Environmental factors affecting transcription of the human L1 retrotransposon. I. Steroid hormone-like agents

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Introduction

Retrotransposons are mobile elements that transpose through an RNA intermediate (Eickbush, 1992). Retrotransposons can be categorized according to whether they contain long terminal repeats (LTRs) and whether they encode retrotransposition enzymes. Non-LTR retrotransposons are found in many species, including maize, fungi, Drosophila, protists, amphibians and mammals, and in mammals have been classified as either short repetitive elements (SINES) or long repetitive elements (LINEs). The human LINE elements are ~6 kb in length and contain a promoter 5′-untranslated region (UTR), a 3′-UTR and remnants of a poly(A) tail. While most of the ~868 000 copies (20% of the human genome) of human LINEs consist of two families (LINE2 and LINE3) that are defective due to truncations at the 5′-end, there is one family, LINE1, that contains active, full-length elements. The human LINE1 family, L1Hs, consists of subsets Ta and Pre-Ta, with 39 and 22 full-length, intact, potentially active members, respectively (Lander, 2001). These numbers closely match estimates of the total number of retrotransposition-capable L1Hs elements in the human genome (Sassaman et al., 1997). L1Hs contains two open reading frames (ORFs), the first of which encodes a multimer-forming protein with nucleic acid chaperone activity that is crucial for retrotransposition (Moran et al., 1996; Hohjoh and Singer, 1997; Martin and Bushman, 2001). The second ORF encodes a protein with endonuclease and reverse transcriptase functions (Mathias and Scott, 1993; Feng et al., 1996).

L1 retrotransposon expression is limited almost exclusively to germ line and embryonic cells (Singer et al., 1993; Tchenio et al., 2000). As a result, L1 activity has significantly shaped the structure and evolution of the human genome. L1Hs is probably the predominant source of endogenous reverse transcriptase (RT) in the human genome, and not only plays a role in its own transposition, but is also thought to mediate the transposition of other retroelements, such as Alu sequences and pseudogenes (Jurka, 1997; Lander, 2001). Even though the number of LINEs and SINEs is likely to be underestimated, ~33% of the human genome has been predicted to be created through L1 activity (Lander, 2001; Venter, 2001). Researchers have speculated that LINE elements may, by direct and indirect means, determine the basic mutation rate in mammals (Edgell, 1994). Consistent with this hypothesis, LINE element insertions have been implicated in both genetic disease and cancer (Kazazian et al., 1988; Miki et al., 1992). The significance of L1Hs to the human genome has also been underscored recently by the finding that reverse transcriptase may be involved in double-strand break repair (Voliva et al., 1984; Teng et al., 1996; Tremblay et al., 2000).

Upon considering the myriad of effects that L1Hs activity has on the sequence content and mutational load of the human genome, one of the central questions is: under what circumstances do human LINE elements retrotranspose? L1Hs transcription is thought to be the rate limiting step in retrotransposition (Skowronski et al., 1988; Mathias and Scott, 1993). However, little is known about the regulation of L1Hs transcription or the relationship between L1Hs transcription and retrotransposition. L1Hs contains an internal promoter that is 952 bp in length (Swergold, 1990) and is a binding site for transcription factors YY1 (Becker et al., 1993; Kurose et al., 1995) and SRY (Tchenio et al., 2000).

The ability of environmental factors to stimulate transposable element activity was first proposed by Barbara McClintock (McCIntock, 1984). McClintock’s ‘genomic shocks’ hypothesis proposes that a variety of environmental stimuli may mobilize transposable elements. McClintock’s hypothesis has been supported by the finding that the copy number of the plant LTR retrotransposon BARE-1 correlates with microclimate aridity, suggesting stress-induced mobilization of this element (Kalander et al., 2000). Recently it was suggested that androgen production in mouse germ cells plays a role in induction of the mouse L1 retrotransposon (Trelogan and Martin, 1995). Regulation of the transcriptional activity of retroelements in diverse organisms is known to be affected by steroid hormones. In cultured Drosophila melanogaster cells the transcriptional
activity of two copia-like retrotransposons, 412 and 1731, is negatively regulated by 20-hydroxyecdysone, the steroid molting hormone of insects (Becker et al., 1991; Ziarczyk and Best-Belpomme, 1991). In mammals there are five major classes of steroid hormones, including progesterone, glucocorticoids, mineralcorticoids, androgens (testosterone and dihydrotestosterone) and estrogens (Stryer, 1995). A stably integrated mouse mammary tumor virus (MMTV)–luciferase construct is transcriptionally activated by glucocorticoids and is up-regulated by progesterone when cells are transiently transfected (Archer et al., 1994). When exposed to steroid hormones, retrovirus-like particles similar to MMTV were also found to be secreted by the human mammary carcinoma cell line T47D (Aff et al., 1992). Expression of VL30 retrotransposons was observed to be greatly elevated by steroid hormone treatment in both male and female gonads (Schiff et al., 1991). Similarly, expression of a VL30 reporter gene construct was dependent upon exposure to luteinizing hormone (Schiff et al., 1991). Transcription of the human endogenous retrovirus HERV K has been shown to be stimulated by treatment with steroid hormones (Ono, 1990). Finally, Trolegan and Martin suggested that L1 transcriptional activity in mouse germ cells may be stimulated by androgens produced by nearby cells (Trolegan and Martin, 1995).

To further investigate the influence of hormones on retrotransposition in humans, we explored the effect of steroid hormone-like agents on the regulation of L1Hs transcription. Human cell lines were established that contained stably integrated plasmids with one of two different active L1Hs promoters fused to the LacZ reporter gene. Expression of the L1Hs promoters was then monitored with a chemiluminescent assay for β-galactosidase. These cell lines were used to conduct a thorough examination of the ability of steroid hormones, serum and environmental hormone-like agents (EHAs) to influence L1Hs transcription.

Materials and methods
Plasmids P1LZ and pL1.2LZ

The plasmid P1LZ (Figure 1A) was obtained from Gary D.Swergold at the FDA (Swergold, 1990) and was resequenced. The L1Hs promoter sequence in plasmid P1LZ was derived from a full-length cDNA and has been named Pito. The plasmid pL1.2LZ (Figure 1A) was constructed similar to P1LZ in order to compare the activity of different L1Hs promoters. pL1.2LZ contains the L1Hs promoter named Pita, which is derived from pL1.2A, a plasmid constructed from an active L1Hs element isolated from a L1Hs retrotransposition into the Factor VIII gene of a hemophilia patient (Dombroski et al., 1991). pL1.2A was obtained from Julie McMillan (NIH) and was not resequenced. Both of these promoters are transcriptionally active, however, only Pita is known to be part of an actively retrotransposing element. Both promoters were fused to a LacZ reporter gene to monitor promoter activity using the Galactolight assay for detection of β-galactosidase. The LacZ reporter gene was fused to the L1Hs 3'-UTR for transcription termination and poly(A) addition sequences. Sequence analysis of the Pito and Pita promoters demonstrated the presence of a variety of transcriptional response elements (Figure 1B), including an androgen response element (ARE) identical to AREs found on androgen-responsive genes (Figure 1C).

Cell culture and transfections

Jeg-3, a human choriocarcinoma cell line, was obtained from the American Tissue Culture Collection (C amend, NJ) and was derived from a tumor of placenta or trophoblast tissue. Jeg-3 was grown in minimum Eagle's medium (Life Technologies) with 10% bovine calf serum (HyClone). The clones used in these experiments, W32 and W8, were derived by transfection of Jeg-3 cells with plasmids P1LZ and pL1.2LZ, respectively. Transfections were performed with 20 µg plasmid using the CaPO4 precipitation method. Selection for cells with an integrated plasmid was performed by cotransfection with pSV2Neo (2 µg) and growth in selective medium containing G418 (400 µg/ml). Individual surviving colonies were confirmed as stable transfectants by long PCR (Stratagene) with primers to the L1Hs promoter and the LacZ gene (data not shown). Transient transfections were conducted with Lipofectin (Life Technologies) using the protocol provided by the manufacturer.

Treatment of cells

Testosterone (TT) and dihydrotestosterone (DHT) (Sigma) were dissolved in 100% ethanol and diluted into medium with or without 10% serum to final concentrations of 0.01, 0.5 and 1 µM. In several experiments these androgen-containing media were added to cells that were previously serum starved for 24 h (indicated in the figure legends as ‘in the absence of serum’). In other experiments, the testosterone was added 2–3 days after plating the cells in serum-containing medium. Lysates were collected 12, 18 and 24 h after addition of TT or DHT. Each time point/treatment, lysis preparation and β-galactosidase reading was performed in triplicate. The standard error of the mean for each time point/treatment is indicated as error bars. The percent increase in the expression of β-galactosidase in the treated samples relative to the appropriate control is presented in each figure. The values that are marked in the figures (dark rectangles) are significantly different from the untreated controls at that time point, with P values of ≤0.05 as determined by ANOVA and the ANOVA post hoc tests of Tukey–Kramer (Kramer, 1956; Keselman and Rogen, 1978) and Dunnnett (Dunnnett, 1964). Each of the statistical tests utilized the same sets of data and was conducted using Statview 5.01 for the Macintosh (www.statview.com). The ANOVA test was used to determine whether the means of a set of variables were equal to each other. The Tukey–Kramer and Dunnett’s tests made multiple comparisons to determine if the means of a set of variables were significantly different from each other. The number of experiments are indicated in the figure legends.

The influence of serum on L1Hs expression in the W32 and W8 clones was determined by first growing the clones in medium without serum for 48 h, after which time the medium was removed and replaced with medium containing either 10 or 20% serum. The serum used was either fetal bovine serum (FBS), donor calf serum (DCS) or FBS stripped of polycyclic carbon compounds (STR) using a proprietary dextran sulfate/charcoal treatment (HyClone). STR treatment is reported to produce a mean decrease of 59% for all steroid hormones except progesterone and estradiol, which showed no change (HyClone certificate of analysis, lot no. AGD6463). These changes in hormone levels are typical for serum treated with charcoal and dextran sulfate (Wilkinson, 1993). Lysates were collected 12 or 24 h after incubation with and without serum.

The influence of organochloride pesticides on L1Hs expression was determined on cell cultures grown without serum for 24 h. The organochloride pesticide mix (OPM) (Sigma) contains 17 pesticides: aldrin (0.685 µM), dieldrin (0.656 µM), endrin (0.586 µM), endrin aldehyde (0.656 µM), endosulfan I (0.094 µM), endosulfan II (0.064 µM), endosulfan sulfate (0.591 µM), endosulfan alpha (0.0895 µM), β-BHC (0.859 µM), γ-BHC (0.085 µM), δ-BHC (0.085 µM), 4,4'-DDE (0.786 µM), 4,4'-DDD (0.781 µM), 4,4'-DDT (0.705 µM), heptachlor (0.669 µM), heptachlor epoxide (0.642 µM) and methoxychlor (2 µM). OPM was dissolved in hexane/toluene (1:1), diluted into medium without serum and then added to the cells at concentrations of 1.7, 10 and 50 µM with respect to methoxychlor (Kcelke et al., 1994, 1995a,b). The cells were incubated for 24 h and then lysates were made and stored at –70°C.

The Galactolight assay

The Galactolight assay (Tropix) was used to determine the level of β-galactosidase expression from the L1Hs promoters. The Galactolight assay was conducted as recommended by the manufacturer (Tropix) with the following alterations. The cells were washed with saline A (137 mM NaCl, 5.36 mM KCl, 0.55 mM dextrose, 0.45 mM NaHCO3) instead of phosphate-buffered saline and 50 µl of DNase I (1 µgm/ml) was added to the lysis buffer to make the pellet more compact. The lysates were centrifuged at 14 000 x g for 10 min and the pellet was centrifuged a second time at 14 000 x g for 2 min. The protein concentrations of the final supernatants were determined using the standard Bradford Assay (Bio-Rad) and luminescence was measured using a monolight 2000 (Analytical Luminescence) luminometer.

Results

P1LZ and pL1.2LZ clones

The limited number of cell lines that are known to express L1Hs were derived from a choriocarcinoma, embryonal carcinoma, breast carcinoma and medulloblastoma. The L1Hs-expressing cell lines utilized in the current studies were derived from the choriocarcinoma cell line Jeg-3, which has previously been demonstrated to express L1Hs (Leibold et al., 1990). Jeg-3 was transfected with the P1LZ and pL1.2LZ plasmids (Figure 1A).
a. Plasmids used

![Diagram of P1LZ and pL1.2LZ plasmids]

**Jeg-3 clone w32**

**Jeg-3 clone w8**

b. Promoter information

![Comparison of Pita and Pito promoters]

c. Androgen Response Element comparison

![Comparison of P1LZ, pL1.2LZ, and consensus AREs]

**Fig. 1.** The structure of the P1LZ and pL1.2LZ plasmids. (A) P1LZ has the L1Hs promoter Pito fused to the LacZ gene and is contained within the Jeg-3 clone W32. pL1.2LZ has the L1Hs promoter Pita fused to the LacZ gene and is contained within the Jeg-3 clone W8. (B) Comparison of sequences of the L1Hs promoters Pito and Pita. The black and white areas show sequence identity and divergence, respectively. The sequence comparison reveals putative transcriptional response elements for androgens (androgen, progesterone, glucocorticoid) (large open triangle), UV/phorbol ester (AP-1) (black triangle) and heat shock (open arrows). (C) Comparison of putative AREs in the two L1Hs promoters with the consensus ARE (consensus) (Carson-Jurica et al., 1990) and the prostate-specific antigen ARE (Prostate Ag) (Cleutjens et al., 1997), prostate-specific antigen androgen response region (ARR) (Cleutjens et al., 1996), prostate-specific antigen low affinity ARE (low affinity) (Cleutjens et al., 1997), Probasin gene ARE (probasin) (Claessens et al., 1996), androgen receptor ARE (AR) (Dai and Burnstein, 1996) and androgen-regulated murine epididymal protein ARE (arMEP24) (Ghyselinck et al., 1993).
The concentrations (0.01 and 1 μM) exposed to TT in the presence of 10% serum (Figure 3A). This steroid hormone receptor-binding site, which is exactly the same as the progesterone and glucocorticoid response element (Carson-Jurica et al., 1990), conveys androgen responsiveness to genes. We therefore determined the effect of different concentrations of two major androgens, TT and DHT, on β-galactosidase expression in the two Jeg-3 clones, W32 and W8.

The Jeg-3 clone W32, containing plasmid P1LZ, was first exposed to TT in the presence of 10% serum (Figure 3A). The concentrations (0.01 and 1 μM) reflect the range of

concentrations examined for androgen-responsive genes (Wong et al., 1995). The concentrations of TT tested produced a small, but statistically significant, increase in β-galactosidase activity at 12 h (25%). Activity was also increased at 0.01 and 1 μM after 18 h (15 and 20%), although only at 1 μM was the increase statistically significant. After 24 h the cells exposed to 0.01 μM TT increased their β-galactosidase activity (~30%), as did those exposed to 1 μM TT (~50%). Notably, only the highest concentration of TT (1 μM) was able to produce a small but statistically significant increase in β-galactosidase activity in clone W32 at 24 h in the absence of serum (Figure 3B). Unlike clone W32, no effect of TT was seen in clone W8 in the absence of serum (Figure 3C). An increase in β-galactosidase activity was observed in the presence of serum, however, this increase was not significantly different from that observed at 0 h and therefore appeared to be due to the serum alone. These results suggest that TT can induce small increases in transcription from a subset of L1Hs promoters.

**The influence of serum on L1Hs expression**

Assessment of the effect of androgens on L1Hs transcriptional activity involved incubating the cells both with and without serum before treatment with the androgen of interest. Serum starvation was conducted because serum is known to contain a very large array of components, including a multitude of steroid hormones, such as TT (MacLeod and Drummond, 1980). In addition to growth factors and steroid hormones, serum also contains steroid hormone-binding proteins which facilitate hormone traversal of the plasma membrane (Fortunati, 1999).

Because the amounts of steroid hormones and growth factors may vary in FBS and DCS, both were examined for their ability to induce L1Hs promoter activity (Figure 2A and B). In addition, STR, which was treated with charcoal/dextran to remove steroid hormones, was also tested (Figure 2A and B). The data from these experiments demonstrate that all three types of serum can reproducibly increase the levels of β-galactosidase activity in both the W32 and W8 clones. The largest increase was apparent 24 h after addition of all three types of serum, although an increase was also observed after 12 h. With clone W32 the increase at 12 h was statistically significant with all three types of serum at both 10 and 20%, although with clone W8 the increase at 12 h was not statistically significant with FBS and STR at 20%. Notably, there was little significant difference in the elevation of activity in the FBS-treated cells compared to that in the DCS-treated cells, although in clone W8 there was a difference between the effect of 20% DCS and 20% FBS at both 12 and 24 h (Figure 2B). STR also increased L1Hs activity in both the W32 and W8 clones (Figure 2A and B), suggesting that the stripping process did not remove the stimulatory molecule(s) responsible for induction. The combined observations of these studies indicates that serum contains a factor(s) that can increase transcription from the two different L1Hs promoters by >2-fold.

**The influence of androgens on L1Hs expression**

Analysis of the L1Hs promoter sequences in the P1LZ and pL1.2LZ plasmids revealed the presence of a putative ARE that matches the human consensus ARE sequence at 12 of 15 bp (Figure 1C). This steroid hormone receptor-binding site, which is exactly the same as the progesterone and glucocorticoid response element (Carson-Jurica et al., 1990), conveys androgen responsiveness to genes. We therefore determined the effect of different concentrations of two major androgens, TT and DHT, on β-galactosidase expression in the two Jeg-3 clones, W32 and W8.

The Jeg-3 clone W32, containing plasmid P1LZ, was first exposed to TT in the presence of 10% serum (Figure 3A). The concentrations (0.01 and 1 μM) reflect the range of

**Fig. 2.** The effect of serum on L1Hs expression in clones W32 and W8. (A) β-Galactosidase activity was determined in extracts of clone W32 after treatment with either 10 or 20% FBS (F), DCS (D) or STR (S) for 12 or 24 h (three experiments). (B) β-Galactosidase activity in extracts of clone W8 after treatment with either 10 or 20% FBS, DCS or STR for 12 or 24 h (two experiments). The values that represent a statistically significant increase compared with the untreated control at that time point are indicated by dark rectangles (see Materials and methods).
The androgen DHT was also tested on both the W32 and W8 clones. In the absence of serum, treatment of the W32 clone with DHT resulted in a small but significant 25% increase in β-galactosidase activity at 1 µM after 18 h (Figure 4A). However, no significant increase in L1Hs promoter activity was seen at other concentrations or times without serum. W32 cultures treated with DHT in the presence of serum showed an increase in β-galactosidase activity at 0 h, typical of cultures treated with serum alone. W32 cultures treated with 0.5–1 µM DHT in the presence of serum produced an additional 25–35% increase in the level of β-galactosidase activity after 24 h, suggesting an additive effect between serum and DHT (Figure 4A).

Similar to clone W32, when clone W8 was exposed to DHT in the absence of serum there was an 30% increase in β-galactosidase activity at 1 µM, which stayed constant at 18 and 24 h (Figure 4B). However, treatment of clone W8 with DHT produced no significant increase in β-galactosidase activity above that caused by serum alone. Thus, both promoters showed small increases in β-galactosidase activity after treatment with 1 µM DHT in the absence of serum, although only the promoter in the P1LZ plasmid produced a small increase in activity after DHT treatment in the presence of serum.

**Treatment of cells with EHAs**

The presence of the putative androgen response element in the L1Hs promoter and the apparent response of the L1Hs promoter to androgens and serum suggests that EHAs may influence L1Hs expression. EHAs are synthetic compounds found in the environment and can act as either agonists or antagonists to androgens found in mammals. An important source of EHAs in the environment is organochloride pesticides and we therefore tested the ability of a complex mixture of 17 organochloride pesticides (OPM) to stimulate expression of the L1Hs promoter. Various concentrations (1.7, 10 and 50 µM) of OPM were tested on the W8 clone containing plasmid pL1.2Lz (Figure 5). In the absence of serum, OPM at a concentration of 1.7 µM produced an increase of 30% in β-galactosidase activity at 24 h. OPM at 10 µM produced an increase of ~50% in β-galactosidase activity at 24 h, while 50 µM OPM produced a nearly 2.5-fold increase at 24 h. However, similar to treatment with DHT in clone W8, treatment with OPM did not appear to increase β-galactosidase activity above that seen with serum alone. Consistent with the effects of serum alone, an increase in β-galactosidase activity was seen at 0 h, however, no additional increase was observed in cultures treated with OPM at 24 h. Thus, organochloride pesticides appear to slightly increase transcriptional activity of the L1Hs promoter, but do not appear to produce additive effects above that seen with serum alone.

**Discussion**

The results presented here demonstrate a small but significant increase in the level of β-galactosidase activity from a LacZ gene under the control of L1Hs promoters following treatment with serum, androgens and EHAs. It is likely that this increase in β-galactosidase activity is a result of changes in transcription...
activity from the L1Hs promoters, although it cannot be ruled out that changes in the turnover rate of mRNA or protein, or changes in translation of the protein are involved. Even small changes in transcription could have a significant effect on retrotransposition, because the transcriptional activity of retrotransposons is thought to be the rate limiting step in retrotransposition (Skowronski et al., 1988; Mathias and Scott, 1993). In fact, it is likely that only small increases in transcription would occur, since there would be strong evolutionary selection against high levels of expression of retrotransposons due to the mutagenic properties of retrotransposition. Previous studies have shown that relatively small increases in expression of retrotransposons can have significant effects on retrotransposition frequency. The influence of small increases in transcription on retrotransposition can be seen in I–R hybrid dysgenesis in D. melanogaster. I–R hybrid dysgenesis involves the transient elevation of I element retrotransposition in the germline of SF female progeny resulting from crosses of inducer and reactive strains. The SF females are sterile, due to high frequencies of I element-mediated insertions and rearrangements (Lim and Simmons, 1994). de La Roche Saint Andre and Bregliano (1998) investigated whether I element transcription correlated with reactivity and retrotransposition frequency. They found that the amount of full-length I element mRNA in SF females from reactive mothers is only 5-fold above the amount of I element mRNA from non-dysgenic progeny. These results indicate that the highest known in vivo retrotransposition frequency is mediated by only a 5-fold increase in transcription over background.

The results presented here suggest that two different L1Hs promoters are subject to the stimulatory effects of various kinds of serum, with the largest effect seen after 24 h (Figure 2). This implies that human blood contains factors that can stimulate active L1Hs promoters in a L1Hs-expressing cell line. L1Hs element-expressing germ cells might therefore be constantly exposed to serum components that stimulate L1Hs expression. These results also demonstrate that previous experiments involving L1Hs transcription in cells grown in 10% serum were conducted in the presence of a L1Hs transcriptional stimulator. Tchenio et al. (1993) reported that a >3-fold increase in mouse LINE retrotransposition frequency could be caused by serum starvation. In this protocol, serum was reduced from 5 to 0.5% over the course of 24 h, at which time serum was again added to the medium. Since it is likely that the re-addition of serum, not serum starvation, stimulated L1Md expression, the interpretation that serum starvation stimulates LINE expression should be modified.

An interesting observation of the work presented here was that STR produced an increase in β-galactosidase activity that was equivalent to regular FBS or DCS with both L1Hs promoters (Figure 2). One possible explanation for this result is that the inducing steroid hormone may not be an androgen, but perhaps...
some other steroid hormone. In this regard, it is interesting to note that the concentrations of some steroid hormones (progesterone, thyroid stimulating hormone and estriol) remained unchanged after the stripping process (Wilkinson, 1993) and that the ARE is identical to the progesterone response element. An alternative possibility is that the steroid hormones most effective at transversing the plasma membrane and stimulating transcription may be that fraction bound to plasma steroid hormone-binding proteins. Therefore, stripping the serum may remove free steroid hormones, but not affect the fraction that is complexed with binding proteins.

In addition to serum, the androgens TT and DHT produced a small increase in β-galactosidase activity, and some differences were noted in the response of the two different clones (Figures 3 and 4). Exposure of clone W32 to both TT and DHT stimulated a small increase in β-galactosidase activity in the presence of serum. DHT also produced a small increase in β-galactosidase activity in clone W32 without serum present (Figure 4A). With serum present the maximum increase was 63% at 1 µM TT at 24 h, while without serum the maximum was 32% at 0.5 µM TT at 18 h. Similar to the studies with serum, there was no dose–response effect. The reason for this is unclear, but may reflect the complexity of the pathways involved in the cellular response to hormones. The results obtained with the androgens TT and DHT in clone W8 were different from those obtained with clone W32. In clone W8 TT produced no significant increase in β-galactosidase activity above that seen in the control cells with or without serum present (Figure 3C). However, when clone W8 was treated with 1 µM DHT in the absence of serum, β-galactosidase activity increased 22% at 18 and 24 h (Figure 4B). Despite this fact, DHT produced no significant increase in β-galactosidase activity in clone W8 treated with serum. Thus, unlike clone W32, clone W8 showed no response to TT with or without serum and no response to DHT in the presence of serum. Since the two promoters tested have exactly the same putative ARE, the differences in the response of the two clones may be due to differences in the other parts of the promoter. The type of androgen may also be important, because DHT has a greater affinity for (and a slower rate of dissociation from) the androgen receptor when compared with TT (Zirkin, 1994). Alternatively, this variability could reflect differences in the two cell clones or the integration sites for the plasmid sequences.

In clone W32 treatment with TT or DHT in the presence of serum resulted in a small increase in β-galactosidase activity above that caused by the presence of serum alone (Figures 3A and 4A). These results suggest that the responses to serum and androgens are additive with the Pito promoter. Thus, the factors in serum responsible for altered expression of the L1Hs promoter may not be androgens, consistent with the observation that STR gave results that were similar to FBS and DCS (Figure 2). However, in contrast to clone W32, the response of clone W8 to serum and androgens was not additive, in that it showed a response to DHT in the absence of serum but no response to DHT in the presence of serum (Figure 4B). Similar to the different responses of these two promoters to TT, these results suggest a fundamental difference in the response of the two different L1Hs promoters to androgens.

The observation that OPM produced a small increase in β-galactosidase activity (Figure 5) suggests that, similar to a previous study studying a different end-point (DeMarini et al., 1996), one or a few of these compounds may have an effect on a cellular steroid hormone receptor(s) binding to the putative ARE in the L1Hs promoter. Some pesticides have previously been shown to act as either agonists or antagonists of steroid hormones (Kelce et al., 1994; Wong et al., 1995; Danzo, 1997; Gaido et al., 1997). Many of the 17 components in OPM (see Materials and methods) are known to have reproductive effects (Chatterjee et al., 1988; Cummings and Laskey, 1993; Cassidy et al., 1994) and have interactions with various steroid hormone receptors (Kelce et al., 1995; Danzo, 1997). Thus, if L1Hs transcription takes place via a steroid hormone receptor-mediated pathway, these compounds might act as antagonists or agonists. An effect on L1Hs retrotransposition may therefore be a mechanism for the mutagenic effects of some of the agents in OPM.

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References

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