | BDNF | [45] |
| | EGF | [35] |
| | aFGF and bFGF | [40, 41] |
| | NGF | [32–36] |
| | PDGF | [35, 44] |
| Peptide receptor | Thyroliberin | [46] |
| | Insulin | [42] |
| | Prolactin | [43] |
| | Thymosin | [47] |
| | VIP | [48, 52] |
| | Neurotensin | [49–51, 53, 54, 56] |

targets for drug action. Obviously, the transduction system of membrane receptors (cyclase, phospholipase C, tyrosine kinase, G protein, etc.) is not an appropriate target for drugs if specific effects are to be elicited. One of the most intriguing features of some receptors is the capacity of the ligand–receptor complex to be translocated from membranes into the nucleus, leading to specific changes of gene expression.

*Nuclear receptors*

Nuclear receptors, well known for steroids, vitamin $D_3$, retinoic acid and thyroid hormone, belong to the receptor superfamily of ligand-activated transcription factors (Table 1) (see Refs. 2, 3 and 10). The recent cloning and sequencing of cDNAs have revealed that these receptors share a similar structure with a high degree of homology. Among the six different domains of the nuclear receptors, two are highly conserved: the DNA binding domain (C) and the ligand binding domain (E). The C region contains zinc finger motifs for binding DNA sequences, which are called hormone responsive elements. The location of the progesterone receptor is essentially nuclear (Fig. 1), whereas estrogen and glucocorticoid receptors are present not only in the nucleus but also in the cytoplasm as a complex with a heat-shock protein. However, recent data indicate that the presence of the progesterone receptor in the nucleus reflects a dynamic situation in such a way that the receptor should diffuse into the cytoplasm and should be transported back into the nucleus, suggesting the existence of a nucleocytoplasmic shuttle mechanism [11]. Ligand binding to estrogen and glucocorticoid receptors results in the dissociation of masked receptors in the cytoplasm and causes dimer formation in the nucleus (Fig. 1). In this regard, three recent papers reported the formation of heterodimers from different nuclear receptors [12–14]; retinoid X receptors may be an auxiliary protein for thyroid hormone, vitamin $D_3$ and retinoic acid receptors in forming heterodimers which are able to greatly enhance the transcriptional activity. This interesting process is reminiscent of interactions reported between the Jun and Fos families of proteins.

A new member in the class of nuclear receptors, recently identified, is the peroxisome proliferator-activated receptor [15]. Several compounds such as clofibrate, leukotriene antagonists, herbicides and plasticizers, which are termed peroxisome proliferators, are the ligands of this new member of the steroid hormone receptor superfamily (Table 1).

Besides the class of hormone receptors whose nuclear nature has been firmly established, there is a group of putative nuclear receptors which appear to be translocated, in certain conditions, from the cell membrane to the nucleus. More attention will be focused on these putative nuclear receptors (Table 1).

The interleukin 1 (IL-1)* receptor, an 80 kDa protein, is a member of the immunoglobulin gene superfamily which binds IL-1$\alpha$ as well as IL-1$\beta$, although there is low amino acid identity between both interleukins (26%). The IL-1 receptor from human T cells has been characterized by molecular cloning as a 552 amino acid transmembrane protein having a single small membrane-spanning segment [16]. The extracellular portion of the IL-1 receptor is composed of three immunoglobulin-like domains with three disulfide bonds while the cytoplasmic domain (~215 aa) does not resemble sequences of known function.

How does IL-1 affect the transcription of specific

* Abbreviations: IL-1, interleukin 1; NLS, nuclear localization signal; and VIP, vasoactive intestinal peptide.

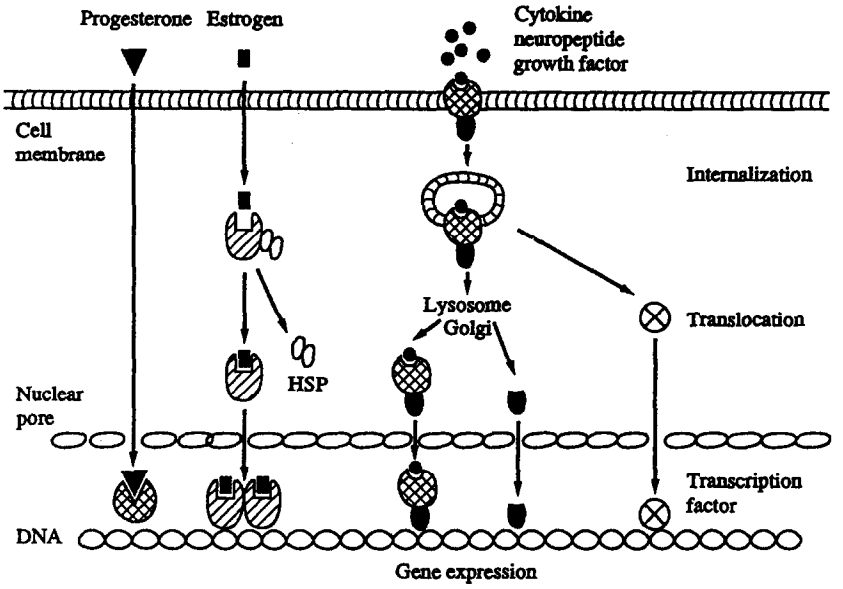


Fig. 1. Schema illustrating different types of nuclear receptors. Progesterone and estrogen cross the cell membrane and then bind to receptors either in the cytoplasm or in the nucleus. In the case of putative nuclear receptors, peptidic ligands induce internalization and nuclear translocation of all or part of the ligand–receptor complex. HSP = heat-shock protein.

genes in the cell by binding to membrane receptors? First, recent experiments have demonstrated that IL-1 can induce, in T cells and fibroblasts, internalization of IL-1 receptors which then undergo extensive down-regulation through degradation in lysosomes [17, 18]. In addition to this, some internalized IL-1 was found to accumulate in nuclei in a temperature-dependent process [17, 18]. Thus, nuclear localization of IL-1 appears to be the consequence of an endocytosis of the ligand–receptor complex (Fig. 2). Interestingly, the IL-1 internalization in EL-4 thymoma cells was correlated with IL-1 signal transduction events to induce IL-2 receptor expression, IL-2 secretion and growth factor production [19, 20]. When internalized IL-1 was extracted from nuclei, it was recovered bound to receptors [20]. Specific and saturable binding of $^{125}$I-IL-1$\alpha$ was demonstrated to occur in purified nuclei isolated from EL-4 cells [21]. That IL-1 receptors were not detected in nuclei in cells not treated with IL-1 strongly suggests that the nuclear translocation is induced by IL-1 binding to cell surface receptors. More evidence on the role of the cytoplasmic domain of IL-1 receptors in signal transduction was provided by using mutant receptors. When murine IL-1 receptor cDNA was modified by almost complete deletion of the cytoplasmic domain and was expressed in CHO cells, IL-1 induced prostaglandin release and colony-stimulating factor production were blocked but the binding of IL-1 to the receptor was not affected [22]. Similarly, mutant human fibroblast IL-1 receptors lacking most of the cytoplasmic domain were expressed at the cell surface and could bind, internalize, and translocate IL-1 to the nucleus but they did not allow IL-1 to mediate the induction of IL-2 and $SV_{40}$ promoters [23]. A critical region of the cytoplasmic domain of IL-1 receptors, i.e. that located between amino acids 477 and 527, is required to transduce the signal-mediated activation of gene expression in T cells.

It is not clear whether the basic amino acids 428–432 (Lys-Lys-Ser-Arg-Arg) of the cytoplasmic region of IL-1 receptors are essential to mediate the translocation of the ligand IL-1 receptor complex to the nucleus as are nuclear targeting sequences of $SV_{40}$ and polyoma large T antigens and nucleoplasmin [24]. In contrast, the putative nuclear localization sequence of IL-1$\beta$ cannot be implicated in the nuclear targeting because three proteins mutated in this sequence, though endowed with lower affinity for the receptor, showed greater biological potency than wild type of IL-1$\alpha$ or IL-1$\beta$ as measured by the induction of IL-2 secretion [25]. More intriguing is that substitution of glycine for arginine at position 127 of the mature human IL-1$\beta$ generates a mutant which stimulates transcription of fibroblast immediate early (*fos* and *jun*) and early (IL-6) genes but, in contrast to wild type IL-1$\beta$ induces minimal or no transcription of late genes, such as those encoding procollagenase and prostromelysin [26]. In conclusion, there are, today, numerous and already convincing data indicating that IL-1 induces nuclear translocation of membrane receptors and that such a process is directly involved in the changes in gene expression caused by IL-1. Of course, further studies are needed to understand how receptors reach the nucleus from endocytotic vesicles. Another problem to be solved concerns the molecular mechanisms at the transcription level. Are DNA responsive elements and other transcription factors involved as they are for the nuclear receptors of the hormone family? The IL-1 receptor already represents the

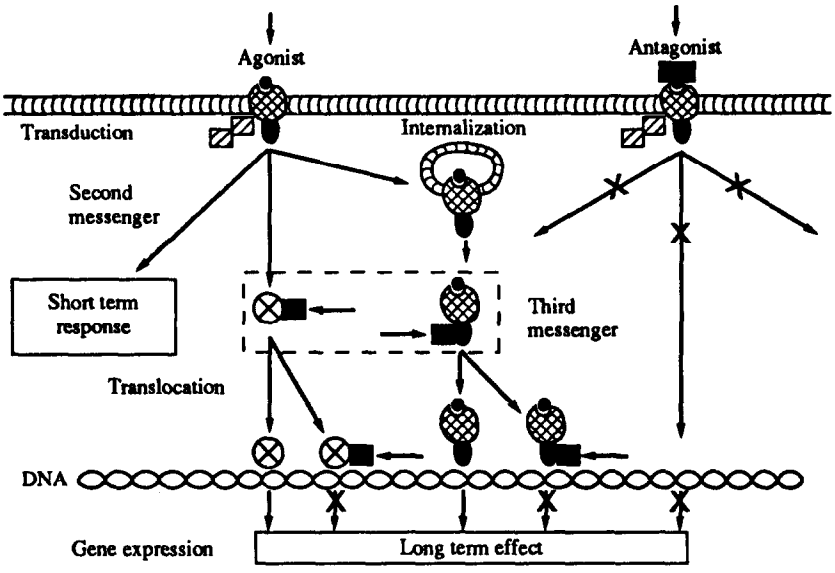


Fig. 2. Potential intracellular targets (black boxes) for drug action. The short-term response (transduction, second messenger) is distinct from the long-term effects (internalization, third messenger, nuclear translocation, gene expression). Antagonists at the cell membrane block both effects. ⊗ represents any cytoplasmic protein that, in addition to the ligand–receptor complex or a part of it, is involved in gene expression.

prototype of a protein which behaves either as a membrane or a nuclear receptor. At the present time, data available with regard to other cytokines, growth factors and neuropeptides are more fragmentary.

A unique feature of the IL-2 receptor is that it consists of two distinct components, the IL-2 R$\alpha$ chain (p55) and the IL-2 R$\beta$ chain (p70–75). IL-2 internalization and signal transduction only occur in cells bearing either the long chain or the two IL-2 receptor forms [27–29]. Like the IL-1 receptor, the IL-2 R$\alpha$ chain contains a large cytoplasmic region consisting of 286 amino acids, that lacks an obvious tyrosine kinase domain. Mutants lacking most of the cytoplasmic domain become deficient in growth signal transduction while the high affinity for IL-2 and the internalization process are not affected [30]. As was the case for the IL-1 receptor, a small portion (46 aa between 267 and 312) of the cytoplasmic domain of the IL-2 receptor is essential for signal transduction. Immuno-staining with two monoclonal antibodies directed against two distinct epitopes of the p55 subunit of the IL-2 receptor revealed that the receptor is transiently present in the nucleus during T cell activation and growth [31]. Here again as for the IL-1 receptor, membrane receptor activation by the ligand was necessary to reveal nuclear localization.

Several growth factors (NGF, BDNF, FGF, EGF and PDGF) interact with specific membrane receptors and behave like broad spectrum mitogens or agents affecting the development and maintenance of specific populations of cells or neurons. As a rule, their long-term effects are better known than the immediate response [32]. One of the major features of NGF is that it can be taken up at nerve terminals and then transported retrogradely to the cell body [33, 34]. Recently, NGF was detected in the nucleus of melanoma cells where it was bound in a non-degraded form to chromatin and where cell surface NGF-receptors were detected by means of monoclonal antibodies [35, 36]. This suggests that the NGF–receptor complex present in the nucleus could play a prominent role at the transcription level to enhance the expression of numerous genes, in particular those of enzymes involved in the synthesis of neurotransmitters (cf. Ref. 32). Interestingly, a soluble form of NGF receptor generated by proteolysis of intact membrane receptors was reported recently [37]; it is tempting to speculate that such a soluble receptor could originate, at least in part, in the course of nuclear translocation as is the case for steroid receptors.

Acidic (aFGF) and basic (bFGF) fibroblast growth factors are the prototypes of a family of polypeptides encoded by at least seven distinct genes (cf. Ref. 38). They exert mitogenic effects on a variety of cells of mesodermal and neuroectodermal origin. Surprisingly, systemic administration of FGF was reported to decrease arterial blood pressure [39]. This rapid response appears to be mediated by EDRF and by ATP-sensitive potassium ion channels. FGF receptors, like many other growth factor receptors, are associated with protein kinase activity. In primary cultures of bovine aortic endothelial cells, exogenously added bFGF was first detected in the cytoplasm and then in the nucleus in a cell cycle-dependent ($G_1$ phase) manner [40]. Indeed, such a nuclear translocation was not observed in quiescent confluent cells. Similar results were recently obtained

in cultured astrocytes and in hippocampal neurons [41]. By autoradiographic studies it has been established that labeled bFGF was internalized in vesicles and was localized to the perinuclear cytoplasm before being translocated to chromatin structures of the nucleus. That a mutant of aFGF lacking a putative nuclear translocation segment failed to induce DNA synthesis and cell proliferation argues that nuclear translocation of FGF is necessary for its mitogenic activity [41].

Other peptide ligands, such as insulin, prolactin, PDGF, EGF and BDNF were also found to be located and/or translocated into the nucleus even though nuclear receptors were not always demonstrated [35, 42–45]. For instance, nuclear prolactin is necessary for IL-2-stimulated proliferation and the ability of prolactin to activate nuclear kinase was totally inhibited by a monoclonal antibody specific for the rat liver prolactin receptor, implying that the activation process is mediated by a prolactin receptor [43]. Thyroliberin and thymosin have also been observed in nuclei by autoradiography or immunonocytochemistry [46, 47].

For the neuropeptides, evidence of nuclear receptors is scarce and often indirect. First, it is important to consider ligand-mediated endocytosis of receptors other than as a purely degradative process. However, partial degradation of receptor bearing vesicles is probably necessary to make membrane receptors more suitable for nuclear translocation. In the case of neuropeptides, a first step of internalization is an absolute prerequisite since, in contrast to steroids, they do not cross the cell membrane. Ligand-mediated internalization has been clearly demonstrated for vasoactive intestinal peptide (VIP) *in vitro* [48] and for the neurotensin *in vitro* [49, 50] and *in vivo* [51]. VIP nuclear receptors were identified in nuclei of a human colonic adenocarcinoma cell line by specific binding and cross-linking of $^{125}$I-labelled VIP [52], whereas labelled neurotensin was detected in nuclei of cholinergic neurons [53] and, more recently, in nuclei of dopaminergic neurons [54]. As a consequence of this, VIP and neurotensin were found to increase tyrosine-hydroxylase mRNA levels in $PC_{12}$ cells and dopaminergic neurons, respectively [55, 56]. For instance, when labelled neurotensin was injected into the rat striatum it was recovered, after retrograde transport, in nuclei of dopaminergic neurons in the substantia nigra; thus, here, a long distance separated endocytosis and nuclear localization [51, 56].

In conclusion, the nuclear translocation of membrane receptors may be pivotal in the regulation of gene expression, which is thought to be responsible for the long-term effects of ligands like cytokines, growth factors and neuropeptides. Much experimental work is needed to clarify the series of events leading to the appearance of nuclear signals. However, the data available today seem to point to a common process operating for a great variety of ligands. If this concept is true, signal molecules are translocated within the cell by mechanisms for which axonal transport and nuclear translocation are the prototypes.

*Intracellular translocation of signal molecules*

Signal molecules undergo at least two main translocation processes (Fig. 2). The first one is intracytoplasmic, from the cell membrane to the perikaryon and may sometimes occur over a very long distance, as in the axon of neurons. The second intracellular movement corresponds to nuclear translocation, whereby signal molecules present in the cytoplasmic compartment enter the nucleus. The neuron represents an ideal model to study intracytoplasmic transport because of the extraordinary eccentricity of nerve terminals with regard to the cell body. Retrograde axonal transport from the synapse to the perikaryon has been clearly demonstrated for three signal molecules: NGF, opiate and neurotensin [33, 51, 57]. Internalization of the ligand–receptor complex is a prerequisite for retrograde transport, a process which may only occur with agonists. Labelled lofentanil, an exogenous synthetic opiate, was found to move retrogradely in the vagus nerve and to accumulate in the nodose ganglion through a process blocked by naloxone (cf. Ref. 57). The two main features of the retrograde axonal transport are: its rapidity and its dependence on microtubule. Colchicine pretreatment was found to prevent the retrograde transport of labelled neurotensin in dopaminergic neurons in rat brain [51]. Interestingly, colchicine was also reported to down-regulate cortical cholecystokinin mRNA levels and to up-regulate mRNA of NPY, galanin, neurotensin, NGF and BDNF [58, 59]. It is tempting to speculate that these changes could be due to a blockade of retrograde transport of signal molecules acting either positively or negatively. Whatever the mechanism, colchicine is known to induce profound changes in the levels of neuropeptides and other modulators and in the number of presynaptic receptors. Therefore, it is not surprising that colchicine has been an effective drug for treating pathologies as diverse as gout, Mediterranean fever, non-infectious pericarditis and biliary cirrhosis [60]. Besides therapeutic applications, colchicine and other microtubule inhibitors are essential tools for analyzing the cytoplasmic translocation of signal molecules. In contrast to the GTP binding $G_{i\alpha}$ for which a retrograde axonal transport was reported recently [61], second messengers such as cAMP, inosital 1,4,5-trisphosphate, diacylglycerol or $Ca^{2+}$ do not move rapidly and retrogradely along axons to the neuronal cell body. This provides more support to the idea that proteins or "third messengers" are pivotal for transferring information molecules from the membrane to the nucleus (Fig. 2). In this regard, it is generally thought that the phosphorylation of these proteins may be of great importance especially at the nerve terminals [62] where it could play a role in dictating the polarity of retrograde axonal transport [57]. In the future, third messengers will probably become more important targets for drug action. Indeed, these proteins when translocated into the nucleus may act as transcription factors presumably in the form of dimers. A good example of such a dimer was provided recently by studying the effects of cyclosporin and FK-506 on the transmission of signals from the T cell antigen receptor [63]. A transcription factor, NF-AT, was formed following the activation of T cells, which was found to induce the nuclear

translocation of a a pre-existing cytoplasmic subunit A and to form the dimer NF-AT with a newly synthesized nuclear subunit B. The translocation of the cytoplasmic component A was blocked by FK-506 and cyclosporin A.

Besides intracytoplasmic transport, there is a continuous exchange of molecules between the cytoplasm and the nucleus through the nuclear envelope which consists of two concentric membranes, the nuclear pore complexes (see Fig. 1) and the nuclear lamina. The nuclear pore complex is the site of protein entry into the nucleus and RNA exits from it. Small proteins cross the nuclear envelope relatively freely, perhaps by diffusion, whereas large proteins (40–60 kDa) are actively transported through a process which is saturable, ATP-dependent, and mediated by a nuclear localization signal (NLS) (also called karyophilic segment) contained within the transported protein itself [64, 65]. The nuclear localization of the progesterone receptor, which reflects a dynamic process rather than a stable state, is an example of continuous exchange by which the receptor diffuses into the cytoplasm and is constantly and actively transported back into the nucleus [11]. In some examples, the nuclear import of protein is regulated through association of the protein with heterologous cytoplasmic factors. For example, the glucocorticoid and estrogen receptors reside in the cytoplasm as a complex with a heat-shock protein (hsp 90) (see Fig. 1). Upon hormone binding, the complex dissociates and the receptor is targeted to the nucleus. Similarly, the transcription factor NF-kB is associated, in the cytoplasm, with a subunit IkB which is released by cell stimulation, allowing NF-kB to enter the nucleus and to activate genes [64]. Another example is the catalytic and regulatory subunits of cAMP-dependent protein kinase (PK-A) which remains associated with the Golgi complex until activation of cAMP elevation whereupon the C or catalytic subunit dissociates and migrates to the nucleus [65].

As already discussed, membrane receptors may undergo nuclear translocation following ligand-induced internalization by a mechanism that remains to be elucidated. However, a major problem arises because the ligand–receptor complex is associated with an endocytotic vesicle, thus necessitating a disassembly process before it can enter the nucleus. Indeed, it is quite unlikely that small vesicles pass through nuclear pores. To solve this problem, two possibilities have already been invoked: a partial degradation in lysosomes and/or a disassembly process in the Golgi apparatus [57]. Another and perhaps more attractive hypothesis is that the receptor-bearing vesicles first bind to specialized proteins of the nuclear pores and then undergo a kind of "exocytosis", the mirror image of endocytosis occurring at the cell membrane for receptor internalization in such a way that either the ligand–receptor complex, the ligand alone or the ligand plus a part of the receptor corresponding to intracellular segments would be delivered into the nucleus (see Fig. 2).

Nuclear localization signals are required for the nuclear translocation of large proteins. These signals consist of short basic sequence motifs with the amino acids arginine (R) or lysine (K), or of two independent basic domains (bipartite NLS) [24, 65–67]. The first demonstration for the existence of NLS came from studies on nucleoplasmin, a nuclear protein of the *Xenopus* oocyte, and on the SV40 large T antigen. Deletion and mutation of the basic sequence PKKKRV of SV40 T antigen and of the bipartite nuclear sequence of nucleoplasmin (see Table 2) were found to cause cytoplasmic accumulation. There are several examples of functional bipartite signals with spacers of about ten amino acids, in particular steroid receptors and transcription factors (Table 2). Interestingly, when short sequences of the basic peptides corresponding to the NLS of the thyroid and steroid hormone receptors were coupled to bovine serum albumin, the peptide–bovine serum albumin conjugates were found to be transported to the nucleus [68].

The existence of NLS has not yet been demonstrated for all of the proteins listed in Table 2 or for many other receptors. However, the bipartite motif of EGF and muscarinic $m_1$ receptors is quite reminiscent of that of nucleoplasmin, steroid receptors and transcription factors, whereas the intracellular domains of muscarinic $m_3$, IL-1, GABA A and neurotensin receptors possess a sequence similar to the NLS of SV40 large T antigen. Some data suggest that the NLS present in HBGF-1$\alpha$ and the PDGF B chain, thus in the ligand itself, may be of importance for nuclear translocation. As a rule a nuclear localization signal binds to proteins located at the nuclear pore complex before being transported to the nucleus. A very large number of karyophilic proteins may operate through such a mechanism; in particular, NLS ought to be present in intracellular domains of numerous membrane receptors such as $\beta$-adrenergic, muscarinic, serotonergic, growth factor, and interleukin. The efficiency of nuclear targeting is not always simply related to the presence or absence of a sequence signal. Indeed, in some cases, phosphorylating or anchoring proteins, such as heat-shock proteins or IkB, can modulate nuclear translocation [64].

Interestingly, the intracellular C terminal segment of membrane receptors which contains basic karyophilic sequences, appears to be one of the less well conserved domains, in contrast to the transmembrane domains which are the most conserved. Such a heterogeneity in the intracellular domains may lead to a great diversity in the long-term effects of receptors or might be simply the expression of the cell phenotype. Thus, a given receptor located on neurons would possess intracellular domains different from those of the same receptor situated on smooth muscle or endothelial cells simply because they have to regulate genes corresponding to the cell phenotype. Such a diversity in the long-term response does not necessarily imply the occurrence of a differential pharmacology, since the amino acid sequence of the ligand binding sites may be the same in the different receptor subtypes. In any case, the receptor subtype diversity that molecular biology has revealed recently [69] is perhaps the expression of the diversity of the intracellular domains of receptors rather than that of the ligand binding sites. Recent data on truncated

Table 2. Comparison of amino acid sequences of (potential) nuclear localization signals (NLS)

| Protein | Source | Position | Signal sequence |
|---|---|---|---|
| Large T antigen | SV40 | | P **KKKRK** V |
| Nucleoplasmin | *Xenopus* | 3144 | **KR** PAATKKAGQA **KKKK** |
| Glucocorticoid R | Human | 479 | **RK** CLGAGMNLEA **RKTKK** |
| Progesterone R | Rabbit | 627 | **RK** CCQAGMVLGG **RK**F**KK** |
| Androgen R | Human | 432 | **RK** CYEAGMTLGA **RK**L**KK** |
| C-Fos | Human | | **RR** ERNKAAAKC **R**N**RRR** |
| | Mouse | | |
| | Rat | | |
| Jun-B | Mouse | 271 | **KR** LRNLAATKC **RKKK** |
| EGF R | Human | 645 | **RRR** HIV **RKR** TL **RR** |
| Muscarinic $m_1$ R | Porcine | 437 | D **KRR** WRKIP **KK** P |
| | Rat | | |
| $GABA_a$ $R_\alpha 3$ | Human | 346 | **KK** VPEALEM **KKK** |
| $GABA_aR_\alpha 2$ | Human | 326 | PSKDKD **KKKK** P |
| Bradykinin $B_2R$ | Rat | 311 | G **KR** F **RKK** S |
| Muscarinic $m_3$ R | Rat | 562 | D **KRKRRK** Q |
| IL-1 R | Human | 427 | V **KK** S **RR** L |
| Neurotensin R | Rat | 386 | RH **RRKKR** P |
| PDGF A chain | Human | | G **KKRKRKR** |
| HBGF-$1_\alpha$ | | 21 | NY **KK** P **K** L |

A single letter amino acid code is used; basic amino acids (R = arginine and K = lysine) are indicated in bold letters. Amino acid clusters presumably important in nuclear targeting appear as one short basic sequence or as two independent basic domains (bipartite NLS). In the first column, R = receptor.

or mutated receptors give more support to the idea that intracellular domains are of greater importance than membrane and extracellular domains for the long-term effects of membrane receptors [23, 30, 70]. Quite intriguing is that mutation of the $\alpha_1$B-adrenergic receptor at the third intracellular loop was found to enhance mitogenesis and tumorigenicity [70]. This is quite compatible with the idea that mutations of the intracellular domains may result in human disease states associated with uncontrolled cell growth. Therefore, the intracellular domains of receptors may become important potential targets for drug action.

*Future prospects*

Genomic pharmacology originates from what we know about the hormone nuclear receptors, the functional dissection of which has been made possible by molecular biology. However, today, genomic pharmacology is gaining new adepts: it spreads to research areas as the neuropeptide, cytokine and growth factor fields but probably also to other domains. The reasons for this are the recent experimental data suggesting that membrane receptors undergo internalization leading to nuclear translocation to play, in the nucleus, the role of transcription factors. As shown in Fig. 2, a membrane receptor may exert a double function in different cell compartments: first, a short-term effect through the transduction mechanisms and the second messengers following ligand binding to membrane receptors; and then a long-term response, i.e. gene expression changes, which are the consequence of receptor internalization and nuclear translocation of third messengers. Therefore, the receptor domains involved in regulating gene expression as well as other third messengers are assumed to become potential targets for drug action.

Further experimental work is needed to identify the nature of these third messengers. Because they are proteins, they contain a great diversity of messages and, in contrast to second messengers, may be transported over a very long distance, for example from synapses to the cell body. Receptors operate in the vicinity of cell membrane for the short-term effects but in the nucleus for the long-term responses. As shown in Figs 1 and 2, the third messengers can be the whole receptor with or without its ligand, or only a part of them corresponding to the intracellular domains in particular the C terminal segment, and/or any other cytoplasmic proteins. One may assume that they act, in the nucleus, as heterodimers. Although the long-term responses of growth factors and cytokines are much better known than their short-term effects, recent data indicate that FGF may elicit hypotension [39] and IL-1 may impair both vascular contraction and relaxation in rabbit isolated aorta [71]. Similarly neurotensin in the striatum enhances dopamine release through presynaptic neurotensin receptors located on dopaminergic nerve terminals whereas the long-term response of neurotensin in the substantia nigra is to increase the synthesis of tyrosine hydroxylase after retrograde axonal transport of the ligand–receptor complex [51, 56]. Conversely opiate inhibits substance P release at nerve terminals and decreases the anterograde axonal transport of substance P through a mechanism not yet elucidated [72].

The long-term response of neurotensin in

dopaminergic neurons is the consequence of an internalization of signal molecules. In *Escherichia coli*, the *lac* operon is regulated by lactose when this signal molecule is present in the external milieu in quite large amounts. By contrast, the blood concentration of steroids is extremely low, which does not prevent these hormones from reaching nuclear receptors. Unlike steroids, neuropeptides cannot cross the cell membrane; thus they have to be internalized if they are to enter the cell to change the gene expression. A few molecules may serve as an "interiorized" message in the latter process which appears to be one of the most economical and quite selective since it is receptor dependent. In the brain such a long distance signalling system is believed to be involved in long-term processes such as development, neurogenesis, neuronal plasticity and presumably in long-term memory [57].

As already mentioned, several transcription factors exist in the form of dimers and even heterodimers [13, 14, 63]. The coexistence of different neurotransmitters in neurons [73] is entirely compatible with the occurrence of heterodimers in the postsynaptic side. Indeed, if two transmitters coexist in a neuron, they will be co-released and then co-internalized at the postsynaptic side so that heterodimers can be formed from the two internalized receptors. Such a process may be of importance in modulating gene expression.

Our hypothesis throughout this commentary has been that more intracellular proteins, third messengers, transcription factors and the intracellular domains of membrane receptors will become potential targets for drug action. These drugs will act, therefore, at a step distal to the cell membrane receptors and known second messengers. The great advantage of drugs that interact within the cell rather than on the ligand binding sites is that they can selectively affect the long-term response of the agonist while preserving the short-term effects (cf. Fig. 2). This approach may yield more selective drugs and perhaps drugs that are specific to a particular cell type owing to the great diversity of receptor subtypes that molecular biology is now revealing. Such a view is not utopian: a recent report has shown that the absence of a single amino acid in the progesterone receptor may render the antiprogesterone RU 486 ineffective in the chick and the hamster [74]. In order to treat proliferative and degenerative diseases, one needs drugs designed for long-term responses that can change gene expression.

## REFERENCES


1. Laduron PM, Towards genomic pharmacology: From membrane to nuclear receptors. In: *Advances in Drug Research* (Ed. Testa B), Vol. 22, pp. 107–148. Academic Press, London, 1992.
2. Green S and Chambon P, Nuclear receptors enhance our understanding of transcription regulation. *Trends Genet* **4**: 309–314, 1988.
3. Beato M, Gene regulation by steroid hormones. *Cell* **56**: 335–344, 1989.
4. Hurley LH and Boyd FL, DNA as a target for drug action. *Trends Pharmacol Sci* **9**: 402–407, 1988.
5. Hélène C and Toulmé JJ, Specific regulation of gene expression by antisense, sense and antigene nucleic acids. *Biochim Biophys Acta* **1049**: 99–125, 1990.
6. Evans BE, Bock MG, Rittle KE, Di Pardo RM, Whitter WL, Veber DF, Anderson PS and Freidinger RM, Design of potent, orally effective, nonpeptidal antagonists of the peptide hormone cholecystokinin. *Proc Natl Acad Sci USA* **83**: 4918–4922, 1986.
7. Garret C, Carruette A, Fardin V, Moussaoui S, Peyronel JF, Blanchard J-C and Laduron PM, Pharmacological properties of a potent and selective nonpeptide substance P antagonist. *Proc Natl Acad Sci USA* **88**: 10208–10212, 1991.
8. Snider RM, Constantine JW, Lowe JA III, Longo KP, Lebel WS, Woody HA, Drozda SE, Desai MC, Vinick FJ, Spencer RW and Hess H-J, A potent nonpeptide antagonist of the substance P ($NK_1$) receptor. *Science* **251**: 435–437, 1991.
9. Wong PC, Chiu AT, Price WA, Thoolen MJMC, Carini DJ, Johnson AL, Taber RI and Timmermans PBMWM, Nonpeptide angiotensin II receptor antagonists. I. Pharmacological characterization of 2-*n*-butyl-4-chloro-1-(2-chlorobenzyl)imidazole-5-acetic acid, sodium salt (S-8307). *J Pharmacol Exp Ther* **247**: 1–7, 1988.
10. Evans RM, The steroid and thyroid hormone receptor superfamily. *Science* **240**: 889–895, 1988.
11. Guiochon-Manel A, Lescop P, Christin-Maître S, Loosfelt H, Perrot-Applanat M and Milgrom E, Nucleocytoplasmic shuttling of the progesterone receptor. *EMBO J* **10**: 3851–3859, 1991.
12. Zhang XK, Hoffmann B, Tran PB-V, Graupner G and Pfahl M, Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. *Nature* **355**: 441–446, 1992.
13. Kliewer SA, Umesono K, Mangelsdorf DJ and Evans RM, Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin $D_3$ signalling. *Nature* **355**: 446–449, 1992.
14. Leid M, Kastner P, Lyons R, Nakshatri H, Saunders M, Zacharewski T, Chen J-Y, Staub A, Garnier J-M, Mader S and Chambon P, Purification, cloning, and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. *Cell* **68**: 377–395, 1992.
15. Issemann I and Green S, Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **347**: 645–650, 1990.
16. Sims JE, Acres RB, Grubin CE, McMahan CJ, Wignall JM, March CJ and Dower SK, Cloning the interleukin 1 receptor from human T cells. *Proc Natl Acad Sci USA* **86**: 8946–8950, 1989.
17. Mizel SB, Kilian PL, Lewis JC, Paganelli KA and Chizzonite RA, The interleukin 1 receptor. Dynamics of interleukin 1 binding and internalization in T cells and fibroblasts. *J Immunol* **138**: 2906–2912, 1987.
18. Qwarnstrom EE, Page RC, Gillis S and Dower SK, Binding, internalization and intracellular localization of interleukin-1$\beta$ in human diploid fibroblasts. *J Biol Chem* **263**: 8261–8269, 1988.
19. Von Hoegen I, Falk W, Kojouharoff G and Krammer PH, Internalization of interleukin 1 (IL1) correlates with IL 1-induced IL 2 receptor expression and IL 2 secretion of EL4 thymoma cells. *Eur J Immunol* **19**: 329–334, 1989.
20. Curtis BM, Widmer MB, de Roos P and Qwarnstrom EE, IL 1 and its receptor are translocated to the nucleus. *J Immunol* **144**: 1295–1303, 1990.
21. Grenfell S, Smithers N, Miller K and Solari R, Receptor-mediated endocytosis and nuclear transport of human interleukin 1$\alpha$. *Biochem J* **264**: 813–822, 1989.
22. Curtis BM, Gallis B, Overell RW, McMahan CJ, de Roos P, Ireland R, Eisenman J, Dower SK and Sims

JE, T-cell interleukin 1 receptor cDNA expressed in Chinese hamster ovary cells regulates functional responses to interleukin 1. *Proc Natl Acad Sci USA* **86**: 3045–3049, 1989.
23. Heguy A, Baldari C, Bush K, Nagele R, Newton RC, Robb RJ, Horuk R, Telford JL and Melli M, Internalization and nuclear localization of IL-1 are not sufficient for function. *Cell Growth Differ* **2**: 311–315, 1991.
24. Robbins J, Dilworth SM, Laskey RA and Dingwall C, Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: Identification of a class of bipartite nuclear targeting sequence. *Cell* **64**: 615–623, 1991.
25. Grenfell S, Smithers N, Witham S, Shaw A, Graber P and Solari R, Analysis of mutations in the putative nuclear localization sequence of IL-1$\beta$. *Biochem J* **280**: 111–116, 1991.
26. Conca W, Auron PE, Aoun-Wathne M, Bennett N, Seckinger P, Welgus HG, Goldring SR, Eisenberg SP, Dayer JM, Krane SM and Gehrke L, An interleukin 1$\beta$ point mutant demonstrates that *jun/fos* expression is not sufficient for fibroblast metalloproteinase expression. *J Biol Chem* **266**: 16265–16268, 1991.
27. Fujii M, Sagamura K, Sano K, Nakai M, Sugita K and Hinuma Y, High affinity receptor mediated internalization and degradation of interleukin 2 in human T cells. *J Exp Med* **163**: 550–562, 1986.
28. Weissman AM, Harford JB, Svetlik PB, Leonard WL, Depper JM, Waldmann TA, Greene WC and Klausner RD, Only high-affinity receptors for interleukin 2 mediate internalization of ligand. *Proc Natl Acad Sci USA* **83**: 1463–1466, 1986.
29. Robb RJ and Greene WC, Internalization of interleukin 2 is mediated by the $\beta$-chain of the high affinity interleukin 2 receptor. *J Exp Med* **165**: 1201–1206, 1987.
30. Hatakeyama M, Mori H, Doi T and Taniguchi T, A restricted cytoplasmic region of IL-2 receptor $\beta$ chain is essential for growth signal transduction but not for ligand binding and internalization. *Cell* **59**: 837–845, 1989.
31. Jothy S, Abadie A, Froussard P, Duphot M and Thèze J, Transient nuclear location of the IL-2 receptor during T cell activation. *Exp Mol Pathol* **50**: 84–91, 1989.
32. Thoenen H and Barde YA, Physiology of nerve growth factor. *Physiol Rev* **60**: 1284–1335, 1980.
33. Hendry IA, Stöckel K, Thoenen H and Iversen LL, The retrograde axonal transport of nerve growth factor. *Brain Res* **68**: 103–121, 1974.
34. Stöeckel K, Schwab M and Thoenen H, Specificity of retrograde transport of nerve growth factor in sensory neurons: A biochemical and morphological study. *Brain Res* **89**: 1–14, 1975.
35. Rakowicz-Szulczynska EM, Rodeck U, Herlyn M and Koprowski H, Chromatin binding of epidermal growth factor, nerve growth factor and platelet-derived factor in cells bearing the appropriate surface receptors. *Proc Natl Acad Sci USA* **83**: 3728–3732, 1986.
36. Rakowicz-Szulczynska EM and Koprowski H, Identification of NGF receptor in chromatin of melanoma cells using monoclonal antibody to cell surface NGF receptor. *Biochem Biophys Res Commun* **140**: 174–180, 1986.
37. Zupan AA and Johnson EM Jr, Evidence for endocytosis-dependent proteolysis in the generation of soluble truncated nerve growth factor receptors by A875 human melanoma cells. *J Biol Chem* **266**: 15384–15390, 1991.
38. Goldfarb M, The fibroblast growth factor family. *Cell Growth Differ* **1**: 439–445, 1990.
39. Cuevas P, Carceller F, Ortega S, Zazo M, Nieto I and Gimenez-Gallego G, hypotensive activity of fibroblast growth factor. *Science* **254**: 1208–1210, 1991.
40. Baldin V, Roman A-M, Bosc-Bierne I, Almaric F and Bouche G, Translocation of bFGF to the nucleus is $G_1$ phase cell cycle specific in bovine aortic endothelial cells. *EMBO J* **9**: 1511–1517, 1990.
41. Imamura T, Engleka K, Zhan X, Tokita Y, Forough R, Roeder D, Jackson A, Maier JAM, Hla T and Maciag T, Recovery of mitogenic activity of a growth factor mutant with a nuclear translocation sequence. *Science* **249**: 1567–1570, 1990.
42. Soler AP, Thompson KA, Smith RM and Jarett L, Immunological demonstration of the accumulation of insulin, but not insulin receptors, in nuclei of insulin-treated cells. *Proc Natl Acad Sci USA* **86**: 6640–6644, 1989.
43. Buckley AR, Crowe PD and Russell DH, Rapid activation of protein kinase C in isolated rat liver nuclei by prolactin, a known hepatic mitogen. *Proc Natl Acad Sci USA* **85**: 8649–8653, 1988.
44. Maher DW, Lee BA and Donorghue DJ, The alternatively spliced exon of the platelet derived growth factor A chain encodes a nuclear targeting signal. *Mol Cell Biol* **9**: 2251–2253, 1989.
45. Wetmore C, Cao Y, Pettersson RF and Olson L, Brain-derived neurotrophic factor: Subcellular compartmentalization and interneuronal transfer as visualized with anti-peptide antibodies. *Proc Natl Acad Sci USA* **88**: 9843–9847, 1991.
46. Laverrière JN, Gourdji D, Picart R and Tixier-Vidal A, Thyroliberin is rapidly transferred to the nucleus of $GH_3$ pituitary cells at both 4°C and 37°C. *Biochem Biophys Res Commun* **103**: 833–840, 1981.
47. Conteas CN, Mutchnick MG, Palmer KC, Weller FE, Luk GD, Naylor PH, Erdos MR, Goldstein AL, Panneerselvam C and Horecker BL, Cellular levels of thymosin immunoreactive peptides are linked to proliferative events: Evidence for a nuclear site of action. *Proc Natl Acad Sci USA* **87**: 3269–3273, 1990.
48. Izzo RS, Scipione RA, Pellecchia C and Lokchander RS, Binding and internalization of VIP in rat intestinal epithelial cells. *Regul Pep* **33**: 21–30, 1991.
49. Mazella J, Leonard K, Chabry J, Kitabgi P, Vincent JP and Beaudet A, Binding and internalization of iodinated neurotensin in neuronal cultures from embryonic mouse brain. *Brain Res* **564**: 249–255, 1991.
50. Vanisberg MA, Maloteaux JM, Octave JN and Laduron PM, Rapid agonist-induced decrease of neurotensin receptors from the cell surface in rat cultured neurons. *Biochem Pharmacol* **42**: 2265–2274, 1991.
51. Castel MN, Malgouris C, Blanchard JC and Laduron PM, Retrograde axonal transport of neurotensin in the dopaminergic nigrostriatal pathway in the rat. *Neuroscience* **36**: 425–430, 1990.
52. Omary MB and Kagnoff MF, Identification of nuclear receptors for VIP on a human colonic adenocarcinoma cell line. *Science* **238**: 1578–1581, 1987.
53. Szigethy E, Leonard K and Beaudet A, Ultrastructural localization of [$^{125}$I]neurotensin binding sites to cholinergic neurons of the rat nucleus basalis magnocellularis. *Neuroscience* **36**: 377–391, 1990.
54. Castel MN, Woulfe J, Wang X, Laduron PM and Beaudet A, Light and electron microscopic localization of retrogradely-transported neurotensin in dopaminergic nigrostriatal neurons of the rat. *Neuroscience*, in press.
55. Wessels-Reiker M, Haycock JW, Howlett AC and Strong R, Vasoactive intestinal polypeptide induces tyrosine hydroxylase in $PC_{12}$ cells. *J Biol Chem* **266**: 9347–9350, 1991.
56. Burgevin MC, Castel MN, Quarteronet D, Chevet T and Laduron PM, Neurotensin increases tyrosine hydroxylase mRNA-positive neurons in subtantia nigra

after retrograde axonal transport. *Neuroscience* **49**: 627–633, 1992.
57. Laduron PM, Axonal transport of neuroreceptors: Possible involvement in long-term memory. *Neuroscience* **22**: 767–779, 1987.
58. Hökfelt T, Cortés R, Schalling M, Ceccatelli S, Pelto-Huikko M, Persson H and Villar MJ, Distribution patterns of CCK and CCK mRNA in some neuronal and non-neuronal tissues. *Neuropeptides* **19** (Suppl): 31–43, 1991.
59. Ceccatelli S, Ernfors P, Villar MJ, Persson H and Hökfelt T, Expanded distribution of mRNA for nerve growth factor, brain-derived neurotrophic factor, and neurotrophin 3 in the rat brain after colchicine treatment. *Proc Natl Acad Sci USA* **88**: 10352–10356, 1991.
60. Levy M, Sprino M, and Read SE, Colchicine: A state of the art review. *Pharmacotherapy* **11**: 196–211, 1991.
61. Hendry IA and Crouch MF, Retrograde axonal transport of the GTP-binding protein $G_{i\alpha}$: A potential neurotrophic intra-axonal messenger. *Neurosci Lett* **133**: 29–32, 1991.
62. Huganir R and Greengard P, Regulation of neurotransmitter receptor desensitization by protein phosphorylation. *Neuron* **5**: 555–567, 1990.
63. Flanagan WM, Corthésy B, Bram RJ and Crabtree GR, Nuclear association of a T-cell transcription factor blocked by FK-506 and cyclosporin A. *Nature* **352**: 803–807, 1991.
64. Baeuerle PA and Baltimore D, IkB: A specific inhibitor of the NF-kB transcription factor. *Science* **242**: 540–546, 1988.
65. Nigg EA, Mechanisms of signal transduction to the cell nucleus. *Adv Cancer Res* **55**: 271–310, 1990.
66. Breeuwer M and Goldfarb DS, Facilitated nuclear transport of histone $H_1$ and other small nucleophilic proteins. *Cell* **60**: 999–1008, 1990.
67. Silver PA, How proteins enter the nucleus. *Cell* **64**: 489–497, 1991.
68. Hamy F, Helbecque N and Henichart JP, Comparison between synthetic nuclear localization signal peptides from the steroid/thyroid hormone receptors superfamily. *Biochem Biophys Res Commun* **182**: 289–293, 1992.
69. Schofield PR, Shivers BD and Seeburg PH, The role of receptor subtype diversity in the CNS. *Trends Neurosci* **13**: 8–11, 1990.
70. Allen LF, Lefkowitz RJ, Caron MG and Cotecchia S, G-protein-coupled receptor genes as protooncogenes: Constitutively activating mutation of the $\alpha_{1B}$-adrenergic receptor enhances mitogenesis and tumorigenicity. *Proc Natl Acad Sci USA* **88**: 11354–11358, 1991.
71. Robert R, Chapelain B, Jean T and Néliat G, Interleukin-1 impairs both vascular contraction and relaxation in rabbit isolated aorta. *Biochem Biophys Res Commun* **182**: 733–739, 1992.
72. De Witte PA, Hamon M, Mauborgne A, Cesselin F, Levy C and Laduron PM, Ethanol and opiate decrease the axonal transport of substance-P like immunoreactive material in rat vagus-nerves. *Neuropeptides* **16**: 15–20, 1990.
73. Hökfelt T, Johansson O, Ljungdahl A and Lundberg JM, Peptidergic neurones. *Nature* **284**: 515–521, 1980.
74. Benhamou B, Garcia T, Lerouge T, Vergezac A, Gofflo D, Bigogne C, Chambon P and Gronemeyer H, A single amino acid that determines the sensitivity of progesterone receptors to RU 486. *Science* **255**: 206–209, 1992.