Pergamon

0960-0760(95)00141-7

J. Steroid Biochem. Molec. Biol. Vol. 54, No. 3/4, pp. 141–146, 1995

0960-0760/95 $9.50 + 0.00

Regulation of Sex Hormone-binding Globulin Secretion and Gene Expression by Cycloheximide *In Vitro*

Mikko Loukovaara,* Marion Carson and Herman Adlercreutz

Department of Clinical Chemistry, University of Helsinki, Meilahti Hospital, SF-00290 Helsinki, Finland

The role of protein synthesis in sex hormone-binding globulin (SHBG) secretion and gene expression was studied in HepG2 cell cultures. Inhibition of protein synthesis by cycloheximide suppressed SHBG levels. Triiodothyronine and estradiol increased SHBG production, and cycloheximide reduced their effects to an extent which correlated with the degree of suppression obtained with the drug alone. Insulin decreased SHBG production, and the effect of the treatment with insulin and cycloheximide together did not differ from that with cycloheximide alone. Cycloheximide did not, alone or with the hormones, decrease SHBG levels more markedly extra- than intracellularly. Therefore, cycloheximide does not impair the secretion of SHBG which is synthesized in the presence of the drug. In contrast to SHBG protein levels, cycloheximide increased SHBG mRNA levels. When the effect of cycloheximide on the rate of SHBG mRNA decay was tested, the drug was found to extend the half-life of SHBG mRNA. Of the hormones, insulin decreased and triiodothyronine modestly increased SHBG mRNA levels, whereas estradiol had no clear effect. Treatment with cycloheximide together with any of the hormones resulted in an increase in SHBG mRNA levels. We conclude that protein synthesis inhibition does not impair the secretion of SHBG produced under such conditions, but stabilizes SHBG mRNA by removing some hepatic protein species involved in the regulation of its degradation.

J. Steroid Biochem. Molec. Biol., Vol. 54, No. 3/4, pp. 141–146, 1995

INTRODUCTION

Sex hormone-binding globulin (SHBG) is a plasma glycoprotein [1] with a single high affinity binding site for estrogens and androgens [2]. In the regulation of plasma SHBG levels, estrogen–androgen balance and thyroid hormone status have been traditionally regarded as important factors [3]. The present opinion suggests, however, that steroid and thyroid hormones may not be physiological regulators of SHBG production, but rather influence it under certain pharmacological and pathological conditions—estrogens and thyroid hormones increasing and androgens decreasing it [4]. Results from recent studies imply that insulin may be the main regulator of SHBG production: fasting plasma insulin levels correlate negatively with plasma SHBG levels in both men [5] and women [6], and insulin decreases SHBG production *in vitro* [7–9].

The hormonal regulation of SHBG production has been studied extensively. The aim of this study was to elucidate the role of protein synthesis in SHBG secretion and gene expression, by utilizing the protein synthesis inhibiting property of cycloheximide in liver cells in culture. The effect of cycloheximide, alone and together with insulin, estradiol and triiodothyronine, on SHBG protein and messenger ribonucleic acid (mRNA) levels was investigated. Further, the effect of cycloheximide on the time course of SHBG mRNA decay was examined.

MATERIALS AND METHODS

Cell cultures

Human hepatoma cells (HepG2 cells, American Type Culture Collection, Rockville, MD) were grown at 37°C in 92% air and 8% CO_2 in Dulbecco's Modified Eagle's Medium without phenol red (Gibco, Paisley, U.K.), supplemented with 10% heat-inactivated fetal calf serum (Gibco), 2% L-glutamine (Gibco), 1% nonessential amino acids (Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin

*Correspondence to M. Loukovaara.
Received 20 Dec. 1994; accepted 3 Apr. 1995.

(penicillin-streptomycin solution, Gibco). Subconfluent cell layers were washed twice with phosphate-buffered saline (PBS), after which the cells were cultivated for 1 day under serum-free conditions. The medium was then replaced by fresh serum-free medium, and cultivation was continued in the presence of the effectors.

To study the effect of protein synthesis inhibition on SHBG secretion, the cells were cultivated for 3 days in the presence of 2 μg/ml cycloheximide (Sigma Chemical Co., St Louis, MO). The chosen concentration of cycloheximide inhibited total protein secretion by 70%, but did not affect cell growth, as seen from the constant DNA content of the cultures. Insulin (Boehringer Mannheim, Mannheim, Germany), 17β-estradiol (Makor Chemicals, Jerusalem, Israel), and triiodothyronine (Sigma Chemical Co.) were added 1 h after the addition of cycloheximide. Subsequently, the cells were refed with the hormones daily. At the end of the incubation, the media were removed and stored at −20°C until assayed. The cells were washed twice with PBS, harvested, and stored at −20°C until homogenized and assayed. Homogenization was accomplished by sonication with a Sonifier cell disrupter B-30 (Branson Sonic Power Co., Danbury, CT).

To study the effect of protein synthesis inhibition on SHBG gene expression, the cultures were treated with cycloheximide and/or the hormones as above. After 2 days, polyadenylated RNA was isolated, and SHBG mRNA was quantified by Northern blot analysis.

The effect of protein synthesis inhibition on SHBG mRNA decay was examined by blocking DNA transcription of the cells with 5 μg/ml actinomycin D (Sigma Chemical Co.), after a 2 day treatment with 2 μg/ml cycloheximide. Thereafter, polyadenylated RNA was isolated at the time points indicated below, and SHBG mRNA was quantified by Northern blot analysis.

SHBG measurement

SHBG concentrations from homogenized HepG2 cells and medium samples were measured by a fluoroimmunometric assay (Delfia SHBG assay, Wallac Oy, Turku, Finland), as described earlier [10].

Extraction of polyadenylated RNA

After the isolation of total hepatocyte RNA by the method of Chomczynski and Sacchi [11] and the RNAzol-B reagent (Cinna/Biotecx Laboratories International, Friendswood, TX), polyadenylated RNA was selected by chromatography on oligo-(deoxythymidine)-cellulose using an mRNA purification kit (Pharmacia, Uppsala, Sweden). RNA was quantified by measuring the absorbance at 260 nm.

Northern blot analysis

Five micrograms of polyadenylated RNA were separated electrophoretically on an 0.8% agarose–formaldehyde gel [12] and transferred to a Pall-Biodyne membrane (Pall, East Hills, NY) in 20 × SSC (1 × SSC is 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0) by capillary action [13]. The membrane was baked, prehybridized and hybridized according to the manufacturer's instructions. SHBG complementary DNA [14] (kindly provided by Dr Geoffrey L. Hammond, London Regional Cancer Center, London,

Table 1. SHBG levels in homogenized HepG2 cells amd culture medium after a 3 day incubation with different effectors

	Cells		Medium	
	SHBG (pg/μg DNA)	SHBG (% of control)	SHBG (pg/μg DNA)	SHBG (% of control)
Control	21.9 ± 0.8	100 ± 4	840 ± 21	100 ± 3
Insulin 15 nM	15.8 ± 0.4	72 ± 2*	617 ± 16	73 ± 2*
Cycloheximide 2 μg/ml	7.0 ± 0.2	32 ± 1†	344 ± 7	41 ± 1†
Insulin + cycloheximide	5.7 ± 0.2	26 ± 1†	331 ± 8	39 ± 1†
Control	21.9 ± 0.8	100 ± 4	840 ± 21	100 ± 3
Estradiol 500 nM	36.4 ± 0.5	166 ± 2‡	895 ± 21	107 ± 3
Cycloheximide 2 μg/ml	7.0 ± 0.2	32 ± 1§	344 ± 7	41 ± 1‖
Estradiol + cycloheximide	12.9 ± 0.3	59 ± 1†	332 ± 8	40 ± 1‖
Control	21.4 ± 1.6	100 ± 8	531 ± 32	100 ± 6
Triiodothyronine 10 nM	31.2 ± 2.3	146 ± 11¶	793 ± 51	149 ± 10**
Cycloheximide 2 μg/ml	7.6 ± 0.6	36 ± 3†	243 ± 9	46 ± 2†
Triiodothyronine + cycloheximide	9.3 ± 0.6	43 ± 3†	316 ± 20	60 ± 4††

*$P < 0.0001$ vs control, vs cycloheximide, vs insulin + cycloheximide.
†$P < 0.0001$ vs control.
‡$P < 0.0001$ vs control, vs cycloheximide, and vs estradiol + cycloheximide.
§$P < 0.0001$ vs control, and vs estradiol + cycloheximide.
‖$P < 0.0001$ vs control, and vs estradiol.
¶$P < 0.01$ vs control; $P < 0.0001$ vs cycloheximide, and vs triiodothyronine + cycloheximide.
**$P < 0.005$ vs control; $P < 0.0001$ vs cycloheximide, and vs triiodothyronine + cycloheximide.
††$P < 0.0005$ vs control.

Canada) was labeled with [α-^{32}P]deoxy-CTP, using Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA), and the labeled probe was purified through a Bio-Spin 6 Chromatography Column (Bio-Rad, Richmond, CA). After hybridization, the membrane was washed [10], and exposed to Kodak X-Omat AR Scientific Imaging Film (Eastman Kodak, Rochester, NY) for 1–5 days at −80°C. The autoradiography signals were quantified by an EDC Electrophoresis Data Center (Helena Laboratories, Beaumont, TX), with reference to the signals obtained by rehybridizing the membranes with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) complementary DNA [15].

DNA measurement

The amount of cellular DNA was determined with a slight modification [10] of Sorger and Germinario's procedure [16].

Statistics

The ratios of SHBG concentrations to DNA content were calculated, and the results from six culture discs for each treatment group were expressed as a percentage of the control value in each case and are presented as the mean ± SEM. The differences in SHBG levels between cultures with and without the effectors were evaluated by factorial analysis of variance, and Fisher's protected least significant difference test was employed for multiple group comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows the effect of cycloheximide and the various hormones, alone and in combination, on intra- and extracellular SHBG levels in HepG2 cell cultures.

Cycloheximide suppressed SHBG levels significantly. In four separate experiments, the suppression was slightly more pronounced within the cells than in the culture medium (67 ± 4% and 57 ± 9%, the mean suppression ± range within the cells and the medium, respectively).

Of the hormones, insulin decreased and triiodothyronine increased, at nanomolar concentrations, the production of SHBG. Estradiol, on the other hand, had no effect on SHBG levels at 10–25 nM concentrations (not shown), but increased the levels within the cells at a concentration of 500 nM. The increase in the culture medium was not significant. Because of the slightly more pronounced effect of cycloheximide on intra- than extracellular SHBG levels, the drug alleviated the estradiol induced imbalance between the intracellular accumulation and secretion of SHBG. Similarly, the effect of triiodothyronine on SHBG levels was reduced by cycloheximide to an extent which correlated with the extent obtained with cycloheximide alone, i.e. the levels reduced slightly more within the cells than in the culture medium. The effect of the combined treatment with insulin and cycloheximide did not differ from that with cycloheximide alone.

In contrast to its effect on SHBG protein levels, cycloheximide increased (by 80 ± 40%, the mean ± range of three experiments) SHBG mRNA levels in HepG2 cells (Fig. 1). To elucidate the mechanism of the increase, the effect of cycloheximide on the rate of SHBG mRNA decay was tested (Fig. 2). During the first 6 h after the blockade of DNA transcription, SHBG mRNA levels remained unaltered in both control and cycloheximide treated cells, which indicates that SHBG mRNA did not degrade substantially. At 12 h, SHBG mRNA levels had declined abruptly in control cells, but were still unaltered in cycloheximide treated cells. The effect of cycloheximide appears to be specific: in both control and cycloheximide treated cells, the initial levels of GAPDH mRNA were 1.4

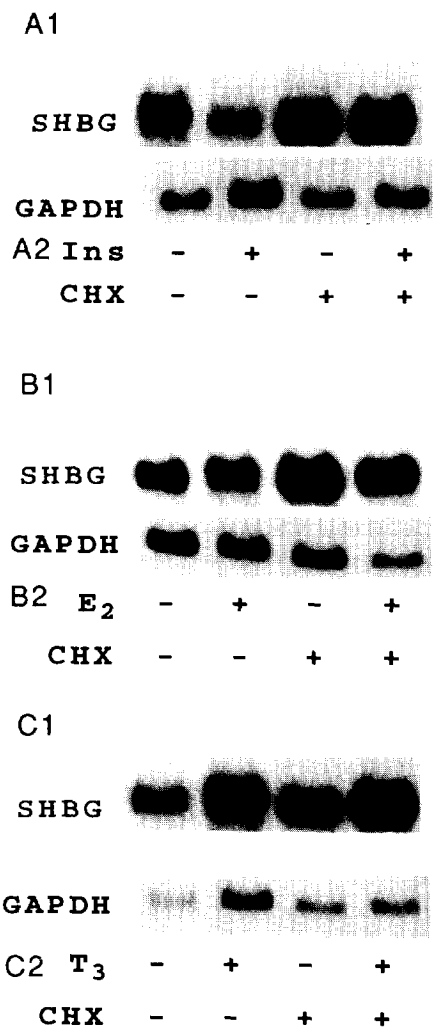

Fig. 1. Effect of a 2 day incubation with (A) insulin (15 nM), (B) estradiol (500 nM), and (C) triiodothyronine (10 nM), in the absence and presence of cycloheximide (CHX, 2 μg/ml), on SHBG mRNA levels in HepG2 cells. SHBG mRNA, 1.6 kb; GAPDH mRNA, 1.3 kb.

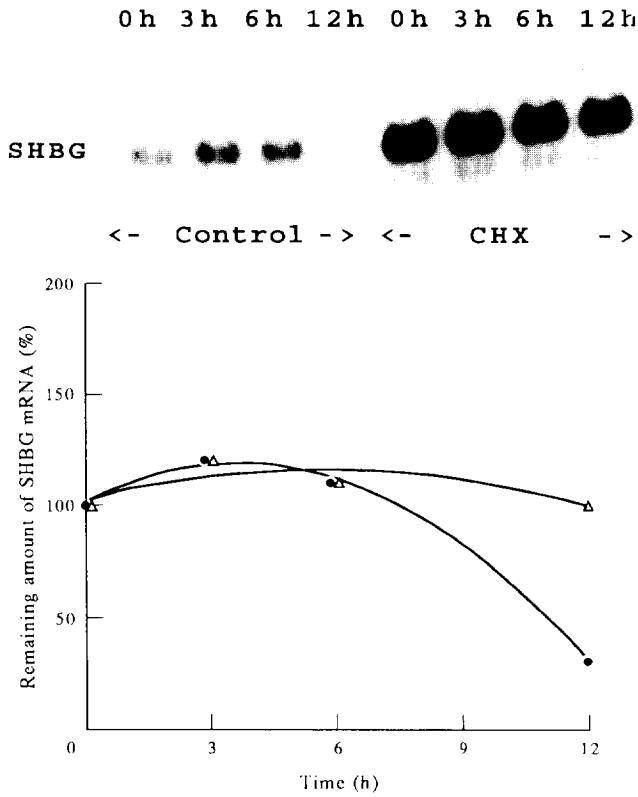

Fig. 2. Effect of cycloheximide on SHBG mRNA turnover rate. HepG2 cells were cultivated for 2 days in the absence or presence of cycloheximide (CHX, 2 μg/ml), after which DNA transcription was blocked with actinomycin D (5 μg/ml). Polyadenylated RNA was isolated at the indicated time points, and SHBG mRNA was quantified by Northern blot analysis. The amount of SHBG mRNA at the time of actinomycin D addition was taken as 100%. ●, control cells; △, cycloheximide treated cells.

times higher than the levels at the end of the incubation.

The effect of each of the hormones on SHBG mRNA levels was tested at concentrations which altered the levels of the protein (Fig. 1). Stimulation with insulin decreased SHBG mRNA levels to $25 \pm 10\%$ (the mean ± range of two experiments) of the levels in unstimulated cells. Triiodothyronine modestly increased the levels of the transcript (by $50 \pm 20\%$, the mean ± range of two experiments), whereas estradiol had no clear effect in two experiments. When the cells were treated with both cycloheximide and one of the hormones, the effect of the hormones was overwhelmed by the drug (a 1.5-, 2.3-, and 2.4-fold increase in SHBG mRNA levels for combined treatments with insulin, estradiol, and triiodothyronine, respectively) (Fig. 2).

DISCUSSION

In the present study, we investigated *in vitro* the effect of protein synthesis inhibition by cycloheximide on the secretion of SHBG, and on the levels and half-life of SHBG mRNA.

Cycloheximide decreased SHBG levels in HepG2 cell cultures, in conjunction with its protein synthesis inhibiting property. Inhibition of protein synthesis does not appear to impair the secretion of SHBG produced under such conditions, as cycloheximide did not decrease SHBG levels more markedly in the culture medium than within the cells. The fact that cycloheximide repeatedly suppressed SHBG levels somewhat more efficiently intra- than extracellularly might suggest that cycloheximide actually assists the secretion of SHBG produced in the presence of the drug. The secretion of SHBG accumulated within the cells prior to the addition of cycloheximide would not alone explain the phenomenon.

As in an earlier study [10], insulin and triiodothyronine altered SHBG levels in parallel intra- and extracellularly, whereas estradiol affected mainly the intracellular SHBG levels. Because cycloheximide affected SHBG levels more potently than the hormones, the results of the treatments with cycloheximide and the hormones together reflected primarily the effect of the former. Thus, SHBG levels decreased as a response to the combined treatments, a result which was again slightly more evident intra- than extracellularly. However, the hampered secretion of SHBG during estradiol stimulation was not completely abolished by the drug.

The imbalance between intracellular accumulation and secretion of SHBG during estradiol stimulation may be explained by the effect of the hormone on the nonsecreted nonsteroid-binding form of SHBG, formed by alternative processing of the SHBG transcript [17, 18]. In addition, changes which are restricted to the glycosylation of SHBG are known to alter the secretion of SHBG, but in this case without effect on the binding affinity of the protein for its ligand [19].

Cycloheximide enhanced SHBG mRNA accumulation in HepG2 cells, which differentiates SHBG mRNA from the mRNA of another steroid binding protein, estrogen receptor, the levels of which remain unaltered as a response to cycloheximide [20]. Induction by protein synthesis inhibition appears to be a feature common to a number of cell-cycle-related mRNAs, resulting from either direct stimulation of transcription, or mRNA stabilization [21]. Cycloheximide has been reported to activate the transcription of genes encoding actin [22], β-interferon [23], and ornithine decarboxylase [24], probably through the activation of enhancer sequences termed serum-responsive elements [25]. However, for the majority of mRNAs described, the mechanism of mRNA induction by protein synthesis inhibition is an increase in the mRNA half-life. Histone mRNA [26] and certain proto-oncogene mRNAs [27, 28], for example, are all stabilized

under such conditions. Of the mRNAs encoding proteins which bind hormones or growth factors, insulin-like growth factor binding protein-1 mRNA has been demonstrated to be stabilized by cyclo-heximide [29].

The present study shows that in the case of SHBG mRNA, decreased turnover rate increases the levels of the transcript during cycloheximide treatment. Thus, our experiments support the concept of a protein regulator of SHBG mRNA decay. Such a protein could be an SHBG mRNA specific RNase or a protein that activates such an RNase or binds to SHBG mRNA to signal its degradation.

The involvement of a repressor protein in the regulation of SHBG mRNA levels would be compatible with our observations that cycloheximide overwhelmed the effects of insulin, estradiol and triiodothyronine on SHBG mRNA levels. Whether these hormones exert their effects on SHBG mRNA levels independently of *de novo* protein synthesis remains unresolved, because the levels were increased by protein synthesis inhibition *per se*. The inference made by other authors [30, 31], that protein synthesis is not necessary for the hormonal induction of SHBG mRNA in hepatic cells, is not justifiable. Namely, they did not examine the effect of cycloheximide on SHBG mRNA levels alone, but only together with either estradiol or triiodothyronine.

With the exception of insulin, the hormones tested in this study did not alter SHBG mRNA levels by a factor of two, a limit suggestive of altered transcription in Northern analysis. The result agrees with the earlier studies carried out *in vitro*. According to three groups, insulin decreases [10, 30, 32], and according to four groups, triiodothyronine slightly increases SHBG mRNA levels [10, 30–34]. The results with estradiol are controversial. As a response to this steroid, SHBG mRNA levels have been reported to slightly increase [10, 30, 33], or to remain unaltered [32]. The steady state levels of the alternatively spliced SHBG transcripts are not necessarily equal. The clearer response of the cells to triiodothyronine than to estradiol might be due to the effect of the latter on the levels of a quantitatively minor transcript. This would correlate with the fact that estradiol primarily increases the levels of intracellular SHBG, a quantitatively minor translation product of SHBG.

We conclude that protein synthesis inhibition does not impair the secretion of SHBG produced under such conditions, but stabilizes SHBG mRNA by removing some hepatic protein species involved in the regulation of its degradation.

REFERENCES

1. Van Baelen H., Heyns W. and De Moor P.: Microheterogeneity of the testosterone binding globulin of human pregnancy serum demonstrated by isoelectric focusing. *Ann. Endocr.* **30** (1969) 199–203.
2. Turner E. E., Ross J. B. A., Namkung P. C. and Petra P. H.: Purification and characterization of the sex steroid binding protein from Macaque serum. Comparison with the human protein. *Biochemistry* **23** (1984) 492–497.
3. Anderson D. C.: Sex-hormone-binding globulin. *Clin. Endocr.* **3** (1974) 69–96.
4. von Schoultz B. and Carlström K.: On the regulation of sex-hormone-binding globulin—a challenge of an old dogma and outlines of an alternative mechanism. *J. Steroid Biochem.* **32** (1989) 327–334.
5. Hautanen A., Sarna S., Pelkonen R. and Adlercreutz H.: Serum sex hormone-binding globulin, cardiovascular risk factors, and adrenal cortisol responses to dexamethasone and corticotropin. *Metabolism* **42** (1993) 870–874.
6. Preziosi P., Barrett-Connor E., Papoz L., Roger M., Saint-Paul M., Nahoul K. and Simon D.: Interrelation between plasma sex hormone-binding globulin and plasma insulin in healthy adult women: The Telecom study. *J. Clin. Endocr. Metab.* **76** (1993) 283–287.
7. Plymate S.R., Matej L. A., Jones R. E. and Friedl K. E.: Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. *J. Clin. Endocr. Metab.* **67** (1988) 460–464.
8. Plymate S. R., Jones R. E., Matej L. A. and Friedl K. E.: Regulation of sex hormone binding globulin (SHBG) production in Hep G2 cells by insulin. *Steroids* **52** (1988) 339–340.
9. Singh A., Hamilton-Fairley D., Koistinen R., Seppälä M., James V. H. T., Franks S. and Reed M. J.: Effect of insulin-like growth factor-type I (IGF-I) and insulin on the secretion of sex hormone binding globulin and IGF-I binding protein (IBP-I) by human hepatoma cells. *J. Endocr.* **124** (1990) R1–R3.
10. Loukovaara M., Carson M. and Adlercreutz H.: Regulation of production and secretion of sex hormone-binding globulin in HepG2 cell cultures by hormones and growth factors. *J. Clin. Endocr. Metab.* **80** (1995) 160–164.
11. Chomczynski P. and Sacchi N.: Single-step method of RNA isolation by acid guanidium thiocyanate–phenol–chloroform extraction. *Analyt. Biochem.* **162** (1987) 156–159.
12. Sambrook J., Fritsch E. F. and Maniatis T.: *Molecular Cloning—a Laboratory Manual*, 2nd edition. Cold Spring Harbor Laboratory, Cold Spring Harbor (1989) pp. 7.43–7.45.
13. Southern E. M.: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Molec. Biol.* **98** (1975) 503–517.
14. Hammond G. L., Underhill D. A., Smith C. L., Goping I. S., Harley M. J., Musto N. A., Cheng C. Y. and Bardin C. W.: The cDNA-deduced primary structure of human sex hormone-binding globulin and location of its steroid-binding domain. *FEBS Lett.* **215** (1987) 100–104.
15. Fort P., Marty L., Piechaczyk M., El Sabrouty S., Dani C., Jeanteur P. and Blanchard J. M.: Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. *Nucl. Acids Res.* **13** (1985) 1431–1442.
16. Sorger T. and Germinario R. J.: A direct solubilization procedure for the determination of DNA and protein in cultured fibroblast monolayers. *Analyt. Biochem.* **131** (1983) 254–256.
17. Sullivan P. M., Petrusz P., Szpirer C. and Joseph D. R.: Alternative processing of androgen-binding protein RNA transcripts in fetal rat liver. Identification of a transcript formed by *trans* splicing. *J. Biol. Chem.* **266** (1991) 143–154.
18. Sullivan P. M., Wang Y-M. and Joseph D. R.: Identification of an alternate promoter in the rat androgen-binding protein/sex hormone-binding globulin gene that regulates synthesis of a messenger RNA encoding a protein with altered function. *Molec. Endocr.* **7** (1993) 702–715.
19. Bocchinfuso W. P., Ma K-L., Lee W. M., Warmels-Rodenhiser S. and Hammond G. L.: Selective removal of glycosylation sites from sex hormone-binding globulin by site-directed mutagenesis. *Endocrinology* **131** (1992) 2331–2336.
20. Saceda M., Lippman M. E., Lindsey R. K., Puente M. and Martin M. B.: Role of an estrogen receptor-dependent mechanism in the regulation of estrogen receptor mRNA in MCF-7 cells. *Molec. Endocr.* **3** (1989) 1782–1787.
21. Rollins B. J. and Stiles C. D.: Serum-inducible genes. *Adv. Cancer Res.* **53** (1989) 1–32.

22. Elder P. K., Schmidt L. J., Ono T. and Getz M. J.: Specific stimulation of actin gene transcription by epidermal growth factor and cycloheximide. *Proc. Natn. Acad. Sci. U.S.A.* **81** (1984) 7476–7480.
23. Ringold G. M., Dieckmann B., Vannice J. L., Trahey M. and McCormick F.: Inhibition of protein synthesis stimulates the transcription of human β-interferon genes in Chinese hamster ovary cells. *Proc. Natn. Acad. Sci. U.S.A.* **81** (1984) 3964–3968.
24. Olson E. N. and Spizz G.: Mitogens and protein synthesis inhibitors induce ornithine decarboxylase gene transcription through separate mechanisms in the BC_3H1 muscle cell line. *Molec. Cell. Biol.* **6** (1986) 2792–2799.
25. Subramaniam M., Schmidt L. J., Crutchfield III C. E. and Getz M. J.: Negative regulation of serum-responsive enhancer elements. *Nature* **340** (1989) 64–66.
26. Stimac E., Groppi Jr. V. E. and Coffino P.: Inhibition of protein synthesis stabilizes histone mRNA. *Molec. Cell. Biol.* **4** (1984) 2082–2090.
27. Dony C., Kessel M. and Gruss P.: Post-transcriptional control of *myc* and p53 expression during differentiation of the embryonal carcinoma cell line F9. *Nature* **317** (1985) 636–639.
28. Thompson C. B., Challoner P. B., Neiman P. E. and Groudine M.: Expression of the c-*myb* proto-oncogene during cellular proliferation. *Nature* **319** (1986) 374–380.
29. Angervo M.: Epidermal growth factor enhances insulin-like growth factor binding protein-1 synthesis in human hepatoma cells. *Biochem. Biophys. Res. Commun.* **189** (1992) 1177–1183.
30. Mercier-Bodard C., Nivet V. and Baulieu E-E.: Effects of hormones on SBP mRNA levels in human cancer cells. *J. Steroid Biochem. Molec. Biol.* **40** (1991) 777–785.
31. Barlow J. W., Crowe T. C., Cowen N. L., Raggatt L. E., Topliss D. J. and Stockigt J. R.: Stimulation of sex hormone-binding globulin mRNA and attenuation of corticosteroid-binding globulin mRNA by triiodothyronine in human hepatoma cells. *Eur. J. Endocr.* **130** (1994) 166–170.
32. Crave J. C., Lejeune H., Zerbib M., Baret C., Brebant C. and Pugeat M.: Tamoxifen increases corticosteroid binding globulin in the human. *The 11th International Symposium of J. Steroid Biochem. Molec. Biol., "Recent Advances in Steroid Biochemistry and Molecular Biology"*. Seefeld, Tirol, Austria (1993) Abstract No. 76P.
33. Mercier-Bodard C., Baville F., Bideux G., Binart N., Chambraud B. and Baulieu E-E.: Regulation of SBP synthesis in human cancer cell lines by steroid and thyroid hormones. *J. Steroid Biochem.* **34** (1989) 199–204.
34. Raggatt L. E., Blok R. B., Hamblin P. S. and Barlow J. W.: Effects of thyroid hormone on sex hormone-binding globulin gene expression in human cells. *J. Clin. Endocr. Metab.* **75** (1992) 116–120.