J. Steroid Biochem. Molec. Biol. Vol. 41, No. 2, pp. 113–123, 1992
Printed in Great Britain.

0960-0760/92 $5.00 + 0.00

ESTROGEN REGULATES EXPRESSION OF THE *jun* FAMILY OF PROTOONCOGENES IN THE UTERUS

CONSTANCE CHIAPPETTA,[1] JOHN L. KIRKLAND,[2] DAVID S. LOOSE-MITCHELL,[1] LATA MURTHY[2] and GEORGE M. STANCEL[1]*

[1]Department of Pharmacology, The University of Texas Medical School at Houston and [2]Division of Endocrinology, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77225, U.S.A.

(Received 30 May 1991)

Summary—Treatment of immature female rats with estradiol increases uterine levels of c-*jun* and *jun-B* mRNAs approx. 10-fold. This effect is specific for estrogenic steroids. The induction of *jun* transcripts is blocked by actinomycin D but not puromycin, suggesting that the hormonal effect is due at least in part to transcriptional activation. The hormone effect is rapid and peak levels of *jun* mRNAs are seen within 3 h after treatment. Inductions of *jun* and *fos* transcripts in the uterus by estradiol exhibit similar dose response curves (maximum responses at 4 μg/kg). Estradiol also elevates uterine levels of *jun-D*, and this induction is insensitive to puromycin. *In vivo* treatment with the phorbol ester TPA rapidly elevates uterine levels of *fos*, *jun*, and *myc* transcripts, indicating that expression of these protooncogenes is under non-estrogenic as well as estrogenic regulation in this target tissue. These results suggest that multiple members of the *jun* and *fos* protooncogene families may play a role in amplifying the uterine response to estrogens.

Estrogens cause a rapid and dramatic growth response of the mammalian uterus that culminates in DNA synthesis and cell division. Many laboratories have used the immature rat uterus as an experimental system to study the underlying mechanism(s) of this hormonally induced growth. In this rodent system, estradiol causes a doubling of uterine weight and an increase in DNA synthesis in all major cell types (epithelium, stroma and myometrium) within 24 h after treatment [1, 2]. The speed and magnitude of the hormonal effect indicate that uterine cells have a mechanism(s) to greatly amplify the signal emanating from the initial estrogen receptor interaction.

Since steroid receptors function as transcriptional regulators, a major goal has been to identify genes that are rapidly activated by estrogen in the uterus and that code for products that could play a role(s) in amplifying the tissue response. Other transcription factors are an attractive possibility for such genes, since it seems unlikely that the estrogen receptor itself would directly regulate the many genes that are activated during the hormonally induced growth process.

*To whom correspondence should be addressed.

The c-*fos* protooncogene seemed to be a potential candidate for such a gene since it functions as a transcriptional regulator and is rapidly activated by serum and numerous growth factors [3–6]. We [7] and others [8] have recently shown that estradiol rapidly increases expression of this gene in the uterus. Since Fos functions in transcriptional regulation as part of a complex with Jun, a second cellular oncogene [3, 9–14], we [15] and others [16–18] have now extended our studies to investigate estrogen regulation of c-*jun* expression in the uterus.

c-*jun* was originally identified as the cellular homolog of v-*jun*, the oncogene in avian sarcoma virus ASV-17 [19]. Subsequent analysis revealed that Jun was identical to the major polypeptide in a family (i.e. AP-1) of previously identified mammalian transcription factors [20–22]. It is now known that the species which function as transcriptional regulators are Jun–Jun homodimers or Jun–Fos heterodimers [3, 9–14].

Since the initial identification of c-*fos* and c-*jun* a number of other genes in these families have been identified. These include *jun-B* [23] and *jun-D* [24, 25] in the Jun family, and *fra-1* [26], *fra-2* [27] and several forms of *fos-B* [28, 29] in the Fos family. The products

of these two gene families can form a variety of homo- and heterodimers with similar DNA binding properties, but different biological activities [28–33]. The specific pattern of Fos and Jun proteins synthesized in a particular situation could thus determine the number and type of dimers formed, and these could in turn determine the subsequent pattern of "secondary" gene expression.

Therefore, to further investigate the possible role of Fos and Jun as mediators of the uterine response to estrogen, we have extended our previous studies on c-*fos* regulation to investigate estrogenic regulation of the *jun* family of genes. The results indicate that estrogen treatment *in vivo* rapidly elevates the levels of c-*jun* and *jun-B* transcripts. In general, this regulation is very similar to the pattern of estrogen induction of c-*fos* mRNA. Estrogen treatment also increases *jun-D* mRNA levels in the uterus, although this induction is less well characterized at present.

EXPERIMENTAL

Animals

Immature female Sprague–Dawley rats (21 days old, 40–45 g, Harlan Sprague Dawley, Indianapolis, IN) were ovariectomized 5 days before use. Animals were injected s.c in the periscapular region with 0.5 ml of estradiol (E2 dissolved in 5% ethanol, 95% normal saline; controls received vehicle injections only. Unless otherwise noted (e.g. the dose response studies), the dose of E2 was 40 μg/kg body wt). The non-estrogenic steroids were administered similarly. Where indicated in the text, puromycin (100 mg/kg) was administered 30 min prior to E2. Actinomycin D was administered in two equal doses (4 mg/kg each). The first dose was given 3 h before E2 and the second dose was given concomitantly with the hormone. TPA (0.25 mg/kg) was administered by i.p. injection in 0.5 ml of 5% DMSO/95% saline.

RNA preparation and blot analysis

RNA was prepared as described by Chirgwin *et al.* [34] as described previously [7, 35]. Briefly, uteri were removed from anesthetized animals and immediately homogenized in 5 M guanididium isothiocyanate using a Polytron homogenizer (Brinkmann, Westbury, NY) set at half-maximal power for 60 s. In most cases, uteri from 2 or 3 animals were pooled for the preparation of a single sample of RNA. RNA was pelleted through 5.7 M CsCl, extracted twice with phenol–chloroform (1:1), once with chloroform, and precipitated with ethanol. RNA was quantified by absorption at 260 nm. Samples of total RNA (10 μg) from control or treated animals were denatured for 30 min in 15 mM methylmercuric hydroxide (Alfa, Salt Lake City, UT) and separated on a 1% agarose gel containing 6% (v/v) formaldehyde. After the electrophoresis, RNA was transferred to Duralon (Stratagene) by electroblotting (16 h at 25 V in 25 mM sodium phosphate, pH 6.5). Membranes were allowed to dry at room temperature and then prehybridized in 0.8 M NaCl, 2 mM EDTA, 0.5% sodium dodecyl sulfate (SDS), 20 mM piperizine-*N*-*N*′-bis[2-ethanesulfonic acid], 50% deionized formamide, and 100 μg/ml denatured salmon sperm DNA for 3 h at 62°C. ^{32}P-labeled antisense RNA probes were synthesized following the manufacturer's recommendation with Sp6 or T7 RNA polymerases, and radiolabeled RNA was added directly to the prehybridization mixture. Blots were hybridized for 16–24 h at 60°C and then washed twice with 2 × SSC, 0.1% SDS (1 × SSC is 0.15 M NaCl, 0.015 mM NaCitrate, pH 7.0) at room temperature for 30 min, twice with 0.1 × SSC, 0.1% SDS at 60°C for 30 min and rinsed with 2 × SSC. After the rinse, blots were treated with RNase A (1 μg/ml) in 2 × SSC for approx. 5 min, washed in 0.1 × SSC and 0.1% SDS for 10 min, and exposed to X-ray film. Where indicated in the text, films were scanned with a Zeineh Soft Laser scanning densitometer.

The *jun* probes used in this work were kindly provided by Dr Daniel Nathans of Johns Hopkins University School of Medicine [23, 36]. The c-*jun* probe is an 1800 bp fragment of murine c-*jun* inserted into pGEM 2 (Promega) at the EcoR1 site. This clone contains the complete coding sequence. Antisense RNA probes were produced from this insert using T7 RNA polymerase (Promega, Madison, WI) as per the manufacturer's recommendations. The murine *jun-B* probe is also approx. 1800 bp in length and was inserted into the pGEM2 vector at the same site. In this case antisense probes were generated with Sp6 RNA polymerase using the recommended procedure provided by the supplier (Promega). The 1700 bp murine *jun-D* probe was originally obtained in the Bluescript (-) vector, and subsequently subcloned into the EcoR1 site of pGEM 3. Antisense probes were generated with Sp6 RNA polymerase (Promega) as per the manufacturer's recommendations.

The mouse c-*fos* probe used to obtain the data in Fig. 8 has previously been described in detail [7]. The murine c-*myc* probe used in that series of experiments was kindly provided by Dr Michael T. Crow (Department of Pharmacology, the University of Texas Medical School at Houston). This is a second exon fragment about 1 kb in length that was subcloned into pGEM 4. Sp6 RNA polymerase was used to generate the antisense riboprobe.

Uterine DNA synthesis

Uterine DNA synthesis (in Fig. 7) was measured as previously described [37]. Briefly, animals were treated with the indicated doses of E2 24 h prior to sacrifice. Uteri were removed and incubated for 1 h at 37°C in media containing tritiated thymidine under an atmosphere of 95% oxygen/5% carbon dioxide. Tissues were then homogenized and treated as previously described [37] to determine tritiated thymidine incorporation per μg of DNA. The values shown in Fig. 7 represent the means of 5 separate determinations; the SEM was $<10\%$ of the mean for all points.

Materials

The [^{32}P]UTP (800 Ci/mmol) was obtained from Amersham Radiochemicals (Arlington Heights, IL) and diluted to 400 Ci/mmol with radioinert UTP (Boehringer Mannheim, Fed. Rep. Germany) for the RNA polymerase reactions. E2 was obtained from Steraloids (Wilton, NH). Guanidine isothiocyanate, cesium chloride and formamide were obtained from International Biotechnologies Incorporated (New Haven, CT). All other chemicals and reagents were obtained from Sigma (St Louis, MO) and were the highest grade available.

RESULTS

Figure 1 illustrates the levels of c-*jun* [Fig. 1(A)] and *jun-B* [Fig. 1(B)] transcripts in the uterus at various times after administration of E2 to immature female rats. Low levels of the 3.2 and 2.7 kb c-*jun* and the 2.1 kb *jun-B* transcripts are present in tissues from animals that did not receive hormone treatment. Shortly after E2 administration, levels of both c-*jun* transcripts and *jun-B* mRNA begin to increase and all three reach maximum levels in 3 h. These increases are transient and return to baseline values by 15 h after hormone treatment.

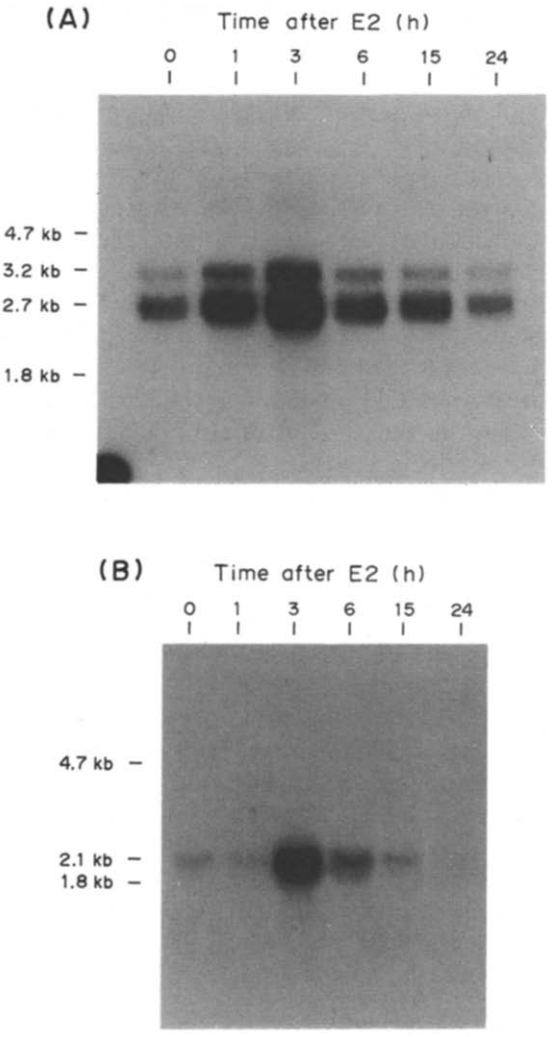

Fig. 1. Time course of E2 induction of c-*jun* (A) and *jun-B* (B) mRNA. Total uterine RNA was prepared from animals (4 animals per sample) at the indicated times after E2 treatment and analyzed by blot analysis as described in the Experimental section. The positions of the 3.2 and 2.7 kb c-*jun* transcripts and the 2.1 kb *jun-B* transcript are indicated.

In order to estimate the quantitative increase in these transcripts following E2 treatment, uterine RNA was prepared from a number of groups of animals treated with E2 for 3 h. The RNA was subsequently analyzed by blot

Table 1. Induction of *jun* transcripts by E2

mRNA species	Fold induction by E2 (+SEM)	No
c-*jun* (3.2 kb)	9.60 ± 1.28	6
c-*jun* (2.7 kb)	8.62 ± 1.16	6
jun-B	10.8 ± 1.70	9

Animals were treated with E2 (40 μg/kg) or the vehicle alone 3 h prior to sacrifice. Total RNA was prepared and analyzed by blot analysis as described in the Experimental section. The resultant films were scanned with a laser densitometer to obtain a quantitative estimate of the fold-induction of each transcript. The values shown represent the means + SEM for the indicated number of determinations. Each determination was made on an RNA sample prepared from 3–4 pooled uteri.

analysis, and the resultant films were scanned with a laser densitometer. The results are summarized in Table 1, and illustrate that both c-*jun* and *jun-B* mRNA levels are elevated 8- to 10-fold following hormone treatment.

Since these experiments were performed in the rat, we conducted a limited study of *jun* induction by E2 in the mouse to determine if this cellular oncogene was also regulated by the hormone in another species. These studies showed a clear increase in c-*jun* and *jun-B* transcripts in the mouse uterus after estrogen treatment, but we did not conduct sufficient studies to make a quantitative comparison to the results in the rat.

The effects of various classes of steroids on *jun* mRNA levels are indicated in Fig. 2. As shown for the 3.2 and 2.7 kb c-*jun* transcripts, neither glucocorticoids, androgens nor progestins elevate *jun* mRNA levels. A similar pattern of hormonal specificity was also observed for the induction of the 2.1 kb *jun-B* transcript (data not shown). These results illustrate that under the conditions of these experiments (e.g. acute treatment, immature animals, etc.) the regulation of *jun* mRNAs is very specific for estrogens.

We next investigated the effects of RNA and protein synthesis inhibitors on the induction of *jun* transcripts by E2. As shown in Fig. 3, actinomycin D abolishes the estrogen stimulated increases in both c-*jun* [Fig. 3(A)] and *jun-B* [Fig. 3(B)] indicating that observed increases represent *de novo* RNA synthesis. On the other hand, the protein synthesis inhibitor puromycin does **not** block the induction of either *jun* transcript (Fig. 4). This suggests that the observed increases in mRNA levels are due at least in part to transcriptional activation and that the cellular components required for the estrogenic induction of *jun* are present in the unstimulated tissue.

At this point, it was of interest to define the dose response relationships for the induction of *jun* transcripts by E2. As seen in Fig. 5, induction of both c-*jun* and *jun-B* is first observed at hormone doses of 0.04 to 0.4 μg of E2 per kg of body wt, and maximum increases occur at doses of 4.0 μg/kg. Both the 3.2 kb c-*jun* mRNA, the 2.7 kb c-*jun* mRNA, and the 2.1 kb *jun-B* mRNA show similar dose response curves.

We [7] and others [8, 18] have previously reported that E2 increases uterine c-*fos* mRNA levels. Since the Fos and Jun proteins are thought to function by formation of heterodimers that act as transcriptional regulators [3, 9–14], it was of interest to compare the induction of both *fos* and *jun* transcripts in the uterus after estrogen stimulation. Figure 6 illustrates the time course for the estrogen induction of both transcripts in the uterus,

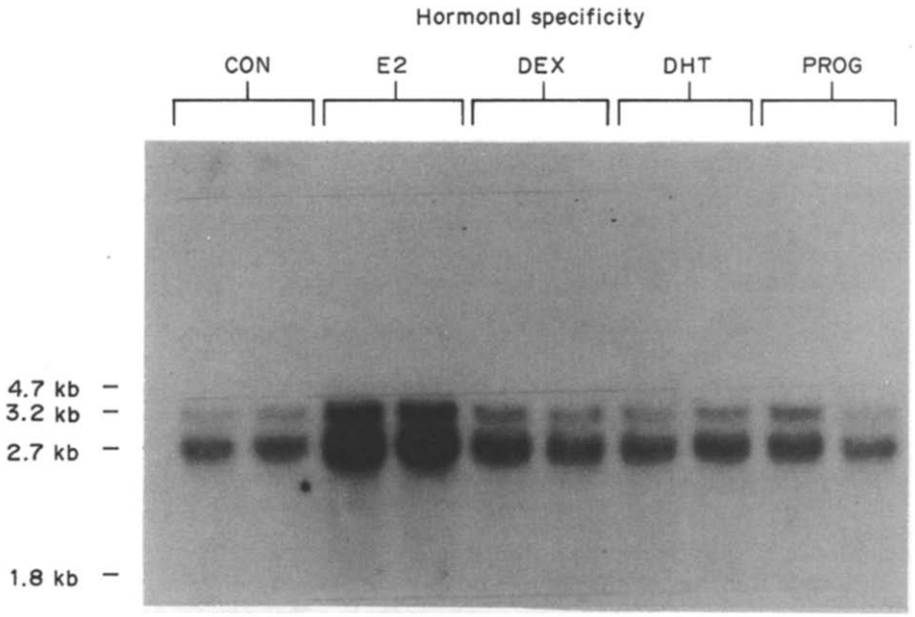

Fig. 2. Hormonal specificity of c-*jun* induction by steroids. Animals were treated with E2 (40 μg/kg), dexamethasone (600 μg/kg), DEX, 5-α-dihydrotestosterone (400 μg/kg), DHT, progesterone (40 mg/kg), PROG, or the vehicle alone, CON, 3 h prior to sacrifice. Total uterine RNA was prepared and analyzed by blot analysis. Each lane represents a separate RNA sample prepared from 3 pooled uteri.

and Fig. 7 illustrates the dose response relationships for hormonally induced increases. While there may be some specific differences, e.g. the increase in c-*jun* mRNA may precede the other two mRNAs illustrated in Fig. 6, the time course and dose response patterns for the *jun* and *fos* transcripts are generally similar. In this regard, it is especially interesting to note the dose response relationship for estrogenic induction of uterine DNA synthesis, which is also shown in Fig. 7. It is clear that induction of all three transcripts by E2 occurs in the same dose range of the hormone as induction of tissue DNA synthesis.

Our data and the work of others [16–18] indicates that E2 increases *jun* mRNA in the uterus, and previous work has indicated that the transcripts of *myc* [3, 38, 39] and *fos* [7, 8] are also elevated by estrogen in this system. In many other cells, these so-called "competence" or "immediate early" genes are regulated by serum, growth factors, and phorbol esters [3–6]. It was thus of interest to determine if such factors could regulate expression of these proto-oncogenes in the uterus, where they are clearly under estrogenic control.

To investigate this possibility, we treated immature ovariectomized rats with the phorbol ester TPA which is known to rapidly activate expression of *fos*, *jun*, and *myc* in other systems. Total uterine RNA was then isolated and used for blot analysis of these transcripts. The results are illustrated in Fig. 8. It is clearly seen that TPA treatment causes a large increase in the

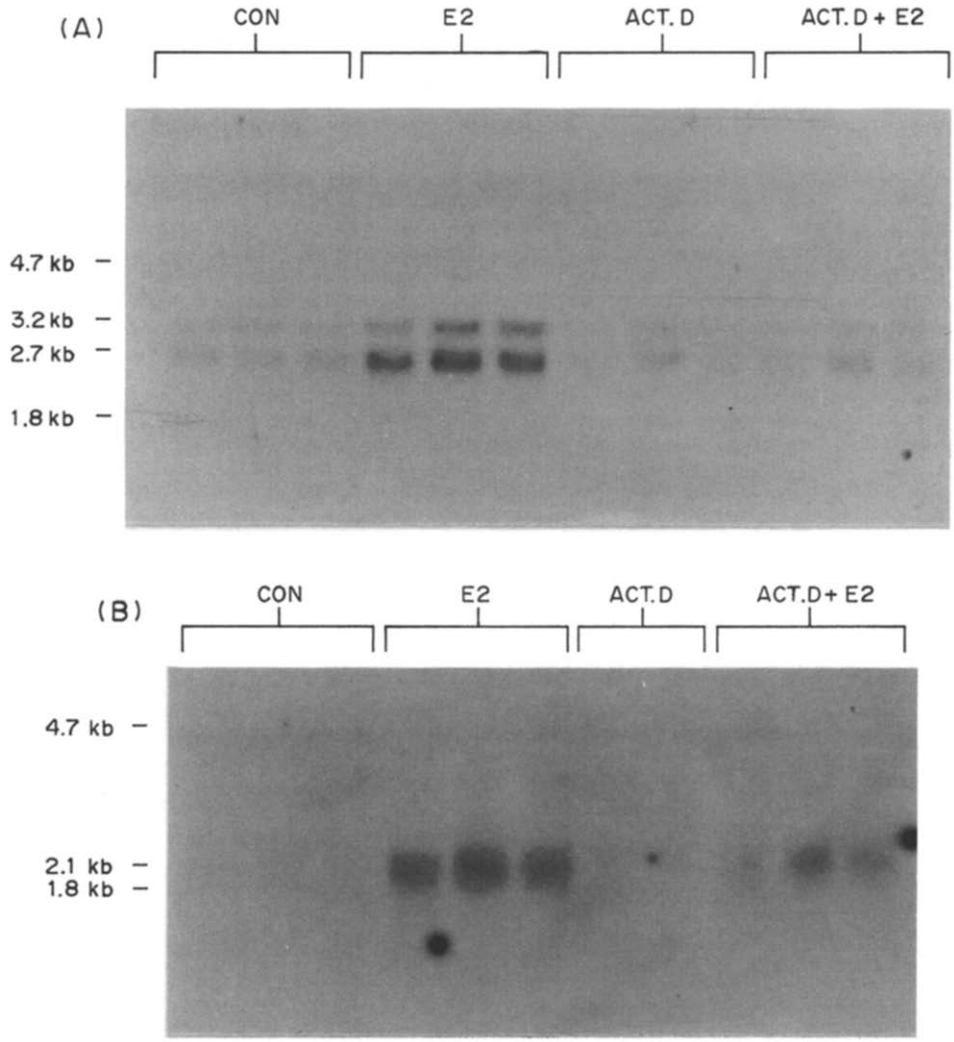

Fig. 3. Actinomycin D blocks induction of c-*jun* (A) and *jun-B* (B) mRNA by E2. Animals were treated with E2 or the vehicle alone (CON) 3 h prior to sacrifice. Actinomycin D was administered in two doses (4 mg/kg each), 3 h before and at the same time as the vehicle alone (ACT. D) or E2 (ACT. D + E2). Total uterine RNA was prepared and analyzed by blot analysis as described in the Experimental section. Each lane represents a separate RNA sample prepared from 3 pooled uteri.

level of all of these mRNAs. Expression of these protooncogenes in the uterus can therefore be regulated by both estrogenic and non-estrogenic stimuli.

The studies to this point were directed toward elucidating the regulation of c-*jun* and *jun*-B mRNA levels. In addition to these two genes, the *jun* family also contains another member, *jun*-D [24, 25]. Therefore, to more completely understand the estrogenic regulation of this gene family by estrogens, we also investigated the effect of estrogen treatment on uterine levels of *jun-D* mRNA. The results of this study are shown in Fig. 9. It is clearly seen that estrogen treatment for 3 h causes an increase in the level of this transcript. As is the case for c-*jun* and *jun-B*, this effect is not abolished by the protein synthesis inhibitor puromycin, suggesting that the hormonal effect is at least in part at the transcriptional level. Taken together with results shown in previous figures, this finding indicates that E2 regulates expression of all three known members of the *jun* family.

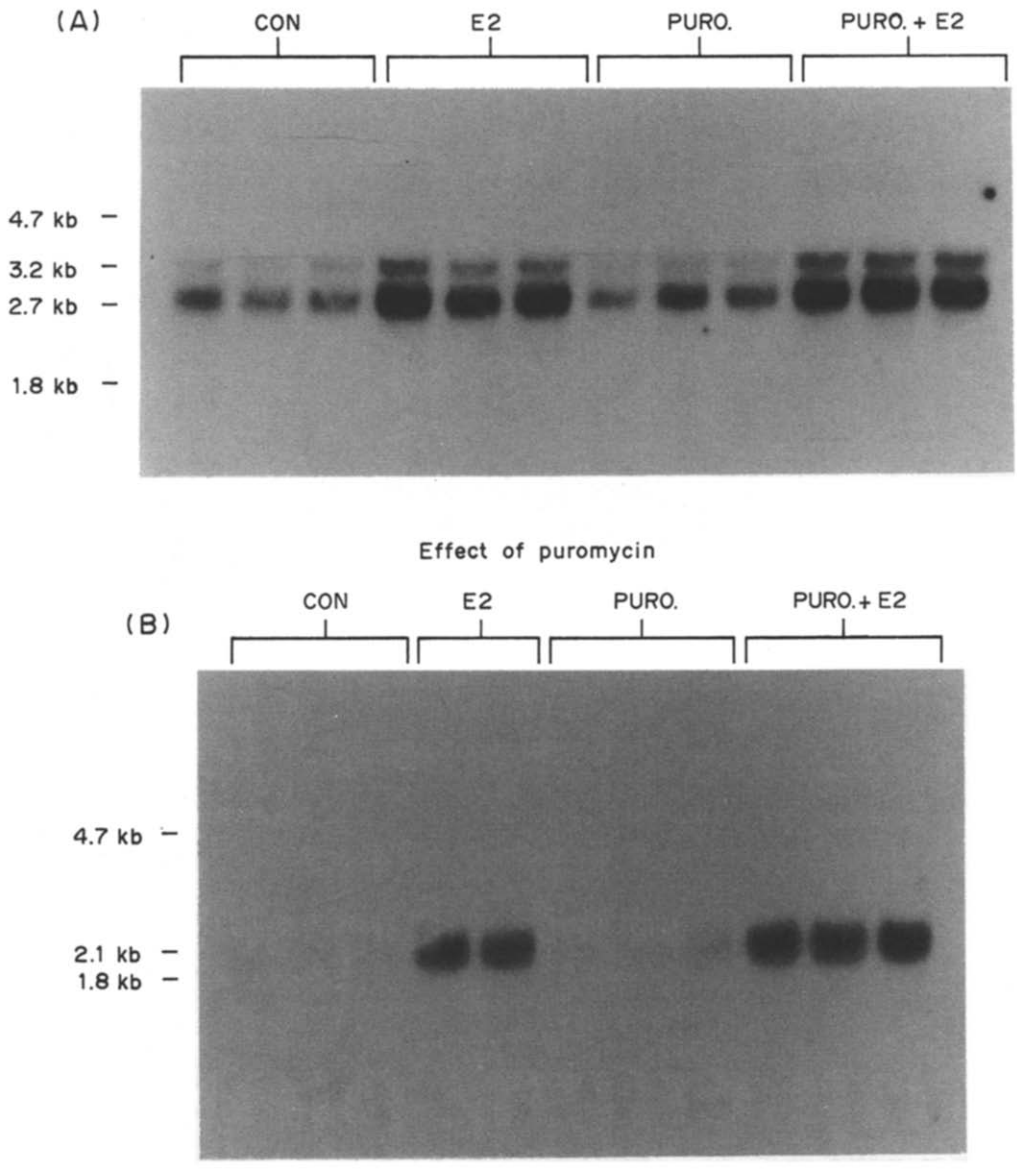

Fig. 4. Puromycin does **not** block induction of c-*jun* or *jun-B* mRNA by E2. Total RNA was prepared from animals (3 uteri per each sample) 3 h after treatment E2, the vehicle alone (CON), puromycin alone (PURO), or E2 plus puromycin (PURO. + E2). RNA was subsequently blotted and hybridized to either c-*jun* (A) or *jun-B* (B) antisense RNA probes as described in Experimental. The puromycin (200 mg/kg) was given 30 min prior to the hormone or control vehicle injections.

DISCUSSION

Several groups have previously reported that E2 increases c-*jun* mRNA levels in the uterus [16–18]. These reports demonstrated that estrogen increases c-*jun* transcript levels in mature [16–18] and immature [17] rat uteri; that the induction is not blocked by the protein synthesis inhibitor cycloheximide [16]; and that the effect is tissue specific since the hormone does not produce this response in organs other than the uterus [16]. Weisz *et al.* [16] also showed that this effect is at the transcriptional level by using nuclear run-on assays. In this report we have confirmed the induction of c-*jun* mRNA by estrogen in the uterus and the insensitivity of this effect to protein synthesis inhibitors. We have further characterized the estrogenic induction of c-*jun* by defining the hormonal specificity and dose response relationship for this hormonal effect. In addition, we have demonstrated that the hormone regulates two other members of the *jun* family, *jun-D* and *jun-B*. The observation that both c-*jun* and *jun-B* are expressed following estrogen may be particularly important for understanding uterine growth, since these two members of the *jun* family can exhibit different biological effects [32, 33].

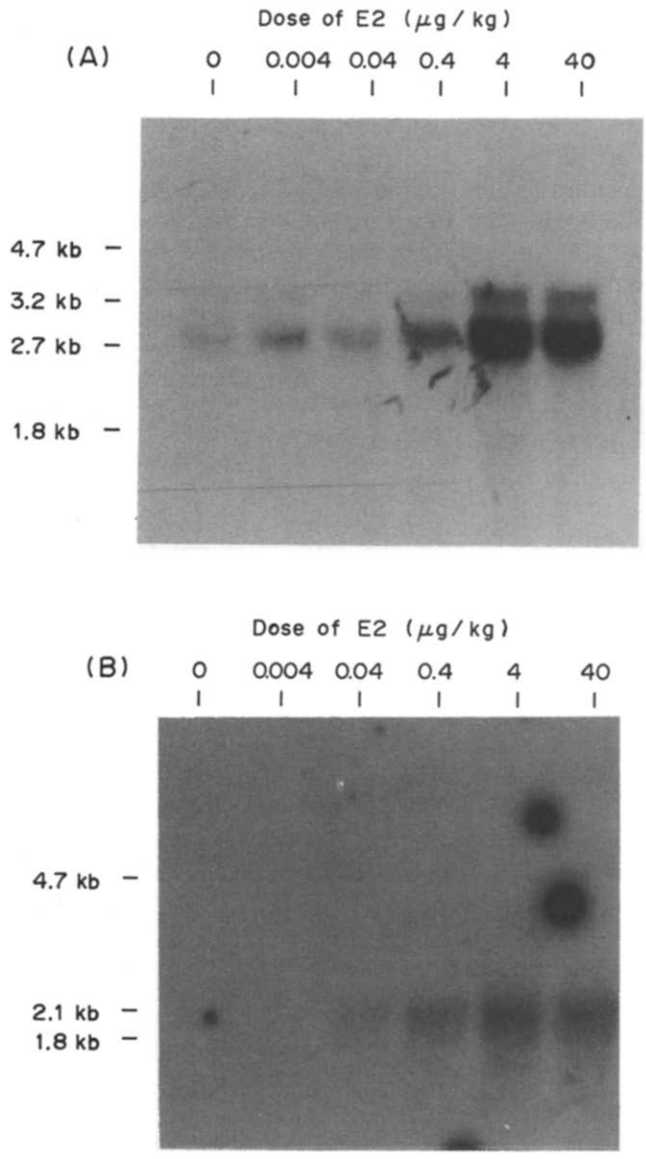

Fig. 5. Dose response relationship for induction of c-*jun* (A) and *jun-B* (B) mRNA by E2. Animals were treated with the indicated doses of E2 3 h prior to sacrifice and the preparation of total uterine RNA. Blotting and hybridization procedures were as described in the Experimental section. Each lane represents a separate RNA sample prepared by pooling uteri from 3 animals.

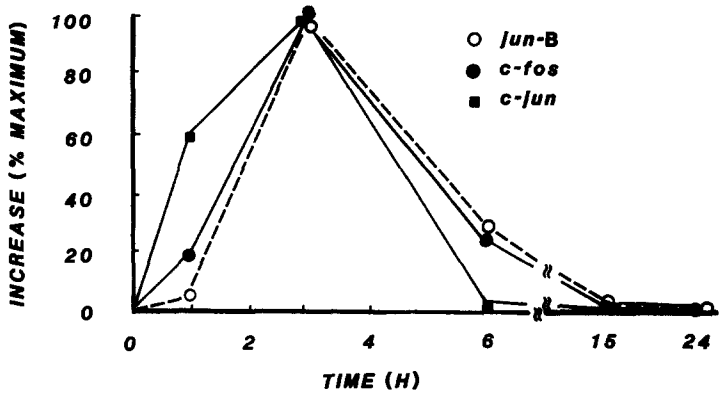

Fig. 6. Time course of induction of c-*fos*, c-*jun* (2.7 kb), and *jun-B* messenger RNA by E2. Animals were treated with E2 for the indicated times prior to sacrifice and preparation of total uterine RNA. The same RNA samples were then subject to blot analysis and hybridization with c-*fos*, c-*jun* or *jun-B* antisense RNA probes. The resultant films were scanned with a laser densitometer to obtain a quantitative estimate of the amount of each message. Each point represents the mean of 3 determinations, with each determination made on an RNA sample prepared by pooling 3 uteri. To facilitate comparisons, the data is presented as % maximum increase (the 3 h value in all cases) for each transcript.

Our time course studies are very similar to those of Webb *et al.* [17] who found that c-*jun* mRNA levels peaked in the uterus of the immature animal 3 h after E2 treatment, although Weisz *et al.* [16] showed that this response is slightly more rapid in the mature animal. The quantitative increases we have observed in uterine levels of c-*jun* transcripts (i.e. 8- to 10-fold) are similar to the 3- to 5-fold maximum increases previously reported by others [16, 17]. Our results have also established that the timing and magnitude of increases in uterine c-*fos*, c-*jun*, and *jun-B* mRNA after estrogen are all very similar.

While all studies to date agree that estrogen increases *jun* mRNA levels in the uterus, it should be noted that the hormone may have other effects on this protooncogene in other species and/or tissues. For example, Lau *et al.* [40] recently reported that estrogen decreases expression of c-*jun* in the chick oviduct system.

Our studies with the protein synthesis inhibitor puromycin suggest that the induction of all three *jun* mRNAs result from transcriptional activation of these genes, at least in part. These observations are similar to the previous report that cycloheximide does not block the increase in uterine c-*jun* after estrogen treatment [16].

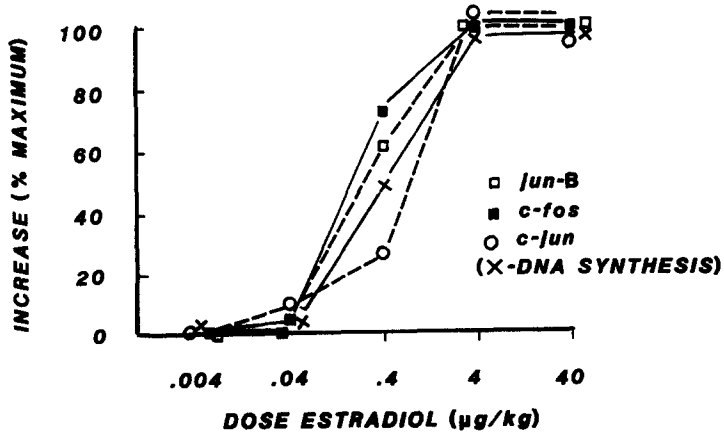

Fig. 7. Induction of c-*fos*, c-*jun* (2.7 kb), and *jun-B* mRNA as a function of E2 dose. Animals were treated with the indicated doses of E2 3 h prior to sacrifice and blot analysis of the indicated transcripts. The resultant films were scanned with a laser densitometer to obtain a quantitative estimate of the amount of each message. Each point represents the mean of determinations on 3 separate RNA samples, with each sample prepared from 3 pooled uteri. The dose response curve for uterine DNA synthesis (measured 24 h after hormone treatment) is also shown. To facilitate comparisons, all the values are presented as the % maximum increase, which was taken as the average of the 4 and 40 µg/kg doses in all cases.

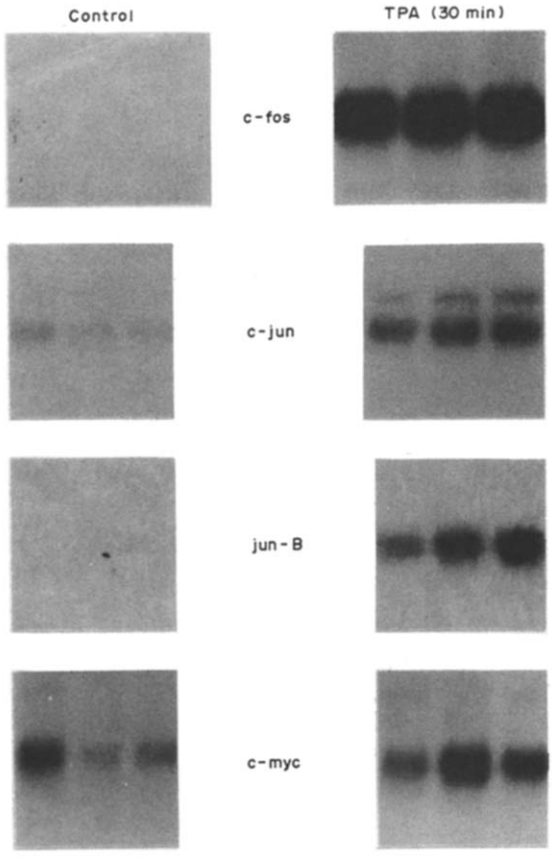

Fig. 8. Induction of protooncogene messenger RNAs by TPA. Animals were sacrificed 30 min after treatment with TPA or the vehicle alone (control) and total uterine was prepared. Aliquots of the samples were electrophoresed on a series of agarose gels, transferred by electroblotting, and hybridized with the indicated antisense riboprobes. Each lane represents a separate sample of uterine RNA, and each sample was prepared by pooling 3 uteri.

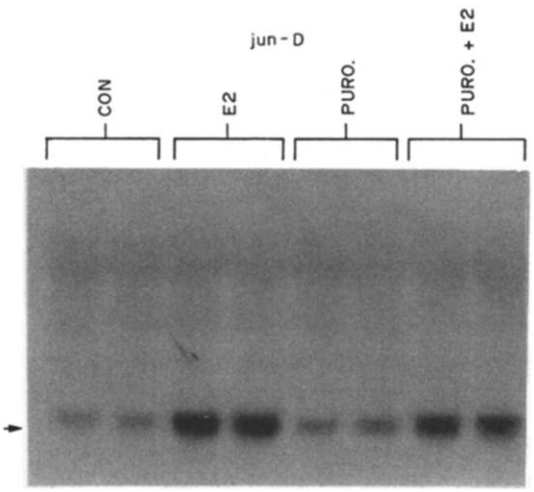

Fig. 9. Induction of *jun-D* mRNA by E2. Total uterine RNA was prepared from animals 3 h after treatment with E2 (40 μg/kg), the vehicle alone (CON), puromycin (200 mg/kg) given 30 min prior to the vehicle (PURO.), or puromycin (200 mg/kg) given 30 min prior to the E2 (PURO + E2). The RNA was subsequently blotted and hybridized to a *jun-D* antisense riboprobe as described in the Experimental section. Each lane represents a separate sample prepared from 3 pooled uteri.

Perhaps more importantly, that study established directly that the transcription rate of c-*jun* in the uterus is increased following estrogen administration [16]. These observations, coupled with the hormonal specificity we have observed, suggest a direct role for the estrogen receptor in the activation of *jun* transcription. However, we are not aware of any published studies using either receptor binding or transfection of reporter genes that have identified the regulatory sequences that mediate estrogenic induction of this gene family. Further studies will thus be required to identify the specific DNA sequences involved. Our studies, which involved acute administration of steroids to immature animals, established a strict hormonal specificity for estrogenic induction of *jun* in the uterus. The uterus, however, contains receptors for progestins [41], glucocorticoids [42] and androgens [43, 44], and it is thus possible that other steroids may affect *jun* transcription under different conditions (e.g. after estrogen priming) or at different ages (e.g. mature animals).

It has been demonstrated that E2 can stimulate transcription of reporter genes containing the TRE/AP-1 response element in hormonally responsive cells containing estrogen receptors [16]. This suggests that *jun* and *fos* may play an important role to amplify the tissue responses to estrogen by secondarily activating genes that do not contain estrogen responsive elements (EREs). This possibility is consistent with the hormonal specificity (Fig. 2 and Ref. [7]) and dose response relationships (Fig. 7) for induction of these cellular oncogenes in the uterus. However, it is clear from published studies with short-acting estrogens that induction of *jun* and *fos* alone is not sufficient for subsequent induction of uterine DNA synthesis [18], and we have recently obtained similar data (Orengo *et al.*, in preparation).

It is clear from our studies (Figs 6 and 7) and the results of others [16–18] that E2 regulates the levels of c-*fos*, c-*jun*, *jun-D*, and *jun-B* in the uterus. Thus, it seems reasonable to suggest that the products of these genes can form various homo- and heterodimers that in turn regulate expression of genes that do not contain EREs, but are involved in the uterine growth response to estrogens. Uterine levels of *fos*, *myc*, and *jun* transcripts can also be elevated by TPA (Fig. 8). This indicates that these genes can be regulated

in the uterus by non-estrogenic as well as estrogenic stimuli. Thus, these genes may represent sites for "cross-talk" between estrogens and other regulatory molecules that function via signal transduction pathways involving protein kinase C activation. This provides a potential site of action for other hormones, growth factors, autocoids, and drugs to modulate the uterine response to estrogens and vice versa.

The overall regulation of uterine gene expression following estrogen administration may be even more complex, however, since at least three other members of the Fos family (Fra-1, Fra-2, and Fos-B) are known [26–28]. In addition, two other proteins (Fos-B and IP-1) that inhibit Fos/Jun transcriptional activity have recently been identified [29, 45], and a protein(s) that stimulates DNA binding by Fos–Jun and Jun–Jun dimers has also been discovered [46]. Since these various genes may be regulated by estrogens and/or other physiological and pharmacological factors, uterine growth and function may involve additional interactions between transcription factors.

Finally, it is especially interesting to note numerous recent reports which indicate that Fos and Jun can interact with steroid receptors to regulate expression of hormone sensitive genes [47–53]. Since estrogens clearly regulate *fos* and *jun* expression, such interactions could provide multiple mechanisms to control the sequence of target tissue gene expression after hormone administration. For example, the estrogen receptor complex could initially activate expression of *fos* and *jun* gene families by interactions with ERE sequences. The resultant protein products could then activate transcription of other genes via two different mechanisms. First, Fos/Jun dimers could independently regulate expression of genes containing TRE/AP-1 sites. Second, the Fos and/or Jun proteins could act in concert with estrogen receptors to regulate the expression of still other genes. This second type of mechanism would enable the estrogen receptor to regulate expression of uterine genes in a sequential fashion, depending upon the level of Fos, Jun and/or other factors. While these are attractive possibilities based upon our current understanding of the cellular functions of *fos* and *jun* families of transcription factors, a role of these transcription factors in estrogen stimulated uterine growth is not yet unequivocally established.

Acknowledgements—This work was supported by NIH Grants HD-08615 and DX-38965 and a grant from the John P. McGovern Foundation.

REFERENCES

1. Kaye A. M., Sheratzky D. and Lindner H. R.: Kinetics of DNA synthesis in immature rat uterus: age dependence and estradiol stimulation. *Biochim. Biophys. Acta* **261** (1972) 475–486.
2. Kirkland J. L., LaPointe L., Justin E. and Stancel G. M.: Effect of estrogen on mitosis in individual cell types of the immature rat uterus. *Biol. Reprod.* **21** (1979) 269–272.
3. Ransone L. J. and Verma I. M.: Nuclear Proto-Oncogenes *FOS* and *JUN*. *A. Rev. Cell Biol.* **6** (1990) 539–557.
4. Rollins B. J. and Stiles C. D.: Regulation of c-*MYC* and C-*FOS* expression by animal cell growth factors. *In Vitro Cell. Dev. Biol.* **24** (1988) 81–84.
5. Curran T.: The *fos* oncogene. In *The Oncogene Handbook* (Edited by E. P. Reddy, A. M. Skalka and T. Curran). Elsevier Science Publishers, Amsterdam (1988) pp. 307–325.
6. Curran T., Bravo R. and Muller R.: Transient induction of c-*fos* and c-*myc* is an immediate consequence of growth factor stimulation. *Cancer Surv.* **4** (1985) 655–681.
7. Loose-Mitchell D. S., Chiappetta C. and Stancel G. M.: Estrogen regulation of c-*fos* messenger ribonucleic acid. *Molec. Endocr.* **2** (1988) 946–951.
8. Weisz A. and Bresciani F.: Estrogen induces expression of c-*fos* and c-*myc* protooncogenes in rat uterus. *Molec. Endocr.* **2** (1988) 816–824.
9. Vogt P. K. and Bos T. J.: The oncogene *jun* and nuclear signalling. *Trends Biochem. Sci.* **14** (1989) 172–175.
10. Curran T. and Franza R. B. Jr: Fos and Jun: the Ap-1 connection. *Cell* **55** (1988) 395–397.
11. Rauscher F. J., Cohen D. R., Curran T., Bos T. J., Vogt P. K., Bohmann D., Tjian R. and Franza B. R. Jr: Fos-associated protein p39 is the product of the *jun* protooncogene. *Science* **240** (1988) 1010–1016.
12. Turner R. and Tjian R.: Leucine repeats and an adjacent DNA binding domain mediate the formation of function cFos-cJun heterodimers. *Science* **243** (1989) 1689–1699.
13. Hirai S.-I., Bourachot B. and Yaniv M.: Both Jun and Fos contribute to transcription activation by the heterodimer. *Oncogene* **5** (1990) 39–46.
14. Abate C., Luk D., Gagne E., Roeder R. G. and Curran T.: Fos and Jun cooperate in transcriptional activation via heterologous activation domains. *Molec. Cell. Biol.* **10** (1990) 5532–5535.
15. Chiappetta C., Stancel G. M. and Loose-Mitchell D. S.: Estrogen induced regulation of c-*jun* messenger ribonucleic acid *in vivo*. *J. Cell Biol.* **109** (1989) 119a.
16. Weisz A., Cicatiello L., Persicot E., Scalona M. and Bresciani F.: Estrogen stimulates transcription of c-*jun* protooncogene. *Molec. Endocr.* **4** (1990) 1041–1050.
17. Webb D. K., Moulton B. C. and Khan S. A.: Estrogen induced expression of the c-*jun* proto-oncogene in the immature and mature rat uterus. *Biochem. Biophys. Res. Commun.* **168** (1990) 721–726.
18. Persico E., Scalona M., Cicatiello L., Sica V., Bresciani F. and Weisz A.: Activation of 'immediate early' genes by estrogen is not sufficient to achieve stimulation of DNA synthesis in rat uterus. *Biochem. Biophys. Res. Commun.* **171** (1990) 287–292.
19. Maki Y., Bos T. J., Davis C., Starbuck M. and Vogt P. K.: Avian sarcoma virus 17 carries the *jun* oncogene. *Proc. Natn. Acad. Sci. U.S.A.* **84** (1987) 2848–2852.

20. Vogt P. K., Bos T. J. and Doolittle R. F.: Homology between the DNA-binding domain of the GCN4 regulatory protein of yeast and the carboxyl-terminal region of a protein coded for by the oncogene *jun*. *Proc. Natn. Acad. Sci. U.S.A.* **84** (1987) 3316–3319.
21. Bohmann D., Bos T. J., Admon A., Nishimura T., Vogt P. K. and Tijan R.: Human proto-oncogene c-*jun* encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. *Science* **238** (1987) 1386–1392.
22. Angel P., Allegretto E. A., Okino S. T., Hattori K., Boyle W. J., Hunter T. and Karin M.: Oncogene *jun* encodes a sequence-specific *trans*-activator similar to AP-1. *Nature* **332** (1988) 166–171.
23. Ryder K., Lau L. F. and Nathans D.: A gene activated by growth factors is related to the oncogene v-*jun*. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1988) 1487–1491.
24. Ryder K., Lanahan A., Perez-Albuerne E. and Nathans, D.: *Jun-D*: third member of the *Jun* gene family. *Proc. Natn. Acad. Sci. U.S.A.* **86** (1988) 1500–1503.
25. Hirai S., Ryseck R., 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.
26. Cohen D.R. and Curran T.: Fra-l: A serum inducible cellular immediate-early gene that encodes a Fos-related antigen. *Molec. Cell. Biol.* **8** (1988) 2063–2069.
27. Nishina H., Sato H., Suzuki T., Sato, M. and Iba H.: Isolation and characterization of *fra-2*, an additional member of the *fos* gene family. *Proc. Natn. Acad. Sci. U.S.A.* **87** (1990) 3619–3623.
28. Zerial M., Toschi L., Ryseck R.-P., Schuermann M., Miller R. and Bravo R.: The product of a novel growth factor activated gene, *FosB*, interacts with JUN proteins enhancing their DNA binding activity. *EMBO Jl* **8** (1989) 805–813.
29. Nakabeppu Y. and Nathans D.: Naturally occurring truncated form of Fos B that inhibits Fos/Jun transcriptional activity. *Cell* **64** (1991) 751–759.
30. Nakabeppu Y., Ryder K. and Nathans D.: DNA binding activities of three murine Jun proteins: stimulation by Fos. *Cell* **55** (1988) 907–915.
31. Cohen D.R., Ferreira P. C. P., Gentz R., Franza B.R. Jr and Curran T.: The product of a *fos*-related gene, *fra-1*, binds cooperatively to the AP-l site with Jun: transcription factor AP-l is comprised of multiple protein complexes. *Genes Dev.* **3** (1989) 173–184.
32. Chiu R., Angel P. and Karin M.: Jun-B differs in its biological properties from, and is a negative regulator of c-Jun. *Cell* **59** (1989) 979–986.
33. Schutte J., Viallet J., Nau M., Segal S, Fedorko J. and Minna J.: *jun-B* inhibits and c-*fos* stimulates the transforming and trans-activating activities of c-*jun*. *Cell* **59** 987–997.
34. Chirgwin J. M., Pryzbyla A. E., MacDonald R. J. and Rutter W. J.: Isolation of biologically active ribonucleic acids from sources enriched in ribonuclease. *Biochemistry* **18** (1979) 5294–5299.
35. Lingham R. B., Stancel G. M. and Loose-Mitchell D. S.: Estrogen induction of the epidermal growth factor receptor mRNA. *Molec. Endocr.* **2** (1988) 230–235.
36. Ryder K. and Nathans D.: Induction of protooncogene c-*jun* by serum growth factors. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1988) 8464–8467.
37. Mukku V. R., Kirkland J. L., Hardy M. and Stancel G. M.: Hormonal control of uterine growth: temporal relationships between estrogen administration and deoxyribonucleic acid synthesis. *Endocrinology* **111** (1982) 480–487.
38. Murphy L.J., Murphy L.C. and Friesen H.G.: Estrogen induction of N-myc and c-myc protooncogene expression in the rat uterus. *Endocrinology* **120** (1987) 1882–1888.
39. Travers M. T. and Knowler J. T.: Oestrogen-induced expression of oncogenes in the immature rat uterus. *FEBS Lett.* **211** (1987) 27–30.
40. Lau C. K., Subramaniam M., Rasmussen K. and Spelsberg T. C.: Rapid inhibition of the c-jun protooncogene expression in avian oviduct by estrogen. *Endocrinology* **127** (1990) 2595–2597.
41. Feil P. D., Glasser S. R., Toft D. O. and O'Malley B. W.: Progesterone binding in the mouse and rat uterus. *Endocrinology* **91** (1972) 738–745.
42. Panko W. B., Clark J. H. and Walters M. R.: Glucocorticoid receptors: documentation in the rat uterus. *J. Recept. Res.* **2** (1981) 29–45.
43. Giannopoulos G.: Binding of testosterone to uterine components of the immature rat. *J. Biol. Chem.* **248** (1973) 1004–1010.
44. Chang S. H. and Tindall D. J.: Physiochemical characterization of the androgen receptor in rat uterine cytosol. *Endocrinology* **113** 1486–1493.
45. Auwerx J. and Sassone-Corsi P.: IP-1 a dominant inhibitor of Fos/Jun whose activity is modulated by phosphorylation. *Cell* **64** (1991) 983–993.
46. Abate C., Luk D. and Curran T.: A ubiquitous nuclear protein stimulates the DNA-binding activity of fos and jun indirectly. *Cell Growth Diff.* **1** (1990) 455–462.
47. Jonat C., Rahmsdorf H. J., Park K.-K., Cato, A. C. B., Gebel S., Ponta H. and Herrlich P.: Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell* **62** (1990) 1189–1204.
48. Schule R., Rangarajan P., Kliewer S., Ransone L. J., Bolado J., Yang N., Verma I. M. and Evans R. M.: Functional antagonism between oncoprotein c-jun and the glucocorticoid receptor. *Cell* **62** (1990) 1217–1226.
49. Schule R., Umesono K., Mangelsdorf D. J., Bolado J., Pike J. W. and Evans R. M.: Jun-Fos and receptors for vitamins A and D recognize a common response element in the human osteocalcin gene. *Cell* **61** (1990) 497–504.
50. Gaub M.-P., Bellard M., Scheuer I., Chambon P. and Sassone-Corsi P.: Activation of the ovalbumin gene by the estrogen receptor involves the Fos–Jun complex. *Cell* **63** (1990) 1267–1276.
51. Diamond M. I., Miner J. N., Yoshinaga S. K. and Yamamoto K.: Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. *Science* **249** (1990) 1266–1272.
52. Yang-Yen H.-F., Chambard J.-C., Sun Y.-L., Smeal T., Schmidt T. J., Drouin J. and Karin M.: Transcriptional interference between c-jun and the glucocorticoid receptor: mutual inhibition of DNA binding by direct protein-protein interaction. *Cell* **62** (1990) 1205–1215.
53. Lucibello F. C., Slater E. P., Jooss K. U., Beato M. and Muller R.: Mutual transrepression of Fos and the glucocorticoid receptor: involvement of a functional domain in Fos which is absent in FosB. *EMBO Jl* **9** (1990) 2827–2834.