Cell Transfection as a Tool to Study Growth Hormone Action (43738)

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Abstract. **The isolation of growth hormone receptor (GHR) cDNA clones has made possible the transfection of GHRs into cultured cells. Our aim in this minireview is to show how the application of such approaches have benefited GHR research. GH stimulation of cells expressing GHR cDNAs can cause an alteration of cellular function that mimic those of the endogenous GHR. GHR cDNA transfected cells also offer a system where the mechanism of GH action can be studied. Such a system has been used to demonstrate that the GHR itself becomes tyrosine phosphorylated and that further phosphorylation of downstream proteins is important in GH action. The GH signals are transmitted to the nucleus and GH regulated genes have now begun to be characterized. The ability to use cell transfection for mechanistic studies of GH action will be instrumental to define domains within the receptor that are of functional importance and to determine pathways whereby GH signals are conveyed within the cell.**

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Molecular endocrinology has benefited enormously from the newly emerged techniques which allow the introduction of foreign DNA into cultured cells. Numerous examples exist where cell transfection has been used to study hormone receptors and the components involved in their signaling pathways. In the following review, the use of cell transfection as a tool to study growth hormone (GH) action will be discussed. There are two main rationales for the transfection of cells with GH receptor (GHR) cDNA. One is simply to obtain cells that respond well to GH. It is known that several cell types lose GHRs *in vitro,* a situation which is unfortunate for functional studies. The intact rat liver is the major source of the GHR but established liver cell lines display a remarkable low receptor capacity. The reason for this receptor loss may be attributed to the correlation of GHR expression to a highly differentiated liver (1). The transfection of mutated forms of GHRs provides a second rationale since this allows the definition of functional domains in the receptor (described by Billestrup *et al.,* this volume).

GHR Expression in Transfected Cells

The cloning of human and rabbit GHR cDNA provided the impetus to evaluate the binding properties of expressed GHR after cell transfection (2). As initially shown in COS-7 cells, the expressed GHR binds GH with similar affinity and specificity as endogenous GHRs (2). The suggestion that GHR dimerization is of functional significance has in part emerged from experiments using cells transfected with GHR cDNA; here, monoclonal antibodies to the GHR function as agonists, whereas their monovalent fragments did not (3). GHR cDNA has subsequently been isolated from a variety of different species (4–6), and cell transfection using rat GHR cDNA has in particular been used to investigate GH induced alterations in cellular function (for review, see 7).

Biological Functions in Cells that Express GHR cDNA

It is now evident that cells expressing transfected GHR cDNA gain a functional GH response. This has been demonstrated using different cell lines such as Chinese hamster ovary cells (CHO), rat insulinoma cells (RIN), buffalo rat liver (BRL) cells, and a pro-

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myeloid cell line (FDC-P1) (8, 12, 15, 16). CHO cells express few endogenous GHRs but stable transfection with GHR cDNA results in high-affinity, low-capacity GH binding (8). GH regulation of protein synthesis has been demonstrated in these cells which provides a starting point for further functional studies (8, 9). Also, lipid synthesis is stimulated by short-term exposure to GH, whereas longer exposure to GH results in lipolysis (10). Furthermore, GH possesses a weak mitogenic activity in these cells (11). RIN cells normally respond to GH and endogenously express both GH and prolactin (Prl) receptors. Stable expression of GHR cDNA increases the GH binding to these cells and decreases the ED50 for GH stimulated functions (12). The biological effects of GH in these cells include stimulation of insulin production and cell division (13). Cells of hepatic origin (BRL) have also been transfected and stable transfection with GHR cDNA confers responsiveness of the lipoprotein lipase (LPL) gene to GH, which is not the case with untransfected cells (14). GHR cDNA can also be functionally expressed in a murine promyeloid cell line FDC-P1; these cells grow in the presence of GH without interleukin-3 (15, 16). As summarized in Table I, several systems now exist where GH functions can be studied in GHR transfected cells. Furthermore, the results indicate that the expressed GHR cDNA closely mimics the function of an endogenous GHR.

Table I. Functional Expression of GH Receptor cDNA[a]

Cell line	Biological function	Reporter gene transfection
CHO (Chinese hamster ovary)	Protein/lipid metabolism (6, 8, 10) Mitogenesis (11) Protein phosphorylation (11, 18, 19)	a (25, 33)
BRL (Buffalo rat liver)	Lipoprotein lipase mRNA (14)	a, c (14, 25)
FDC-P1 (myeloid precursor)	Proliferation (15, 16)	
RIN (Rat insulinoma)	Proliferation (12)	b (27)
	Insulin production (12, 13)	

[a] Examples of cell lines used for transfection with GH receptor cDNA. The end point biological functions used to test GH function are indicated. GH regulation of transfected reporter genes have been evaluated using reporter genes fused with regions from the 5′ flank derived from the following genes: a, Serine protease inhibitor (SPI) gene; b, somatostatin gene; c, lipoprotein lipase gene. For more information see text.

GHR Signal Transduction in GHR cDNA-Transfected Cells

After GH binding, the ligand receptor complex is internalized. The ultimate fate of the receptor is not known, but it is interesting to note the existence of GHRs in nuclei (17). We have recently been able to demonstrate nuclear localization of GHRs also in cases of GHR cDNA-transfected cells (Lobie *et al.*, in preparation). Stimulation of the GHR in transfected cells results in the appearance of multiple tyrosine phosphorylated proteins (18), some of which have now been identified. Furthermore, from a functional viewpoint, it is known that inhibitors of phosphorylation, such as staurosporine, inhibit GH effects (e.g., lipogenesis) (10). One protein that becomes tyrosine phosphorylated in a GH-dependent manner is the GHR itself (18). Because the receptor itself is not a tyrosine kinase, one hypothetical cause for GHR phosphorylation was the existence of a receptor associated kinase (18). Cell transfection with the GHR cDNA, which yielded a shorter form of GHR, provided the first evidence that this hypothesis could be correct (19), and the identity of a GHR-associated kinase has recently been disclosed (20). This kinase, Janus kinase 2 (JAK2) belongs to a distinct family of tyrosine kinases (21), and the association of JAK2 with the GHR may be of fundamental importance to the understanding of GH signal transduction. Downstream of the receptor, the GH induced phosphorylation cascade contains members yet to be identified, one of which is mitogen activated protein (MAP) kinases (11). MAP kinase becomes maximally tyrosine phosphorylated 10 min after GH stimulation. It is also interesting to note that MAP kinase is GH activated in some GHR transfected cells (CHO) but not in other systems such as BRL cells (unpublished results). Gene expression provides one suitable measurement of the effects of GH in that several genes have been shown to be regulated by GH (for a review, see 22). In our experience, attempts to show GH regulation of insulin-like growth factor I (IGF-I) gene expression in receptor transfected cells have not yet been successful. However, other genes show GH regulation and lipoprotein lipase (LPL) mRNA is increased in GHR transfected BRL cells (BRL-4) (14). To further understand the nature of these signaling pathways, the approach of transfecting putative GH responsive reporter genes has been attempted. GH regulates a construct consisting of 1713 bp of the LPL 5′ flank fused to a chloramphenicol acetyltransferase (CAT) reporter gene when transfected into BRL-4 cells (14). As another example, BRL cells have been transfected with both GHR cDNA and a GH response element (GHRE) in a reporter gene construct. This GHRE, initially described by Yoon *et al.* (23), consists

of 50 base pairs and was identified in the 5′ flank of the GH responsive serine protease inhibitor (SPI) gene expressed in liver (24). A GH responsive reporter plasmid has been made in which six repeats of the GHRE from the SPI gene were fused to a heterologous promoter in front of a CAT reporter. As shown in Table II, this construct is markedly GH responsive in a variety of receptor transfected cells (25). It is interesting to note that the reporter gene also responds to GH in receptor transfected cells of nonhepatic origin such as CHO and neuroblastoma cells. Studies have also been carried out using the promoter region of the somatostatin gene fused to a CAT reporter plasmid. Such fusion genes are GH regulated in RIN cells (26). The somatostatin promoter −44 to −33 contains a GH responsive element with the following sequence: CAGAGAGAGA. This element binds nuclear protein(s) in gel retardation assays without any apparent GH dependency. The size of the protein binding to this element has an estimated molecular weight of 56 kDa (27). Sequence comparisons between the GH regulated SPI and insulin genes show that these genes contain areas of similarity to the GHRE in the somatostatin promoter. The nature of GH-dependent transcription factor(s) is yet unclear, but it is of potential interest that sequence similarities exist between interferon/interleukin target genes (e.g., the SPI GHRE) (for a review, see 28). Interferon gamma, as one example, activated genes by phosphorylation of preexisting transcription factor(s) (29), and it is interesting to speculate that GH could have a similar type of mechanism for gene regulation. It is likely that different genes are regulated by GH by a variety of different mechanisms. The IGF-I gene does not contain the GHRE described above; instead, a single GH-dependent DNase hypersensitive site has been mapped in chromatin to the second IGF-I intron (30). Another set of GH-regulated genes belong to the family of hepatic cytochrome P450s. Within this family some genes (e.g., P450 2C12) are regulated in a sex-specific manner by GH (for a review, see 31). It is possible that GH regulates the P450 2C12 by mechanisms that are different from both SPI and IGF-I. The expression of P450 2C12 cannot be influenced by GH in receptor transfected liver derived cell lines (unpublished observations) but primary hepatocytes respond to GH by a marked induction of P450 2C12 (32). One may thus argue that the GHR activates different types of signal transduction pathways in the cell. Part of the explanation may reside in findings linking different receptor domains to different functions (see Billestrup *et al.*, this volume) but GH regulation of gene expression could also depend on as of yet unclear factors, such as cell differentiation and/or chromatin structure.

Table II. GH Regulation of a Transfected GH Responsive SPI-CAT Gene[a]

Cell line	GH-Induced CAT activity (fold increase)
HepG2-GHR	1.6
BRL-GHR	33.0
FAO-GHR	7.4
CHO-GHR	3.3
SKN-GHR	54

[a] Stable GH receptor expressing clones of cells transfected with rat GH receptor cDNA (GHR) was obtained after neomycin selection. The cells used were derived from liver in the case of HepG2, BRL, and FAO. Other cell types were CHO and the human neuroblastoma cell line SKN. The cells were transiently transfected with a fusion gene containing a 6 mer repeat of a GH responsive element from the SPI gene (23) fused to a TK-CAT expression vector (25). Cells were stimulated with GH (100 n*M*) and CAT activity measured in protein extracts. The fold induction demonstrates the difference in CAT activity compared to unstimulated controls. The table is modified from Ref. 25.

Conclusions

Different cellular model systems now exist where GHR cDNA has been functionally expressed. These cells allow an analysis of different steps involved in the mechanism of GH action such as the function of different receptor domains. GHR transfected cells can also be used to study GH regulation of gene expression and to analyze different components in a GH induced signal transduction chain. The GHR in cells transfected with GHR cDNA becomes phosphorylated in a ligand dependent manner and GH causes phosphorylation of JAK2 and MAP kinases. Protein phosphorylation thus seems to play an important role in GH action. One type of GH signal is conveyed to the nucleus where GH responsive DNA elements have begun to be characterized. Functional expression of the GHR by cell transfection of GHR cDNA thus represents one route to a more detailed knowledge of the mechanism of GH action.

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