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Developmental activation of an episomic *hsp70* gene promoter in two-cell mouse embryos by transcription factor Sp1

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ABSTRACT

To investigate the control of zygotic genome expression in two-cell mouse embryos, we studied transcription factors required for transient expression of microinjected DNA constructs driven by the promoter of one of the earliest genes activated after fertilization in this system, the heat shock gene *hsp70*. *Cis*-acting elements required for *hsp70* activation were first investigated by mutational analysis. Mutation of the TATA box and a proximal GC box strongly inhibited construct expression, while that of a CCAAT box had no effect. Transcription factors binding the wild-type *hsp70* promoter were then titrated *in vivo* by coinjecting the construct with double-stranded oligodeoxyribonucleotides containing definite consensus sequences. Wild-type GC box oligonucleotides strongly inhibited construct expression, while those containing mutated GC boxes, wild-type CCAAT boxes, and heat shock elements had no effects. Finally, construct expression was challenged by coinjecting antibodies to specific transcription factors. Antibodies to factor Sp1 depressed construct expression in a dose-dependent manner, while those to Sp2, HSF1 and HSF2 were ineffective. These results pinpoint the Sp1 transcription factor as an absolute requirement for activation of the *hsp70* gene promoter in two-cell mouse embryos, and make this factor a candidate for a major regulator of the onset of murine zygotic genome expression.

INTRODUCTION

Transcriptional activation of the zygotic genome in mammals occurs at very early developmental stages. In the mouse, the first major burst of transcription is observed at the early G_1 phase of the two-cell stage with expression of a number of genes, such as those encoding for a complex of nuclear-associated proteins (TRC) (1) and several heat shock genes, including the heat shock cognate 70 gene (2,3), *hsp80* (4) and the major stress-inducible heat shock gene *hsp70* (formerly *hsp68*; *hsp70.1* according to Hunt and Calderwood, 5) (2,6). The G_2 phase of the one-cell stage, however, is already transcriptionally competent (7), and *hsp70*, among other genes, is activated at this time in the male pronucleus (6).

Since standard biochemical techniques are made difficult in mouse preimplantation embryos by the paucity of biological material, molecular mechanisms activating zygotic genome transcription following fertilization have been largely investigated by transient expression analysis of microinjected DNA constructs (8–18). Such studies have provided a wide body of information on aspects such as, among others, the differential repression of gene expression in the maternal versus the paternal pronucleus at the one-cell stage (14,19), and the role played by basal promoter and enhancer sequences in one-cell embryos versus that played at later preimplantation stages (12,17,20). However, direct information on embryonic factors actually involved in the regulation of early transcriptional activity is still scarce, except for the demonstration that transcription factors Sp1 and TATA box binding protein (TBP) accumulate in one- and two-cell embryos (21), and that factors binding the Sp1 consensus sequence activate microinjected promoters in early cleaving embryos (16,22).

Spontaneous developmental activation of *hsp70* in early mouse embryos, together with its refractoriness to stress-induction until the late morula stage (23), provides a unique model to study the control of gene expression at these stages. In this paper, we have pursued such a study by transient expression analysis of a microinjected DNA construct driven by an *hsp70* gene promoter (p*hsplacZ*) that is regulated in development according to the pattern of spontaneous expression of endogenous *hsp70* (15,18). We have used this construct to analyze the role played by different *cis*-acting elements of the *hsp70* proximal promoter and to directly identify the *trans*-activating factor(s) that bind such elements in intact embryos. This study was performed by the different criteria of construct mutational analysis, *in vivo* transcription factor titration by injected double-stranded oligodeoxyribonucleotides, and *in vivo* inhibition of specific transcription factor activity by injected antibodies. The results obtained indicate that the GC box proximal to the TATA box is a genetic element essential for construct spontaneous activity, and identify Sp1 as the transcription factor responsible for such activation.

MATERIALS AND METHODS

DNA constructs and mutagenesis

Construct p*hspPTlacZpA* (p*hsplacZ*) (24) contains an *Escherichia coli lacZ* gene driven by 620 bp of promoter sequences from the murine *hsp70.3* gene (25,26). Nucleotide sequences of this gene

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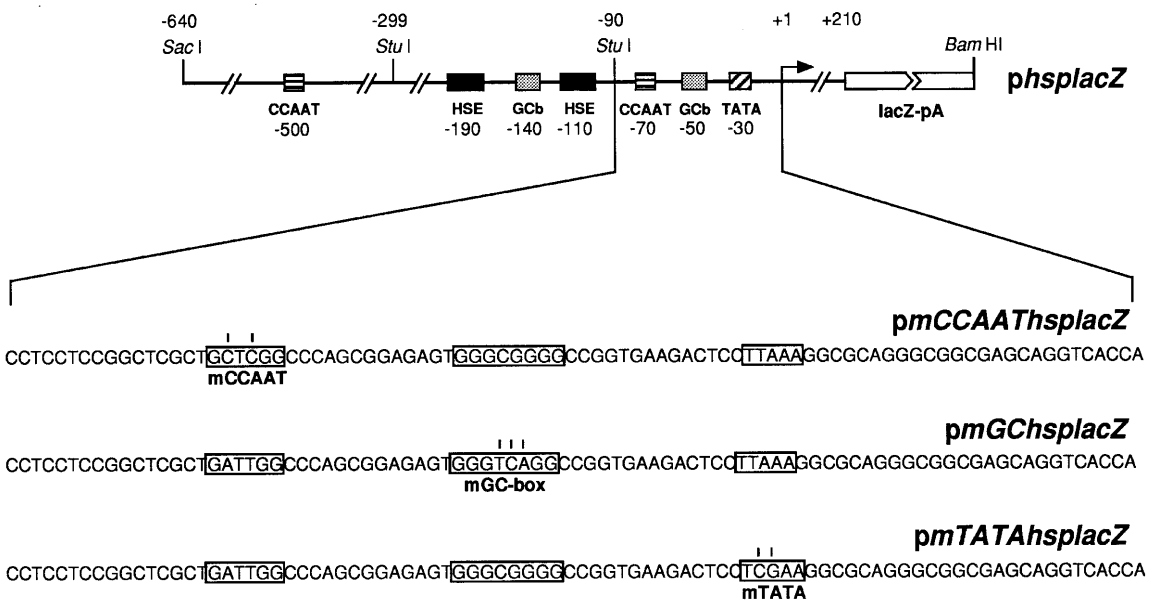

Figure 1. Proximal promoter region of p*hsplacZ*-derived constructs used in the present study. Genetic elements, identified according to their consensus sequences, were mutated as described in Material and Methods. Simian virus 40 polyadenylation signal is indicated by pA. Numbers indicate nucleotide positions relative to the transcriptional start site (arrow). Vector sequences (pUC19) are not represented.

are virtually identical to those of *hsp70.1*, which is expressed in early mouse embryos (6). In fact *hsp70.1* and *hsp70.3* have highly conserved coding regions and identical proximal promoter sequences, including the TATA box (–30 bp from the transcriptional start site), a GC box (–50), a CCAAT box (–70), and two tetrameric heat shock elements (HSEs) (–110 and –190), while divergencies between the two genes are found upstream of the HSEs and in the 3′-untranslated region. Previous work performed in our laboratory has shown that, when injected into one- and two-cell mouse embryos, p*hsplacZ* is developmentally expressed according to the pattern of transcription of endogenous *hsp70.1*, and that construct activity at these stages depends on its proximal, but not distal, promoter sequences (18).

Site-directed mutagenesis of p*hsplacZ* proximal promoter consensus sequences of TATA box, GC box and CCAAT box was performed by the overlapping recombinant PCR method of Higuchi (27), modified by the use of Vent DNA polymerase (New England Biolabs, Beverly, MA). The TATA box was mutated from TTAAA to TCGAA (p*mTATAhsplacZ*); the GC box from GGGCGGGG to GGGTCAGG (p*mGChsplacZ*); and the CCAAT box from GATTGG to GCTCGG (p*mCCAAThsplacZ*). Nucleotide sequences of the mutated constructs are reported in Figure 1. For microinjections, constructs were dissolved in 10 mM Tris, 0.1 mM EDTA, pH 7.4 (TE) at a final concentration of 1.6 ng/μl, corresponding to ~250 construct copies/pl.

Transient construct expression in L-cells

L-cell monolayers were transfected with various constructs by standard calcium phosphate precipitation (28), and heat-shocked at 44°C for 20 min, as previously described (18). Transfection efficiency was monitored by cotransfection with pCAT-Control Vector (Promega, Madison, WI). Biochemical assays of CAT and β-galactosidase activities were performed as previously described (18).

Embryo culture and intranuclear microinjection

Fertilized eggs were obtained from B6D2F1/JIco hybrid mice (Charles River Italia, Calco, Italy), using hormonally primed, 30- to 40-day-old females (29). Fertilization time was considered midnight after mating. Eggs were collected 10–12 h post fertilization (p.f.) as described (18) and cultured *in vitro* at 37°C under a humidified atmosphere of 5% CO_2 in air until the appropriate developmental time. Construct injection (~2 pl DNA solution) was performed into the male pronucleus of one-cell embryos (13–14 h p.f.), or the nucleus of a single blastomere of two-cell embryos (32–34 h p.f.), as previously described (18). Following injection, embryos were cultured *in vitro* as needed, and assayed for β-galactosidase activity 20 h following injection at the one-cell stage, or 5 h following injection at the two-cell stage. Biochemical enzymatic assays were performed on single embryos (18).

In vivo titration assays with double-stranded oligodeoxyribonucleotides

Complementary oligodeoxyribonucleotide pairs carrying consensus sequences for the appropriate wild-type/mutated genetic element are reported in Table 1. Oligonucleotide pairs were annealed at a concentration of 0.2 μg/μl in TE buffer containing 0.025 N NaCl, giving origin to double-stranded oligonucleotides having *Bam*HI cohesive ends. When needed, double-stranded oligonucleotides were blunt-ended by treatment with Klenow enzyme prior to microinjection. For microinjection, double-stranded oligonucleotides were mixed with construct p*hsplacZ* in TE (1.6 ng/μl), to obtain molar ratios of the specific genetic element to construct of 25:1, 50:1, and 75:1. Microinjection of p*hsplacZ*-oligonucleotide mixtures and subsequent β-galactosidase assays were carried out according to the schedules described above.

Table 1. Sequences of oligodeoxyribonucleotide pairs used for *in vivo* titration of transcription factors binding the GC box, the CCAAT box and the heat shock element in two-cell mouse embryos

Wild-type GC box	sense:	5′-GATCCGGATGGGCGGGGCCGGGGATGGGCGGGGCCG-3′
	antisense:	5′-GATCCGGCCCCGCCCATCCCCGGCCCCGCCCATCCG-3′
Mutated GC box	sense:	5′-GATCCGGATGGTCAGGTCCGGGGATGGTCAGGGCCG-3′
	antisense:	5′-GATCCGGCCCTGACCATCCCCGGACCTGACCATCCG-3′
CCAAT box	sense:	5′-GATCCGCTGATTGGCCCAGCGGCTGATTGGCCACG-3′
	antisense:	5′-GATCCGTGGCCAATCAGCCGCTGGGCCAATCAGCG-3′
Heat shock element	sense:	5′-GATCCAGAAACTTCTGGAAGATTCCTG-3′
	antisense:	5′-GATCCAGGAATCTTCCAGAAGTTTCTG-3′

In vivo titration assays with antibodies

Affinity purified mouse monoclonal IgG_1 reacting with amino acids 520–538 of human Sp1, and affinity purified rabbit polyclonal IgG reacting with amino acids 4–23 of human Sp2, were purchased from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany). Rabbit polyclonal antisera reacting with murine heat shock factor 1 (HSF1) and murine heat shock factor 2 (HSF2), respectively, (30) were a kind gift of Dr Vincenzo Zimarino. For microinjection, antibodies were microdialized against TE through MF filters (Millipore, Rome, Italy) and eventually diluted to a final IgG concentration of 12.5–50 ng/µl in TE containing 1.6 ng/µl p*hsplacZ*. Final IgG concentrations of all antibodies were comparable. For control experiments, Sp1 antibody was preadsorbed with its immunogenic peptide (Santa Cruz Biotechnology) at a molar ratio of 1:10. Microinjection of p*hsplacZ*-antibody solutions and β-galactosidase assays were carried out according to the schedules reported above.

Chemicals

Restriction enzymes were purchased from BRL (Gaithersburg, Germany), Pharmacia (Milano, Italy) and Promega (Madison, WI); hyaluronidase from Boehringer Mannheim Italia (Milano, Italy); hormones and other chemicals from Sigma Chemical Co. (St. Louis, MO).

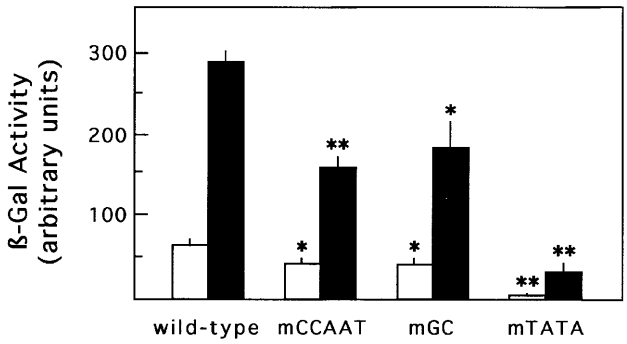

Figure 2. Transient expression of construct p*hsplacZ* and its derivatives in transfected L-cells. L-cells were transfected with p*hsplacZ* (wild-type), p*mCCAAThsplacZ* (mCCAAT), p*mGChsplacZ* (mGC), and p*mTATAhsplacZ* (mTATA), as indicated under the bars. Transfection efficiency was monitored by cotransfection with pCAT-control vector. Following transfection, cells were maintained at 37°C (open bars) or subjected to heat shock (solid bars). Transfection, heat shock, and biochemical assays of CAT and β-galactosidase activities were performed as described in Materials and Methods. Values represent arbitrary units of β-galactosidase activity (mean ± SEM) normalized for transfection efficiency with CAT activity as the internal control, obtained in three independent experiments. Open bars: cells were maintained under normal temperature conditions; solid bars: cells were heat-shocked prior to the assay. Asterisks above bars indicate a significant difference with cells transfected with the wild-type construct, calculated by Student *t*-test: **P* <0.025; ***P* <0.001.

RESULTS

Construct mutational analysis

Effects of site-specific mutations of p*hsplacZ* promoter on construct expression were first evaluated in transfected L-cells, both under normal temperature conditions and after heat shock (Fig. 2). In these experiments, L-cell monolayers were cotransfected with pCAT-Control Vector and wild-type/mutated constructs as described in Materials and Methods. β-Galactosidase activity produced by different constructs was then expressed in terms of the ratio to CAT activity. Mutation of the TATA box had the strongest effect, with ~90% inhibition of construct expression in both unstressed and stressed cells. A less severe, but still highly significant inhibiting effect (50–60% under both temperature conditions) was also observed with mutations of either the GC box or the CCAAT box. It was therefore concluded that *hsp70* promoter activity in L-cells strongly depends on the TATA box, and that proximal CCAAT and GC boxes play a positive role in its transcription both in stressed and unstressed cells.

Mutational analysis was then performed in mouse embryos (Fig. 3) by microinjecting constructs p*hsplacZ*, p*mTATAhsplacZ*, p*mGChsplacZ* and p*mCCAAThsplacZ* at either the one- or the two-cell stage, as described in Materials and Methods. With both injection schedules, mutation in the TATA box effectively inhibited construct expression, but its effect was less relevant than that previously observed in L-cells. The major difference with somatic cells, however, was that in two-cell embryos, mutational inactivation of the GC box had a dramatic inhibitory effect, almost suppressing construct expression. Finally, mutation of the CCAAT box had no apparent effect. These results showed that in two-cell mouse embryos, the proximal GC box plays a major role in *hsp70* promoter activation, and that maximal transcription efficiency also depends on the presence of a functional TATA box, while the proximal CCAAT box appears dispensable.

In vivo titration of transcription factors by injection of double-stranded oligodeoxyribonucleotides

In these experiments, transcription factors binding the microinjected *hsp70* promoter in living embryos were titrated by using 27–40mer synthetic double-stranded oligodeoxyribonucleotides that contained CCAAT box, HSE, or wild-type/mutated GC box consensus sequences (Fig. 5). Oligonucleotides with mutated GC boxes contained the same base substitutions of construct p*mGChsplacZ*. Wild-type p*hsplacZ* construct and increasing

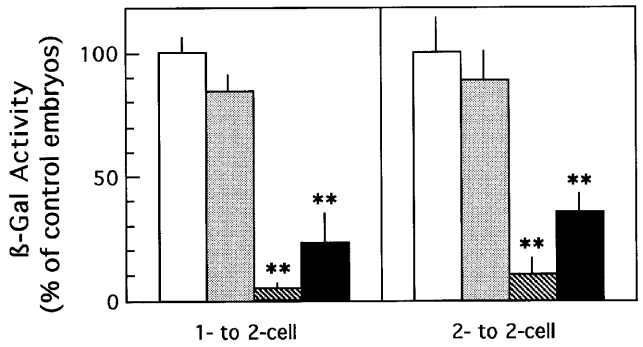

Figure 3. Transient expression of construct p*hsplacZ* and its derivatives in two-cell mouse embryos. Histograms indicate β-galactosidase activity of embryos that received constructs p*mCCAAThsplacZ* (stippled), p*mGChsplacZ* (striped), or p*mTATAhsplacZ* (solid), expressed as percentage of the enzyme activity of embryos injected with the wild-type construct (open). Injection and assay schedules were as follows: 1- to 2-cell, eggs were subjected to DNA injection 14 h p.f., cultured for 20 h to the two-cell stage and biochemically assayed for β-galactosidase activity; 2- to 2-cell, eggs were cultured for 22–24 h until the two-cell stage, subjected to DNA injection, cultured for additional 5 h, and then biochemically assayed for β-galactosidase activity. Values represent the mean ± SEM of 20–40 embryos, pooled from at least three independent experiments. Double asterisk above bars indicates a significant difference (P <0.001) with embryos injected with the wild-type construct, calculated by Student *t*-test.

amounts of competing oligonucleotides were coinjected according to the injection schedules followed in previous experiments, and β-galactosidase assays were carried out consequently.

Oligonucleotides carrying canonical GC boxes effectively inhibited p*hsplacZ* expression in two-cell embryos in a dose-dependent manner, regardless of the schedule of injection. Construct expression was decreased to 15% of the normal values by a 75-fold molar excess of GC boxes. In contrast, no significant effect was observed with oligonucleotides carrying mutated GC boxes, canonical CCAAT boxes, or HSEs, regardless of their fold-excess. Similar results were obtained with either blunt- or cohesive-end oligonucleotides (not shown), suggesting that possible concatemerization of these DNA fragments did not influence transcription factor-binding activity, and/or that early mouse embryos very efficiently concatemerize blunt-ended DNA fragments. In light of the excellent agreement between results of titration experiments and those obtained by mutational analysis, it was concluded that a transcription factor(s) binding the GC box, but not the CCAAT box nor the HSEs, was the major activator of the injected *hsp70* gene promoter in two-cell mouse embryos.

In vivo identification of transcription factor activity by microinjection of specific antibodies

Since oligonucleotide titration of transcription factors could not identify the specific nature of the factors involved, we challenged the activity of possible *trans*-activators of p*hsplacZ* by microinjection of antibodies raised to different transcription factors, including Sp1, Sp2, HSF1 and HSF2. Wild-type p*hsplacZ* construct and increasing amounts of antibodies were coinjected according to the experimental protocols employed so far, and β-galactosidase assays were carried out consequently. In these experiments (Fig. 5), while injection of antibodies to Sp2, HSF1 and HSF2 did not affect construct expression at any concentration used, injection of antibody to Sp1 had no apparent effect at 12.5 ng/μl concentration, but produced dose dependent degrees of inhibition in construct expression in the range 25–50 ng/μl. Anti-Sp1 antibody preadsorbed with a 10-fold excess of the immunogenic peptide showed no inhibiting activity. On the basis of these results we concluded that transcriptional recruiting of the *hsp70* promoter in two-cell embryos strictly depends on activity of Sp1 transcription factor, and not on other factors possibly present in early mouse blastomeres.

DISCUSSION

In the present study we have investigated transcriptional regulation at the onset of zygotic gene expression in early mouse embryos by use of a microinjected DNA construct, p*hsplacZ*, that contains the coding region of an *E.coli lacZ* gene under control of the promoter region of the murine *hsp70.3* gene. As shown previously, this construct is spontaneously activated in early cleaving embryos both in episomic form and after genomic integration in transgenic mice (15,18), and thus analysis of its regulation may provide direct information on transcription factors involved in embryonic transcriptional activation. Deletion analysis of this construct has previously revealed that its developmental activation in early mouse embryos is independent of the presence of HSEs, but only requires the presence of a 90 bp proximal region that includes three major genetic elements: a CCAAT box, a GC box and the TATA box (18). We have further investigated the transcriptional regulation of such a construct in two-cell mouse embryos by analysis of molecular mechanisms acting on its proximal promoter region.

Mutational analysis of *hsp70* gene promoter in transfected L-cells produced results in substantial agreement with previous observations on the activity of the human *hsp70* gene promoter in HeLa cells (31). Murine and human *hsp70* are conserved genes, sharing a very high degree of homology in their proximal promoter regions. Similarly to the human promoter, the murine *hsp70* gene promoter was virtually inactive when devoid of a functional TATA box, confirming its strong need for TATA-binding protein (TBP) and the basal transcription machinery for activity under both normal temperature and stress conditions. CCAAT and GC box mutations produced degrees of promoter inactivation in both unstressed and stressed L-cells milder than those observed for the human *hsp70* gene promoter (31). However, this observation is not surprising since it was previously found that *hsp70* is subject to slightly different regulation by murine and human cells (32).

Experiments on early mouse embryos showed that the need for a functional TATA box in *hsp70* promoter activation was also observed at the two-cell stage. Presence of TBP at cleavage stages has been previously shown (21), although it was also found that TATA-less promoters, including the Simian virus 40 (SV40) early promoter (15,22) can be expressed in early mouse embryos (16). It is interesting to note that levels of β-galactosidase activity expressed by the TATA-less *hsp70* promoter here described (p*mTATAhsplacZ*) were found to be strikingly similar to those produced by construct p*eSV40lacZ* in previous observations (15). Expression of p*eSV40lacZ* is controlled by the TATA-less SV40 early promoter, whose activity is exclusively driven by GC boxes. Promoters of p*mTATAhsplacZ* and p*eSV40lacZ* thus represent very similar promoters, in which a GC box-binding factor(s) is sufficient to induce formation of the transcriptional initiation complex (33,34). It therefore appears that at the beginning of

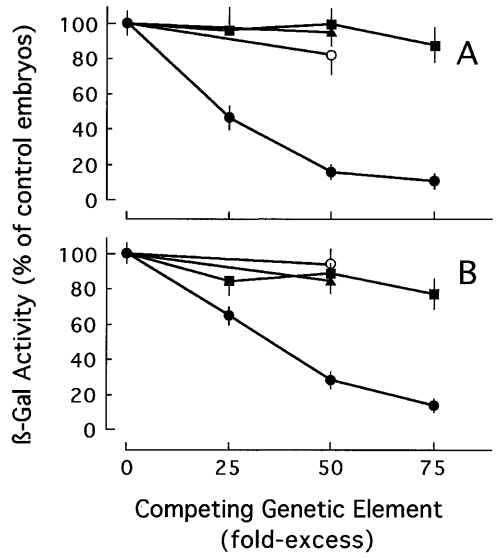

Figure 4. Effect of oligonucleotide injection on p*hsplacZ* expression in two-cell mouse embryos. Symbols indicate β-galactosidase activity of embryos that received 500 copies of construct p*hsplacZ* and increasing amounts of double-stranded oligonucleotides, equal to 12 500 copies (25-fold excess), 25 000 copies (50-fold excess) and 37 500 copies (75-fold excess) of the appropriate genetic element. (**A**) Embryos were injected at the one-cell stage and assayed for β-galactosidase activity at the two-cell stage. (**B**) Embryos were injected and assayed at the two-cell stage. Embryos received wild-type (●) or mutated (○) GC box oligonucleotides, wild-type CCAAT box oligonucleotides (▲), or wild-type HSE oligonucleotides (■). Values represent the mean ± SEM of 20–40 embryos, pooled from at least three independent experiments. Effect of wild-type Sp1 oligonucleotides: (A) $r = -0.753$, $P < 0.0001$; (B) $r = -0.786$, $P < 0.0001$.

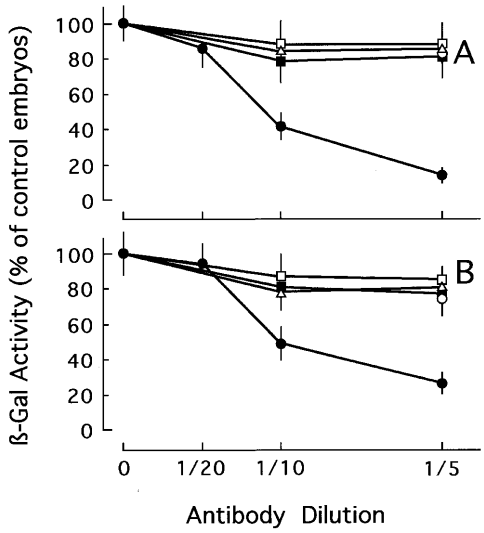

Figure 5. Effect of antibody injection on p*hsplacZ* expression in two-cell mouse embryos. Symbols indicate β-galactosidase activity of embryos injected with 500 copies of construct p*hsplacZ* and increasing amounts of antibodies, corresponding to IgG concentrations of 12.5 ng/µl (1/20 dilution), 25 ng/µl (1/10 dilution) and 50 ng/µl (1/5 dilution). (**A**) Embryos were injected at the one-cell stage and assayed for β-galactosidase activity at the two-cell stage. (**B**) Embryos were injected and assayed at the two-cell stage. Symbols indicate the antibody injected: (●) Sp1; (○) Sp1, preadsorbed with the immunogenic peptide; (△) Sp2; (□) HSF1; (■) HSF2. Values represent the mean ± SEM of 20–40 embryos, pooled from at least three independent experiments. Significance of the effect of Sp1 antibodies in the interval 1/20–1/5: (A) $r = -0.586$, $P < 0.0001$; (B) $r = -0.661$, $P < 0.0001$.

mouse development, such a factor(s) alone is capable of inducing transcription of a TATA-less promoter, and that its cooperative interaction with TBP on a TATA box-containing promoter increases transcriptional efficiency 2- to 3-fold (compare histograms of Fig. 3). The dependency of *hsp70* gene promoter activity on the TATA box confirms previous observations indicating that factors of the basal transcription apparatus in early mouse embryos are in common with those of somatic cells (16).

A substantial difference with L-cells, however, was found analyzing the effects of mutations in the CCAAT and GC boxes on activation of the *hsp70* promoter in two-cell embryos. In fact, while the presence of the CCAAT box was dispensable, the presence of the GC box was essential for activation at the two-cell stage. This result identified the proximal GC box as the main controlling element of the *hsp70* promoter in early mouse embryos. Since other promoters active at the earliest stages of mouse development have been found to depend on GC boxes, including those for the herpes simplex virus (HSV) thymidine kinase gene (16) and the SV40 early genes (15,22,35), it appears that a factor(s) binding such genetic elements is among the earliest transcription factors active after fertilization.

In order to demonstrate presence of the GC box-binding activity, expression of microinjected p*hsplacZ* construct was competed with increasing amounts of displacing genetic elements in intact embryos. *In vivo* titration of transcription factors by specific genetic sequences was originally developed in cleaving sea urchin embryos by Livant and coworkers (36), who injected the 9.3 kb promoter of the *CyIIIa* gene into transgenic embryos and observed a strong reduction of expression of a *CyIIIa*-CAT fusion transgene, although expression of endogenous *CyIIIa* was not affected. More recently, a GC box-binding factor was titrated in early mouse embryos by coinjecting a reporter construct that carried two such genetic elements, with competing amounts of another plasmid with 12 tandem copies of the same element (16).

The novelty of our approach was the use of purified, double-stranded, 27–40mer oligodeoxyribonucleotides, instead of entire plasmids or full-length promoters (Fig. 4). In our experiments, we have coinjected p*hsplacZ* with increasing amounts of oligonucleotides containing canonical/mutated GC box, canonical CCAAT box or HSE sequences. Canonical GC box oligonucleotides effectively inhibited expression of p*hsplacZ* in a dose-dependent manner and regardless of the injection schedule. In addition, factor binding was strongly specific for canonical GC box oligonucleotides, since no effects were observed with either mutated GC box or canonical CCAAT box, or HSE sequences. Oligonucleotide titration experiments fully confirmed results of mutational analysis, pinpointing the proximal GC box as the genetic element of the *hsp70* gene promoter essential for its developmental activation pattern. Dispensability of the proximal CCAAT box for expression at the two-cell stage was not surprising, being in agreement with our previous finding that expression of an episomic *lacZ* gene driven by the human β-*actin* promoter, which is mainly controlled by a CCAAT box, is initiated in mouse embryos only at the four-cell stage (18). These observations may suggest that CCAAT box binding activities appear in early mouse embryos only after transcription is initiated. Absence of effects by HSE oligonucleotides was also expected on the basis of our previous observations that *hsp70*

promoter sequences containing such elements are dispensable for full activity of the injected construct in early mouse embryos(18).

The conclusive finding that a GC box-binding factor(s) was involved in activation of *hsp70* promoter at the beginning of mouse development prompted us to identify such factor(s) by titration experiments with antibodies to Sp1 and Sp2 factors. Control experiments were performed by use of antibodies to HSF1 and HSF2.

Microinjection of antibodies to block specific protein functions has been particularly successful in the study of gap junctional communication during murine preimplantation development (37). Experiments reported in the present study show evidence that microinjection of antibodies to a specific transcription factor into early mouse embryos results in abolition of activity of a promoter which is under the control of that factor. Blocking effect of antibody to Sp1 was dose dependent, since it was not observed at the lowest concentration employed, but increased progressively by increasing antibody concentration in the range 25–50 ng/µl. In addition, the antibody effect was highly specific, since it was suppressed by preadsorption with the immunogenic peptide, and since microinjection of antibody to the related factor Sp2 at the same concentrations had no effect on activation of the *hsp70* promoter. Finally, use of antibodies to other transcription factors such as HSF1 and HSF2, which were expected not to play a significant role in early p*hsplacZ* transcription on the basis of previous deletion analysis (18) and present oligonucleotide titration assays, did not alter the pattern of *hsp70* promoter activation.

Antibody microinjection confirms previous observations on the presence of Sp1 transcription factor in two-cell mouse embryos (21) and demonstrate that this factor is directly responsible for transcriptional activation of a gene promoter at this developmental stage. Sp1 thus represents one of the earliest factors active at the onset of embryonic genome expression in the mouse. It remains to be elucidated whether this factor is involved in the transcriptional activation of endogenous genes and thus plays a role as a general activator of gene expression in mouse embryos.

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