Inducible expression of an hsp68–lacZ hybrid gene in transgenic mice

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Summary

Transgenic mice have been generated that express the E. coli β-galactosidase gene under the control of the promoter from the mouse heat-shock gene, hsp68. Sequences from −664 to +113 relative to the start of transcription of the hsp68 gene were sufficient to direct stress-induced expression of the β-galactosidase gene in adult tail tissue and various tissues of fetal stages of development. Expression was detected in situ by staining with the chromogenic substrate, X-gal. The hybrid gene was refractory to induction in preimplantation embryos until the blastocyst stage of development, as reported for the endogenous hsp68 gene. No constitutive expression was observed by in situ staining or Northern analysis at any stage of development, even in tissues that constitutively express the endogenous hsp68 gene. We conclude that the hsp68 promoter region included in the construct contains sufficient sequence information for heat and arsenite inducibility, but it does not contain sequences controlling tissue-specific expression during development. This tightly regulated inducible promoter may provide a useful tool for short-term inducible gene expression in transgenic mice.

Key words: transgenic mice, heat shock, lacZ hybrid gene, developmental regulation, expression.

Introduction

The ubiquitous heat-shock response is a conserved genetic program presumed to be required to repair thermal damage. The major induced protein is approximately 70×10^3 M₀ (hsp70) and every organism has at least one hsp70-related gene. In eukaryotic cells there are usually several such genes and they can be roughly divided into two groups; those that are constitutively expressed (cognate genes), and those that are stress inducible. The mouse hsp70 multigene family consists of five or six genes and as many pseudogenes (Lowe et al. 1983; Lowe & Moran, 1984; S. Shtang, M. D. Perry & L. A. Moran, unpublished). Two of these, the heat-shock cognates hsc70 and BiP (hsc74, grp78), are expressed constitutively in most cell types and are presumably essential for viability. Proteins related to both hsc70 and BiP have been identified in other mammals (O'Malley et al. 1985; Munroe & Pelham, 1986; Sorger & Pelham, 1987). One of the activities of hsc70 is to disassemble clathrin-coated vesicles (Ungeewickell, 1985; Chappell et al. 1986). BiP (hsc74) was originally identified as an endoplasmic reticulum (ER)-localized, immunoglobulin heavy chain binding protein (Haas & Wabl, 1983) and is identical to the glucose-regulated protein grp78 (Lee et al. 1984; Munroe & Pelham, 1986; Ting et al. 1987).

The stress-inducible genes are usually transcribed only in stressed cells but some of the mouse hsp70 genes are also developmentally regulated. One of these genes, hsp68 (formerly called hsp70B1), has been isolated recently (Perry & Moran, 1987; Perry et al. manuscript submitted) and we have shown that it is constitutively expressed in the extraembryonic tissues of day 8.5 to day 18.5 mouse conceptuses, and in fetuses from day 15.5 to day 18.5 (Kothary et al. 1987).

In order to obtain a better understanding of the cis-acting sequences involved in regulation of the mouse hsp68 gene during embryogenesis, we have generated several transgenic mice carrying an hsp68–lacZ hybrid gene. This hybrid gene contained about 800 bp of 5′ nontranscribed and nontranslated sequences which was sufficient to confer heat and sodium arsenite inducibility at all stages of postimplantation development tested. The only exceptions were placentae from late stage conceptuses, where the hsp68–lacZ hybrid gene was refractory to induction. The construct was also refractory to induction in early preimplantation em-
bryos but was inducible in blastocysts. Constitutive expression of hsp68–lacZ was not seen in any embryonic or extraembryonic tissues analysed, indicating that developmental regulatory sequences lie in a different region from heat-inducible elements in the hsp68 promoter.

Materials and methods

Generation of transgenic mice and embryos
Outbred CD-1 mice (purchased from Charles River Canada) were used for most experiments. In some microinjection experiments, B6CBA F1 × CD-1 embryos were used. Natural matings were established by placing estrus females with males and checking for vaginal plugs on the following morning (designated day 0.5 of pregnancy). One-cell zygotes were collected on the morning of the plug and hsp68–lacZ DNA at a concentration of 3 ng μl−1 was microinjected into one of the pronuclei. After retransfer into pseudopregnant recipients, the embryos were allowed to go to term. Offspring were weaned at 3 weeks of age at which time a tail biopsy was taken for DNA extraction and Southern blot analysis, or for β-galactosidase assay.

After mating transgenic offspring to CD1 mice, embryos were recovered at various stages of postimplantation development, collected in sterile phosphate-buffered saline (PBS), and dissected into the embryo, placenta and yolk sac. The tissues were transferred to α-MEM (Gibco) + 10% fetal calf serum (FCS) and subjected to various treatments for one hour: control (37°C), heat shock (42°C), or sodium arsenite (0-5 mm). They were then allowed to recover for 2h prior to either β-galactosidase assay or RNA analysis. Preimplantation embryos were also recovered from the same crosses and subjected to the same treatments in embryo culture medium.

Primary cell cultures
Tail tissue from transgenic mice was minced with scissors and incubated in α-MEM + 10% FCS. After an overnight culture period, attached cells were subjected to a heat shock at 42°C for 2h, and subsequently fixed and stained for β-galactosidase activity.

E. coli β-galactosidase assay
A histochemical staining procedure for β-galactosidase activity (Dannenberg & Suga, 1981) was used to detect lacZ expression in both cultured cells and intact embryos. Samples were rinsed with PBS and then fixed for 5–20 min at 4°C in 0.2% glutaraldehyde in PBS. After two washes in PBS, samples were incubated in a staining mix that consisted of 0.04% 4-chloro-5-bromo-3-indolyl-β-D-galactopyranoside (X-gal, Sigma), 1 mm-MgCl2, 10 mm-potassium ferrocyanide, and 10 mm-potassium ferricyanide in PBS. Agarose at 1% was included for staining of cultured cells. Incubation was from 12–36 h at 37°C.

In some cases, stained embryos were postfixed in 2.5% glutaraldehyde and processed for paraffin embedding. Sections were cut at 10 μm and examined for blue cells in unstained sections.

RNA analysis
Tissues were frozen in liquid nitrogen and stored at −70°C prior to RNA extraction. Extraction of RNA and Northern blot analysis were performed as described (Kothary et al. 1987). pCH126, a derivative of pCH110 (Hall et al. 1983), pMHS243 (Lowe & Moran, 1986), and pMOBA, a mouse β-actin cDNA clone (R. Zirngibl, unpublished results) were used as probes. Plasmid DNA was radioactively labelled with [α-32P]dCTP (Amersham Corp.) by the random priming method of Feinberg & Vogelstein (1983).

Expression assay of hsp68–lacZ constructs
A comparison was made of the efficiency of expression of the original hsp68–lacZ construct, which contains bacterial sequences for the initiation of translation, and a construct containing the Kozak consensus sequences (Kozak, 1983) for eukaryotic translational initiation. This plasmid (phspPTacZpA) was made by fusing the hsp promoter to the lacZ gene derived from the plasmid pMC 1871 (Pharmacia) such that the start of translation of the lacZ gene was derived from the eukaryotic hsp68 gene. Transfection of Rat II cells (105 cells/100 mm plate) was carried out by calcium phosphate precipitation using 10 μg of purified insert DNA/plate. After 48 h, half the plates were heat shocked at 42°C for 2h, followed by recovery at 37°C for 2h. These plates and control, untreated plates were then stained for β-galactosidase activity.

Expression of the constructs was also assayed in permanently transfected cell lines. Insert DNA from the two hsp68–lacZ constructs (10 μg) plus 3 μg linearized human β-actin–neo plasmid (W. Skarnes, unpublished) was introduced into Rat II cells by electroporation. G418 (GIBCO) was added to the culture medium 24 h following electroporation. After 12 days, neo+ colonies were subjected to heat shock and stained with X gal. Blue colonies were counted and then all colonies were stained with methylene blue to facilitate counting of neo+ colonies.

Results

Generation of hsp68–lacZ transgenic mice
A 4.55 kb BamHI fragment of DNA (Fig. 1) comprising

![Fig. 1. Structure of the mouse hsp68–lacZ gene construct. The 4.55 kb BamHI fragment was microinjected into fertilized eggs. The different segments of the hybrid gene are sequences −664 to +113 of the mouse hsp68 gene (solid), the Eco.gpt TrpS–E. coli lacZ fusion gene (light stripe and white), and the SV40 polyadenylation signal (striped).](image-url)
Table 1. Transgene copy number versus lacZ activity

<table>
<thead>
<tr>
<th>Transgenic line</th>
<th>hsp68-lacZ copy number</th>
<th>Relative lacZ activity*</th>
</tr>
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<tbody>
<tr>
<td>4</td>
<td>16</td>
<td>+++</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
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<td>10</td>
<td>20</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>+</td>
</tr>
</tbody>
</table>

* Activity assessed visually by intensity of X-gal staining of tail tissues.

Table 2. Expression of hsp68-lacZ in preimplantation embryos

<table>
<thead>
<tr>
<th>Stage of development</th>
<th>Control</th>
<th>Heat shock</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-cell</td>
<td>1*/57</td>
<td>0/44</td>
</tr>
<tr>
<td>8-cell</td>
<td>0/13</td>
<td>1*/36</td>
</tr>
<tr>
<td>Blastocyst</td>
<td>1*/48</td>
<td>23/62</td>
</tr>
</tbody>
</table>

* Embryos with very weak, equivocal staining.

Expression of the hybrid gene was assayed by a sensitive enzymic in situ detection procedure for E. coli β-galactosidase, in which the chromogenic substrate, X-gal, is enzymically cleaved by β-galactosidase to release an insoluble blue dye. Tail biopsies were obtained from the seven founder transgenic mice and subjected to a heat stress at 42°C in PBS for 1 h. After recovery at 37°C for 2 h to allow for expression, the tissues were fixed in glutaraldehyde and stained with X-gal. In all cases, lacZ was expressed only in the heat-stressed sample and not in its control counterpart. No lacZ expression was observed in nontransgenic samples. Thus all seven transgenic mice had apparently integrated intact functional copies of the hybrid gene.

Heat inducibility of the hybrid gene in tail tissue

Expression of the hybrid gene was assayed by a sensitive enzymic in situ detection procedure for E. coli β-galactosidase, in which the chromogenic substrate, X-gal, is enzymically cleaved by β-galactosidase to release an insoluble blue dye. Tail biopsies were obtained from the seven founder transgenic mice and subjected to a heat stress at 42°C in PBS for 1 h. After recovery at 37°C for 2 h to allow for expression, the tissues were fixed in glutaraldehyde and stained with X-gal. In all cases, lacZ was expressed only in the heat-stressed sample and not in its control counterpart. No lacZ expression was observed in nontransgenic samples. Thus all seven transgenic mice had apparently integrated intact functional copies of the hybrid gene.

Fig. 2. Heat inducibility of hsp68-lacZ in blastocysts. Blastocysts from line 7 matings were stained for β-galactosidase activity after heat-shock treatment. Several cells in all putative transgenic embryos stained blue. No expression was observed without heat shock (not shown). Bar, 50 μm.
Transgenic offspring were identified in future generations by using this tail-staining assay. The seven transgenic lines differed in the level of heat-inducible β-galactosidase activity as estimated visually in tail tissue. There was no obvious correlation between transgene copy number and enzyme activity (Table 1). For example, line 7 had the lowest copy number (one) but the strongest staining whereas line 10 contained approximately 20 copies of hsp68-lacZ but stained very lightly. When primary tail fibroblast cultures were analysed, a similar variability in heat-inducible expression of lacZ was observed (not shown).

Further it was noted that only about 80% of the cells in each culture were stained after heat stress, although they should all be genotypically identical. This variability in staining has been reported by others when transfecting lacZ hybrid genes (MacGregor et al. 1987) or in retroviral infection experiments with cells in culture (Price et al. 1987).

Expression of the hybrid gene in preimplantation development

Preimplantation embryos were recovered at the 2-cell, 8-cell and blastocyst stages of development after mating hemizygous male mice from lines 4, 7, 8 and 10 to CD-1 females. Reciprocal crosses used in some cases had no effect on the results observed. Half of the embryos obtained from these matings were expected to be hemizygous for the transgene and half wild type. The wild-type embryos served as controls for endogenous β-galactosidase activity. As is reported to be the case for the endogenous hsp68 gene, the hybrid gene was refractory to induction by heat shock at the 2-cell and 8-cell stage. Heat-inducibility was first apparent at the blastocyst stage (Table 2, Fig. 2) in all four lines. Although there was some variation in the level of expression between lines, there was no variation in the time of onset of heat inducibility, and so the data in Table 2 represent pooled data from all four lines. By the blastocyst stage, the number of embryos that stained blue after heat shock was not significantly different from the expected 50%. Heat-shocked blastocysts did not show equal levels of expression in all cells (Fig. 2) but the hybrid gene was induced in both the inner cell mass (ICM) and the trophectoderm. Intense blue staining of both ICM and trophectoderm cells was also seen after stress treatment of blastocysts outgrown in culture for 3 days (not shown). In none of the lines tested was there any constitutive expression of the hsp68-lacZ construct at any stage of preimplantation development (Table 2).

Expression of the hybrid gene during postimplantation development

Analysis of expression of the transgene during postimplantation development was performed by subjecting the entire fetus and its membranes to stress conditions in vitro. Expression could then be monitored in a variety of tissues by visual observation followed by sectioning to score internal tissues.

Male mice from the same lines used to generate preimplantation embryos were used to produce post-implantation embryos for in situ analysis. At least 3 litters were analysed at each time point between 6.5 and 15.5 days of development. Lines 7, 8 and 10 showed no evidence of constitutive expression of the lacZ transgene in either embryonic or extraembryonic tissues, although prolonged incubation in the staining mixture resulted in low level detection of the endogenous mouse β-galactosidase, particularly in the yolk sac. This background staining could be eliminated by altering the stain conditions (not shown). Hemizygous transgenic embryos from line 4 showed constitutive expression of the lacZ gene in the spinal cord but no other tissue. Presumably this reflects the integration site of the gene since it was not observed in any other line (Kothary et al. 1988). After heat shock or arsenite treatment in vitro, fetuses of all four transgenic strains showed expression of the hsp68-lacZ gene at all stages of development tested (not shown) although each line responded to different extent. Southern blot analysis of placental DNA confirmed that all transgenic fetuses stained blue, while all nontransgenic fetuses were negative. Arsenite treatment was a more effective inducer than heat shock, as judged by the degree of X-gal staining. As expected from the tail assay, embryos from line 7 showed the highest level of β-galactosidase expression as assessed visually.

The patterns of expression at different stages were similar for all four lines, although the low level of expression in lines 8 and 10 made exact tissue localization of expression difficult. At 6.5 days and 7.5 days of development, heat-induced lacZ expression occurred sporadically in cells of the embryonic and extraembryonic regions. Interestingly, the most intense expression at 7.5 days was observed in the developing mesoderm cells of the primitive streak (Fig. 3A). At 8.5 days, intense staining was apparent in the entire embryo and extraembryonic membranes. On sectioning, however, it was apparent that there was considerable variation in expression from tissue to tissue. Both layers of the amnion, the epidermis, the neural folds, the notochord, the heart and head mesenchyme showed extensive expression but internal tissues, particularly the somites, did not show much β-galactosidase activity (Fig. 3B).

At 9.5 days, intense external staining of the embryo was apparent, but histological examination revealed that expression was almost entirely confined to the epidermis (not shown). Examination and dissection of stained 12.5-day fetuses revealed that the lacZ gene was strongly expressed over almost the entire skin surface, with some additional expression in the heart, lungs and liver (Fig. 4).

Inducible expression of lacZ was observed in the visceral yolk sac but no expression of the lacZ gene was detected in the placenta of any line after either heat shock or arsenite treatment (Fig. 4).

Comparison of transcription of hsp68-lacZ and endogenous hsp68 gene

Northern blot analysis of total RNA extracted from the fetus, placenta and yolk sac of hemizygous day-13.5
Fig. 4. hsp68–lacZ expression during embryogenesis. Embryos (panels A,D,G), placenta (panels B,E,H) and yolk sac (panels C,F,I) at day 12.5 of development were derived from mating hemizygous males from line 7 with CD1 females. Tissues were treated, fixed and subsequently assayed in situ for β-galactosidase activity. Panels A–C: control; Panels D–F: heat-shock treated samples (42°C 1 h, followed by 2 h at 37°C); Panels G–I: sodium arsenite treated samples (0.5 mM for 1 h, recovery for 2 h). In Panel E staining is only observed in visceral and parietal endoderm remnants around the placenta. Bar, 1 mm.
embryos from line 4 showed that lacZ mRNA could be detected in stressed tissues (Fig. 5A). LacZ mRNA levels were readily detectable in the embryo and the yolk sac after stress, whereas little if any lacZ mRNA was detectable in placenta even after long exposure (2 weeks) of the autoradiogram. Levels of lacZ RNA were slightly higher after arsenite rather than heat-shock treatment (Fig. 5A). Although we did not directly compare levels of lacZ message and endogenous hsp68 mRNA levels, a rehybridization of the same Northern blot (Fig. 5B) with an endogenous hsp68 probe of similar specific activity revealed apparently similar levels of expression of the endogenous gene as judged by the similar exposure time required to reveal the two messages. The endogenous gene was induced at similar levels in embryo and yolk sac but, like the hsp68–lacZ hybrid gene, was expressed at lower levels in placenta after stress treatment (Fig. 5B).

As previously shown (Kothary et al. 1987), the endogenous hsp68 gene was expressed at low levels constitutively in the placenta and yolk sac, but not in the embryo proper (see Fig. 5B). These same tissues

![Fig. 3. hsp68–lacZ expression in early postimplantation development. Conceptuses were derived from mating hemizygous males from line 7 with CD1 females, subjected to heat shock and subsequently assayed for β-galactosidase activity. (A) Three 7.5-day conceptuses, two transgenic and one nontransgenic (centre). A few isolated cells in the ectoplacental cone and extraembryonic region stained blue but extensive induction of lacZ was only observed in the developing primitive streak (arrows). (B) Section of embryo at 8.5 days showing that lacZ expression is not uniform throughout the embryo. he, heart; m, mesenchyme; n, notochord; nf, neural folds, s, somites. Bar, 1 mm.](image-url)
Fig. 5. Expression of hsp68-lacZ mRNA during embryogenesis. Total RNA was extracted from day-13.5 embryos. Matings were set up as described in Fig. 3. Embryo (lanes a, d, and g), placenta (lanes b, e, and h), and yolk sac (lanes c, f, and i). Lanes a–c; control (uninduced); lanes d–f; heat-shocked samples, lanes g–i: sodium-arsenite-treated samples. Approximately 5μg of each sample was assayed on a Northern gel and probed with labelled lacZ DNA (A), MHS243 DNA (B), and subsequently with pMOBA DNA (C), open triangle, lacZ mRNA; closed triangle, hsp68 mRNA; open circle, β-actin mRNA. Note that lanes a–c in panel B were exposed for 5 days while the rest of the lanes were exposed for 2 days.

contained no constitutively expressed hsp68-lacZ mRNA, even after long exposures, implying that the hybrid gene does not respond to the developmental signals affecting the hsp68 gene. We assume that this is due to lack of necessary sequence information on the hybrid gene.

Expression of hsp68-lacZ constructs in a fibroblast cell line

A comparison of a number of blue colonies observed in a transient expression assay after transfection of hsp68-lacZ and phspPTlacZpA into Rat II cells revealed that the construct containing a eukaryotic translational start site was more efficiently expressed after heat shock. In two different experiments, 621 and 589 blue colonies were observed with hsp68-lacZ while over 1600 (too many to accurately count) were observed with phspPTlacZpA. This threefold enhancement of detectable expression was confirmed in permanently transfected colonies. Of 1650 neo<sup>+</sup> colonies obtained by cotransflecting hsp68-lacZ and β-actin-neo, 273 stained blue after heat shock (16%). However, 611 of 1452 neo<sup>+</sup> (42%) colonies stained blue after transfection of phspPTlacZpA.

Discussion

We have shown that transgenic mice carrying a mouse hsp68-lacZ hybrid gene show no constitutive expression of the lacZ gene but show readily detectable β-galactosidase activity in embryonic and adult tissues after induction by heat or arsenite. Thus all of the information necessary for inducibility is contained in the hybrid gene. Approximately 800 bp of 5' upstream sequence from the mouse hsp68 gene (Perry & Moran, 1987; Perry et al. manuscript submitted), including the putative TATA box, the CAAT box and at least five copies of the heat-shock consensus element (HSE) were included in the construct. These HSE sequences consist of a conserved palindromic sequence which has been shown to confer heat inducibility to heat-shock gene promoters in other systems (Mirault et al. 1982; Pelham, 1982; Pelham & Bienz, 1982; Dudler & Travers, 1984; Wu et al. 1986; Kay et al. 1986; Slater & Craig, 1987). All seven transgenic lines produced had functional copies of hsp68-lacZ as monitored by β-galactosidase activity in heat-shocked adult tail tissue stained with X-gal. The apparent level of activity after heat stress varied from line to line, but did not seem to correlate with the copy number of hsp68-lacZ in any of the lines tested. Variations in level of inducible activity probably result from position effects due to different sites of integration. However, heat inducibility per se did not vary between lines; all lines showed stress-induced β-galactosidase activity.

During embryonic development, the hsp68-lacZ construct could be activated at most stages by heat stress or arsenite treatment in vitro. In postimplantation stages of development, several tissues, both internal and external, of the transgenic fetuses showed inducible β-galactosidase expression. Activation of the gene was clearly not uniform in all tissues and at all stages. The variation observed was probably partly due to tissue variation in ability to respond to heat stress and partly due to inadequate exposure to the stress stimulus. Cells of egg-cylinder-stage and early-somite-stage embryos were presumably equally exposed to the stress stimulus and yet not all cells stained blue. The localization of blue staining to the primitive streak at 7.5 days was
particularly indicative of a tissue-specific response. At later stages, blue staining was almost entirely confined to external structures and this probably largely reflects the inaccessibility of internal structures to the stress stimulus. None of the variation could be attributed to position effects related to transgene insertion site since all lines tested showed similar patterns of expression. None of the transgenic lines generated here showed stress-inducible lacZ expression in the placenta. Northern blot analysis of endogenous hsp68 expression showed that the endogenous gene was also weakly induced in this tissue under the stress conditions used.

These results indicate that the construct contained sufficient 5' sequence information for correct stress inducibility in a variety of tissues. The construct also showed the predicted refractoriness to stress induction during early preimplantation development (Wittig et al. 1983; Morange et al. 1984; Muller et al. 1985; Hahnel et al. 1986). Embryos at the 2- and 8-cell stages from several lines showed no inducible β-galactosidase expression but all contained some blue-staining ICM and trophectoderm cells at the blastocyst stage. Thus the acquisition of heat inducibility at the blastocyst stage is a property of both ICM and trophectoderm. It is not clear why the hybrid gene and the endogenous hsp68 gene are refractory in early mouse embryos. Plausible explanations include: insufficient concentrations of requisite transcription factors (e.g. HSTFs or HAP, Parker & Topol, 1984; Wu, 1984); hsp68 is in an inaccessible condensed chromatin domain, or a negative regulator is specifically repressing hsp68. A similar quiescence has been observed in the embryos of other organisms (Graziosi et al. 1980; Bientz, 1984; Heikkila et al. 1985).

Thus, the construct used here contains sequences involved in both stress inducibility and refractoriness to induction in early embryogenesis. It does not, however, appear to contain sequences required for tissue-specific developmental expression. No constitutive expression of lacZ mRNA or lacZ protein could be detected in the placenta and yolk sac where previous studies have demonstrated transcription of the endogenous hsp68 gene (Kothary et al. 1987). This separation of tissue-specific and developmental regulatory regions increases the utility of this promoter as an inducible promoter for developmental studies. Further studies using constructs with additional 5' and 3' flanking regions will be required to identify controlling sequences for developmental expression of the mouse hsp68 gene.

The E. coli lacZ gene has become increasingly popular as a reporter gene for in situ analysis of patterns of gene expression in transgenic mice (Goring et al. 1987) and cell lineage relationships in mammalian development (Sanes et al. 1986; Price et al. 1987). As a sensitive in situ marker it distinguishes marked and unmarked cells at the single cell level. However, there have been reports that expression of lacZ hybrid constructs may be 'patchy' in tissue culture cells (MacGregor et al. 1987). The construct used here also resulted in some patchy expression in primary fibroblast cultures and stably transfected fibroblast cell lines. We also observed patchy expression in vivo even in tissues that responded well to stress induction of the hsp68–lacZ gene (Fig. 3B). Much of this patchy expression may be attributable to inefficient translation of the lacZ transcripts. The hybrid hsp68–lacZ gene used to generate the transgenic lines contains a bacterial translational start site which is not surrounded by the usual consensus sequence for eukaryotic translational initiation (Kozak, 1983). When the hsp68 translational start site was utilized in the pshpPPlacZpA construct, both transient assays and permanent transfections into fibroblast cell lines indicated that at least a 3-fold increase in translational efficiency could be achieved over the original hsp68–lacZ construct. Further, permanently transfected colonies showed no patchy expression: all cells stained blue. Whether this construct will eliminate patchy expression and allow detection of expression in apparently negative tissues remains to be tested in vivo.

There is considerable interest in identifying promoters inducible during mouse embryogenesis. Such promoters could be used to control expression of various developmentally regulated genes in an attempt to assign functions to these genes. The hsp68 promoter region we have described here has the advantage over most other inducible promoters that its activity is very stringently regulated. Expression is absent in all unduced tissues and widely distributed in induced tissues. Heat shock or arsenite treatment of pregnant mice could potentially be used to activate the hsp68 promoter in vivo in embryos during selected windows of development. Thus the hsp68 promoter would be an excellent candidate for the perfect inducible promoter, were it not for the fact that it has not yet proved possible to induce promoter activity without subjecting the embryos to stresses that may be deleterious to subsequent normal development (Bellve, 1972; German et al. 1986; and reviewed in Edwards, 1986). However, it may be useful for short-term studies where such deleterious effects are not manifest.

We thank Achim Gossler for useful discussions, Ralph Zirngibl for technical assistance and Roger Pedersen for the β-galactosidase in situ staining protocol. R.K. and S.D. were supported by postdoctoral fellowships and J.R. by a Research Associateship from the National Cancer Institute of Canada. M.P. was a recipient of a University of Toronto Open Doctoral fellowship. This work was supported by grants from the MRC, NSERC and NCI of Canada to L.M. and J.R.

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(accepted 20 December 1988)