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Steroids and Growth Factors in Breast and Uterus

OESTROGEN DIRECTLY STIMULATES GROWTH FACTOR SIGNAL TRANSDUCTION PATHWAYS IN HUMAN BREAST CANCER CELLS

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Summary—We have studied the mechanism by which 17β-oestradiol (E2) stimulates breast cancer proliferation using the MCF7 cell line as a model system. We provide evidence that E2 directly stimulates cellular proliferation by inducing, like many growth factors, the c-*fos* proto-oncogene. E2 by itself, however, is poorly mitogenic and it does not induce genes from the *jun* family, whose gene products are necessary for heterodimerization with the c-*fos* encoded protein (Fos), leading to an important step in growth factor signalling pathways, stimulation of the 12-*O*-tetradecanoyl-phorbol-13-acetate responsive element (TRE)-dependent transcriptional activity. In combination with insulin-like growth factors (IGFs), efficient inducers of c-*jun* in breast cancer cells, E2 synergistically stimulates TRE-activity and proliferation. This direct stimulation by E2 of growth factor signalling pathways suggest that E2 can directly induce proliferation, independent from autocrine growth factors.

INTRODUCTION

Approximately one third of human breast tumors are 17β-oestradiol (E2)-dependent in initial stages of the disease, showing a regression following hormonal therapy. Growth stimulation by E2 is thought to be an indirect process mediated by autocrine acting E2-induced polypeptide growth factors, such as insulin-like growth factors (IGFs) and transforming growth factory α (TGFα) (reviewed in [1]). In this hypothesis, autonomous production of these growth factors would lead to loss of E2-dependence, as associated with progression of the disease.

To unravel the process leading to loss of hormone-dependency we are studying the mechanism by which E2 and polypeptide growth factors stimulate proliferation of the human breast cancer cell line MCF7. In order to be able to perform such studies under steroid- and growth factor-defined conditions a novel culture system was developed. In the light of the difficulties of culturing human breast cancer cells under strict serum-free conditions [2], we used media supplemented with serum in which growth factors were chemically inactivated and steroids are removed [3]. The thus obtained medium appears to be a very suitable general-purpose medium, since it supports growth of all 11 breast cancer cell lines tested so far [3–5; Van der Burg B. *et al.*, unpublished], and has been used in primary cultures of solid canine mammary tumors to isolate cell lines [4, Van der Burg B. *et al.*, unpublished].

As a result of this mitogen depletion the E2-dependent human breast cancer cell line MCF7 becomes quiescent in the G1/G0 phase of the cell cycle, from which it can be stimulated to enter the cell cycle by using defined mitogens [3]. This contrasts with the situation in conventional serum that is treated only to remove endogenous steroids, and that still contains growth factors causing background mitogenic activity. Thus, under our conditions MCF7 cells displayed their strict hormone-dependence that has been established *in vivo* (in nude mice), under physiological conditions [6, 7].

Surprisingly, under these defined conditions E2 alone was a poor mitogen but synergistically stimulated proliferation in combination with low concentrations of insulin or IGFs. At much higher concentrations insulin and related factors

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are fully mitogenic and, unlike growth factors TGFα, EGF, FGF, PDGF and TGFβ, are able to bypass E2-requirement [3]. Therefore, IGFs seemed the prime candidates to serve an autocrine function. Kinetic studies, however, showed that E2 stimulates DNA synthesis without a delay-period compared to IGFs, suggesting that no growth factor production is involved in E2-induced mitogenesis [8]. Moreover, binding proteins [5] or antibodies [9, Van der Burg *et al.*, unpublished] that efficiently blocked IGF-induced mitogenesis did not inhibit the E2-response. In addition, in a recently developed bioassay for IGFs [10] no activity was found in conditioned media of E2-treated MCF7 cells or E2-independent human breast cancer cell lines, again suggesting a minor role for autocrine produced growth factors in human breast cancer proliferation [5]. Recent results obtained in other laboratories also suggest that breast cancer cells generally do not react to the growth factors which they produce (reviewed in [11]).

MCF7 cell proliferation *in vivo* seems to be mediated by E2 in combination with endocrine insulin and IGFs. Since human fibroblasts were found to produce IGFs in a biological active form [5], the large amount of stromal cells often present in breast tumors may be an additional source.

Nuclear proto-oncogenes like c-*fos* and c-*myc* are a primary target of the signal transduction that leads from growth factor receptors to the nucleus (reviewed in [12]). The products of these genes are among the first to be induced after mitogenic stimulation and are involved in transforming the incoming signal into a change in gene expression. The expression of these transcription factors is rapidly downregulated and constitutive expression can lead to cellular transformation.

The product of c-*fos* (Fos) by itself does not bind to DNA but interacts with other transcription factors. Recently it has been shown that Fos can form heterodimers with Jun, a major component of the transcription factor AP-1 [13–17]. This interaction results in a greatly enhanced affinity for the AP-1 target sequence, the 12-*O*-tetradeconoyl-phorbol-13-acetate (TPA)-responsive element (TRE), and a stimulation of TRE-dependent transcriptional activity [13, 17, 18]. The gene coding for Jun (*jun*) is also growth factor inducible [18–21]. Induction of TRE activity is thought to be a critical step in the onset of cellular proliferation and transformation by polypeptide growth factors (reviewed in [12]). In this process c-*fos* expression is essential since suppression of expression by antisense RNA abolishes the TRE-dependent induction of genes, and cellular proliferation and transformation.

Here, we report the direct stimulation by E2 of c-*fos* expression and TRE activity, giving additional support to the idea that E2 directly stimulates proliferation, without inducing autocrine loops.

EXPERIMENTAL

Materials

A phenol red-free 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium (DF) was obtained from Gibco (Grand Island, NY). Trypsin and the EDTA used for cell culture were obtained from Flow Laboratories (Irvine, Scotland). Fetal calf serum (FCS) was purchased from Hyclone Laboratories (Logan, UT), bovine insulin, E2, human transferrin and bovine serum albumin (BSA) were from Sigma (St Louis, MO). [methyl-^{3}H]-Thymidine (77.9 Ci/mmol) was purchased from NEN (Boston, MA). Yeast recombinant human IGF-I was a gift from Dr Anna Skottner (KabiVitrum, Stockholm, Sweden). DCC-FCS was prepared by treating FCS with dextran coated charcoal (DCC) to remove steroids, as described [3]. DCC-SH-FCS was prepared by a treatment with dithiothreitol to inactivate polypeptide growth factors [22], followed by a DCC-treatment, as described previously [3].

Cell culture

MCF7 cells were kindly provided by Dr C. Quirin-Stricker (Institut de Chimie Biologique, Faculté de Médicine, Strasbourg, France), and were cultured in phenol red-containing DF medium supplemented with 5% FCS and buffered with bicarbonate. The cells were passaged twice a week using trypsin and EDTA, and they were grown in a humidified atmosphere containing 7.5% CO_2. For experiments only exponentially growing, 4-day-old cultures were used. The cells were free of *Mycoplasma* contamination.

Cell growth experiments

The effect of mitogens on MCF7 cells on DNA synthesis was treated in the absence of phenol red [23], essentially as described pre-

viously [8]. In short, cells were plated at a density of $2 \times 10^4/cm^2$ in DF containing 30 nM selenite, 10 μg/ml transferrin and 0.2% BSA (referred to as DF^+), supplemented with 5% DCC-FCS. After 24 h this medium is changed to DF^+, in which the cells become quiescent [3]. After another 24 h this medium is replaced by DF^+ supplemented with 10% DCC-SH-FCS. Subsequently the compounds to be tested were added. After 20 h [^{3}H]thymidine (1.0 μCi/ml) was added, and after another 8 h incubation the cells were washed with trichloro-acetic acid (TCA) and then lysed with NaOH, followed by liquid scintillation counting.

RNA extraction and Northern blot analysis

The cells were plated at a density of $2.0 \times 10^4/cm^2$ in 5% DCC-FCS, and were growth arrested by 24 h of serum deprivation, followed by lysing the cells in guanidine isothiocyanate, and by centrifugation through a CsCl cushion [24]. Northern blots were prepared as described previously [25]. For hybridization the following probes were used: a 1.0 kb PstI mouse c-*jun* genomic [W. Kruijer *et al.*, unpublished] fragment homologous to v-*jun* and a 0.8 kb PstI fragment of v-*fos*.

Transfection and CAT (chloramphenicol acetyl transferase) assays

MCF7 cells, suspended in 5 ml DF^+ supplemented with 5% DCC-FCS, were plated at a density of 2×10^4 cells/cm^2 in a 6 cm dia tissue culture dish. After 24 h the cells were incubated for 5 h with calcium phosphate-precipitated plasmid DNAs [26]. The reporter plasmid used to determine TRE-dependent transcriptional activity contained 5 copies of a synthetic oligonucleotide containing the collagenase TRE [27] flanked by Hind III/Bam HI linker sequences, upstream of the HSV-tk promoter and the CAT gene. The oligonucleotide was ligated 5 times head-to-head and cloned into Hind III/Bam HI cut pBL-CAT2 [28]. Directly after transfection the cells were washed with DF^+ and growth-arrested by a 24 h-incubation in 10% DCC-SH-FCS medium. Subsequently, the medium was replaced by fresh medium supplemented with the compounds to be tested. The cells were incubated for another 24 h after the beginning of the hormonal treatment. The cells were then harvested and CAT activity was measured [29]. The % conversion of ^{14}C labelled chloramphenicol was calculated by dividing the activity assessed in the acetylated reaction products by the total amount of radioactivity on TLC. Transfection efficiencies were determined by measuring the placental alkaline phosphatase (PAP) activity of cotransfected pSV_2 PAP constructs, as described by Henthorn *et al.* [30].

RESULTS

We and others showed that growth stimulation by E2 of MCF7 cells leads to a rapid induction of the growth-related proto-oncogenes c-*fos* [8, 31] and c-*myc* [8, 32]. This induction was a direct effect, independent of protein synthesis, showing that no growth factor production is involved in E2-induced proto-oncogene expression. To further evaluate the importance of E2-induced c-*fos* expression, we studied the induction of c-*jun* expression in quiescent MCF7 cells. Using Northern blot analysis a rapid induction of c-*jun* by high concentrations of insulin (10 μg/ml) was observed [Fig. 1(A)]. In contrast, stimulation of the cells with E2 did not result in elevation of c-*jun* mRNA in these E2-dependent cells [Fig. 1(B)]. Also a suboptimal concentration of insulin (25 ng/ml) that is sufficient to synergistically stimulate proliferation together with E2 (Fig. 2), was able to elevate c-*jun* mRNA levels slightly. We showed that 100 ng/ml of insulin is sufficient to maximally induce c-*jun* in these cells [25].

The synergism between insulin and E2, as observed in MCF7 cells, might be caused by a step in signal transduction prior to the induction of proto-oncogene expression, resulting in a cooperative stimulation of expression of these genes. The additional presence of E2, however, did not enhance the weak induction of expression of c-*jun* by low concentrations of insulin, 1 h after stimulation [Fig. 2(A)]. Furthermore, E2-induced c-*fos* expression was not influenced by the additional presence of insulin. Apparently, E2 is responsible for the induction of c-*fos* under synergistic conditions at this time point. Also at 30 min after stimulation, when the stimulatory effect of insulin on c-*fos* expression is at its maximum in these cells [8], low concentrations of insulin did not influence E2-induced c-*fos* expression (data not shown). Therefore it seems that synergism is determined by an effect distal to receptor activation and the pathway leading to the induction of proto-oncogene expression. This is consistent with a model in which the interaction of insulin-induced Jun and E2-induced Fos leads to syner

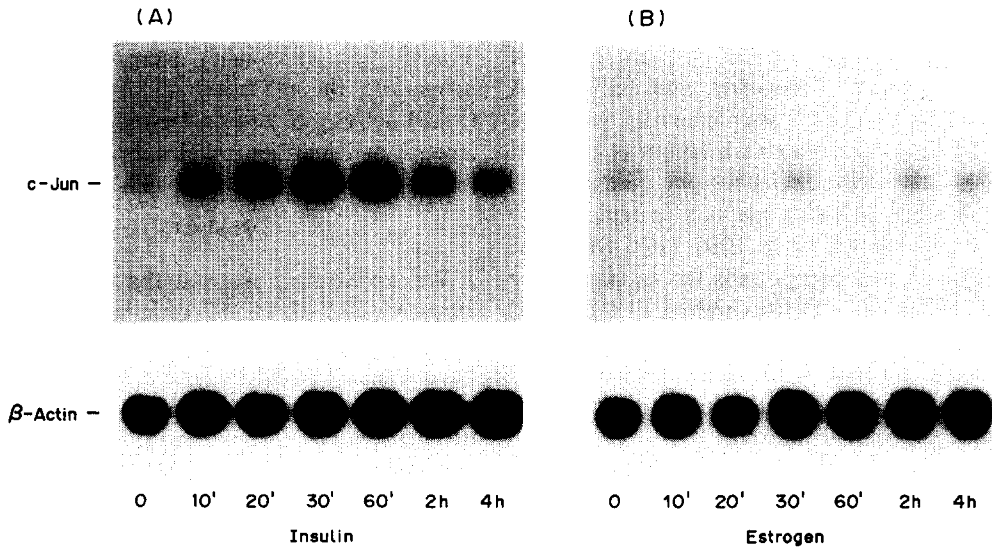

Fig. 1. Time-course of *c-jun* expression after stimulation of quiescent MCF7 cells with insulin (10^4 ng/ml; A) or E2 (10^{-9} M; B). Northern blot analysis of total RNA (20 μg/lane).

gistic stimulation of gene expression, ultimately leading to cellular proliferation. If this hypothesis holds true, the synergistic combination of insulin and E2 should induce the formation of a functional Fos/Jun complex in these cells, resulting in transactivation of target genes.

To study if the mitogens were able to induce TRE transactivating activity in the cells, we used CAT constructs linked to 5 TREs. Prior to hormonal stimulation the cells were cotransfected with these TRE constructs, together with control constructs containing the PAP gene linked to the early SV40 promoter to monitor the transfection efficiency. TPA, an efficient stimulator of the TRE, was used as a positive control. In MCF7 cells this factor stimulated CAT activity almost 10-fold (Fig. 3). Insulin treatment of the transfected cells also resulted in an elevation of TRE activity. It seems likely that the elevated TRE transactivating function

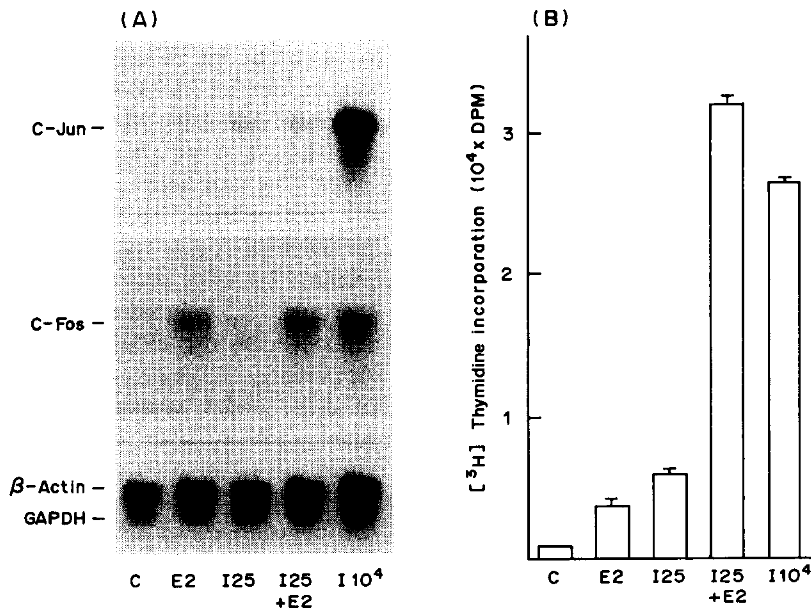

Fig. 2. Induction of *c-fos* and *c-jun* expression and DNA synthesis by the synergistic combination of insulin at low concentration and E2. A, Northern blot analysis of total RNA (20 μg/lane), extracted 60 min after stimulation. B, DNA synthesis as determined by measuring [^{3}H]thymidine incorporation 24 h after mitogenic stimulation in parallel cultures. Control cultures (C) received ethanol vehicle only. The mitogens were added at the following concentrations: E2, 10^{-9} M; insulin at low concentrations, 25 ng/ml (I 25); insulin at high concentrations, 10^4 ng/ml (I 10^4).

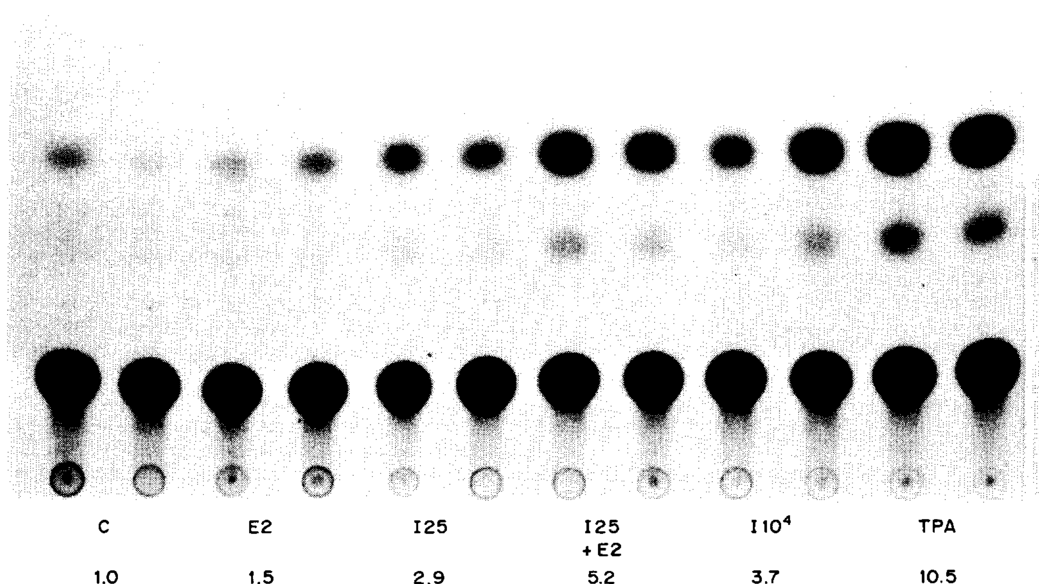

Fig. 3. Hormonal induction of activity of TRE-CAT constructs transfected into MCF7 cells. TPA was added in a concentration of 100 ng/ml. The concentrations of the other compounds are given in Fig. 2. The mean value of the total percentage conversion of chloramphenicol is indicated. This is a representative experiment from three separate experiments.

observed is a result of the formation of a complex between the two products of the c-*fos* and c-*jun* genes, although possibly products of recently identified c-*jun* and c-*fos* related genes contribute to this activity, since they can also form TRE binding heterodimers [15, 33, 34]. Although inducing c-*fos* expression, E2 alone does not induce TRE activity, confirming that no induction of c-*jun*, or related genes with similar function occurs. In contrast, E2 cooperatively induced TRE activity together with suboptimal concentration of insulin. In this synergistic situation the cooperative induction of TRE activity seems to be mediated by E2-induced c-*fos* expression, and c-*jun* expression by low concentrations of insulin.

DISCUSSION

TRE-dependent transcriptional activity is induced when MCF7 cells are optimally stimulated to proliferate, either by insulin at high concentrations or the synergistic combination of low concentrations of insulin together with the steroid hormone E2. In addition, this activity is also efficiently induced by the phorbol ester TPA, a compound that leads to growth arrest in these cells, probably by inducing differentiation [35, 36]. Therefore, TRE activity appears to be obligatory, but no guarantee for the induction of cellular proliferation. Also in other cells it probably plays a role in induction of differentiation [37]. A differential induction of other early response genes or differences in post-translational modifications may influence the control of important growth regulatory genes by the mitogens and differentiation inducing agents.

These results show that by inducing c-*fos* expression E2 is able to directly interact with nuclear events that are also induced by polypeptide growth factors and that form a critical step in the induction of cellular proliferation and tumorigenesis [12]. This implicates that growth factor secretion may not be an essential part of this mitogenic pathway elicited by E2, as was initially proposed [1]. This is in line with recent data which suggest that MCF7 cells do not react to the growth factors which they secrete [11]. Stimulation of TRE activity in cells by TPA treatment or transfection with activated *ras* oncogenes (reviewed in [38]), results in growth factor secretion [39–41]. Therefore growth factor secretion by MCF7 cells may be a consequence rather than a cause of growth stimulation [11, 25]. Interestingly, when MCF7 cells are transfected with the potent v-Ha-*ras* oncogene, a loss of E2-dependence concomitant with elevated growth factor secretion has been observed [42, 43]. We propose that not this elevated growth factor secretion but the direct activation of TRE activity is the cause of the

autonomous proliferation. It is tempting to speculate that in human breast tumors a similar process underlies the conversion towards hormone-independence that is associated with progression.

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REFERENCES

1. Dickson R. B. and Lippman M. E.: Estrogenic regulation of growth and polypeptide growth factor secretion in human breast carcinoma. *Endocrine Rev* **8** (1987) 29–43.
2. Barnes D. W.: Serum-free cell culture of MCF7 human mammary carcinoma. In *Methods for Serum-Free Culture of Cells of the Endocrine Systems* (Edited by D. W. Barnes, D. A. Sirbasku and G. H. Sato). Alan R. Liss, New York (1984) pp. 201–216.
3. Van der Burg B., Rutteman G. R., Blankenstein M. A., de Laat S. W. and van Zoelen E. J. J.: Mitogenic stimulation of human breast cancer cells in a growth factor-defined medium: synergistic action of insulin and estrogen. *J. Cell Physiol.* **123** (1988) 101–108.
4. Van der Burg B., van Selm-Miltenburg A. J. P., van Maurik P., Rutteman G. R., Misdorp W., de Laat S. W. and van Zoelen E. J. J.: Isolation of autonomously growing dog mammary tumor cell lines cultured in medium supplemented with serum treated to inactivate growth factors. *J. Natn. Cancer Inst.* **81** (1989) 1545–1551.
5. Van der Burg B., Isbrücker I., van Selm-Miltenburg A. J. P., de Laat S. W. and van Zoelen E. J. J.: The role of estrogen-induced insulin-like growth factors in the proliferation of human breast cancer cells. *Cancer Res.* **50** (1990) 7770–7774.
6. Shafie S. M. and Liotta L. A.: Formation of metastasis by human breast carcinoma cells (MCF-7) nude mice. *Cancer Lett.* **11** (1980) 81–87.
7. Soule H. D. and McGrath C. M.: Estrogen responsive proliferation of clonal human breast carcinoma cells in athymic mice. *Cancer Lett.* **10** (1980) 177–189.
8. Van der Burg B., van Selm-Miltenburg A. J. P., de Laat S. W. and van Zoelen E. J. J.: Direct effect of estrogen on *c-fos* and *c-myc* proto-oncogene expression and cellular proliferation in human breast cancer cells. *Molec Cell. Endocr.* **64** (1989) 223–228.
9. Arteaga C. L. and Osborne C. K.: Growth inhibition of human breast cancer cells *in vitro* with an antibody against the type I somatomedin receptor. *Cancer Res.* **49** (1989) 6237–6241.
10. Van Zoelen E. J. J., Ward-van Oostwaard Th. M. J., Nieuwland R., van der Burg B., van den Eijnden-van Raaij A. J. M., Mummery C. L. and de Laat S. W.: Identification and characterization of polypeptide growth factors secreted by murine embryonal carcinoma cells. *Dev. Biol.* **133** (1989) 272–283.
11. Van der Burg B. and de Laat S. W.: Molecular action of steroids and growth factors in human breast tumor cells. In *Recent Advances in Cell to Cell Signals in Plants and Animals* (Edited by V. Neuhoff and J. Friend), Springer Verlag (1991) pp. 256–287.
12. Herrlich P. and Ponta H.: "Nuclear" oncogenes convert extracellular stimuli into changes in the genetic program. *TIG* **5** (1989) 112–116.
13. Chiu R., Boyle W. J., Meek J., Smeal T., Hunter T. and Karin M.: The *c-fos* protein interacts with *c-jun*/AP-1 to stimulate transcription of AP-1 responsive genes. *Cell* **54** (1988) 541–552.
14. Halazonetis T. D., Georgopoulos K., Greenberg M. E. and Leder P.: *c-jun* Dimerizes with itself and with *c-fos*, forming complexes of different DNA binding affinities. *Cell* **55** (1988) 917–924.
15. Nakabeppu Y., Ryder K. and Nathans D.: DNA binding activities of three murine Jun proteins: stimulation by Fos. *Cell* **55** (1988) 907–915.
16. Rauscher F. J., Sambucetti L. C., Curran T., Distel R. J. and Spiegelman B. M.: Common DNA binding site for Fos protein complexes and transcription factor AP-1. *Cell* **52** (1988) 471–480.
17. Sassone-Corsi P., Lamph W. W., Kamps M. and Verma I. M.: *fos*-Associated cellular p39 is related to nuclear transcription factor AP-1. *Cell* **54** (1988) 553–560.
18. Lamph W. W., Wamsley P., Sassone-Corsi P. and Verma I. M.: Induction of proto-oncogene JUN/AP-1 by serum and TPA. *Nature* **334** (1988) 629–631.
19. Quantin B. and Breathnach R.: Epidermal growth factor stimulates transcription of the *c-jun* proto-oncogene in rat fibroblasts. *Nature* **334** (1988) 538–539.
20. Ryder K. and Nathans D.: Induction of proto-oncogene *c-jun* by serum growth factors. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1988) 8464–8467.
21. Ryseck R.-P., Hirai S. I., Yaniv M. and Bravo R.: Transcriptional activation of *c-jun* during the GI/GO transition in mouse fibroblasts. *Nature* **334** (1988) 535–537.
22. Van Zoelen E. J. J., van Oostwaard Th. M. J., van der Saag P. T. and de Laat S. W.: Phenotypic transformation of normal rat kidney cells in a growth factor-defined medium: induction by a neuroblastoma-derived transforming growth factor independently of the EGF receptor. *J. Cell. Physiol.* **123** (1985) 151–160.
23. Berthois Y., Katzenellenbogen J. A. and Kazenellenbogen B. S.: Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 2496–2500.
24. Chrigwin J. M., Przybyla A. E., MacDonald R. J. and Rutter W. J.: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18** (1979) 5294–5299.
25. Van der Burg B., de Groot R. P., Isbrücker L., Kruijer W. and de Laat S. W.: Stimulation of TRE activity by a cooperative action of insulin and estrogen in human breast cancer cells. *Molec. Endocr.* **4** (1990) 1720–1726.
26. Graham F. L. and van der Eb A. J.: A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52** (1973) 456–467.
27. Angel P., Bauman I., Stein B., Delius H., Rahmsdorf H. J. and Herrlich P.: 12-*O*-tetradecanoyl-phorbol-13-acetate induction of the human collagenase gene is mediated by an inducible enhancer element located in the 5′-flanking region. *Molec. Cell Biol.* **7** (1987) 2256–2266.
28. Luckow B. and Schütz G.: CAT constructions with multiple restriction sites for the functional analysis of eukaryotic promoters and regulatory elements. *Nuleic Acids. Res.* **15** (1987) 5490.
29. Gorman C. M., Moffat L. F. and Howard B. H.: Recombinant genomes which express recombinant chloramphenicol acetyltransferase in mammalian cells. *Molec. Cell Biol.* **2** (1982) 1044–1051.
30. Henthorn P., Zervos P., Raducha M., Harris H. and Kadesh T.: Expression of a human placental alkaline phosphatase gene in transfected cells: use as a reporter for studies of gene expression. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1988) 6342–6346.

31. Wilding G., Lippman M. E. and Gelman E. P.: Effects of steroid hormones and peptide growth factors on proto-oncogene c-*fos* expression in human breast cancer cells. *Cancer Res.* **48** (1988) 802–805.
32. Dubik D. and Shiu R. P. C.: Transcriptional regulation of c-*myc* oncogene expression in hormone-responsive human breast cancer cells. *J. Biol. Chem.* **236** (1988) 12705–12708.
33. Hirai S. I., Ryseck R. P., Mechta F., Bravo R. and Yaniv M.: Characterization of *junD*: a new member of the *jun* proto-oncogene family. *EMBO Jl* **8** (1989) 1433–1439.
34. Zerial M., Toschi L., Ryseck R. P., Schuemann M., Müller R. and Bravo R.: The product of a novel growth factor activated gene, *fos B*, interact with JUN proteins enhancing their DNA binding activity. *EMBO Jl* **8** (1989) 805–813.
35. Abe M. and Kufe D.: Effects of maturational agents on expression and secretion of two partially characterized high molecular weight milk-related glycoproteins in MCF-7 breast carcinoma cells. *J. Cell Physiol.* **126** (1986) 126–132.
36. Valette A., Gas N., Jozan S., Dupont M. A. and Bayard F.: Influence of 12-*O*-tetradecaoylphorbol-13-acetate on proliferation and maturation of human breast carcinoma cells (MCF-7): Relationship to cell cycle events. *Cancer Res.* **47** (1987) 1615–1620.
37. Bartel D. P., Sheng M., Lau L. F. and Greenberg M. E.: Growth factors and membrane depolarization activate distinct programs of early response gene expression: dissociation of *fos* and *jun* induction. *Genes Dev.* **3** (1989) 304–313.
38. Imler J. L. and Wasylyk B.: A composite transcription factor implicated in abnormal growth control. *Prog. Growth Factor Res.* **1** (1989) 69–77.
39. Bjorge J. D., Paterson A. J. and Kudlow J. E.: Phorbol ester or epidermal growth factor (EGF) stimulates the concurrent accumulation of mRNA for the EGF receptor and its ligand transforming growth factor-α in a breast cancer cell line. *J. Biol. Chem.* **264** (1989) 4021–4027.
40. Pittelkow M. R., Lindquist P. B., Abraham R. T., Graves R. T., Derynck R. and Coffey R. J.: Induction of transforming growth factor-α expression in human keratinocytes by phorbol esters. *J. Biol. Chem.* **264** (1989) 5164–5171.
41. Colamonici O. R., Trepel J. B., Vidal C. A. and Neckers L. M.: Phorbol ester induces c-*sis* gene transcription in stem cell line K-562. *Molec. Cell Biol.* **6** (1986) 1847–1850.
42. Dickson R. B., Kasid A., Huff K. K., Bates S. E., Knabbe C., Bronzert D., Gelmann E. P. and Lippman M. E.: Activation of growth factor secretion in tumorigenic states of breast cancer induced by 17β-estradiol or v-Ha-*ras* oncogene. *Proc. Natn. Acad. Sci. U.S.A.* **84** (1987) 837–841.
43. Kasid A., Knabbe C. and Lippman M. E.: Effect of v-Ha-*ras* oncogene transfection on estrogen-independent tumorigenicity of estrogen-dependent human breast cancer cells. *Cancer Res.* **47** (1987) 5733–5738.