Expression of the Aryl Hydrocarbon Receptor Is Regulated by Serum and Mitogenic Growth Factors in Murine 3T3 Fibroblasts*

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The aryl-hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates biological responses to planar aromatic hydrocarbons such as benzo[a]pyrene. However, no endogenous physiological ligand for the AhR has been identified. Since the AhR regulates bioactivity to common environmental pollutants, and since it is predicted to play an important physiological function, we have investigated the expression of the AhR during the cell cycle of normal 3T3 fibroblasts. We show here that stimulation of growth-arrested 3T3 cells with serum results in increased expression of AhR protein. Serum-induced expression of AhR in synchronized, serum-stimulated cells occurs at the onset of DNA synthesis (S phase) and is maximal at time points corresponding to late S phase. Transient transfections with an AhR-promoter-luciferase construct demonstrate that reporter gene transcription from the AhR promoter is regulated in a serum-dependent manner. Serum-dependent induction of AhR expression is prevented by an inhibitor of tyrosine kinase activity. Ligand-activated growth factor receptors (platelet-derived growth factor receptor basic fibroblast growth factor receptor) as well as an ectopically expressed tyrosine kinase receptor (v-Src oncogene) induce AhR expression in the absence of serum. Therefore, tyrosine kinase signaling is both necessary and sufficient for induction of AhR expression. Studies with the G1 blocker sodium butyrate show that the signal transduction pathways mediating serum-stimulated progression through the cell cycle are distinct from those that induce AhR expression. These data suggest that transcriptional regulation of the AhR is important in determining cellular sensitivity to the actions of AhR ligand(s) and that the AhR may play a role during the cellular proliferative response.

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor of the basic helix-loop-helix family that displays high affinity binding to certain planar aromatic compounds. Such compounds include polycyclic aromatic hydrocarbons (typified by benzo[a]pyrene, or B[a]P) and halogenated aromatic hydrocarbons (typified by 2,3,7,8-tetrachlorodibenzo-p-dioxin). Polycyclic aromatic hydrocarbons such as B[a]P are generated during the combustion of fossil fuels and are present in tobacco smoke and smoked meats. Halogenated aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-p-dioxin are formed as contaminants during the manufacture of several commercial products, including the chlorophenols. These chemicals are persistent high level environmental pollutants and cause a variety of toxic and carcinogenic effects that are largely mediated by activation of the AhR (for reviews see Refs. 1 and 2 and references therein).

The unliganded AhR exists in the cytosol, in a complex with the 90-kDa heat shock protein (HSP 90). Upon binding to arylic hydrocarbons, the AhR dissociates from HSP 90 and translocates to the nucleus, where it is thought to form a complex with the aryl hydrocarbon receptor nuclear transporter (ARNT) protein (1, 4). The ligand-activated AhR-ARNT complex binds to specific enhancer sequences (termed xenobiotic-response elements; XREs), present within the promoter region of aromatic hydrocarbon-inducible genes. The most widely studied AhR-responsive genes are the AhR-inducible members of the P450 cytochrome family. Ligand activation of the AhR results in transcriptional activation of genes of the P450 subfamily, specifically P4501A1/1A2 and P4501B1 (1, 2, 5). The DNA-bound AhR-ARNT heterodimer is thought to facilitate the recruitment of other transcription factors to the promoter, thereby promoting transcription (1, 2). However, the ligand-activated AhR can also inhibit transcription of certain genes by binding to XREs that overlap other positive regulatory elements (6).

The AhR is widely expressed and is postulated to play a role in normal growth and development based upon patterns of AhR expression in developing mouse embryos (7, 8). Although it is likely that a physiological ligand for the AhR does exist, no such molecule has yet been identified. Nevertheless, inappropriate activation of the AhR by aromatic hydrocarbons induces a variety of cell-specific effects. These include increased proliferation (e.g. parenchymal cells, epithelial cells of the urinary tract, the intestine, and the interfollicular epidermis), inhibition of differentiation (e.g. the generative cells of the gastric glands) as well as tumor promotion in experimental animals (Refs. 1 and 2 and references therein). Thus, AhR activation can have profound effects on cell growth and differentiation. These data support the notion that the AhR plays a part in normal growth and development and that deregulation of AhR signal transduction pathways by aromatic hydrocarbons interferes with normal growth processes.

Regulation of expression of the AhR is a potentially important mechanism for determining the physiological responsiveness to AhR ligands during normal growth and development.

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¶ The abbreviations used are: AhR, aryl hydrocarbon receptor; B[a]P, benzo[a]pyrene; ARNT, aryl hydrocarbon receptor nuclear transporter; XRE, xenobiotic-response elements; PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; EMSA, electrophoretic mobility shift assay.
Regulation of Aryl Hydrocarbon Receptor Expression

Moreover, regulated expression of the AhR may underlie the cell-specific responsiveness to AhR ligands and may be an important determinant of aryl hydrocarbon-induced toxicity. Here we report that expression of the AhR is stringently regulated during the course of the mammalian cell cycle. These data establish a further link between the AhR and cell proliferation and lend support to other studies implying a role for the AhR during the life cycle of mammalian cells.

Murine 3T3 fibroblasts have provided a valuable cell system for the study of growth factor-regulated signal transduction and cell cycle-regulated events (for reviews see Refs. 9 and 10). 3T3 fibroblasts are immortal, nontransformed cell lines, originally derived from mouse embryos. The growth properties and growth factor requirements of 3T3 cells have been well characterized. Moreover, much is known regarding the molecular mechanisms that regulate cell cycle progression in these cells. For exponential growth, 3T3 fibroblasts require exogenously added growth factors, usually supplied by supplementation of the growth medium with 10% donor calf serum. Upon serum withdrawal, 3T3 cells undergo growth arrest and remain in a quiescent state termed G0. However, when stimulated with fresh serum or certain defined mitogenic growth factors (such as platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF)), the cells synchronously re-enter the G1 phase of the cell cycle. G1 is defined as the time interval between G0 (or mitosis) and the initiation of DNA synthesis (S phase) and is characterized by a cascade of growth factor-stimulated signaling events including growth factor-induced synthesis of second messenger molecules, activation of small GTP-binding proteins, protein kinase cascades, and the transcriptional activation of “immediate early” and “delayed response” genes whose protein products are thought to be necessary for progression through the cell cycle (reviewed in Refs. 9–12). Thus, the cell cycle comprises a complex program of temporally ordered events that mediate cellular responses to external mitogenic stimuli.

We have investigated the expression of the AhR during the 3T3 fibroblast cell cycle. We show here that expression of the AhR is regulated at the transcriptional level in response to serum and purified growth factors during the course of the cell cycle. These data suggest that regulation of AhR expression may be an important mechanism for sensitizing cells to AhR ligands and strengthen the idea that the AhR serves an important function during normal cell growth and development.

MATERIALS AND METHODS

Cells and Culture—Swiss 3T3 cells were obtained from the ATCC and were grown in Dulbecco’s modified Eagle’s medium supplemented with penicillin and streptomycin containing 10% donor calf serum. To induce growth arrest, cells were placed in culture medium containing 0.5% serum for 24 h. v-scr-expressing Swiss 3T3 cells were obtained as described previously (13).

Fluorescence-activated Cell Sorter Analysis and [3H]Thymidine Incorporation Assays—These experiments were carried out as described previously (13).

Preparation of Cytosolic Extracts and Nuclei for Immunoblotting—Monolayers of Swiss 3T3 cells in 10-cm culture dishes were rinsed with 10 ml of phosphate-buffered saline. The washed monolayers were detached by the addition of 1.5 ml of 10 mM Tris (pH 7.0), 150 mM NaCl, 1 mM EDTA (TNE) for 10 min at room temperature. Detached cells were scraped off the dish, placed in a microcentrifuge tube, and centrifuged at 10,000 × g for 10 s. The washed cell pellets were lysed in 100 µl of lysis buffer (20 mM Hepes (pH 7.4), 150 mM NaCl, 5 mM MgCl2, 1 mM EGTA, 25% Nonidet P-40), vortexed vigorously, and incubated on ice for 5 min. The lysates were centrifuged at 10,000 × g for 5 min. The resulting supernatants (cytosolic extracts) were removed and frozen at −70 °C prior to immunoblotting. Nuclear pellets were resuspended and washed in 200 µl of lytic buffer and then repelleted at 10,000 × g. The washed nuclear pellets were resuspended in 100 µl of lysis buffer containing 2 units of RNase I (Promega). Chromatin was digested on ice for 10 min. Digested nuclei were then frozen at −70 °C prior to SDS-polyacrylamide gel electrophoresis.

Anti-AhR Antisera—All experiments shown were performed with two independently generated antibodies. Dr. Gary Perdew kindly provided us with a monoclonal anti-AhR antibody (clone RPT-1). A polyclonal antibody to recombinant murine AhR was also purchased from Biomol. Both antisera recognized a single 95-kDa band on immunoblots that was blocked by excess recombinant AhR. Identical results were obtained using both antibodies.

SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting—25-µg aliquots of each sample were denatured by heating to 90 °C in SDS-reducing buffer and were separated by electrophoresis on 7.5% SDS-polyacrylamide gels. After transfer to nitrocellulose, the filters were probed with antisera to murine AhR. The blots were developed using an ECL kit (Amersham Corp.).

Transient Transfections—Exponentially growing cultures of 3T3 cells were transfected with 10 µg of the appropriate plasmid DNA plus 20 µg of salmon sperm carrier DNA by calcium phosphate co-precipitation. After 12 h, the transfection medium was removed and replaced with fresh culture medium. 12 h later, the cells were placed in culture medium containing 10% or 0.5% serum. 18 h later, the transfected monolayers were washed with phosphate-buffered saline and detached with TNE as described above. Cell extracts were prepared by detergent lysis and were assayed for luciferase activity using a commercially available kit (Promega).

Preparation of Nuclear Extracts and Electrophoretic Mobility Shift Assays (EMSA)—To prepare nuclear extracts, monolayers of 3T3 cells were rinsed and then incubated in 5 ml of phosphate-buffered saline and pelleted by centrifugation at 1000 × g for 5 min. Washed cells (approximately 107) were resuspended in 5 ml of hypotonic ice-cold lysis buffer (20 mM Hepes (pH 7.9), 1.5 mM MgCl2, 10 mM KCl, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamide, 0.5 mM dithioreitol), left on ice for 10 min, and then homogenized with 10 up-down strokes of a hand-held glass/homogenizer. The lysate was centrifuged at 5000 × g for 5 min. After aspirating the supernatant, the nuclear pellet was resuspended in approximately 0.3 ml of salt extraction buffer (20 mM Hepes (pH 7.9), 25% glycerol, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamide, 0.5 mM dithioreitol). Extraction of nuclear proteins was carried out by incubation on ice for 20 min. The salt-extracted nuclei were removed by centrifugation at 10,000 × g for 10 min. The resulting supernatants containing the salt-extracted nuclear proteins were stored at −70 °C prior to EMSA analysis. XRE binding reactions were performed in a volume of 30 µl containing 10 mM Hepes (pH 7.9), 50 mM KCl, 5 mM MgCl2, 1 mM dithiothreitol, 5% glycerol, 5 µg of poly(dI:dC), and 5 µg of nuclear extract protein. All components were incubated on ice for 10 min prior to addition of double-stranded 32P-labeled oligonucleotide probe specifying the XRE (14). The incubation was continued for a further 20 min after the addition of the probe. Reaction mixes were loaded onto a 6% polyacrylamide gel and electrophoresed at 200 V. Protein-DNA complexes with retarded electrophoretic mobility were detected by autoradiography of the dried gel.

RESULTS

Growth Arrest Is Associated with Elevated AhR Expression—Exponentially growing cultures of Swiss 3T3 cells were placed in fresh growth medium containing 10% serum or were transferred to medium containing reduced (0.5%) serum for 24 h. Fluorescence-activated cell sorter analysis of propidium iodide-stained nuclei confirmed that the cells underwent growth arrest following serum starvation, as evident by reduced levels of cells in S and G2 + M relative to cells in 10% serum (Fig. 1A). Immunoblot analysis of detergent lysates from parallel cultures of fibroblasts with two independent anti-AhR antibodies (see “Materials and Methods”) indicated greatly reduced expression of a 95-kDa immunoreactive protein in cytosol from serum-starved cells relative to cells growing in 10% serum (Fig. 1B). Although prolonged development of immunoblots did eventually enable detection of the 95-kDa band in extracts from serum-starved cells (see, for example, Figs. 4, 6, 7, and 8), serum starvation consistently resulted in 4–10-fold induction of levels of this protein.

To establish that the immunoreactive 95-kDa protein corresponded specifically to the AhR (and not a related PAS protein)
we tested the effects of a specific AhR-activating ligand on the subcellular localization and cellular levels of the protein. Exponentially growing 3T3 cells were treated with the AhR ligand B[a]P. Nuclear and cytosolic extracts were prepared at frequent intervals following B[a]P treatment. Cell extracts were then separated by SDS-polyacrylamide gel electrophoresis and probed with anti-AhR antisera (Fig. 2). As would be expected for the AhR, brief (0.5-h) exposure to 1 \( \mu \text{M} \) B[a]P resulted in nuclear translocation of the immunoreactive 95-kDa species, and prolonged (4-h) treatment with ligand resulted in complete down-regulation of this protein (Fig. 2). These data are similar to the findings of Swanson and Perdew, who also demonstrated ligand-induced down-regulation of the AhR (15), and further confirm the identity of the 95-kDa immunoreactive protein in our experiments as the AhR.

We tested whether the growth state-associated changes in AhR expression were of sufficient magnitude to affect the extent of ligand-dependent AhR nuclear translocation. Exponentially growing 3T3 cells (in 10\% serum) and serum-starved cells were stimulated for 45 min with B[\( \alpha \)]P. Proteins present in nuclear extracts from the cells were tested for XRE binding activity by EMSA. As shown in Fig. 3, nuclear extracts from proliferating cells contained increased amounts of XRE binding activity following B[\( \alpha \)]P stimulation relative to growth-arrested fibroblasts. These experiments demonstrate that the state of growth arrest resulting from serum starvation is associated with reduced expression of the AhR and consequent reduced cellular responsiveness to an AhR ligand.

We examined the kinetics of AhR induction following read- dition of 10\% serum to quiescent (serum-starved) cultures of cells. Immunoblot analysis of AhR expression at various time intervals following the addition of serum showed that serum-induced expression of AhR was detectable after a lag period of approximately 13 h and was maximal by 18 h (Fig. 4A). By comparison, the onset of DNA synthesis (determined by measurements of \([^{1}H]\) thymidine incorporation into genomic DNA) began approximately 13 h following serum stimulation of quiescent cells and was complete after 22–24 h (Fig. 4B). Therefore, AhR expression was induced at the G1/S phase boundary and peaked in late S phase.

**Mechanism of AhR Induction by Serum**—Serum-dependent induction of the AhR protein may have potentially resulted from increases in steady state levels of the AhR mRNA. However, AhR transcripts were undetectable by RNA blot analysis...
Serum: 0.5% 10%

B[a]P: – + – +

Fig. 3. Ligand-dependent AhR nuclear translocation in growth-arrested and proliferating fibroblasts. Parallel cultures of fibroblasts were placed in fresh medium containing 10 or 0.5% serum for 24 h. Cultures were treated with 1 μM benzo[a]pyrene (added as a 1000 × stock in Me2SO) for 45 min. Nuclear extracts were prepared from the cells as described under “Materials and Methods.” EMSA binding reactions were performed by incubating nuclear extracts with a double-stranded oligonucleotide probe specifying the XRE. The position of the B[a]P-induced band with retarded mobility is indicated by the arrow. This experiment was repeated twice with similar results.

Fig. 4. Kinetics of induction of AhR expression and DNA synthesis following serum-stimulation. Cultures of fibroblasts were made quiescent by starvation in 0.5% serum for 24 h. The growth-arrested cultures were left untreated or were given 10% serum for the indicated times. Cells were assayed for entry into S phase by determinations of [3H]thymidine incorporation (A) or for expression of the AhR by immunoblotting of cytosolic extracts (B). Each data point in A represents the mean of duplicate determinations that differed by less than 10%. Error bars are omitted for clarity. –, –serum; ●, +serum. These data are representative of three separate experiments.

Fig. 5. Serum-dependent AhR promoter-driven reporter gene activity. Exponentially growing cultures of Swiss 3T3 cells were transiently transfected with the indicated plasmids by calcium phosphate co-precipitation. After 12 h, the transfected cells were fed with fresh medium containing 0.5% serum (○) or 10% serum (__). 18 h later, the cells were harvested, and cytosolic extracts were assayed for luciferase activity as described under “Materials and Methods.” Plasmid p5K600f contains 600 base pairs of the promoter region of the murine AhR gene upstream of a luciferase reporter gene; pGL2b is a promoterless luciferase construct; pSVLUC contains the luciferase gene inserted downstream of a strong constitutive promoter. The values shown are means of duplicate determinations. These data are representative of two separate experiments.

Promoter region of the murine AhR gene upstream of a luciferase reporter gene (16). The transfected cells were fed with fresh medium containing 10% serum or were placed in culture medium containing 0.5% serum. After 18 h, the cells were harvested, and cytosolic extracts were assayed for AhR promoter-driven luciferase activity. As shown in Fig. 5, extracts from p5K600f-transfected cells maintained in 0.5% serum expressed reduced levels of luciferase activity (greater than 90% reduction) relative to cells that were given 10% serum. By contrast, cytosolic extracts from cultures of cells that were transfected in parallel with a promoterless luciferase construct (pGL2b) or a vector containing the luciferase gene downstream of a strong constitutive promoter (pSVLUC) did not show such serum-dependent changes in luciferase activity (Fig. 5). These data show that serum-dependent expression of the AhR results, at least in large part, from transcriptional regulation.

Tyrosine Kinase Signaling Is Necessary and Sufficient for AhR Induction—Serum comprises a poorly defined mixture of growth-stimulatory (as well as growth-inhibitory) factors. Serum-induced mitogenesis in 3T3 cells is thought to result in large part (but not entirely) from the action of polypeptide growth factors (such as PDGF) present in serum (9, 10). Consequently, serum stimulation of fibroblasts results in ligand occupancy and activation of growth factor receptors of the tyrosine kinase family (including the PDGF receptor). Downstream events resulting from ligand activation of growth factor receptors in 3T3 cells include activation of other intracellular tyrosine kinases (members of the src family; see Ref. 17), as well as elevated levels of signaling molecules (such as diacylglycerol, Ca2+, and cAMP), which mediate activation of appropriate serine/threonine kinases (protein kinase C, calmodulin-dependent kinase, and cAMP-dependent protein kinase, respectively). These intracellular signaling events are thought to contribute to the cellular mitogenic response (reviewed in Refs. 9 and 10). Since our experiments indicated a correlation between serum-dependent progression through the cell cycle
and induction of AhR expression, we investigated the role of these individual signaling events in AhR induction.

Quiescent cultures of 3T3 cells were stimulated with 10% serum for 18 h in the absence or presence of the tyrosine kinase inhibitor herbimycin-A. As shown in Fig. 6, herbimycin-A inhibited serum-induced AhR expression. As expected, the concentration of herbimycin-A used in these experiments (0.5 μg/ml) prevented serum-induced DNA synthesis but did not result in cell death during the course of the experiment (not shown). Therefore, tyrosine kinase signaling was necessary for serum-dependent AhR expression. To directly test whether tyrosine kinase activity could induce AhR expression we ectopically expressed the v-Src oncprotein in Swiss 3T3 cells. As shown in Fig. 6, the v-Src-expressing Swiss 3T3 cells expressed constitutively high levels of AhR, even under conditions of serum-starvation. By contrast, the parental Swiss 3T3 fibroblasts only expressed high levels of AhR after serum stimulation (Fig. 6).

Since protein kinase C, cAMP-dependent protein kinase, and calcium/calmodulin-dependent proteins are downstream targets of activated tyrosine kinase receptors (10), we investigated the potential role of these effectors in AhR induction. Separate cultures of quiescent fibroblasts were stimulated with 100 nM phorbol 12-myristate 13-acetate (a protein kinase C activator), 2 μM A23187 (a calcium ionophore that elevates intracellular calcium levels), or a combination of 1.75 mM dibutyryl-cAMP (a cell-penetrant cAMP analogue) and 0.5 mM 3-isobutyl-1-methylxanthine (a cyclic nucleotide phosphodiesterase inhibitor). 18 h later, cytosolic extracts were prepared and analyzed for AhR expression by immunoblotting. As shown in Fig. 6, none of the treatments induced detectable levels of AhR expression, although phorbol 12-myristate 13-acetate and dibutyryl-cAMP/3-isobutyl-1-methylxanthine both elicited a mitogenic response in parallel assays of [3H]thymidine incorporation (data not shown), as has been reported by other investigators (10).

Therefore, activation of serine/threonine kinase activity (protein kinase C, cAMP-dependent protein kinase, and Ca2+/calmodulin-dependent protein kinase) was insufficient to induce AhR expression. However, tyrosine kinase signaling was specifically required for induction of AhR expression in response to serum, and a constitutively active tyrosine kinase (v-Src) was sufficient to confer high level expression of AhR in the absence of serum.

Since certain defined fibroblast mitogens (PDGF or FGF) activate receptors of the tyrosine kinase family (and are known to activate c-Src, see Ref. 17), we asked whether they would also induce AhR expression. Separate cultures of serum-starved 3T3 cells were stimulated with serum, PDGF, and bFGF. The mitogen-stimulated cells were then analyzed for AhR expression (by immunoblotting) and for entry into the cell cycle (by measurements of [3H]thymidine incorporation). PDGF and bFGF were effective mitogens, as shown by their ability to induce [3H]thymidine incorporation in serum-starved 3T3 cells (Fig. 7A). Both PDGF and bFGF induced AhR expression, albeit to lesser levels than were induced by serum (Fig. 7B). Therefore, defined fibroblast mitogens were able to induce cytosolic AhR expression. We also performed immunoblot analysis on preparations of washed nuclei from quiescent and serum/growth factor-treated cells. As shown in Fig. 7B, nuclei from mitogen-treated cells also contained increased levels of AhR relative to nuclei from quiescent cells. Therefore, the amount of AhR present in the nuclear compartment may be regulated by serum and mitogens during the course of the proliferative cell cycle.

Cycle Cell Progression Is Dissociable from AhR Induction—Since our experiments indicated a good correlation between the mitogenic potential of serum/growth factors and their abilities to induce AhR expression, we asked whether progression through the cell cycle was necessary for AhR induction. Therefore, we tested the effect of the cell cycle inhibitor sodium butyrate on cell cycle progression and AhR expression. Cultures of serum-starved 3T3 fibroblasts were stimulated with serum in the presence or absence of 2 mM sodium butyrate and assayed for entry into the cell cycle (by determining [3H]thymidine incorporation).
blasts are broadly categorized as immediate early, delayed early, or late based upon their kinetics of induction following serum/growth factor stimulation of quiescent cells (9–12). Immediate early genes are induced early in G1, even in the presence of protein synthesis inhibitors (e.g. c-myc, c-fos), whereas delayed early genes are expressed later in G1 (but prior to S phase) and require new protein synthesis (e.g. ornithine decarboxylase). Late genes are those activated at or after the onset of DNA synthesis. The AhR, therefore, must be considered a late gene, since its expression peaks after the onset (and completion) of DNA synthesis.

Like the AhR, many other serum/growth factor-induced proteins are transcription factors (e.g. c-myc, c-fos, c-jun). The protein products of many immediate early genes are transcription factors that are themselves thought to mediate expression of delayed early genes and late genes, thereby achieving an ordered sequential program of transcriptional events. Interestingly, the AhR promoter contains binding sites for transcription factors that are expressed earlier in the cell cycle, namely AP-1 (composed of c-fos and c-jun dimers) and c-myc. It is noteworthy that, like the AhR, c-myc expression is elevated in exponentially growing cultures of fibroblasts relative to growth-arrested cells (18, 19) and is induced in response to serum as well as purified mitogenic growth factors such as PDGF and FGF (20). The possibility exists, therefore, that the AhR promoter is a physiologically relevant target for the c-myc proto-oncogene. More detailed analysis of the AhR promoter is under way to identify the cis acting elements and trans-acting factors that mediate serum-dependent transcription of the AhR gene.

Induction of AhR expression during the replicative program of 3T3 cells most likely serves to sensitize proliferating cells to the actions of AhR ligands. Indeed, our EMSA analysis demonstrates that B[a]P stimulation results in greater nuclear accumulation of the AhR in proliferating 3T3 cells relative to growth-arrested fibroblasts. Moreover, our finding that nuclear receptor expression is present in nuclei of serum/growth factor-stimulated cells suggests that nuclear translocation and perhaps even transcription factor activity of the AhR is directly modified by mitogens.

Mouse embryos transcribe AhR mRNA (7, 8), and AhR −/− “knockout” mice are reported to be developmentally impaired (21), although the latter study is now controversial (22). Those studies suggest that the AhR plays an important role in normal growth and development. The 3T3 fibroblasts used in our studies were derived from Swiss mouse embryos. Our finding that expression of the AhR is transcriptionally regulated by mitogenic stimuli (serum and purified growth factors) suggests that this transcription factor may play a role in embryonic cell proliferation and differentiation. Phillips et al. (23) have found that the AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin can inhibit terminal adipocyte conversion of 3T3-L1 fibroblasts. Interestingly, the 3T3-L1 fibroblast subline used in the latter study was originally derived from Swiss 3T3 cells (24). Therefore, AhR activation can directly influence the balance between cell proliferation and differentiation. It is likely to be significant that this critical growth-regulatory gene is itself subject to stringent transcriptional control. In the living organism, fibroblasts and their proliferation in response to growth factors play important roles in organogenesis, the maintenance of connective tissue, and wound healing. Our finding that the AhR is expressed in a regulated manner in these cells raises the possibility that the AhR is involved in these vital physiological processes. Moreover, many of the serum-induced genes originally identified in fibroblasts are now known to play ubiquitous roles in growth and differentiation.
is likely, therefore, that the findings described here will be of general significance.

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