Role of the CCAAT/Enhancer Binding Protein-α Transcription Factor in the Glucocorticoid Stimulation of p21wafl/cip1 Gene Promoter Activity in Growth-arrested Rat Hepatoma Cells*

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The preceding paper (Cha, H. H., Cram, E. J., Wang, E. C., Huang, A. J., Kasler, H. G., and Firestone, G. L. (1998) J. Biol. Chem. 273, 0000–0000(478563) defined a glucocorticoid responsive region within the promoter of the p21 CDK inhibitor gene that contains a putative DNA-binding site for the transcription factor C/EBPα (C/EBPα). Wild type rat BDS1 hepatoma cells as well as as4 hepatoma cells, which express antisense sequences to C/EBPα and ablate its protein production, were utilized to investigate the role of this transcription factor in the glucocorticoid regulation of p21 gene expression. The stimulation of p21 protein levels and promoter activity, as well as inhibition of CDK2-mediated retinoblastoma protein phosphorylation, by the synthetic glucocorticoid, dexamethasone, required the expression of C/EBPα. Overexpression of C/EBPα in as4 cells rescued the dexamethasone responsiveness of the p21 promoter. Site-directed mutagenesis of the p21 promoter revealed that dexamethasone stimulation of p21 promoter activity required the C/EBPα consensus DNA-binding site. Furthermore, in glucocorticoid receptor-defective EDR1 hepatoma cells, dexamethasone failed to stimulate C/EBPα and p21 protein expression and promoter activities. Our results have established a functional link between the glucocorticoid receptor signaling pathway that mediates a G1 cell cycle arrest of hepatoma cells and the transcriptional control of p21 by a cascade that requires the steroid induction of C/EBPα gene expression.

Treatment with glucocorticoids, one class of steroid hormones, can inhibit both the in vivo and in vitro growth of many different types of normal and transformed cells. Normal hepatocytes and certain hepatoma cell lines are acutely sensitive to the anti-proliferative effects of glucocorticoids (1–9). We have previously established that in specific types of steroid responsive rat hepatoma cells (10, 11) and rat mammary tumor cells (12), glucocorticoids induce an early G1 block in cell cycle progression suggesting the existence of a unique hormone-regulated G1 restriction point in these transformed cells. Furthermore, the loss of G1 cell cycle control has been implicated in the uncontrolled proliferation of a variety of neoplastically transformed cells (13, 14). Given that steroid receptors are transcriptional regulators (15–20), this G1 cell cycle arrest is likely to be controlled in part by a glucocorticoid-mediated transcriptional cascade in which the glucocorticoid receptor directly alters the transcription of a small subset of genes which then regulate the subsequent expression and/or activity of specific sets of downstream proteins. Important final targets of this glucocorticoid growth suppression pathway are likely to be G1-acting components of the cell cycle which define a critical checkpoint in cell cycle progression. However, the molecular basis for the functional relationship between the glucocorticoid control of early events within this signaling cascade and the final cell cycle arrest of hepatoma cells is poorly understood.

To investigate the cellular signaling pathways mediating the glucocorticoid growth arrest of epithelial cells, we have isolated glucocorticoid growth suppressible and non-suppressible hepatoma cell proliferation variants derived from the rat Reuber hepatoma (2, 9). Characterization of these hepatoma cell variants revealed that the anti-proliferative effect of glucocorticoids is a receptor-dependent process that does not affect cell viability, decrease total cell number, or induce an apoptotic response (3, 4). Moreover, glucocorticoids induce an early G1 block in cell cycle progression within one cell doubling time in BDS1 hepatoma cells (4). By using this cell system, we have defined some of the earliest transcriptional events associated with the G1 arrest of the tumor cell line. Most significantly, the CCAAT/enhancer binding protein-α (C/EBPα) gene expression is specifically required for the glucocorticoid-mediated G1 cell cycle arrest of hepatoma cells. Ablation of C/EBPα protein by expression of antisense sequences precluded glucocorticoids from inducing the G1 cell cycle arrest and overexpression of C/EBPα suppressed hepatoma cell growth in the absence of glucocorticoids (11). In addition to the well established role of the C/EBP family of transcription factors in normal liver function (21, 22), the glucocorticoid stimulation of C/EBPα gene expression is a rapid response that represents an early and crucial intermediate in the glucocorticoid-stimulated anti-proliferative cascade that governs the cell cycle of liver-derived epithelial tumor cells (11).

G1-acting cell cycle components may be important downstream targets of the glucocorticoid growth suppression pathway in rat hepatoma cells. For example, C/EBPα, or other steroid responsive transcriptional regulators, may inhibit the transcription of components necessary for cell cycle progres-
**Glucocorticoid Cell Cycle Control by C/EBPα**

Glucocorticoid-sensitive rat hepatoma (2) and vector control (vc) cell lines were derived by transfection of BDS1 cells with the pCD expression vector or the pBCMGneo parental vector, and were selected for expression with antisense sequences for this transcription factor, as4 cells (11), and a glucocorticoid-resistant hepatoma cell line, EDRI (2), we demonstrate that the glucocorticoid stimulation of p21 promoter activity and induction of protein levels required the regulated expression of the C/EBPα transcription factor.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dulbecco’s modified Eagle’s/F12 (1:1) medium, fetal bovine serum (FBS), and trypsin-EDTA were supplied by BioWhittaker (Walkersville, MD). Dexamethasone was obtained from Sigma. [3H]Thymidine (84 Ci/mmol), [3H]acetyl coenzyme A (200 Ci/mmol), [α-32P]dCTP, [α-32P]dATP, and [α-32P]dGTP (3,000 Ci/mmol) were obtained from NEN Life Science Products. Anti-p21, anti-C/EBPα, anti-C/EBD2, and horse-radish peroxidase-conjugated donkey anti-goat antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase-conjugated goat anti-rabbit antibodies were purchased from Bio-Rad. The enhanced chemiluminescence protein detection system was purchased from Amersham. All other chemicals were reagent grade.

**Hepatoma Cell Lines and Methods of Culture**—Glucocorticoid-sensitive BDS1 cells are epithelial tumor cells derived from the rat Reuber hepatoma and were selected for a glucocorticoid-responsive rat hepatoma cells in which we abrogated expression with antisense sequences for this transcription factor, as4 cells (11), and a glucocorticoid-resistant hepatoma cell line, EDRI (2), we demonstrate that the glucocorticoid stimulation of p21 promoter activity and induction of protein levels required the regulated expression of the C/EBPα transcription factor.

**Stable Transfections**—Logarithmically growing hepatoma cells were transfected by electroporation essentially as described in the preceding paper (57) with the following procedural variations for blotting with the anti-C/EBPα antibodies. For each sample, 30 µg of protein were mixed with 15 µl of sample buffer (62.5 mM Tris-HCl, pH 6.8, 8% glycerol, 5%β-mercaptoethanol, 3% SDS, 0.01% bromphenol blue) prior to resolution by electrophoresis and Western blotting (described below).

**Assay of DNA Synthesis by [3H]Thymidine Incorporation**—Triplicate samples of asynchronously growing BDS1, vc, and as4 cells were treated with dexamethasone as indicated below and pulse-radioabeled as described in the accompanying paper (57).

**Western Blot Analysis**—Western blots were prepared essentially as described in the accompanying paper (57) with the following procedural variations for blotting with the anti-C/EBPα antibodies. For each sample, 30 µg of protein were mixed with 15 µl of sample buffer and fractionated on 10% polyacrylamide, 0.1% SDS resolving gels by electrophoresis. Blots were subsequently incubated in TBST at room temperature with 1 mg/ml rabbit anti-C/EBPα for 1 h. Horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies were diluted in TBST, 1% non-fat dry milk 1:10000, and membranes were incubated with the diluted antibodies for 1 h at room temperature.

**Transfection Procedures**—Logarithmically growing hepatoma cells were transfected by electroporation essentially as described in the preceding paper (57) with the following procedural variations for blotting with the anti-C/EBPα antibodies. For each sample, 30 µg of protein were mixed with 15 µl of sample buffer (62.5 mM Tris-HCl, pH 6.8, 8% glycerol, 5%β-mercaptoethanol, 3% SDS, 0.01% bromphenol blue) prior to resolution by electrophoresis and Western blotting (described below).

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Glucocorticoid Cell Cycle Control by C/EBPα

RESULTS

Dexamethasone Stimulation of p21 Protein Levels and Inhibition of CDK2 Activity Requires the Expression of the C/EBPα Transcription Factor—We have previously demonstrated that the G1 block in cell cycle progression in rat hepatoma cells induced by the synthetic glucocorticoid, dexamethasone, requires the steroid-regulated expression of C/EBPα. This analysis was accomplished using hepatoma cells in which C/EBPα expression had been ablated by stable transfection of an antisense C/EBPα expression vector, generating the as4 hepatoma cells. In contrast, in as4 hepatoma cells, which fail to induce C/EBPα protein due to the expression of antisense C/EBPα sequences, dexamethasone treatment had no effect on p21 protein levels. In each of these three cell lines, CDK2 levels did not change and provided a loading control for the Western blot. The incorporation of [3H]thymidine was determined relative to cells not treated with dexamethasone (11), and the presence of a canonical C/EBP DNA-binding site in its promoter. Therefore, as4 and vc hepatoma cells, as well as the BDS1 parental cell line, were utilized to functionally examine the mechanistic relationships between C/EBPα expression and the regulated expression of the p21 CDK inhibitor gene within the glucocorticoid growth suppression response.

The levels of p21 and C/EBPα protein were initially examined by Western blots of cell extracts isolated from dexamethasone-treated or untreated BDS1, as4, and vc hepatoma cells. As shown in Fig. 1, dexamethasone induced the level of p21 protein by approximately 5-fold in nontransfected BDS1 cells and in vc hepatoma cells, which also show a 5–10-fold induction of C/EBPα protein. In contrast, in as4 hepatoma cells, which fail to induce C/EBPα protein due to the expression of antisense C/EBPα sequences, dexamethasone treatment had no effect on p21 protein levels. In each of these three cell lines, CDK2 levels did not change and provided a loading control for the Western blot. The incorporation of [3H]thymidine was determined relative to cells not treated with dexamethasone (11), and the presence of a canonical C/EBP DNA-binding site in its promoter. Therefore, as4 and vc hepatoma cells, as well as the BDS1 parental cell line, were utilized to functionally examine the mechanistic relationships between C/EBPα expression and the regulated expression of the p21 CDK inhibitor gene within the glucocorticoid growth suppression response.

To functionally test if the glucocorticoid-mediated increase in p21 protein in hepatoma cells had an effect on CDK activity, the in vitro activity of immunoprecipitated CDK2 was examined in dexamethasone-treated and untreated BDS1, as4, and vc cells. Consistent with the effects on p21 protein levels, dexamethasone inhibited the ability of CDK2 immunoprecipitated from extracts of BDS1 and vc cells, but not as4 hepatoma cells, to phosphorylate Rb (Fig. 2). In addition, significantly less p21 protein co-immunoprecipitated with CDK2 from dexamethasone-treated as4 cells compared with either vc or BDS1 cells. Taken together, our results show a functional correlation between expression of the C/EBPα transcription factor in growth suppressible hepatoma cells and the glucocorticoid-induced expression of p21 and the p21 cell cycle inhibitor.

Dexamethasone Activation of p21 Promoter Activity Can Be Functionally Complemented by Overexpression of C/EBPα in as4 Cells That Lack Induction of This Transcription Factor—The preceding paper (57) demonstrated that the dexamethasone stimulation of p21 gene products in BDS1 hepatoma cells resulted from the transcriptional stimulation of the p21 gene. The requirement of C/EBPα transcription factor expression for the induction of p21 protein, the induction of C/EBPα by dexamethasone (11), and the presence of a canonical C/EBP DNA-binding site at ~1270 bp in the p21 promoter implicated this transcription factor in the activation of p21 promoter activity. To test directly this possibility, as4, vc, and BDS1 hepatoma cells were transiently cotransfected with a C/EBPα expression vector (11). As described in the accompanying paper (57), glucocorticoids stimulate the expression of the p21 CDK inhibitor gene which contains a canonical C/EBP DNA-binding site in its promoter. Therefore, as4 and vc hepatoma cells, as well as the BDS1 parental cell line, were utilized to functionally examine the mechanistic relationships between C/EBPα expression and the regulated expression of the p21 CDK inhibitor gene within the glucocorticoid growth suppression response.

2 Taken together, our results show a functional correlation between expression of the C/EBPα transcription factor in growth suppressible hepatoma cells and the glucocorticoid-regulated expression of p21 and the p21 cell cycle inhibitor.

2 E. J. Cram and G. L. Firestone, unpublished results.
Glucocorticoids stimulate p21 promoter activity in hepatoma cells in which C/EBPa is glucocorticoid responsive. BDS1 hepatoma cells, vector control (vc) transfected cells, and antisense C/EBPa expressing as4 hepatoma cells were transiently transfected with the −2.326 p21-CAT reporter plasmid and treated with or without 1 μM dexamethasone (Dex) for 48 h. CAT activity was assayed by a quantitative method that measures the conversion of [3H]acetyl coenzyme A and unlabeled chloramphenicol into [3H]acetyl chloramphenicol. CAT-specific activity is the CAT activity produced per μg of protein present in the corresponding cell lysates and is described under “Experimental Procedures.” The reported values are an average of three independent experiments of triplicate samples, and the error bars represent the standard deviation.

**Fig. 3.** Glucocorticoid stimulation of p21 promoter activity in hepatoma cells in which C/EBPa is glucocorticoid responsive. BDS1 hepatoma cells, vector control (vc) transfected cells, and antisense C/EBPa expressing as4 hepatoma cells were transiently transfected with the −2.326 p21-CAT reporter plasmid and treated with or without 1 μM dexamethasone (Dex) for 48 h. CAT activity was assayed by a quantitative method that measures the conversion of [3H]acetyl coenzyme A and unlabeled chloramphenicol into [3H]acetyl chloramphenicol. CAT-specific activity is the CAT activity produced per μg of protein present in the corresponding cell lysates and is described under “Experimental Procedures.” The reported values are an average of three independent experiments of triplicate samples, and the error bars represent the standard deviation.

vector (11, 26) and a chimeric reporter plasmid containing 2326 bp of the p21 promoter upstream of the transcription start site linked to the CAT reporter gene. Hepatoma cells transfected with an empty expression vector served as a negative control. Analysis of CAT activity in cells treated with or without dexamethasone for 48 h revealed that in vc and BDS1 hepatoma cells, this p21 promoter fragment conferred glucocorticoid responsiveness to the CAT reporter gene (Fig. 3, right and left panels). In contrast, in as4 cells, which lack C/EBPa, dexamethasone treatment failed to induce p21 promoter activity (Fig. 3, middle panel). Importantly, cotransfection of a C/EBPa expression vector into as4 cells rescued the defective glucocorticoid induction of p21 promoter activity (Fig. 3, middle panel) and caused a modest increase in the absolute level of both the basal and steroid-induced levels of p21 promoter activity in BDS1 or vc hepatoma cells (Fig. 3, left and right panels). This functional complementation of glucocorticoid-inducible p21 promoter activity demonstrates the requirement for expression of the C/EBPa transcription factor in this steroid response.

**Dexamethasone-stimulated Activity of the p21 Promoter Fragment Requires the C/EBPa DNA-binding Site and Expression of the C/EBPa Transcription Factor—**Sequence analysis of the promoter region of p21 revealed no canonical glucocorticoid response elements, but the promoter does contain a putative C/EBP DNA-binding site between nucleotides −1270 to −1256. As detailed in the preceding paper (57), this transcription factor site is located within one of the glucocorticoid-responsive subfragments of the p21 promoter. To test directly if the C/EBP DNA-binding site in the p21 promoter contributes to the glucocorticoid-regulated transcriptional activity, the ATCCTCTGCAATTT wild type C/EBP DNA-binding site in the −1.380- to −1.184-bp fragment of p21 promoter was mutated to ATCCTCCCATGTTT eliminating the key nucleotides required for transcription factor binding (27). These p21 promoter fragments, containing either the wild type sequence or the C/EBP DNA-binding site mutation, were linked immediately upstream of the thymidine kinase (tk) minimal promoter sequences driving the bacterial CAT gene, forming −1.380/−1.184 p21-tkCAT and C/EBPmut-1.380/−1.184 p21-tkCAT, respectively (see diagrams in Fig. 4). The vc control cells and C/EBPa-deficient as4 hepatoma cells were cotransfected with one of the reporter plasmids in the presence or the absence of a C/EBPa expression vector. As shown in Fig. 4, dexamethasone failed to induce CAT activity from either of the p21 promoter fragments in transfected as4 cells. Transient ectopic expression of C/EBPa reinstated the dexamethasone activation of p21 promoter activity only in cells transfected with the wild type p21 promoter fragment and not in cells transfected with the reporter plasmid mutated in the C/EBP DNA site. In vc hepatoma cells, the wild type −1.380/−1.184 p21-tkCAT reporter plasmid was dexamethasone inducible in the presence or absence of cotransfected C/EBPa, whereas the reporter plasmid containing the mutated C/EBPa DNA-binding site was nonresponsive to glucocorticoids. In all experiments, reporter gene activity in cells transfected with the minimal promoter pTk-CAT alone was low and unaffected by dexamethasone treatment (data not shown). Thus, the presence of an intact C/EBP DNA-binding site and expression of a functional C/EBPa transcription factor is required for dexamethasone to confer glucocorticoid responsiveness of the −1.380 to −1.184 p21 promoter fragment to a heterologous promoter.

**Dexamethasone Stimulation of C/EBPa and p21 Promoter Activities and Gene Expression Are Ablated in a Glucocorticoid-resistant Hepatoma Cell Variant—**A glucocorticoid-resistant hepatoma cell variant, EDRI, that was selected for its inability to be growth-suppressed (2), and which produces a glucocorticoid receptor with a point mutation in its zinc finger region, was utilized to test the functional relationship between the glucocorticoid stimulation of C/EBPa and p21 gene expression. Western blot analysis of dexamethasone-treated and untreated hepatoma cells revealed that under conditions in which both C/EBPa and p21 protein are stimulated by glucocorticoids in BDS1 hepatoma cells, neither protein was induced in the EDRI

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3 R. A. Ramos and G. L. Firestone, unpublished results.
hepatoma cell variant (Fig. 5, upper panel). BDS1 and EDR1 hepatoma cells were transiently transfected with luciferase reporter plasmids containing either fragments of the p21 promoter (−2.4 p21-Luc) or the C/EBPα promoter (−350 C/EBPα-Luc). Determination of relative luciferase-specific activity in both sets of transfections revealed that dexamethasone stimulated both the p21 promoter and the C/EBPα promoter activity to approximately the same extent in growth suppressible BDS1 hepatoma cells (Fig. 5, lower panel). In the EDR1 hepatoma cell line, which does not undergo a G1 cell cycle arrest (11), neither promoter was regulated by dexamethasone (Fig. 5, lower panel). These results suggest a direct functional connection between the glucocorticoid stimulation of C/EBPα gene expression and that the steroid responsiveness of p21 promoter activity is a key process involved in the cell cycle control of hepatoma cells.

**DISCUSSION**

An intricate network of growth inhibitory and stimulatory signals transduced from the extracellular environment converge on specific sets of cell cycle components which, through their concerted action, either drive cells through critical cell cycle transitions or inhibit cell cycle progression (28–32). Our results have established that the glucocorticoid receptor-mediated signaling pathway induces a G1 cell cycle arrest of rat hepatoma cells (4) with the coordinate transcriptional control of the p21 gene. The precise interactions between C/EBP family members contain a leucine zipper subregions of the p21 promoter that contain DNA recognition sequences for members of transcription factor families known to interact with the glucocorticoid receptor (see preceding accompanying paper (57)). The p21 promoter does not contain a canonical glucocorticoid response element, suggesting that the glucocorticoid receptor does not act through direct DNA binding (16, 18, 19, 33–35). Consistent with our results, it is well established that protein-protein interactions of steroid receptors with other transcription factors and accessory factors can effectively regulate gene transcription in the absence of DNA-binding sites for the receptor (15, 17, 19, 36, 37).

We hypothesize that there is a second branch of the glucocorticoid signaling pathway in which the promoter of the p21 CDK inhibitor gene is a direct downstream target of the glucocorticoid responsive C/EBPα transcription factor (Fig. 6). The proposed requirement for the C/EBPα transcription factor in this pathway is based on our observation that antisense ablation of C/EBPα production in as4 hepatoma cells abolished the glucocorticoid stimulation of p21 promoter activity. Moreover, mutation of the C/EBPα DNA-binding site eliminated glucocorticoid responsiveness in wild type hepatoma cells, and ectopic expression of C/EBPα rescued the defective glucocorticoid stimulation of p21 promoter activity in as4 hepatoma cells. Although the antisense C/EBPα expressing as4 cells have functional glucocorticoid receptors (11), dexamethasone was unable to induce p21 promoter activity. This suggests that the glucocorticoid receptor alone is not sufficient to activate the p21 promoter and functionally requires C/EBPα or an additional factor which interacts with C/EBPα. The ability of dexamethasone to induce p21 transcripts in the presence of cycloheximide likely reflects biological redundancy between the two branches of the cellular cascade that target the glucocorticoid responsive region of the p21 gene promoter. The precise interactions between C/EBPα, p21 promoter elements, and promoter bound proteins may be complex because C/EBP family members contain a leucine zipl-
or the 2EDR1 cells were transiently transfected with either the members of the Ets transcription factor family (20, 38). There-

transcription factors including the glucocorticoid receptor and de novo responsive region of the promoter. In a mechanism that does not require signaling.

pression and cell cycle control by glucocorticoid receptor sig-

tations, p21 gene expression is stimulated by glucocorticoids which causes DNA sequence within the p21 promoter. As a result of these interac-

ions, the glucocorticoid-induced cell cycle arrest, whereas in other growth suppressible cells (9, 12, 40, 41), such as mammary cells, osteosarcoma cells, fibroblasts, and lymphoid-derived cells, glucocorticoid receptors may induce the expression or interact with other tissue-specific transcription factors to affect cell cycle control.

The activities of the cyclin-dependent kinases (CDKs), which drive progression through the cell cycle, are regulated in part by the formation of protein complexes with appropriate cyclin and the cyclin-dependent kinase inhibitor binding partners (30). A key consequence of the glucocorticoid growth arrest pathway in hepatoma cells is an elevation in the level of p21, which inhibits the ability of the G1-acting CDKs to phosphor-

tylate the Rb protein, and thereby helps induce the G1 cell cycle arrest. Of the two families of CDK inhibitors, the p21 family (p21, p27, and p57) forms complexes with a wider range of cyclins and CDKs (42, 43). The expression of p21 appears to be important in normal liver development because the targeted expression of this gene in hepatocytes of transgenic mice re-

sulted in an aberrant organization of liver tissue, a decreased number of adult hepatocytes, reduced liver growth, and the failure of partial hepatectomy to stimulate liver cell proliferation (44). Mice deficient in C/EBPα also have defects in the control of hepatic growth, as well as lung development (45). We have observed that glucocorticoids induce both C/EBPα and p21 protein production in normal liver, suggesting a shared in vivo mechanism of regulation. Thus, consistent with our observ-

ations in rat hepatoma cells, which demonstrate a direct func-

tional connection between C/EBPα and expression of p21, these in vivo studies suggest a potential mechanistic link between C/EBPα and p21 in the growth and development of normal liver tissue.

Only a few studies have directly assessed the regulation of p21 promoter activity and the function of the many potential transcription factor binding sites in the p21 promoter (46–51). For example, both vitamin D3 and retinoic acid, which act through members of the steroid/thyroid hormone receptor family, directly stimulate p21 transcription through their cognate DNA-binding sites in the p21 gene promoter (52–54). In addition, C/EBPα has been shown to stimulate p21 promoter activity in adipocytes (55). Our results have established that the p21 promoter is regulated by glucocorticoids through two distinct transcriptional mechanisms, one of which utilizes the regu-

lated expression of C/EBPα to induce a G1 block in cell cycle progression of liver epithelial tumor cells (11). Because C/EBPα plays a key role in normal liver and adipocyte function (45, 56), it is tempting to speculate that the glucocorticoid-regulated production of p21 may play a key role in the differentiated functions of normal and transformed cells. We are currently attempting to identify other targets of C/EBPα that may com-

per domain and can selectively interact with a variety of other transcription factors including the glucocorticoid receptor and members of the Ets transcription factor family (20, 38). There-

fore, specific transcriptional regulators of the p21 promoter may be utilized by both branches of the glucocorticoid receptor transcriptional cascade, although the molecular details of this signaling mechanism have not been explored.

The transcriptional effects of steroid receptors on specific target genes can be enhanced, reduced, or inhibited depending on the availability of other transcription factors and accessory factors that target the promoter (15, 37, 39). We propose that C/EBPα represents a tissue-specific factor that is involved in the glucocorticoid regulation of p21 promoter activity and that its use as a transcriptional regulator of the cell cycle is restricted to a subset of cell types. Consistent with this possi-

bility, our preliminary evidence has shown that different regions of the p21 promoter are glucocorticoid responsive in growth-arrested mammary tumor cells compared with the hepatoma cells. In liver-derived cells, C/EBPα plays a unique role in the glucocorticoid-induced cell cycle arrest, whereas in other growth suppressible cells (9, 12, 40, 41), such as mammary cells, osteosarcoma cells, fibroblasts, and lymphoid-derived cells, glucocorticoid receptors may induce the expression or interact with other tissue-specific transcription factors to affect cell cycle control.

FIG. 5. Glucocorticoid stimulation of p21 and C/EBPα pro-

moter activity and protein production is defective in a glucocor-

ticoid-resistant hepatoma cell variant. Upper panels, glucocorticoid responsive BDS1 hepatoma cells and glucocorticoid-resistant EDR1 cells were treated with or without 1 μM dexamethasone for 6 h, and cell extracts were fractionated by polyacrylamide gel electrophoresis. Western blots were probed with antibodies to p21, C/EBPα, or CDK2 and protein levels detected by autoradiography. Lower panels, BDS1 and EDR1 cells were transiently transfected with either the −2.4 p21-Luc or the −350 C/EBPα Luc reporter plasmids, treated with or without 1 μM dexamethasone (DEX) for 48 h, and luciferase-specific activity measured as described under “Experimental Procedures.” The relative light units per μg of protein were calculated as an average of three independent experiments of triplicate samples, and the error bars represent the standard deviation.

FIG. 6. Model for the transcriptional control of p21 gene ex-

pression and cell cycle control by glucocorticoid receptor sig-

naling. We propose that glucocorticoids stimulate p21 gene expression through two distinct signaling pathways that target a glucocorticoid responsive region of the promoter. In a mechanism that does not require de novo protein synthesis, glucocorticoid receptors (GR) are proposed to interact with preexisting transcription factors (ovals) located within the glucocorticoid responsive region of the promoter and participate in the formation of an active transcription complex. A second cycloheximide-sensitive pathway (CHX) involves the glucocorticoid induction of the C/EBPα transcription factor that subsequently binds to its consensus DNA sequence within the p21 promoter. As a result of these interac-

tions, p21 gene expression is stimulated by glucocorticoids which causes an inhibition of CDK activity and facilitates the G1 cell cycle arrest.
plement the function of glucocorticoid responsive cell cycle components such as p21, thereby mediating or maintaining the growth-arrested and differentiated state of hepatic-derived tissue.

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