In vitro manipulation of early mouse embryos induces HIV1-LTRlacZ transgene expression

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SUMMARY

We report here that the transcriptional activity of early mouse embryos is affected by their manipulation and culture in vitro, using transgenic embryos that express the reporter gene lacZ. We examined the pattern of expression of the lacZ gene fused to the human immunodeficiency virus type 1 long terminal repeat during the preimplantation stages. Transgene expression is induced as early as the two-cell stage in embryos developed in vitro, while there is no constitutive expression at the same stage in embryos developed in vivo. We have established a relation between this inducible expression occurring in vitro and an oxidative stress phenomenon. Indeed, when the culture medium is supplemented with antioxidants such N-acetyl-cysteine or CuZn-superoxide dismutase the transgene expression is markedly reduced. We also present evidence that the transgene expression in vitro coincides with the onset of the embryonic genome activation as attested by the synthesis of the 70x10^3 M_r protein complex. Therefore, this transgene expression could prove to be a useful tool in our understanding of the molecular mechanisms involved in this crucial developmental event.

Key words: embryonic transcription, genome activation, oxidative stress, HIV1-LTR, transgenic mice

INTRODUCTION

In many species, including man, fertilized oocytes can develop into blastocysts when cultured in vitro in chemically defined media (Bavister, 1987; Kishi et al., 1991). Despite the fact that some embryos exposed to in vitro conditions can develop to term after transfer to recipient females, embryo development is generally delayed (Wright and Bondioli, 1981). Since most of our knowledge concerning preimplantation development at the molecular level relies on the ability of embryos to develop normally under in vitro conditions, the environmental variables that may potentially influence preimplantation development have to be carefully considered. Amongst these variables, the oxygen tension between the in vitro and in vivo environments is drastically different. Indeed, oxygen levels reported in the oviduct or uterus of various mammals are approximately 5-8% (Mastroianni and Jones, 1965; Yochim and Mitchell, 1968; Mass et al., 1976) compared with 20-21% in air. It has been reported for several mammalian species that reduced O_2 levels (5%) have beneficial effects on embryonic development (Bavister, 1987; Umaoka et al., 1992). In particular, the reduction of oxygen tension overcomes the stage-specific developmental block frequently observed in vitro (Thompson et al., 1990; McKiernan and Bavister, 1990; Batt et al., 1991; Kishi et al., 1991). Furthermore, several studies in mouse embryos indicate that protection against oxidative stress, mediated via a potent scavenger of oxygen radicals, (the enzyme superoxide dismutase : SOD), significantly improves embryonic development in vitro (Umaoka et al., 1991, 1992; Legge and Sellens, 1991; Nonogaki et al., 1991; Noda et al., 1991). Additionally, it has been recently reported that exposure of embryos to in vitro conditions clearly affects the intracellular level of H_2O_2. Indeed, when embryos were cultured in vitro from the one-cell or early two-cell stage, the production of hydrogen peroxide increased transiently at the two-cell stage, whereas this phenomenon was not observed in vivo (Nasr-Esfahani et al., 1990).

Since oxygen-free radicals can elicit the transcriptional activation of a set of genes encoding stress proteins (Keyse and Tyrrell, 1989; Donati et al., 1990), these results prompted us to test whether the in vitro culture of early embryos might affect their transcriptional activity. It has been reported that the Human Immunodeficiency Virus type 1 long terminal repeat (HIV1-LTR) is highly susceptible to different stresses (Valerie et al., 1988; Geelen et al., 1988; Stein et al., 1989; Stanley et al., 1990; Legrand-Poels et al., 1990; Schreck et al., 1991). We and others have previously demonstrated its activation by UV light in transgenic mice (Cavard et al., 1990; Frucht et al., 1991; Morrey et al., 1991). In our transgenic animals, the HIV1-LTR promoter directs the expression of the reporter gene lacZ (Cavard et al., 1990). We report here that the HIV1-
LTRlacZ transgene expression is observed in early embryos developed in vitro and coincides with zygotic genome activation. This expression does not appear in vivo and is probably induced in vitro by oxidative stress. Thus, this transgene expression analysis has allowed us to demonstrate that the pattern of gene expression in early embryos cultured in vitro does not necessarily reflect in vivo events.

MATERIALS AND METHODS

Recovery of transgenic embryos

Mice carrying the HIV-1-LTRlacZ transgene were generated as described previously (Cavard et al., 1990). Two homozygous transgenic lines, 10 and 17, were used in this study. They presented the same pattern of expression in adult mice and in embryos during postimplantation development. In all experiments (except where mentioned), transgenic embryos were obtained from adults (C57BL/6J × DBA2) F1 females mated with homozygous transgenic males. Female mice were injected i.p. with 5 I.U. of pregnant mare’s serum gonadotrophin (PMS; Intervet) and 5 I.U. of human chorionic gonadotrophin (hCG; Intervet) at an interval of 48 hours to induce superovulation.

The transgene expression was analysed either in freshly recovered embryos or in embryos cultured in vitro. In the first case, two-cell embryos, four-cell embryos, morula and blastocysts were recovered at 38-44 hours, 50-53 hours, 68-72 hours and 90-94 hours post-hCG, respectively. In the second case, fertilized eggs were released from the oviduct 20 hours post-hCG. After removal of cumulus cells with hyaluronidase (0.5 mg/ml), eggs were washed in M2 medium (Pratt, 1987), then incubated in M16 medium (Pratt, 1987) or T6 medium (Wood et al., 1987) at 37°C under 5% CO2 for 2 minutes at room temperature in M2 medium supplemented with 7% ethanol (Barton et al., 1987). Eggs were incubated in M16 at 37°C under 5% CO2 and in air and, after 4-5 hours in culture, the haploid eggs that had extruded the second polar body and had a visible pronucleus were selected and kept in culture.

Preparation of parthenogenetic embryos

Ovulated eggs were collected from superovulated homozygous transgenic females 17-18 hours after hCG injection in M2 medium. The cumulus cells were dispersed by incubation in 0.5 mg/ml hyaluronidase (0.5 mg/ml), eggs were washed in M2 medium (Pratt, 1987), then incubated in M16 medium (Pratt, 1987) or T6 medium (Wood et al., 1987) at 37°C under 5% CO2 in air (M2, M16 and T6 media contain BSA at 4 mg/ml). In some experiments, transgenic eggs were cultured after recovery under a lower oxygen tension, in M16 medium gassed with a mixture of 5% O2, 5% CO2 and 90% N2.

Preparation of parthenogenetic embryos

E. coli β-galactosidase assay and statistical analysis

The procedure for in situ detection of E. coli β-galactosidase using X-gal staining was as previously described (Sanes et al., 1986). To quantify E. coli β-galactosidase activity in eggs and embryos, a fluorogenic substrate of β-galactosidase, 4-methylumbelliferyl-β-D-galactoside (MUG) (Sigma Chemical Co.) was used. Briefly, 10 eggs or embryos were pooled in 5 µl of M2 medium and incubated for 16 hours at 37°C in 100 µl reaction mixture containing 0.5 mM MUG, 6 mM NaHPO4, 4 mM NaH2PO4, 1 mM KCl, 0.1 mM MgSO4 pH 7, 0.3% β-mercaptoethanol, and 0.5% Triton X-100. The reaction was stopped by adding 400 µl of 100 mM glycine pH 10. Quantitation of enzyme activity was obtained by measuring methylumbelliferone fluorescence (emission at 446 nm) by a spectrofluorimeter (Perkin Elmer LS-2 fluorimeter). The values were arbitrarily expressed in fluorescence units per 10 eggs or embryos (f.u.). Data were analyzed after logarithmic transformation using one-way analysis of variance. After verification of homogeneity of the variances by Bartlett’s test, the means were compared using Tukey’s and Fisher-Snedecor tests. In all comparisons, P<0.001.

Embryo labelling and one-dimensional denaturing polyacrylamide gel electrophoresis

Eggs were cultured for 3 hours in [35S]methionine (1 mCi/ml) M16. Eggs were then washed three times with protein-free M2 and placed in 15 μl of SDS sample buffer (Laemmli, 1970), boiled for 5 minutes and stored at −80°C. PAGE was performed as previously described in Vernet et al. (1992).

Separation of polypeptides by SDS-polyacrylamide gel electrophoresis in two dimensions.

Groups of 30 embryos were labelled by a 3 hour incubation in [35S]methionine (1 mCi/ml) diluted in M16 medium. They were rinsed three times in 0.9% NaCl containing 0.4% PVP 40 (polyvinylpyrrolidone) and stored at −20°C. N-ethylmaleimide (NEM) was purchased from Sigma Chemical Co. A 1 M stock solution in PBS was freshly prepared and NEM was added to a final concentration of 20 mM in M16 medium. The medium was equilibrated with NaOH to pH 7.5-7.6. CuZn-super oxide dismutase (CuZn-SOD) from bovine erythrocytes (4200 U/mg) was purchased from Sigma Chemical Co. The enzyme was diluted in M16 medium to a final concentration of 6000 U/ml. Before use the medium containing enzyme was pre-equilibrated (20 minutes at 37°C in 5% CO2 in air).

RESULTS

The characteristics of HIV-1-LTRlacZ transgene expression are those expected for a marker of embryonic genome activation

Expression of the HIV-1-LTRlacZ transgene in early mouse embryos was initially assayed in situ using X-gal staining.Embryos cultured in vitro were tested for expression at different stages (one-cell, two-cell and four-cell stages). Experiments were carried out on two transgenic lines (10 and 17). In preimplantation mouse embryos, the transgene expression appeared as early as the two-cell stage and persisted at least until the four-cell stage for the two lines analysed (Fig.1A,B). This expression was independent of the parental origin of the transgene, since the same results were obtained for transgenic embryos derived from reciprocal crosses (data not shown).

It is known that in mouse embryos the embryonic genome...
is activated at the two-cell stage, as shown by modifications in the pattern of protein synthesis (Sawicki et al., 1981; Flach et al., 1982; Bensauè et al., 1983; Bolton et al., 1984). In particular, the appearance of a complex of $70 \times 10^3 M_r$ proteins whose synthesis is inhibited by $\alpha$-amanitin is usually taken as a marker for transcriptional activation of the zygotic genome (Bensauè et al., 1983; Poueymirou and Schultz, 1987, 1989; Conover et al., 1991; Latham et al., 1992). This $70 \times 10^3 M_r$ protein synthesis is also independent of both DNA synthesis and cell division (Howlett, 1986a).

To assess any correlation between this early transgene expression and zygotic genome activation, we examined the transgene expression in comparison with the $70 \times 10^3 M_r$ protein synthesis. To this end, transgenic embryos recovered at the one-cell stage were incubated in presence of $\alpha$-amanitin (11 $\mu$g/ml). This inhibitor of RNA polymerase blocks the activation of the embryonic genome and consequently arrests the embryonic development at the two-cell stage. Under such conditions, the transgene expression was no longer observed even after 48 hours in culture, while the proportion of untreated embryos expressing lacZ reached 86% (Table 1). The pattern of protein synthesis confirmed the absence of the $70 \times 10^3 M_r$ protein complex in transgenic eggs exposed to $\alpha$-amanitin and labelled for 3 hours (47-50 hours post-hCG) with $^{35}$S-methionine (Fig. 2 lane 1). On the contrary, in the absence of the drug and for the same labelling period, the $70 \times 10^3 M_r$ protein complex was clearly present (Fig. 2 lane 2).

To establish whether transgene expression is independent of both DNA replication and cell division, transgenic eggs were cultured after recovery in the presence of aphidicolin (2 $\mu$g/ml), an inhibitor of DNA polymerase. After 24-28 hours in culture, transgenic embryos arrested at the one-cell stage by aphidicolin expressed the transgene to the same extent as their control counterparts, which have reached the two-cell stage (Table 1). The pattern of protein synthesis of transgenic eggs exposed to aphidicolin was compared to that of untreated embryos. When embryos were labelled at the one-cell stage (before activation of the zygotic genome) for 3 hours (25-28 hours post-hCG) with $^{35}$S-methionine, the $70 \times 10^3 M_r$ proteins were absent in both untreated and embryos exposed to aphidicolin (Fig. 3, lanes 1 and 2). In contrast, when embryos were labelled after the onset of genome activation between 44-47 or 47-50 hours post-hCG, the $70 \times 10^3 M_r$ proteins were clearly detected in control embryos (Fig. 3, lanes 3 and 5), although slightly reduced in embryos arrested at the one-cell stage by aphidicolin (Fig. 3, lanes 4 and 6). To support further the hypothesis that transgene expression coincided with the onset of transcriptional activation, we examined whether the transgene could be activated in parthenogenetic embryos. Indeed, as demonstrated by $70 \times 10^3 M_r$ protein synthesis, the female genome can be activated independently of the male genome (Barra and Renard, 1988). Parthenogenetic embryos were obtained after ethanol activation of ovulated transgenic oocytes derived from females of either line 10 or 17. The lacZ gene expression was detected in the parthenogenetic embryos that had undergone cleavage 48 hours post-hCG. This expression was no longer observed when activated oocytes were incubated with $\alpha$-amanitin (11 $\mu$g/ml, after ethanol activation) indicating that transgene expression was dependent on the transcriptional activity (Table 2).

Thus, HIV1-LTRlacZ expression appears as early as the two-cell stage and is dependent on transcriptional activity.

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**Table 1. The HIV1-LTR lacZ transgene expression requires transcription but does not necessitate the completion of the first DNA replication**

<table>
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<th>Percentage of $\beta$-gal$^+$ embryos (total number)</th>
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<tr>
<td>Embryos exposed to $\alpha$-amanitin (arrested at the two-cell stage)</td>
<td>0% (43)</td>
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<tr>
<td>Embryos exposed to aphidicolin (arrested at the one-cell stage)</td>
<td>84% (52)</td>
</tr>
<tr>
<td>Control embryos</td>
<td>86% (54)</td>
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Pronuclear transgenic eggs were incubated in the absence (control embryos) or in presence of either 2 $\mu$g/ml aphidicolin, which blocks both replication and the first segmentation of eggs, or 11 $\mu$g/ml $\alpha$-amanitin, which blocks the activation of the embryonic genome. Control embryos and those exposed to $\alpha$-amanitin were X-gal stained after 24-28 hours in culture. Embryos exposed to aphidicolin were X-gal stained after 47-50 hours in culture. The results are expressed as the percentage of $\beta$-galactosidase-positive embryos and the total number of embryos tested is indicated in brackets.

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Fig. 1. Expression of HIV1-LTRlacZ transgene in early embryos.

Fertilized transgenic eggs were obtained after mating wild-type females with transgenic males either hemizygous (white bars) or homozygous (hatched bars). Embryos were X-gal stained at the one-cell, two-cell and four-cell stages, 26, 44, and 68 hours post-hCG injection respectively. The result presented for each stage is the percentage of embryos that were $\beta$-galactosidase positive. The total number of embryos analyzed is indicated above each column. (A,B)
However expression is independent of both DNA replication and the first round of cytokinesis and occurs in parthenogenetic embryos. Since the synthesis of the $70 \times 10^3 M_r$ proteins shows a similar pattern, we propose that this transgene expression may be also considered as a marker for embryonic genome activation.

Exposure of embryos to in vitro conditions induces HIV1-LTRlacZ transgene expression

To test whether the rise in $H_2O_2$ level at the two-cell stage could affect the expression of the lacZ gene in HIV1-LTRlacZ transgenic mouse embryos, we compared the pattern of transgene expression of mouse embryos developed in vitro with those developed in vivo, since this increase appears strictly under in vitro conditions (Nasr-Esfahani et al., 1990). β-galactosidase activity was assayed at different stages of preimplantation development. At all stages examined, the basal expression measured in freshly recovered transgenic embryos did not differ from the background levels obtained with nontransgenic embryos (Fig. 4). These results indicated that preimplantation transgenic embryos developed in the female reproductive tract showed no constitutive expression of the lacZ gene. In contrast, and as previously shown using X-gal staining, when embryos were recovered at the pronuclear stage and allowed to develop in vitro, the transgene expression was clearly detected. This quantitative analysis showed that expression of the HIV1-LTRlacZ gene occurs only transiently through the second and third cell cycles. These results suggest that exposure of embryos to in vitro conditions during recovery and handling induces transgene expression. We could modulate the magnitude of transgene activation in vitro by altering culture conditions, in particular the culture medium. When M16 medium was replaced by an optimal culture medium such as T6, the transgene expression in four-cell embryos was significantly reduced in T6 medium (662 f.u.±130) versus M16 medium (2088 f.u.±214). In contrast, at the same stage when we lowered the oxygen tension from 20 to 5%, the transgene expression was not significantly different (1311 f.u.±416) compared with the control group incubated under 5% CO$_2$ in air (2088 f.u.±214).

**Table 2. Expression of HIV1-LTRlacZ in transgenic oocytes activated parthenogenetically by ethanol**

<table>
<thead>
<tr>
<th>LINE 10</th>
<th>(-) α-amanitin</th>
<th>(+) α-amanitin</th>
<th>nd</th>
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<tr>
<td>LINE 17</td>
<td>16% (44)</td>
<td>0% (21)</td>
<td></td>
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Homozygous transgenic oocytes (lines 10 and 17) activated parthenogenetically by ethanol were incubated in absence or in presence of α-amanitin (11 µg/ml) referred as (-) α-amanitin, and (+) α-amanitin respectively. Transgene expression was determined by X-gal staining 48 hours post-hCG in divided parthenogenetic eggs. The results are expressed as the proportion of β-galactosidase-positive embryos and the total number of embryos tested is indicated in brackets. (nd, not determined).
The induction of embryonic transcription in vitro

The expression of the HIV1-LTR lacZ is inhibited by N-acetyl-L-cysteine or CuZn-superoxide dismutase

The correlation between the inducible expression of the HIV1-LTR lacZ hybrid gene in early embryos developed in vitro and the increase in hydrogen peroxide concentration previously reported at the same stages (Nasr-Esfahani et al., 1990), suggested that transgene expression could reflect the activation of the HIV1-LTR by oxidative stress. To test this hypothesis, we examined the ability of an antioxidant, N-acetyl-L-cysteine (NAC) to inhibit transgene expression. Transgenic embryos recovered at the pronuclear stage were incubated in M16 medium supplemented with 20 mM NAC. The β-galactosidase activity was assayed at the four-cell stage where the highest activity was observed (Fig. 4). As shown in Fig. 5, the HIV1-LTR lacZ hybrid gene expression was significantly reduced in the presence of NAC.

Direct involvement of oxygen radicals in the HIV1-LTR activation was investigated by assessing the protective effect of the CuZn-SOD enzyme on this phenomenon. This enzyme is a key member of the enzymatic system developed by living cells as protection against free radicals. It catalyzes the dismutation of the superoxide anion ($O_2^-$) to hydrogen peroxide ($H_2O_2$) (McCord and Fridovich, 1969; Fridovich, 1972). Pronuclear embryos were incubated in the presence of bovine CuZn SOD enzyme (6000 U/ml) and were cultured until the four-cell stage before being processed for β-galactosidase dosage. Under these conditions, we observed a striking inhibitory effect of CuZn-SOD on transgene expression similar to the absence of an oxidant. Under these conditions, we observed a striking inhibitory effect of CuZn-SOD on transgene expression. Direct inhibition by CuZn-SOD on transgene expression is still observed, and the induction of the enzyme gene is probably due to oxidative stress.

The CuZn-SOD does not affect synthesis of HSP70, a hallmark of the transcriptional activation of the genome

The comparison of the HIV1-LTRlacZ expression in preimplantation mouse embryos developed in vivo and in vitro has allowed us to demonstrate that the manipulation of eggs in vitro is sufficient to induce gene activation, probably via oxidative stress. In addition, this transgene expression coincides with the onset of transcriptional genome activation as demonstrated by the synthesis of the 70×10^3 $M_r$ protein complex. Using two-dimensional gel electrophoresis and comparative peptide mapping, two proteins of this complex have been identified as heat-shock proteins including both the heat-inducible and the cognate 70×10^3 $M_r$ heat-shock proteins (Bensaude et al., 1983; Mezger et al., 1991) referred to here as HSP 70. Recently three other members of the 70×10^3 $M_r$ protein complex distinct from HSP 70 have been characterized and presented as the major products of zygotic gene activation (Conover et al., 1991).
From these observations, it is possible that the synthesis of HSP 70 might also be stress-induced, resulting from the manipulation of embryos in vitro. Indeed, promoters of genes encoding HSP proteins are highly susceptible to induction by stress, including oxidative stress (Donati et al., 1990), as described for the HIV1-LTR (Legrand-Poels et al., 1990).

To test this hypothesis, the effect of the CuZn-SOD on the HSP70 synthesis was examined. Transgenic eggs were incubated (from 25 hours post-hCG) in the presence of bovine CuZn-SOD enzyme (6000 U/ml). Embryos exposed to CuZn-SOD and their untreated counterparts were placed in a labelling medium containing $^{35}$S]methionine at 45 hours post-hCG for 3 hours. Two-dimensional gel electrophoretic analysis of proteins synthesized in embryos at the two-cell stage was performed. A similar analysis was carried out on embryos labelled at the one-cell stage to control the absence of HSP 70 before genome activation (Fig. 6A). Radioactive spots corresponding to HSP 70 (Bensaude et al., 1983) were present at the two-cell stage in both control embryos (Fig. 6B) and CuZn-SOD-incubated embryos (Fig. 6C), demonstrating that this enzyme does not affect the synthesis of HSP 70.

In conclusion, as measured by an attempt to inhibit HSP 70 synthesis by the addition of CuZn-SOD, there is no direct correlation between this synthesis at the onset of zygotic genome activation and an oxidative stress phenomenon.

**DISCUSSION**

In this paper, we present evidence that exposure of mouse embryos to in vitro conditions has consequences on transcriptional activity. Previous studies have already shown that there is a transient increase of $\text{H}_2\text{O}_2$ levels when embryos were cultured in vitro, although no equivalent $\text{H}_2\text{O}_2$ increase was seen at the same stage in embryos recovered directly from the female reproductive tract (Nasr-Esfahani et al., 1990). Using transgenic embryos carrying the HIV1-LTR$\text{lacZ}$ transgene, we observed that, while there is no constitutive expression of this transgene in vivo, its expression is induced when embryos are cultured in vitro. Further analysis of transgene expression in vitro has allowed us to propose that this expression is probably induced by oxidative stress. Several arguments support this interpretation. Firstly, in all cases where an increase in $\text{H}_2\text{O}_2$ has been reported, a concomitant transgene expression has been observed: at the two-cell stage in embryos obtained from either sperm-activated or ethanol-activated eggs, and also in one-cell embryos arrested by aphidicolin (Nasr-Esfahani et al., 1990; Nasr-Esfahani and Johnson, 1991). Secondly the reducing agent NAC or the radical scavenger enzyme CuZn-SOD exert an inhibitory effect on transgene expression. Although we have shown that modifying the culture medium can modulate the magnitude of transgene activation, the lowering of oxygen tension was ineffective on transgene expression. Therefore we suspect that 5% $\text{O}_2$ is sufficient to induce an oxidative stress. This hypothesis is supported by results previously obtained by Nasr-Esfahani et al., (1992) who demonstrated that decreasing oxygen tension (5% $\text{O}_2$) did not affect the $\text{H}_2\text{O}_2$ rise observed in embryos developed in vitro.

We have also shown that the $\alpha$-amanitin-inhibited transgene expression at the two-cell stage is correlated to the activation of the zygotic genome. In mouse embryos, genome activation takes place at the two-cell stage and is
usually considered to occur in two phases. Early in two-cell stage (G1 phase) a set of $70 \times 10^3 Mr$ proteins is synthesized (minor activation) followed later (G2 phase) by the synthesis of many proteins (major activation). The major activation is affected by the suppression of the first DNA replication whereas the minor activation is not. Since we demonstrated that the transgene is efficiently expressed in embryos arrested at the one-cell stage by an inhibitor of DNA replication, transgene expression appears to be related to the minor activation. Further supporting evidence for this correlation comes from the observation that transgene expression was also detected in parthenogenetically activated oocytes. It has already been reported that the female genome can be activated in such embryos, as attested by the $70 \times 10^3 Mr$ protein synthesis taken a marker for activation of the embryonic genome (Barra and Renard, 1988). These results indicate that HIV1-LTRlacZ transgene behaviour is that expected for a marker of transcriptional activation of the zygotic genome.

The clear switch of the HIV1-LTR expression in vitro points towards the fact that the gene expression observed in vitro under conventional conditions used for the analysis of endogenous gene expression in early embryos, may not necessarily reflect their activity in vivo. In particular, one could ask whether the HSP70 proteins that have been identified as products of the minor activation of the genome might be also synthesized in response to the oxidative stress previously reported, as expected for such stress-inducible genes. In this context, the presence of HSP70 and hsp70 mRNA has been demonstrated in all cases where embryonic development occurred in vitro (Bensaude et al., 1983; Bolton et al., 1984; Manejwala et al., 1991; Conover et al., 1991). Although we have demonstrated that, unlike the transgene expression, HSP70 synthesis was not affected by the addition of an antioxidant (CuZn-SOD) in the culture medium, this possibility cannot be completely ruled out. Despite the beneficial effects of antioxidants on embryonic development in vitro, they are unlikely to overcome all possible consequences of oxidative injury and, in particular, the increase of denatured, unfolded or ‘abnormal’ proteins, which have been suggested to represent the signal sensed by the cell for transcriptional activation of heat-shock genes (Ananthan et al., 1986).

In conclusion, our observations emphasize that the transcriptional activity assessed in early embryos developed in vitro is at least partially influenced by environmental variables. Therefore the results of molecular studies, which in most cases require in vitro culture of embryos, have to be extended to context to embryos developed in vivo. Finally, the HIV1-LTRlacZ hybrid gene expression which coincides with the onset of zygotic genome activation remains a useful tool to access the molecular mechanisms which regulate transcription in early mouse embryos.

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