Integration of the Non-genomic and Genomic Actions of Estrogen

Membrane-Initiated Signaling by Steroid to Transcription and Cell Biology

Ali Pedram‡§, Mahnaz Razandi‡§, Mark Aitkenhead‡§¶, Christopher C. W. Hughes‡§, and Ellis R. Levin‡§**

From the ‡Division of Endocrinology, Veterans Affairs Medical Center, Long Beach, Long Beach, California 90822 and the Departments of §Medicine, ¶Pharmacology, and **Molecular Biology and Biochemistry, University of California, Irvine, Irvine, California 92717

Estrogen binds to receptors that translocate to the plasma membrane and to the nucleus. The rapid, non-genomic actions of this sex steroid are attributed to membrane action, while gene transcription occurs through nuclear receptor function. However, gene transcription cannot result from estrogen signals initiated at the membrane, but the relative importance of this mechanism is not known. In vascular endothelial cells (EC), estradiol (E2) activates several kinase cascades, including phosphatidylinositol 3-phosphate (PI3K)/Akt, a signaling pathway that impacts EC biology. We determined here by DNA microarray that 40-min exposure to E2 significantly increased 250 genes in EC, up-regulation that was substantially prevented by the PI3K inhibitor, LY294002. This coincided with maximum E2-induced PI3K activity at 15–30 min. An important vascular gene strongly up-regulated by E2 in our array produces cyclooxygenase-2 (Cox-2). In cultured EC, E2 induced both Cox-2 gene expression and new Cox-2 protein synthesis by 40 and 60 min, respectively, and rapidly stimulated the secretion of prostaglandins PGL2 and PGE2. The up-regulation of gene expression reflected transcriptional transactivation, shown using Cox-2 promoter/luciferase reporters in the EC. Soluble inhibitors or dominant negative constructs for PI3K and Akt prevented all these actions of E2. Functionally, EC migration was induced by the sex steroid, and this was significantly reversed by NS-398, a Cox-2 inhibitor. Gene transcription and cell biological effects of estrogen emanate from rapid and specific signaling, integrating cell surface and nuclear actions of this steroid.

Estradiol (E2) and other steroid hormones are traditionally considered to transactivate target genes after binding nuclear receptors (1). However, E2 also has rapid, non-genomic effects (2–4), and these have recently been attributed to cell membrane-initiated signaling. At the cell surface, a small population of ER binds E2 and activates G proteins (5–7). Multiple signaling pathways are then rapidly stimulated by E2 in target cells that express endogenous ERα and ERβ, and these pathways have been linked to discrete cellular actions of the steroid (8–11). In this respect, a truncated MTA1 protein was recently found to be highly expressed in aggressive breast cancer (12). This protein sequesters ER away from the nucleus and strongly reduces E2-activated transcription yet promotes increased ERK signaling and aggressive behavior of the tumor. It is proposed therefore that the integration of cell surface and nuclear signaling impacts overall cell biology (13).

Signaling from the membrane leads to the post-translational modification of important structural and functional proteins in the cell. In EC, E2 activates the p85-PI3K kinase pathway; MAPKAP-2 phosphorylates and modifies the function of heat shock protein 27 (14). This important modification leads to the morphological preservation and survival of the EC and stimulates primitive capillary tube formation. In breast cancer, E2 acts as a cell survival factor, in part by preventing chemotheraphy or radiation-induced JNK activation (9) and thus phosphorolytes and inactivates Bcl2 and Bcl-xL, leading to the assembly of the apoptosome and caspase-mediated cell death. By preventing JNK activation and Bcl2/Bcl-xl phosphorylation, E2 rescues the breast cancer cells (9). This provides a mechanism for the ability of E2 to oppose therapeutic interventions in this malignancy.

In addition to post-translational protein effects, E2 is recognized to stimulate transcription through signaling typically initiated at the membrane. As precedent for this effect of E2, growth factor tyrosine kinase receptors (insulin-like growth factor-1 receptor and epidermal growth factor receptor) exist in the plasma membrane and signal through common kinase cascades to gene transcription. E2 effects may involve the G protein-initiated, signaling-induced synthesis or activation of transcription factors. E2 stimulates c-fos through ERK- or PI3K-dependent pathways (15–17), the BCL-2 gene through the modification of Sp-1 transcription factor (18), and the prolactin gene through ERK (19). E2 activation of PI3K in EC results from the membrane ER-p85 regulatory subunit (PI3K) association, and this signaling to nitric oxide production rescues rats from ischemia-reperfusion injury (20). However, insight is largely lacking as to the full extent and importance of the specific integration of membrane signaling and nuclear effects of the sex steroid.

We therefore identified the transcriptional targets in EC that are rapidly up-regulated from E2 signaling through the activation of PI3K. We also describe in depth the ability of
E2-induced PI3K activation to up-regulate one specific gene, Cox-2, leading to the production of the enzyme, secretion of products of Cox-2 activation, and EC migration. This approach can be used to identify programs of gene activation that result from membrane-initiated steroid signaling (MISS) by E2.

MATERIALS AND METHODS

PI3K Activity Assay—EC were incubated with/without 10 nM E2, and 10 μL LY294002 for up to 6 h. Cells were then lysed, and the lysates pelleted then dissolved in SDS sample buffer, boiled, separated, and transferred onto nitrocellulose. Phosphorylated Akt was detected using phospho-specific monoclonal antibodies (Santa Cruz Biotechnology) and the ECL Western blot kit. Equal samples from the cells were also immunoprecipitated, and immunoblots of the precipitated kinase protein from each experimental condition were determined to show equal gel loading. All experiments were repeated two or three times.

DNA Arrays—Human umbilical vein endothelial cells were incubated without or with 10 nM E2 with or without the specific PI3K inhibitor, LY294002 (10 μM), or LY294002 alone, for 40 min. For validation, the experiment was repeated a second time. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) and RNeasy columns (Qiagen, Valencia, CA). Total RNA was adjusted to 1 μg/μL, and first-strand cDNA, followed by double-stranded cDNA was synthesized from poly(A) mRNA by the MicroArray Facility at the University of California, Irvine. This was done using the SuperScript double-stranded cDNA synthesis kit (Invitrogen) and poly(T) nucleotide primers that contained a sequence recognized by T7 RNA polymerase. A portion of the resulting double-stranded cDNA was used as a template to generate biotin-tagged cRNA from an in vitro transcription reaction (IVT) using the BioArray High-Yield RNA transcript labeling kit (T7 (Enzo Diagnostics, Farmingdale, NY)). 15 μg of the biotin-tagged cRNA was fragmented to strands of 35–200 bases in length following prescribed protocols (Affymetrix GeneChip Expression Analysis Technical Manual). 10 μg of the fragmented cRNA was hybridized with rotation at 45 °C for 16 h to probe sets present on an Affymetrix human U95a array (Affymetrix, Santa Clara, CA). The arrays were automatically washed and stained with streptavidin-phycocerythrin. Probes were then scanned on a Hewlett-Packard GeneArray scanner. Affymetrix Microarray Suite 5.0 was used to quantify and analyze the output from CyberT analysis, a stringent, Bayesian-based statistical analysis program. Duplicate determinations for all conditions were utilized for the analysis. Genes in red are the upper 2.5% of all genes positively regulated by E2 and mostly inhibited by LY294002.

FIG. 1. Time course of PI3K activation in endothelial cells by estradiol (E2). Cells were incubated with 10 nM E2 with or without 10 μM LY294002 for the times indicated, and Akt phosphorylation as a function of PI3K activity was determined by Western blot. Immunoblot of total Akt protein is shown below each condition. A representative study of two completed is shown.

David Dixon (Vanderbilt). The liposomemediated transfection was carried out as previously described (6, 9), and the cells were recovered in serum overnight, then synchronized without serum over 24 h before experiments (in phenol red-free medium). Cells were then incubated for 6 h with/without 10 nM E2 with or without either LY294002 (10 μM), a soluble inhibitor of NfFb (SN50M), or the corresponding inactive control (SN50), each at 20 μM. All inhibitors were from Calbiochem (San Diego, CA). Assays were quantified by luminometer, and the results were adjusted for expression of co-transfected Renilla luciferase. Triplet determinations per condition were carried out in each of two experiments. The combined data were analyzed by analysis of variance plus Scheffe’s test, at p < 0.05 level of significance.

FIG. 2. Estrogen-induced genes in endothelial cells, dependent upon phosphatidylinositol 3-kinase signaling. E2 were incubated/ not incubated with 10 nM E2 with or without LY294002 (PI3K inhibitor), or LY294002 alone, for 40 min. Total RNA from the four conditions was then extracted for purposes of microarray analysis after cDNA/cRNA synthesis (see “Materials and Methods”). The figure is the output from CyberT analysis, a stringent, Bayesian-based statistical analysis program. Duplicate determinations for all conditions were utilized for the analysis. Genes in red are the upper 2.5% of all genes positively regulated by E2 and mostly inhibited by LY294002.

For reporter assays, BAEC were transiently transfected with 10 μg of the PGL-3 plasmid containing 1.8 kb of the human Cox-2 promoter driving a luciferase reporter fusion protein (22), kindly provided by Dr.
linked immunosorbent assay (Cayman Chemical). The study was repeated three times, with the results reflected in the bar graph.

**EC Migration Studies**—EC were grown to monolayer confluence on six-well plates and synchronized for 24 h in the absence of serum. A "wound" was created by scraping the monolayer with a single-edge razor blade, and cells were removed to the left of the wound. Serum-free Dulbecco’s modified Eagle’s medium containing 10 nM E2 with or without NS-398, SC-560, LY294002, wortmannin, or alone as control was added to separate dishes of wounded EC for 24 h at 37°C. The cells were then fixed in 3.7% formaldehyde and assessed for migration (14).

BAEC migration was measured using an image analyzer system composed of an inverted microscope and a 20- to 24-inch digitizing board (Jandel Scientific, Corte Madera, CA) attached to a computer. The Sigma Scan program (Jandel) was used for analysis of measurements of the distance traveled by the cells within the calibrated area adjacent to the wound. Five measurements in each well were taken, and results from three separate experiments contributed to create the bar graph.

**RESULTS AND DISCUSSION**

**Estrogen Stimulates PI3K Activity in EC**— E2 induced substantial Akt phosphorylation by 5 min, reflecting PI3K activation, because the phosphorylation was totally prevented by a PI3K inhibitor (Fig. 1). The peak activity occurred at 15 min and lasted for the 6-h duration of the experiment. We therefore carried out our array studies to assess genes rapidly induced by E2 via PI3K signaling, based upon these results. E2 Rapidly Induces Many Genes via PI3K Activation—EC were incubated with 10 nM E2 or without steroid (control) for 40 min, in the presence or absence of LY294002, a PI3K inhibitor.

cRNA from each experimental condition was used for microarray gene analysis, as delineated under “Materials and Methods.” The DNA array hybridization pattern was analyzed by both Affymetrix statistical software and the CyberT Bayesian-based program, and 250 genes were identified as being significantly up-regulated by E2 (upper 2.5% of all genes) (Fig. 2). This occurred in both E2-inducible and PI3K-reversible fashion. In comparing control cRNA (no treatment) to cRNA from cells treated with LY294002 alone, no differences were detected. We list the genes that were 1) up-regulated in this fashion by more than 2-fold and 2) identified to have some known function (Table I). In contrast, few genes were down-regulated by E2 in the quiescent EC, and none depended upon PI3K/Akt activation, whereas several genes were stimulated by E2 and further enhanced upon PI3K inhibition. Genes discussed in the text are given in boldface in Table I.

As might be predicted from the time chosen, many transcription factors were rapidly up-regulated. These included the fos, myc, and jun genes previously known to be stimulated by estrogen (15, 26, 27), which thereby validate our results. Here we extend the findings and implicate PI3K action in protooncogene up-regulation by E2. This sex steroid was recently shown to activate c-fos transcription in MCF-7 cells, via a PI3K/Akt pathway, targeting the serum response factor motif in the proximal fos promoter (16). We also found stimulation of many transcription factors not previously known to be regul-

### Table I

| Description                                      | Accession # | Fold Increase |
|--------------------------------------------------|-------------|---------------|
| Cat/Tpl-2 kinase                                | MAP3K       | D14497        |
| GTPase                                           | gem         | U10550        |
| protein-synthesis phosphatase CL 108             | M68777      | 11.07         |
| corticosteroid releasing factor receptor         | CRHR1       | X73204        |
| putative toppomerase III                       | TOPP3       | D87012        |
| insulin receptor substrate-1                   | IRS-1       | D05239        |
| STAT3-induced STAT inhibitor-2                  | STAT2       | A977999       |
| protein tyrosine phosphatase                    | FPF-U       | Z48441        |
| Spred-2                                         | SPRY2       | A938643       |
| G protein-coupled receptor                      | RDC1        | U67678        |
| SRC-like protein kinase                         | FKR         | U08003        |
| glycogen kinase                                | GTKB        | X78711        |
| Toll like receptor 2                            | TILA        | A95118        |
| serine/threonine protein kinase                 | ukg         | Y10032        |
| G protein-coupled receptor                      | CNR1        | U73004        |
| Wnt-5a                                          | Wnt-5a      | 1,2061        |
| P3K regulatory 1                                | GRB1        | M61906        |
| GSK355                                          | GSK355      | D87119        |
| Wnt-7a                                          | Wnt-7a      | D87375        |
| chapsin-11                                      | DLG2        | U32736        |
| serotonin receptor 3                            | kC          | S76475        |
| indium kinase                                   | SNK         | A95967       |
| FOX receptor                                    | P2Y6R       | A907803       |
| intra-acrosomal protein                         | SP-10       | S65583        |
| SA (n=hyperstim-associated) homolog SA          | X80062      |
| type II initiator-1 receptor                    | IL-1R2      |
| Dual Specificity protein phosphatase 6          | DUSP6       |
| guanine nucleotide exchange factor SOSP         | L13857      |
| Nuclear Factor of Activated cells x4            | NFA4x       |
| platelet membrane calcium ATPase                | PMCA2       |
| SNF1-like protein kinase                        | SNFLPK       |
| MAP kinase phosphatase                          | MKP-2       |
| insulin polyphosphatase 4-phosphatase type II   | PPPI2A       |
| multiple P20 domain protein                     | MUP1F1      |
| Choline channel protein                         | CLCN3       |
| putative g protein-coupled receptor             | TMMFSF1     |
| G protein-linked receptor                       | GPCHR       |
| kappa opioid receptor                           | OPKR1       |
| thrombospondin precursor                       | THBD        |
| receptor phosphine FCP-2                        | PCF-2       |
| cRNA                                             | X97198       | 2.02          |

**Integration of the Non-genomic and Genomic Actions of Estrogen**

Genes that were induced at least 2-fold in endothelial cells after 40-min exposure to E2, but were not significantly induced after exposure to E2 and LY294002 are listed. Differentially expressed genes were identified by both Affymetrix analysis software and the CyberT Bayesian-based program, and 250 genes were identified as being significantly up-regulated by E2 (upper 2.5% of all genes) (Fig. 2). This occurred in both E2-inducible and PI3K-reversible fashion. In comparing control cRNA (no treatment) to cRNA from cells treated with LY294002 alone, no differences were detected. We list the genes that were 1) up-regulated in this fashion by more than 2-fold and 2) identified to have some known function (Table I). In contrast, few genes were down-regulated by E2 in the quiescent EC, and none depended upon PI3K/Akt activation, whereas several genes were stimulated by E2 and further enhanced upon PI3K inhibition. Genes discussed in the text are given in boldface in Table I.
lated by E2; these genes are therefore implicated in further E2 transcriptional action. In some situations, linked gene programs could be tentatively identified, based upon the existing literature. As an example, bone morphogenetic protein 2 (Table I, cytokines) stimulates osteoblast precursor-cell differentiation in part via up-regulating the AREB6 transcription factor (29). Estrogen induces osteoblast differentiation (30), and we found that E2 stimulates both genes here, via a PI3K-induced mechanism. Another transcriptional target for AREB6 is the Na+/H+-ATPase gene (31). E2 is known to stimulate the activity of this enzyme (32), potentially linking these observations to upstream signaling. As shown here, the HZF2 transcription factor is induced by E2 in PI3K-dependent fashion (Table I). HZF2 has been reported to be up-regulated by nitric oxide (NO) (33), and E2 strongly and rapidly stimulates NO production in EC in PI3K-dependent fashion (20). NO induction by E2 prevents the deleterious blood vessel response to ischemia-reperfusion injury (20). Together, these results potentially identify a linked cell biological program in EC. Several members of the EGR family of transcription factors were identified as induced by E2 in our array. Egr-1 is up-regulated (and important) in the response to acute and chronic vascular injury, where it may serve a protective function (34, 35). E2 mitigates the acute injury response to carotid angioplasty (36), perhaps in part through inducing Egr-1 in the EC, as shown here.

Signaling molecules were also rapidly induced by E2, including both kinases and phosphatases. Steroid and glucoorticoid-inducible kinase activity is known to be a target for PI3K/Akt signaling (37) and has roles in both steroid-induced memory (38) and sodium transport (39), which are both functions of E2 (40, 41). We also found that, via PI3K, E2 up-regulates Cot/Tpl-2, a transformation-associated factor and serine/threonine kinase. Akt phosphorylation of Cot induces NFκB-dependent transduction (42), important for various functions, including cell survival. E2 potently inhibits hypoxia-induced EC apoptosis (14).

Genes coding for structural proteins, cytokines, or enzymes were identified as being stimulated by E2. Two members of the NGFI-B subfamily of nuclear orphan receptors, TR3 and MINOR, were rapidly and strongly induced by E2 via PI3K. A cytokine gene, CYR61, was previously demonstrated to be up-regulated by E2 in breast cancer but through an unknown mechanism (43). In EC, we implicate signaling via PI3K. Up-regulation of the PTTG gene by estrogen (found here) was previously shown to contribute to the pathogenesis of E2-induced pituitary tumor formation and the stimulation of bFGF secretion (44). E2 and bFGF are recognized angiogenesis factors (14, 45), and therefore a potential linkage in EC of PTTG-induced bFGF could be important for the recognized neovascularization function of E2. The PLAP (phospholipase A2-activating protein) gene was found to be stimulated in our array (Table I). This protein has been reported to up-regulate the Cox-2 gene and stimulate prostaglandin E2 (PGE2) production (46).

Based on our results, we propose a...
linkage of E2-induced, PI3K-dependent up-regulation of PLAP, contributing to E2-induced Cox-2 up-regulation and PG secretion/production (see below). Thus, many relevant genes are rapidly induced by this sex steroid in response to one signal pathway typically initiated at the plasma membrane.

**Confirmation by RT-PCR of E2-induced Gene Up-regulation**—To confirm the array studies, we carried out semi-quantitative RT-PCR for several of the genes identified (Fig. 3). For the four genes examined (Cox-2, JunB, CREM, and EGR2), there was increased expression in EC after 40-min exposure to 10 nM E2 (lane 2), compared with the control (no E2) (lane 1). Furthermore, this increase was significantly abrogated by addition of the PI3K inhibitor, LY294002 (lane 3), whereas the inhibitor alone had no effect compared with control levels (lane 4). The results provide validation of the array data for these specific genes.

**Cox-2 Gene and E2/ER Interactions Result from Rapid Signaling by the Steroid**—One of the genes strongly up-regulated by E2 in our EC gene array codes for the Cox-2 enzyme (Table I). Cox-2 activity gives rise to PGL2 and PGE2 production, important for various aspects of vascular function (47, 48). To explore the interactions between E2 signaling through PI3K to Cox-2 in greater depth, we first confirmed the array results by RT-PCR (Fig. 3). We then further investigated transcriptional regulation by E2. We therefore expressed in BAEC a plasmid containing a 1.8-kb human Cox-2 promoter driving a luciferase reporter. This was significantly responsive to 10 nM E2, in a PI3K-dependent fashion (Fig. 4).

One known target for PI3K/Akt signaling is the activation of the NFκB transcription factor (49, 50). We found that a soluble NFκB inhibitor (but not its inactive control) completely reversed the E2 stimulation of the Cox-2 promoter (Fig. 4). These data support the microarray studies and the idea that the up-regulation of the Cox-2 gene by E2 is transcriptional. Furthermore, this E2 action depends on signaling via PI3K and NFκB. NFκB binding sites are present within the human Cox-2 promoter at −580 and −358. Supporting this mechanism, we found that E2 significantly activated both 0.8 and 0.4 kb Cox-2 promoters driving luciferase reporter constructs, suggesting that the NFκB binding site at −358 in particular is important. Previous studies indicate that PI3K/Akt can regulate Cox-2 mRNA production or stability in positive or negative fashion, dependent upon the stimulus and cellular context (51, 52).

**Cox-2 Protein Synthesis and Prostaglandin Secretion Are Stimulated by E2**—Cox-2 protein synthesis was then determined by Western blot. After 1-h exposure of EC to E2, the Cox-2 protein was nearly 3-fold increased in relevant E2 concentration-responsive fashion (Fig. 5A). The PI3K inhibitors, LY 294002 and wortmannin, each caused an 80% reduction in Cox-2 protein synthesis, as did ICI 182,780, an ER antagonist. Expression of dominant negative constructs for the p85 subunit of PI3K (DN-PI3K) or Akt (DN-Akt) also resulted in substantial inhibition of E2-induced Cox-2 protein expression. The dominant negative constructs had no effects alone (data not shown).

In preliminary studies, we determined a time course for PGE2 and PGL2 secretion in response to E2. Compared with basal secretion (0 time), E2 stimulated an initial 2-fold PGE2 release at 10 min (first point assessed), reaching a maximum increase at 30 min and plateauing thereafter (data not shown). Based upon these results, BAEC were then incubated with 10 nM E2 with or without inhibitors of ER, PI3K, and Cox-1 or Cox-2 for 30 min. As seen in Fig. 5B, E2 stimulated a 13-fold increase of PGE2 secretion, 75% prevented by ICI 182,780. Co-incubation of the cells with NS-398 (a specific Cox-2 inhibitor) reversed the E2 effect by 86%. A specific Cox-1 inhibitor (SC-560) also provided a 33% inhibition of the E2 effect, suggesting that PGE2 synthesis was mainly dependent upon the Cox-2 enzyme. It is recognized that PGE2 and PGL2 can both result from either Cox-1 or Cox-2 enzymatic action, but in a given cell type or in response to a specific stimulus, one cyclooxygenase activity may predominate over the other (53).

Importantly, the PI3K inhibitors, wortmannin and LY 294002 each prevented E2-induced PGE2 secretion by 87%. This is consistent with the ability of E2 to activate the Cox-2 gene in a PI3K-dependent fashion, as identified by microarray and reporter studies. Interestingly, the PGE2 receptor was also found in our array to be up-regulated by E2 via PI3K (Table I).

As for PGL2, E2 induced a 4-fold increase in secretion (Fig. 5B). This was 60% reversed by ICI 182,780, 58% by the Cox-2 inhibitor, and 36% by the Cox-1 inhibitor. The PI3K inhibitors also reduced the E2 induction of PGL2 by 60%. Our results

---

1 A. Pedram, M. Razandi, M. Aitkenhead, C. C. W. Hughes, and E. R. Levin, unpublished observations.
greatly extend the observations of others that E2 can rapidly stimulate the secretion of PGI2 from endothelial cells (54, 55). Here we show transcriptional up-regulation of the Cox-2 gene, increased protein production, and stimulation of both PGE2 and PGI2 secretion, in a PI3K-dependent fashion. An observation that is relevant for EC is that Cox-2 and cAMP-signaling enhances angiogenesis through the induction of vascular endothelial growth factor (56). Cox-2 and cAMP are up-regulated by
E2 signaling from the membrane (2, 6, and here). This may be significant to E2-modulated induction and developmental function of vascular endothelial growth factor, in the formation and permeability of the blood vessels of the ovary and uterus (56, 57).

EC Migration—We then examined possible roles for Cox-2-derived prostaglandins and E2 in mediating EC migration. E2 is known to induce several aspects of angiogenesis, including EC migration (14), and Cox-2 also importantly participates in these processes (28). Cultured EC were "wounded," and the migration of EC across this wound barrier was determined after 24-h exposure to various conditions. As seen in Fig. 6, 10 nM E2 (panel B) induced a 3-fold increase in migration area compared with control EC (panel A). The effect of E2 was reduced 75% by a Cox-2 inhibitor (panel C), but not significantly by a Cox-1 inhibitor (panel D). The effect of E2 was also prevented by LY294002 (panel E) and by wortmannin (PI3K inhibitor) (panel F). The inhibitors by themselves had no effects on EC migration (data not shown). Thus, we link the Cox-2 up-regulation in EC, induced by E2 and PI3K signaling, to a cell biological outcome.

Increasingly, the integration of membrane and nuclear actions of steroids is recognized. An important mechanism demonstrated here is that steroids rapidly induce through kinase signaling many genes coding for transcription factors. We determined that the identified transcription factor gene promoters usually lack any form of estrogen response elements. Transcription factor up-regulation presumably then leads to the induction of additional genes that regulate steroid-induced cellular function. However, we also show that, by the same signaling mechanism, steroids rapidly up-regulate genes coding for enzymes or signaling molecules. The protein products of these genes both directly impact cell functions (i.e. cell migration) and induce additional transcription. Through membrane-initiated steroid signaling (MISS), we propose that estrogen affects overall cellular processes by post-translationally modulating the functions of existing proteins (9, 14) and by activating discrete programs of gene expression.

REFERENCES
1. Truss, M., and Beato, M. (1993) Endocrine Rev. 14, 459–479
2. Aronica, S. M., Kraus, W. L., and Katznellenbogen, B. S. (1994) Proc. Natl. Acad. Sci. 91, 8517–8521

Fig. 6. E2 stimulates EC migration in PI3K and Cox-2 related fashion. Cultured EC were cut with a surgical blade, and all cells were removed on the left side of the wound with a scraper. The remaining cells were then cultured overnight with/without 10 nM E2 with or without PI3K and Cox-2 inhibitors. Panel A is control cells (no E2 or serum); panel B is E2, panel C is E2 + NS-398, panel D is E2 + SC-560, panel E is E2 + LY294002, and panel F is E2 + wortmannin (50 nm). The inhibitors alone had no effects (data not shown). The bar graph below the composite reflects three experiments combined. *, p < 0.05 for control versus E2; +, p < 0.05 for E2 versus E2 plus inhibitor.
Integration of the Non-genomic and Genomic Actions of Estrogen:
MEMBRANE-INITIATED SIGNALING BY STEROID TO TRANSCRIPTION
AND CELL BIOLOGY
Ali Pedram, Mahnaz Razandi, Mark Aitkenhead, Christopher C. W. Hughes and Ellis R.
Levin

J. Biol. Chem. 2002, 277:50768-50775.
doi: 10.1074/jbc.M210106200 originally published online October 7, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M210106200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 56 references, 17 of which can be accessed free at http://www.jbc.org/content/277/52/50768.full.html#ref-list-1