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REVIEW ARTICLE

Control of gene expression by glucocorticoid hormones

Guy G. ROUSSEAU
International Institute of Cellular and Molecular Pathology and University of Louvain Medical School, Box 7529, Avenue Hippocrate 75, B-1200 Brussels, Belgium

Introduction

Glucocorticoid hormones are essential for life. They play a regulatory rôle in tissue differentiation during development. In the adult organism, they control intermediary metabolism and co-ordinate the adjustment of physiological processes in response to stress (for a review, see Baxter & Rousseau, 1979).

In target cells, glucocorticoids bind non-covalently and reversibly to about 25000 intracellular receptor sites. These sites are borne by a thermodynamically homogeneous population of oligomeric phosphoproteins. The receptor is found in the cytosol when the steroid is absent (as discussed later, this does not prove that, in intact cells, steroid-free receptors are located in the cytoplasm). Binding of a glucocorticoid agonist is thought to promote dissociation and dephosphorylation of the receptor. This process, called 'activation' or 'transformation', enables the steroid–receptor complex to become associated with chromatin in the cell nucleus. The biochemical mechanisms of this nuclear transfer are unknown. One way in which glucocorticoid antagonists act is by binding to the receptor to form a complex that cannot undergo the 'transformation' process (Fig. 1).

The receptor monomer contains a single polypeptide chain of M_r 90000. The steroid- (one per monomer) and DNA-binding sites occur on a receptor region no greater than about half the

Abbreviations used: MTV, mouse mammary tumour virus; bp, base pairs; LTR, long terminal repeat; GRE, glucocorticoid response element; TIE, transcription initiation element.

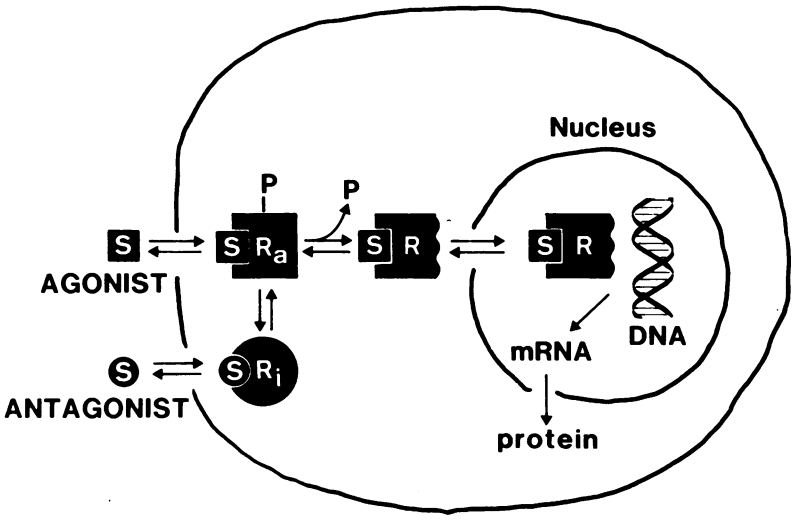

Fig. 1. *First steps in glucocorticoid hormone action*
The figure shows an idealized target cell in which glucocorticoid agonists (small squares) or antagonists (small circles) penetrate rapidly and combine reversibly with the glucocorticoid receptor to form an active (R_a) or inactive (R_i) complex. In order to interact with chromatin, the receptor–agonist complex must become 'transformed' (R), a step that probably involves dephosphorylation (P). The possibility that all the intracellular steps described actually occur within the nucleus has not been entirely discounted (see the text for discussion).

molecule. The other half is called the 'specifier' region because it is required for receptor action (Vedeckis, 1983).

Most of the glucocorticoid effects amenable to experimentation have been shown to result from a receptor-mediated change in concentration or activity of key proteins. This change is a consequence of an action of the receptor–steroid complex on the molecular machinery that controls gene expression. Recent advances such as recombinant-DNA and monoclonal-antibody techniques have offered new ways of exploring this machinery. Since the glucocorticoid receptor qualifies as one of the few proteins known to control gene expression in eukaryotes, it is not surprising that glucocorticoids have become a fashionable tool for the molecular biologist.

In this Review I will attempt, by relying largely on the current literature, to review the progress in the field and to show how the study of glucocorticoid hormone action has shed a new light on important biochemical tissues that can be as remote from endocrinology as transcriptional control or retrovirus expression.

Glucocorticoids modulate mRNA concentration

A physiological consequence of the formation of a glucocorticoid–receptor complex in the intact cell is an increase or a decrease in the amount of a small number of gene products (less than 1%) that are specified by the differentiation of the cell (Ivarie *et al.*, 1980; Voris & Young, 1981). Depending on the so-called 'glucocorticoid domain' of the target cell, such gene products include enzymes (tyrosine aminotransferase), 'house-keeping' proteins (metallothionein), structural proteins (fibronectin, collagen), membrane hormone receptors (insulin receptor), secretory proteins (casein, α_{2u}-globulin), hormones (somatotropin), or even retroviruses such as the mouse mammary tumour (Bittner) virus (MTV).

The activity or concentration of those gene products changes as a result of changes in the concentration of their own mRNA (Table 1), or of the mRNA of regulatory factors which, in turn, control the activity of the gene products. These regulatory factors often remain unidentified. Their involvement is postulated when the glucocorticoid-induced change in enzyme activity results from a change in the properties of enzyme molecules, as is the case for the inhibition of plasminogen activator (Coleman *et al.*, 1982). Another example is induction of alkaline phosphatase, which has been ascribed, at least in part, to a glucocorticoid-induced dephosphorylation mechanism (Cox & Elson, 1971). Glucocorticoid-induced intermediates should also be suspected when the lag period

Table 1. *Glucocorticoids change mRNA concentration*

Gene product	Effect*	System	Reference
Tyrosine aminotransferase†	I	Rat liver and hepatoma cells	Diesterhaft *et al.* (1980), Olson *et al.* (1980)
Tryptophan oxygenase†	I	Rat liver	Danesch *et al.* (1983)
Glutamine synthetase	I	Chick embryo retina	Sarkar & Griffith (1976)
Glycerol phosphate dehydrogenase	I	Rat glial cells	McGinnis & de Vellis (1978)
α_{2u}-Globulin	I	Rat liver	Chen & Feigelson (1979)
Metallothionein†	I	HeLa cells, mouse sarcoma cells	Karin *et al.* (1980), Mayo & Palmiter (1981)
Lysozyme	I	Chick oviduct	Renkawitz *et al.* (1982)
Mouse mammary tumour virus†	I	Mouse and rat (HTC) cells	Groner *et al.* (1983*a*)
Somatotropin†	I	Rat pituitary cells (+thyroid hormone)‡	Spindler *et al.* (1982), Evans *et al.* (1982)
β-Casein	I	Rabbit mammary gland (+prolactin)‡	Teyssot & Houdebine (1981)
Phosphoenolpyruvate carboxykinase	I	Rat liver (+glucagon)‡	Salavert & Iynedjian (1982)
α_1-Acid glycoprotein	I	Rat hepatoma cells	Feinberg *et al.* (1983)
Pro-opiomelanocortin	D	Mouse and rat pituitary cells	Roberts *et al.* (1982)
Procollagen type I	D	Chick fibroblasts	Sterling *et al.* (1983)
Prolactin	D	Rat pituitary cells	Ivarie *et al.* (1982)
α_1-Foetoprotein†	D	Developing rat liver	Guertin *et al.* (1983)
β-Globin†	D	Differentiating mouse (Friend) cells	Mierendorf & Mueller (1982)

* I, increase; D, decrease.

† The lack of effect of protein synthesis inhibitors on glucocorticoid action suggests that the latter is a primary event.

‡ The hormones mentioned in parentheses are required for (prolactin, glucagon) or amplify (thyroid hormone) the glucocorticoid effect.

observed before the activity of the gene product rises is abnormally long. In rat hepatoma (HTC) cells, for instance, we have found that stimulation of the plasma membrane enzyme alkaline phosphodiesterase I by glucocorticoids requires RNA and protein synthesis (Rousseau *et al.*, 1980), but becomes detectable long after induction of tyrosine aminotransferase, a transcriptional effect, has taken place (Fig. 2).

In some cases, ongoing protein synthesis is required for the full glucocorticoid effect on mRNA to be seen. Cycloheximide abolishes in rat liver cells the induction of α_{2u}-globulin (Chen & Feigelson, 1979) and of phosphoenolpyruvate carboxykinase (Iynedjian & Jacot, 1981) mRNA; it reduces the accumulation of α_1-acid glycoprotein mRNA (Feinberg *et al.*, 1983). In rat glial cells, the glucocorticoid-induced accumulation of mRNA for glycerol phosphate dehydrogenase is inhibited by cycloheximide (McGinnis & de Vellis, 1978). Another interesting example of indirect effects is the regulation by glucocorticoids, in MTV-infected rat hepatoma cells, of post-translational processing and compartmentalization of viral glyco- and phosphoproteins, under conditions when their transcription is not affected by the hormone (Firestone *et al.*, 1982).

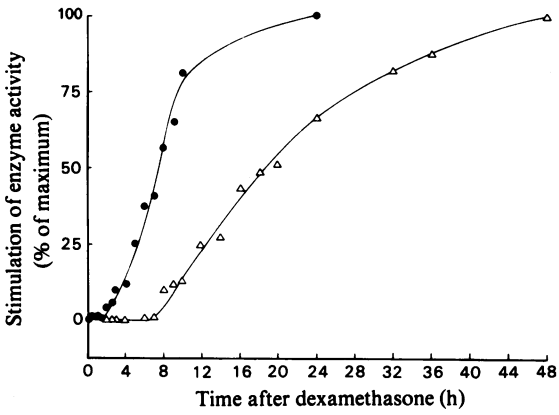

Fig. 2. *Time-course of the glucocorticoid stimulation of enzyme activity in rat hepatoma cells*

Suspension cultures of rat hepatoma (HTC) cells in serum-free medium were divided to receive, at zero time, either 0.5 μM-dexamethasone (a semisynthetic glucocorticoid), or the vehicle (ethanol). Samples were taken at the times indicated for determination (Rousseau *et al.*, 1980) of the specific activity of tyrosine aminotransferase (●) and of alkaline phosphodiesterase I (△). Respective enzyme activities were 2 and 93 munit/mg of protein in unstimulated cells, and 26 and 216 munit/mg of protein in maximally stimulated hormone-treated cells (M. Verhaegen & G. G. Rousseau, unpublished work).

Whether they account directly or indirectly for the observed changes at the protein level, the receptor-induced changes in mRNA concentration could, theoretically, result from transcriptional or (and) post-transcriptional effects. Although they have not been ruled out, direct effects of the glucocorticoid–receptor complex on the translational or post-translational machinery remain to be demonstrated. For instance, glucocorticoids inhibit both the transcription (Mierendorf & Mueller, 1982) and the translation (Papaconstantinou *et al.*, 1983) of globin mRNA in Friend erythroleukaemia cells stimulated to differentiate by dimethyl sulphoxide. The effect on translation can be observed under conditions when transcription of the globin genes is unaffected. However, transcription of some other genes remains glucocorticoid-sensitive and therefore a product of those genes might be responsible for the translational effect of the hormone.

In all the experimental models studied in detail, glucocorticoids up- or down-regulate the expression of genes already transcribed at a certain rate which is not suppressible by glucocorticoid antagonists. Thus, rather than being true inducers or repressors, glucocorticoids behave as modulators of gene activity.

Defined DNA regions are required for glucocorticoid action

Recombinant DNA technology has provided new ways of exploring the problem of how glucocorticoids control gene expression. A first type of experiment was to examine whether glucocorticoid-sensitive genes remain under hormonal control once they have been removed from their normal 'genomic' environment. Another approach has been to try and confer glucocorticoid sensitivity to 'naive' genes by linking them with defined DNA sequences from glucocorticoid-sensitive genes (Table 2).

Retention of glucocorticoid control after gene transfer

The most popular model has been the MTV (Fig. 3), the transcription of which can be stimulated rapidly (15 min) and up to 100-fold by glucocorticoids (Ringold *et al.*, 1977*b*), independently of protein synthesis (for a review, see Groner *et al.*, 1983*b*). This glucocorticoid effect was retained in non-murine (i.e. exempt from endogenous provirus) cells infected with the virus and expressing it (Ringold *et al.*, 1977*a*). Experiments with rat hepatoma cell clones containing only one viral copy showed that stimulation did not result from read-through of flanking host DNA, since expression of the latter was not affected by glucocorticoids (Ucker *et al.*, 1981). Transfection of thymi-

Table 2. *Glucocorticoid control of transfected genes*

Abbreviations used: MTV, mouse mammary tumour virus; LTR, long terminal repeat; HSV, herpes simplex virus I; Mo-MSV, Moloney mouse sarcoma virus; Ha-MuSV, Harvey murine sarcoma virus; SV40, simian virus 40; tk, thymidine kinase; dhfr, dihydrofolate reductase. For references, see the text.

	Transfected genetic material		
Glucocorticoid-sensitive DNA	Selection or indicator gene	Covalent linkage	Recipient cell
Whole MTV	–	–	Rat hepatoma
MTV or fragments thereof	HSV tk	–	tk⁻ L Mouse fibroblast
Rat α_{2u}-globulin	HSV tk	–	tk⁻ L Mouse fibroblast
Rat and human somatotropin	HSV tk	–	tk⁻ L Mouse fibroblast
Rat somatotropin	Mo-MSV	–	Mouse 3T3 fibroblasts
MTV LTR	HSV tk	+	tk⁻ L Mouse fibroblast
MTV LTR	Mouse dhfr	+	dhfr⁻ Hamster ovary
MTV LTR	Ha-MuSV *ras*	+	Mouse 3T3 fibroblast
Chicken lysozyme promoter region	SV40 T antigen	+	Chick oviduct cells
Human somatotropin promoter region	HSV tk	+	tk⁻ L Mouse fibroblast

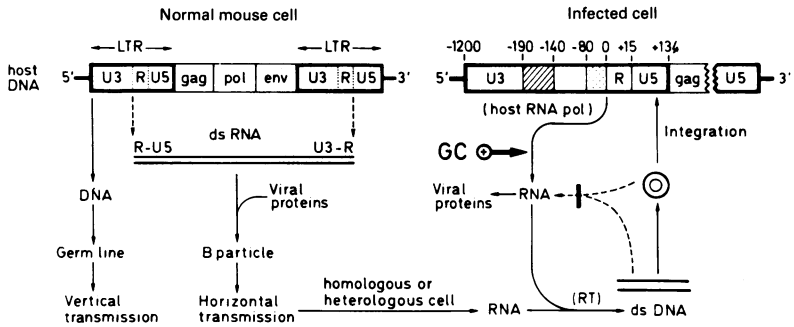

Fig. 3. *The mouse mammary tumour virus (MTV), a model for glucocorticoid action*

The MTV has two genomic nucleic acids, a DNA and a RNA. The DNA is 10 kbp (see numbers at the top) long and occurs as endogenous provirus (boxes) in multiple, methylated, copies in the mouse genome (left-hand side of the Figure). This DNA contains at both ends an identical 1328 bp sequence called 'long terminal repeat' (LTR), which may be analogous to bacterial transposons (Finnegan, 1981). The LTR (Temin, 1981) is made of a unique sequence (~1200 bp) copied from the 3' end of the viral RNA (U3), a unique sequence (120 bp) from the 5' end of the viral RNA (U5), and a sequence (16 bp) repeated at both ends of the RNA (R). The first 959 bp of U3 could code for an unidentified gene product (orf) of unknown function. The five viral core proteins (group-specific antigens) are encoded in the *gag* sequence, the reverse transcriptase (RT) is encoded in the *pol* sequence, and the two envelope proteins are encoded in the *env* sequence. Replication of the provirus with the genome of the host, where it is normally non-tumourigenic, ensures its vertical transmission. A rare event is the production in mammary gland cells of 70S double-stranded (ds) RNA transcripts which become packaged as 'B particles' containing reverse transcriptase (RT). These milk-borne infectious particles account for the horizontal transmission of the virus. In infected cells (right-hand side), the viral RT catalyses the production of linear and circular dsDNA molecules which cannot be transcribed but are able to integrate in the host genome as single or multiple non-methylated copies. This can lead, via unknown mechanisms, to cellular transformation. The integrated viral DNA can now be transcribed by the host RNA polymerase into viral RNA, a process which is stimulated by glucocorticoids (GC). Viral proteins are produced from the transcribed viral RNA (see Ringold *et al.*, 1978, for review). The 'transcription initiation element' (TIE), i.e. the viral promoter region (stippled area) which contains the TATA box (at about −30) is within 80 bp upstream from the transcription initiation (cap) site. The glucocorticoid-sensitive region, also called 'glucocorticoid response element (GRE), (hatched area) maps in the region −200 to −100, most probably between −190 and −140 (see the text and Groner *et al.*, 1983*b*). The MTV box on the right-hand side has been expanded to show the left LTR in more detail.

dine kinase (tk)-deficient mouse fibroblasts (tk⁻ L cells) with cloned viral DNA allowed a definition of the glucocorticoid-sensitive viral DNA regions to be made. In these experiments, unintegrated viral DNA from infected hepatoma cells (Buetti & Diggelman, 1981) or proviral DNA from a mouse library (Hynes *et al.*, 1981*b*) was packaged in bacteriophage λ and cloned in *Escherichia coli*. This

material was mixed with herpes simplex virus I tk^+ plasmid DNA to co-transfect the L cells. The stable transformants, selected with a suitable (HAT) medium, were sensitive to glucocorticoids in terms of viral induction. There was no induction after aberrant or mock transfer, showing that glucocorticoids did not activate provirus endogenous to the mouse recipient cell. Further transfection experiments using the same technique with cloned viral sequences obtained by restriction cleavage showed that glucocorticoid sensitivity was retained in a 1450 base pairs (bp) fragment containing the viral long terminal repeat (LTR) (Fasel *et al.*, 1982). The LTR contains the viral transcription initiation site (Fig. 3).

These experiments led to the conclusion that (i) sequences are present within the proviral DNA itself that are required for hormonal induction of MTV transcription; (ii) receptor action appears to stimulate the rate of initiation events from a single pre-existing transcriptional start site within the viral DNA; (iii) a specific subregion (LTR) of the proviral DNA is competent to mediate hormonal regulation upon its re-introduction into cellular genomes by DNA transformation.

Glucocorticoid-sensitive genes other than viral genes have been successfully transferred and induced by the hormone in heterologous, glucocorticoid receptor-positive, cells. The cloned human metallothionein-IIA gene has been introduced into rat fibroblasts and found to retain its responsiveness to both glucocorticoids and heavy metals (Karin & Richards, 1982). Two rat α_{2u}-globulin genes introduced by co-transfection with the herpes virus–thymidine kinase gene in the tk^- L cells were transcribed and expressed as secreted α_{2u}-globulin. Both the mRNA and the protein levels rose in response to the steroid (Kurtz, 1981). Similar results in the same system were obtained with the rat (Moore *et al.*, 1982) and human somatotropin genes and showed that DNA sequences required for induction reside within 500 bp of 5′-flanking DNA (Robins *et al.*, 1982). Finally, a rat somatotropin gene, possessing at least 2 kbp of adjacent cellular sequences at both ends of the coding region, was introduced in mouse NIH-3T3 cells, by using a plasmid vector containing Moloney mouse sarcoma virus. The transformed cells form foci upon infection and can thus be selected. These cells not only expressed rat somatotropin mRNA and secreted mature rat somatotropin, they responded to glucocorticoids by increased somatotropin production (Doehmer *et al.*, 1982). These studies confirm the conclusion drawn from the experiments with MTV, but do not rule out a post-transcriptional effect of the steroid since, in all cases, only steady-state concentrations of mRNA were measured.

Conversion of glucocorticoid-insensitive genes into inducible genes

Transfection of cells with chimaeric DNA molecules showed that glucocorticoid-unresponsive genes, such as the tk gene, can become responsive when linked to DNA sequences from hormonally inducible genes. L cells (tk^-) transfected with MTV LTR–thymidine kinase recombinant DNA correctly transcribed the chimaeric DNA into thymidine kinase RNA in the absence of hormone and into both LTR–thymidine kinase RNA and thymidine kinase RNA, in increased amount, in presence of the hormone (Hynes *et al.*, 1983). Thus, the LTR was sufficient to confer glucocorticoid sensitivity to a gene located at its 3′-side. This conclusion confirmed earlier experiments using other glucocorticoid-insensitive genes. Mouse dihydrofolate reductase (dhfr) was expressed and its production was stimulated by glucocorticoids in $dhfr^-$ Chinese hamster ovary (CHO) cells transfected with plasmids containing LTR-dihydrofolate reductase recombinant DNA (Lee *et al.*, 1981). The p21 transformation (*ras*) gene from Harvey murine sarcoma virus, another glucocorticoid-unresponsive gene, was removed from its normal promoter, linked downstream of the MTV LTR, and introduced into NIH-3T3 cells which became transformed. The levels of p21 RNA and protein were increased in the presence of glucocorticoids (Huang *et al.*, 1981). It should be noted that retroviral LTRs may behave as strong 'promoters' (in the broad sense) which exhibit the general property of activating, irrespective of glucocorticoid stimulation, the expression of heterologous gene coding sequences from which the natural promoter has been deleted (Joyner *et al.*, 1982). On the other hand, the lack of glucocorticoid sensitivity of the Rous sarcoma virus (Majors & Varmus, 1983) indicates that not all LTRs are glucocorticoid-sensitive. Thus, it was of interest to find that the promoter region of the chicken lysozyme gene, covalently linked to the coding region of the SV40 T antigen, could confer glucocorticoid sensitivity to the expression of this foreign antigen (detected by immunofluorescence) after microinjection into individual chick oviduct cells (Renkawitz *et al.*, 1982). Similarly, a 500 bp sequence of 5′-flanking DNA from the human somatotropin gene rendered the thymidine kinase gene responsive to glucocorticoids (Robins *et al.*, 1982).

Cell transfection by chimaeric DNA also helped to delineate the DNA regions that confer to genes their glucocorticoid sensitivity. A 'functional' mapping of the MTV LTR was attempted by studying how deletions in this LTR affected expression of LTR–thymidine kinase constructs. Stepwise deletion of nucleotide sequences 5′ to the

viral initiation site, which is within the LTR at about 1200 bp from the 5′ end, showed that hormonal inducibility of the chimaeric DNA (but not expression of the thymidine kinase gene, which contained its own promoter) was fully retained when only 200 bp were left upstream from the initiation site, but was lost when less than 50 bp were left (Hynes *et al.*, 1983). Similar studies with a promoter-less thymidine kinase gene linked to LTR with deleted sequences suggested that the steroid-sensitive region maps between −204 and −105 (Buetti & Diggelman, 1983) or −190 and −140 bp (Majors & Varmus, 1983) from the initiation site and is distinct from the LTR promoter region which is responsible for the constitutive levels of thymidine kinase expression and which is located downstream from −80 (Fig. 3). Moreover, regulation was maintained when the latter region was replaced by analogous (but non-inducible) Rous sarcoma virus sequences or when the interval between these regions was reduced by 20 bp or increased by 4 bp (Majors & Varmus, 1983). Thus one can distinguish, in MTV DNA, a 'glucocorticoid response element' (GRE) which is distinct from the 'transcription initiation element' (TIE). MTV fragments containing the GRE but not the TIE, when fused to a thymidine kinase gene containing its own TIE, conferred to the latter glucocorticoid sensitivity. This activity of GRE persisted even when its location and orientation relative to the thymidine kinase TIE differed from that relative to the MTV TIE (Chandler *et al.*, 1983). Using a similar approach, Karin *et al.* (1984) have characterized the DNA sequences through which glucocorticoids and cadmium induce the human metallothionein-IIA gene. Rat fibroblasts were transfected with a chimaeric gene consisting of 800 bp of the 5′ flanking region, containing the promoter elements, the cap site and the 5′ untranslated region of the metallothionein gene, fused to the transcribed portion of the herpes simplex virus–thymidine kinase gene. Transcription from the metallothionein promoter generated a functional thymidine kinase mRNA at levels increased, by a transcriptional mechanism, upon treatment of the transfected cells with either dexamethasone or cadmium. Study of deletion mutants defined three separate regulatory elements. One (−70 to −90) is important for maintenance of the basal level of expression; it contains a sequence capable of forming a Z-DNA structure. The second, a dodecameric sequence present in two copies (−38 to −50 and −130 to −150), is involved in induction by heavy metals. The third is the glucocorticoid-sensitive element and its location (−237 to −268) clearly shows that both types of inducers act at distinct sites on DNA.

Glucocorticoid receptor binds to defined DNA regions

Early experiments had shown that the receptor binds to total DNA but had failed to demonstrate preferential binding to specific DNA regions (Rousseau *et al.*, 1975). This suggested that low-affinity non-specific DNA binding could preclude detection of a small number of physiologically relevant DNA binding sites, as is the case for prokaryotic transcriptional regulatory proteins (Yamamoto & Alberts, 1976). The availability of purified receptor, together with the discovery in MTV of defined DNA sequences involved in the glucocorticoid control of gene expression, made it possible to re-assess the issue.

DNA–receptor interactions were assayed using nitrocellulose filters which retain double-stranded DNA only if it is protein-bound. Receptor-bound radioactive DNA can be characterized, after elution from the filter, by agarose-gel electrophoresis followed by autoradiography. Glucocorticoid-bound receptor purified from rat liver was 'transformed' (see the Introduction) and incubated with radioactive cloned fragments of MTV DNA. Specific receptor binding was detected with LTR-containing DNA but not with phage or plasmid DNA (Payvar *et al.*, 1981; Govindan *et al.*, 1982). Experiments with LTR deletion mutants showed that the receptor binding region was restricted to a DNA segment located between 202 and 50 bp upstream from the cap (i.e. initiation) site (Scheidereit *et al.*, 1983). It is precisely within this segment that one finds the GRE defined by the transfection experiments discussed above. DNA-bound receptors were visualized by electron microscopy as clusters of putative tetramers occupying discrete regions along the DNA molecule (Payvar *et al.*, 1983).

The receptor also bound to MTV regions outside the LTR and to mouse genomic sequences flanking endogenous proviral copies (Geisse *et al.*, 1982). The fact that a 45000 M_r receptor fragment, presumably devoid of the 'specifier' domain (see the Introduction), displayed DNA binding in these experiments casts doubt on the physiological significance of such receptor–DNA interactions. In addition to the receptor-binding region in the LTR upstream from the cap site, four binding regions were detected within transcribed sequences between 4 and 8 kbp downstream from the cap site. The functional significance of these binding sites is unknown (Payvar *et al.*, 1983). The actual DNA sequences recognized by the receptor were identified by 'footprinting' experiments. Several regions within each of the DNA fragments specifically retained by the receptor were protected by the purified receptor against digestion by deoxyribonuclease I. Recent experiments with the

chimaeric metallothionein-thymidine kinase constructs described above (Karin *et al.*, 1984) showed that the purified rat receptor binds preferentially to, and protects against nuclease digestion, a DNA sequence (−248 to −263) located within the GRE. This sequence has a high degree of homology with two regions (−186 to −170 and −129 to −113) of the MTV-LTR that are protected against nuclease digestion (Scheidereit *et al.*, 1983). A comparison of these segments yields the consensus sequence T(C)GGTA(T)CAA(C)A(T)TGTT(C)CT. This sequence is different from that involved in induction of metallothionein by heavy metals.

Specific binding of purified LTR-containing DNA to the receptor in crude cell extracts could also be shown by using a DNA–cellulose competition assay (Pfahl, 1982) or by 'fishing out' the DNA–receptor complex with an anti-receptor antibody (Scheidereit *et al.*, 1983). This allowed one to show that inhibition of receptor transformation by molybdate inhibited DNA binding and that mouse receptor mutants deficient in nuclear transfer displayed decreased affinity for DNA.

Mechanisms of receptor action

Involvement of the receptor and of the steroid

The molecular mechanism by which glucocorticoids modulate the concentration of mRNAs of specific genes is unknown. A good correlation has been found between nuclear receptor binding and mRNA stimulation, for somatotropin (Samuels *et al.*, 1979) and metallothionein (Mayo & Palmiter, 1981) induction. Furthermore, a glucocorticoid antagonist decreased both the binding of receptors to LTR DNA and the stimulation by glucocorticoids of LTR transcription (Groner *et al.*, 1983*a*). These findings strongly suggest that the receptor is directly involved in the glucocorticoid effect.

Whether the steroid itself plays a rôle other than maintaining the receptor in an 'active' conformation is not known. Models have been put forward (Sluyser, 1983) in which both the receptor and its steroid ligand functionally interact with the genome, as has been proposed for the cyclic AMP–catabolite activator protein (CAP) complex in prokaryotes (Ebright & Wong, 1981). Such models would predict that some glucocorticoid antagonists might act at the nuclear level by blocking the receptor in an inactive complex with DNA because they cannot contribute properly to the DNA-binding interaction. Indeed, receptors complexed with the antagonists promegestone (Golaz & Beck, 1983) or dexamethasone mesylate (Simons *et al.*, 1983) bind to the nucleus of target cells. In this context, it must be pointed out that the cytosolic localization of the receptor in extracts of steroid-free cells does not prove that the unbound receptor belongs to the cytoplasm in the intact cell. It may well be that the receptor is a nuclear protein which leaks out of the nucleus upon homogenization in hypo-osmotic buffers, unless it is steroid-bound. Association of the glucocorticoid antagonists with the nucleus would then not be surprising. This view is supported by recent experiments on the oestrogen receptor (for discussion, see Schrader, 1984). However, immunofluorescence microscopy of intact cells shows that receptors labelled with anti-receptor antibodies do actually 'translocate' from the cytoplasm to the nucleus upon association with a glucocorticoid (Govindan, 1980). Glucocorticoids and oestrogens do not necessarily act through identical molecular and cellular mechanisms. Indeed, the 'transformation' process involves receptor dissociation in the case of glucocorticoids (Vedeckis, 1983; Raaka & Samuels, 1983) and receptor dimerization in the case of oestrogens (Notides *et al.*, 1975).

In any case, the possibility that all the intracellular events depicted in Fig. 1 do actually occur within the nucleus in intact cells does not challenge the basic 'two-step' model of glucocorticoid action according to which the receptor exists under two distinct states, an inactive conformation (unbound or antagonist-bound receptor) and a 'gene-active' conformation (agonist-bound receptor).

Transcriptional and post-transcriptional effects

The mechanisms of receptor action on mRNA must account for both the transcriptional and the post-transcriptional effects of glucocorticoids. Post-transcriptional processes include capping, methylation, polyadenylation, splicing, cytoplasmic transfer, and turnover of mRNA (Nevins, 1983). Glucocorticoids stabilize α_1-foetoprotein mRNA, despite the fact that they decrease its rate of transcription (Guertin *et al.*, 1983). They stabilize metallothionein mRNA in HeLa cells transfected with the metallothionein gene (Mayo *et al.*, 1982). Stabilization of mRNA, in addition to increased transcription, might also account for the increased somatotropin mRNA levels seen in pituitary cells stimulated by glucocorticoids (Lan *et al.*, 1984). The glucocorticoid-induced increase in casein mRNA levels, seen in mammary glands stimulated with prolactin, seems to involve a post-transcriptional effect (Guyette *et al.*, 1979).

Most observations, however, are best explained by a transcriptional effect of glucocorticoids, as has been discussed with the MTV paradigm (for a review, see Yamamoto, 1984*a*). A kinetic study of the glucocorticoid induction of LTR–thymidine kinase chimaeric constructs (Groner *et al.*, 1983*a*) showed that initiation of LTR RNA synthesis,

absent from hormone-free transfected cells, was simulated within 7.5min after hormone addition. This was independent of protein synthesis, and required a functional steroid–receptor complex since it was inhibited by a glucocorticoid antagonist. The half-life of LTR RNA (30min) was unchanged by dexamethasone. Recent experiments in this system suggest that glucocorticoids affect solely the efficiency of utilization of the MTV promoter, leaving unchanged initiation, splicing, and polyadenylation (Ucker *et al.*, 1983). This simple model is contrasting with the more complex mechanism thought to be involved in the regulation by steroid hormones of egg white gene transcription in the chick oviduct (Palmiter *et al.*, 1981).

A transcriptional effect of glucocorticoids might hold true even when mRNA levels are decreased by glucocorticoids. Indeed, kinetic and cell-free transcription experiments on the glucocorticoid inhibition of α_1-foetoprotein (Guertin *et al.*, 1983) and of β-globin synthesis (Mierendorf & Mueller, 1982) suggest that the decrease in mRNA levels, which can be observed within 30min following glucocorticoid administration, results from a direct inhibition of transcription, most probably at the initiation step. An inhibition of transcription is also the most likely interpretation of the near-complete inhibition of rRNA synthesis seen in cells that undergo mitotic arrest after glucocorticoid treatment (Cavanaugh & Thompson, 1983). Whether receptor action is transcriptional or post-transcriptional, it could involve a receptor–protein or (and) a receptor–nucleic acid interaction, as is discussed below.

Involvement of DNA

The experiments with MTV and metallothionein–thymidine kinase constructs reported above demonstrate that the receptor can interact with DNA, presumably at sites distinct from the TIE. The results obtained with the latter system clearly show that the glucocorticoid receptor can act as a positive regulatory factor. Indeed, deletion of the GRE, which also contains the receptor-binding site, does not affect the basal level of metallothionein promoter activity or its ability to be induced by heavy metals. The functional and physical relationships of the putative glucocorticoid target (GRE) with the TIE have led to the concept that GREs are 'receptor-dependent transcriptional enhancers' (proto-enhancers) (Chandler *et al.*, 1983; Yamamoto, 1984*a*). Enhancers are DNA regions, found in viral and cellular genomes, that increase, through unknown mechanisms, the transcriptional efficiency of genes in a manner relatively independent of their position and orientation with respect to those genes (Khoury & Gruss, 1983). In this context, receptor action would mediate a change in DNA or (and) chromatin configuration such that its functional consequence is propagated from the GRE to the TIE. No DNA sequences specific to enhancers have been identified yet (Fried *et al.*, 1983). Still, a 70% consensus sequence 10bp long (TXAGXTCTXA) has been found in putative GRE regions for four glucocorticoid-responsive genes: MTV, somatotropin, tryptophan oxygenase, and pro-opiomelanocortin (Buetti & Diggelmann, 1983).

Recent experiments using cells transfected with chimaeric LTR–thymidine kinase genes (these cells express the thymidine kinase gene) showed that initiation at the LTR cap site in the absence of glucocorticoids required the presence of the two LTRs, as is the case in intact provirus. In constructs containing only one LTR, glucocorticoids both stimulated thymidine kinase transcription and allowed initiation at the LTR cap site, as if the hormone could substitute for the missing, second, LTR (Groner *et al.*, 1983*b*). The complexity of glucocorticoid action is further illustrated by the facts that it superimposes to that of viral (SV40 or murine sarcoma) enhancers (Majors & Varmus, 1983), and that it appears to stimulate transcription of repetitive sequences homologous to the *Alu* family (Feinberg *et al.*, 1983) (*Alu* sequences are short, repetitive, stretches of DNA that are complementary to sequences found in small cytoplasmic RNAs such as the 7S RNA component of the 'signal recognition particles').

The receptor–steroid complex could act on DNA by physically perturbing it (e.g. the receptor destabilizes the double helix and facilitates RNA polymerase entry), or by chemically modifying it (e.g. the receptor is an enzyme). Since expressed genes appear to be less methylated than silent genes, glucocorticoids could act through DNA methylation. No change in DNA methylation, however, could be detected during tyrosine aminotransferase induction by glucocorticoids in hepatoma cells, a short-term event (Lapeyre *et al.*, 1980). Yet, experiments with MTV-infected lymphoid cells suggest that long-term treatment with glucocorticoids could lead to demethylation of the induced gene, and this might play a rôle in cell differentiation events induced by these hormones (Mermod *et al.*, 1983). In any case, glucocorticoids induce the non-methylated transfected forms of MTV, but not their methylated endogenous forms (Groner *et al.*, 1983*b*). From these and other experiments with lymphoma cells sensitive or resistant to lysis by glucocorticoids, it appears that hypomethylation of a gene could be a condition necessary, but not sufficent, for glucocorticoid sensitivity (Gasson *et al.*, 1983).

Involvement of RNA

There is evidence that the receptor can interact with RNA. Treatment with ribonuclease converts the oligomer receptor into its monomeric, DNA-binding, form (Tymoczko *et al.*, 1983; Rossini & Barbiroli, 1983). This effect can be reversed by a cytosolic ribonuclease-sensitive factor (Hutchens *et al.*, 1982) or by purified RNA (Chong & Lippman, 1982). Preliminary data from our laboratory (I. V. Economidis & G. G. Rousseau, unpublished work) suggest that the receptor can occur as a ribonucleoprotein particle. One interpretation is that the DNA binding site in 'untransformed' receptor is protected by an RNA molecule. One might also imagine that this RNA allows receptor translocation across the nuclear membrane, much in the same way as the RNA-containing 'signal recognition particle' participates in injection of secretory proteins into the endoplasmic reticulum. Another possibility is that DNA recognition by the receptor involves a receptor-associated RNA molecule. Alternatively, the putative receptor-associated RNA might play a rôle in the post-transcriptional effects of the receptor, such as mRNA stabilization. Clarification of these speculative but exciting issues (small RNAs can behave as biochemical catalysts; see Altman, 1984) obviously requires further characterization of this RNA.

Involvement of chromatin

MTV-infected rat hepatoma cells, containing a single copy of the provirus stably integrated into their DNA, may or may not respond to glucocorticoids by increased MTV transcription (Ucker *et al.*, 1981, 1983). This points to the importance, for hormone action, of the site of retrovirus integration and, presumably, of chromatin conformation. This is consistent with the observation (André *et al.*, 1980) that in the normal host (mouse mammary tumour) cell, mild nuclease treatment of the nuclei preferentially releases the receptor together with a small proportion of MTV DNA. This fraction, containing the few glucocorticoid-sensitive copies of MTV DNA, would correspond to 'active chromatin'. Enrichment of the active chromatin fraction in receptors and inducible (i.e. somatotropin) genes has also been reported in pituitary cells (Levy-Wilson, 1983).

Thus, a hormone-sensitive gene might be rendered insensitive by an unfavourable chromatin enrichment. Such a mechanism has been proposed to explain the loss of glucocorticoid inducibility of the metallothionein gene once it has been amplified in mouse sarcoma cells by selection for resistance to cadmium (Mayo & Palmiter, 1982), or after it has been transfected as recombinant DNA into human or mouse cells (Mayo *et al.*, 1982). Interestingly, the amplified or transfected gene is still inducible by heavy metals. Butyrate, which inhibits histone deacetylase (but also histone acetyltransferase) and alters chromatin structure, reversibly reduces the accumulation of tyrosine aminotransferase mRNA seen in response to glucocorticoids, without affecting the basal levels (Tichonicky *et al.*, 1981). Plesko *et al.* (1983) have shown that this short-term inhibitory effect of butyrate on transcription is specific for a few mRNA species, most of which are glucocorticoid-inducible. Moreover this was correlated with inhibition (not stimulation) of the rapid form of histone acetylation, which is compatible with the notion that this form of acetylation 'opens' chromatin structure around steroid-sensitive genes.

Chromatin, in turn, might be functionally altered as a result of glucocorticoid action. Glucocorticoids increase within 2h the phosphorylation of H2A histones in intact mouse cells (Prentice *et al.*, 1978). In intact rat pituitary cells, human skin fibroblasts, and mouse lymphoma cells, glucocorticoids alter within 15min chromatin structure, as revealed by a change in the number of initiation sites of *E. coli* RNA polymerase assayed under cell-free conditions (Johnson *et al.*, 1979). Depending on the metabolic state of the cell, this number is increased or decreased, and gene expression is respectively increased or decreased, by glucocorticoids. This suggests that the receptor might act enzymically. Glucocorticoids decrease within 15min the number of endogenous ADP-ribose moieties on the non-histone high-mobility-group 14 and 17 proteins in mouse mammary tumour cells. This is concomitant with the hormonal stimulation of MTV RNA synthesis in these cells. Since these proteins are associated with actively transcribed genes, it is likely that ADP-ribosylation of chromosomal proteins serves as a negative regulator for MTV gene expression and that glucocorticoid action involves a change in ADP-ribose metabolism (Tanuma *et al.*, 1983).

Whatever the respective rôle of nucleic acids and chromatin proteins in glucocorticoid regulation of gene transcription, the mechanism of action of the glucocorticoid–receptor complex must account for several puzzling observations. First, glucocorticoids may increase mRNA by acting synergistically with other hormones (Table 1) as is the case for induction of somatotropin mRNA by glucocorticoid and thyroid hormone in cultured pituitary cells (Wegnez *et al.*, 1982). Moreover, in these cells, a given gene product can be regulated by one hormone but not the other, or by the two hormones in one of three ways: co-induction, co-repression or induction by one hormone and repression by the other (Ivarie *et al.*, 1980, 1982).

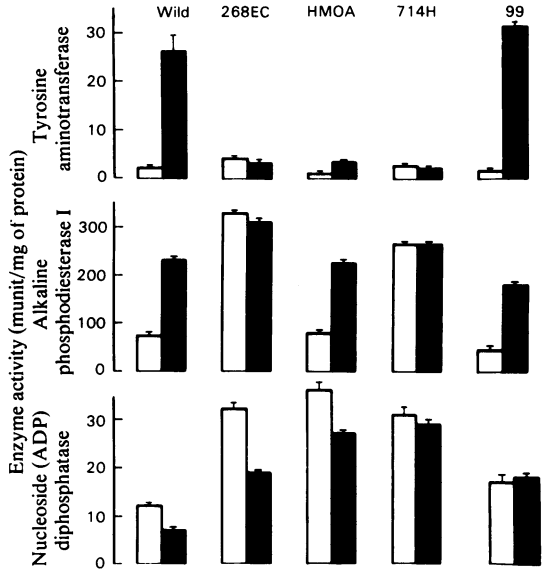

Fig. 4. *Co-ordinated differences in glucocorticoid control of gene expression in rat hepatoma cell variants*
Rat hepatoma (HTC) cells in monolayer cultures were exposed to 0.5 μM-dexamethasone (filled bars) or the vehicle, ethanol (open bars) for 48 h, following which specific enzyme activity (mean ± S.E.M. for three samples) was determined as described elsewhere (Rousseau *et al.*, 1980). The tyrosine aminotransferase-inducible variants HMOA and 99 were kindly provided by Dr. P. Mamont. The variants 268EC and 714H, non-inducible for tyrosine aminotransferase, were kindly provided by Dr E. B. Thompson. (G. G. Rousseau, unpublished work.)

Also, the same gene product (e.g. phosphoenolpyruvate carboxykinase) can be stimulated in one tissue (the liver) and inhibited in another (white adipose tissue) by glucocorticoids (Feldman, 1977). Another phenomenon is the co-ordinate regulation of the glucocorticoid 'domain of response'. In rat hepatoma cells, pharmacological and genetic data show that induction of tyrosine aminotransferase, glutamine synthetase, glycoprotein Belt I, and MTV is mediated by a single glucocorticoid stimulation pathway (Vannice *et al.*, 1983). Interestingly, loss of inducibility of one gene product (e.g. tyrosine aminotransferase) can be accompanied by 'constitutive' expression of another inducible (e.g. alkaline phosphodiesterase I) gene product (Fig. 4).

Summary and conclusions

Glucocorticoids control the expression of a small number of transcriptionally active genes by increasing or decreasing mRNA concentration. Either effect can result from a transcriptional or a post-transcriptional mechanism. Induction of mouse mammary tumour virus RNA results from a stimulation of transcription initiation and depends on the presence of defined regions in proviral DNA. These regions bind the glucocorticoid receptor and behave functionally as proto-enhancers. Glucocorticoid-inducible genes can retain their sensitivity to the hormone after transfer to a heterologous cell by transfection techniques. Non-inducible genes can become inducible when linked to the promoter region of an inducible gene.

The mechanisms by which the receptor–steroid complex stimulates or inhibits transcription or influences mRNA stability are unknown. Receptor binding to nucleic acids appears to be a necessary but not sufficient condition. It is likely that the receptor also interacts with chromatin proteins. This might lead to a catalytic modification of these proteins, resulting in a modulation of gene expression. Development of glucocorticoid-sensitive, biochemically defined, cell-free transcription systems should provide a tool to delineate the molecular determinants of this essential regulatory mechanism.

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