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The SINE-encoded mouse B2 RNA represses mRNA transcription in response to heat shock

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Cells respond to changes in environmental conditions via orchestrated modifications in gene expression. For example, in response to heat shock, cells execute a program of gene-specific transcriptional activation and repression. Although the activation of genes upon heat shock has been widely studied, the mechanism of mRNA transcriptional repression upon heat shock is unexplained. Here we show that during the heat shock response in mouse cells, a small noncoding RNA polymerase III transcript, B2 RNA, associates with RNA polymerase II and represses transcription of specific mRNA genes. These studies define a unique transcriptional regulatory mechanism involving an RNA regulator and reveal how mRNA transcription is repressed upon heat shock. Moreover, we identify a function for B2 RNA, which is transcribed from short interspersed elements that are abundant in the mouse genome and historically considered to be 'junk DNA.'

A critical control point for regulating gene expression is at the level of mRNA transcription. mRNA is transcribed by RNA polymerase II (Pol II) during a complex process that is tightly regulated by the coordinated actions of activators and repressors. The vast majority of transcriptional regulators are proteins; however, RNA regulators of transcription have recently been identified[1–5]. While studying the mechanism of early transcription by Pol II *in vitro*, we found that a single-stranded polyguanosine RNA oligonucleotide potently inhibits a specific step in transcription by binding tightly to Pol II[6]. Accordingly, we hypothesized that natural RNAs exist that bind to Pol II and repress mRNA transcription in mammalian cells. Of the natural eukaryotic RNA regulators of transcription identified to date, none function via direct interaction with Pol II.

One condition under which general Pol II transcription is transiently repressed is during the heat shock response, but the mechanism by which this occurs is not understood. Total Pol II transcriptional activity decreases in response to heat shock in eukaryotic cells[7–13]. When the transcription of specific *Drosophila melanogaster* genes was investigated using pulse-chase, nuclear run-on, and *in vivo* cross-linking experiments, it was found that transcription of the actin and histone H1 genes markedly decreased within minutes after heat shock, whereas transcription of genes encoding heat shock proteins (such as hsp70) markedly increased[14–16]. In extracts from non-heat-shocked HeLa cells, a non-heat-shock-responsive promoter (the adenovirus major late promoter, AdMLP) was shown to be active whereas the hsp70 promoter was not[17]. When an extract from heat-shocked cells was titrated into the extract from non-heat-shocked cells, it inhibited transcription from the AdMLP and supported transcription from the hsp70 promoter[17]. These results indicate that extracts from heat shocked cells contain a negative regulator that represses RNA synthesis from a non-heat-shock promoter, but this regulator has not been identified.

Changes in RNA polymerase III (Pol III) transcription have also been observed in response to heat shock. For example, upon heat shock in mouse cells two small, untranslated Pol III transcripts are upregulated: B1 RNA and B2 RNA[18–22]. B1 and B2 RNAs are transcribed from short interspersed elements (SINEs) and have no known function[23]. SINEs, which are abundant in most eukaryotic genomes, are mobile elements that can relocate in the genome via retrotransposition[24]. The increase in B1 and B2 RNAs in response to cell stress such as heat shock is controlled at the level of Pol III transcription[20,22,25]. This is specific to SINE genes and is not a general characteristic of all Pol III transcription; for example, 7SK, 7SL and U6 RNAs do not increase upon heat shock[18]. In addition to mouse B1 and B2 RNAs, SINE transcripts from other organisms increase with heat shock[18,26]. Why SINE transcripts are upregulated during heat shock has remained unclear; however, it has been proposed that these RNAs play a critical role in regulating the cellular response to heat shock[18].

We hypothesized that B1 and/or B2 RNA would associate with Pol II and repress mRNA transcription in mouse cells after heat shock. To test this, we investigated the Pol II and III transcriptional responses to heat shock in mouse cells. We found that Pol III transcription was required for the repression of Pol II transcription in response to heat shock, that B2 RNA represses Pol II transcription in heat shocked cells and *in vitro*, and that B2 RNA associates with Pol II specifically after heat shock. Our experiments suggest a model whereby upon heat shock in mouse cells, B2 RNA is upregulated, binds Pol II and represses mRNA transcription.

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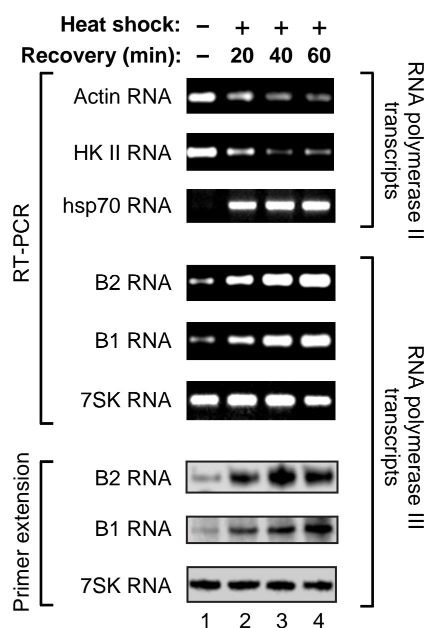

Figure 1 After heat shock in NIH 3T3 cells, nuclear B1 and B2 RNA levels decreased and nuclear actin and hexokinase II mRNA levels increased concomitantly. RNA polymerase II and III transcripts were monitored by RT-PCR or primer extension at the times indicated after heat shock.

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RESULTS

Transcription changes rapidly after heat shock

We first asked whether a correlation exists between the rate at which Pol II transcription is downregulated and the rate at which Pol III transcription is upregulated after heat shock. Based on previous studies we anticipated that the decrease in mouse Pol II transcription after heat shock would be rapid[14–16]; however, it had not been determined whether the upregulation of B1 and B2 RNAs occurs rapidly. Moreover, it was unclear whether B1 and B2 RNAs accumulate in the nucleus after heat shock; this would be necessary if they were involved in repressing Pol II transcription. To investigate these phenomena, we isolated nuclei from NIH 3T3 (mouse fibroblast) cells and examined the abundance of several mRNAs and Pol III transcripts at early time points after heat shock. In this way, only newly synthesized RNA was detected, as opposed to steady-state levels of mRNA (which are predominately cytoplasmic), thereby allowing changes in transcription to be detected. Using RT-PCR we monitored levels of three Pol II transcripts in response to heat shock and found that actin and hexokinase II rapidly decreased while hsp70 rapidly increased (**Fig. 1**). Of the three Pol III transcripts monitored, B1 and B2 RNAs rapidly increased in response to heat shock while 7SK RNA remained constant. Notably, the rates at which nuclear actin and hexokinase II mRNA levels decreased were similar to the rates at which nuclear B1 and B2 RNA levels increased. Control reactions carried out in the absence of reverse transcriptase produced no detectable PCR products (data not shown). To ensure that the experiments were done in a linear response range, nuclear RNA was titrated into the reverse transcription reactions, and the resulting cDNA was titrated into the PCR reactions. We also monitored the Pol III transcripts using primer extension to ensure that B1 and B2 RNAs with the correct 5′ ends increased upon heat shock, as opposed to mRNAs containing B1 and/or B2 sequences. Indeed, B1 and B2 RNAs with the correct 5′ ends increased upon heat shock, whereas 7SK RNA remained constant (**Fig. 1**). We conclude that the decreases and increases in Pol II and III transcription, respectively, are rapid and concurrent.

Tagetitoxin blocks mRNA decreases after heat shock

To directly test whether Pol III transcription is involved in the repression of Pol II transcription after heat shock, we treated mouse cells with tagetitoxin, which specifically inhibits Pol III by pausing elongation complexes[27]. No effect on transcription by RNA polymerases I or II has been observed with tagetitoxin[28]. We treated NIH 3T3 cells with tagetitoxin for 1 h before heat shock. The increases in levels of nuclear B1 and B2 RNAs that normally occur in response to heat shock were attenuated by tagetitoxin treatment (**Fig. 2**). Notably, in cells treated with tagetitoxin, nuclear actin and hexokinase II mRNA levels remained constant during the response to heat shock. The level of 7SK RNA was not appreciably affected by tagetitoxin. Moreover, tagetitoxin had no effect on nuclear hsp70 mRNA before and after heat shock. Together, these results show that during the heat shock response in mouse cells (i) ongoing Pol III transcription is required for the inhibition of mRNA transcription (ii) the upregulation of hsp genes does not depend on ongoing Pol III transcription, and (iii) tagetitoxin does not affect Pol II transcription nonspecifically (that is, hsp70 transcript levels were unaffected). These results show that Pol III transcription and Pol II transcription are coupled in regulating the cellular response to heat shock.

B2 RNA inhibits Pol II *in vitro*

Because B1 and B2 RNAs are the only Pol III transcripts known to increase with heat shock in mouse cells[18], we next determined whether B1 and/or B2 RNA could directly repress Pol II transcription *in vitro*. To do so we generated recombinant B1 RNA (136 nt) and B2 RNA (178 nt) by *in vitro* transcription (**Fig. 3a**). The purified B1 and B2 RNAs were titrated into Pol II transcription reactions using nuclear extracts prepared from NIH 3T3 cells. Primer extension was used to monitor GAL4-p53 activated transcription from a GAL4 responsive promoter. B2 RNA inhibited Pol II transcription with an IC_{50} of

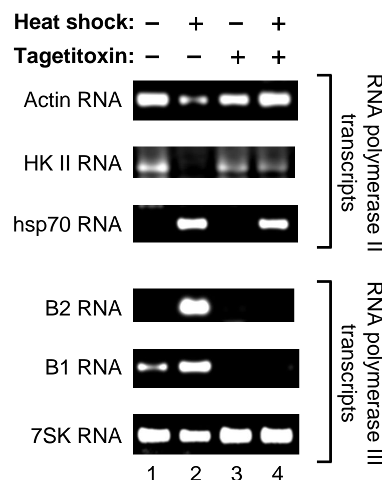

Figure 2 Ongoing Pol III transcription is required for the decrease in mRNA transcription after heat shock. NIH 3T3 cells were treated with tagetitoxin to block Pol III transcription, and the cells were subsequently heat-shocked. Nuclear B1 and B2 RNA levels no longer increased and nuclear actin and hexokinase II levels no longer decreased upon heat shock when compared with untreated cells. RNAs were monitored by RT-PCR.

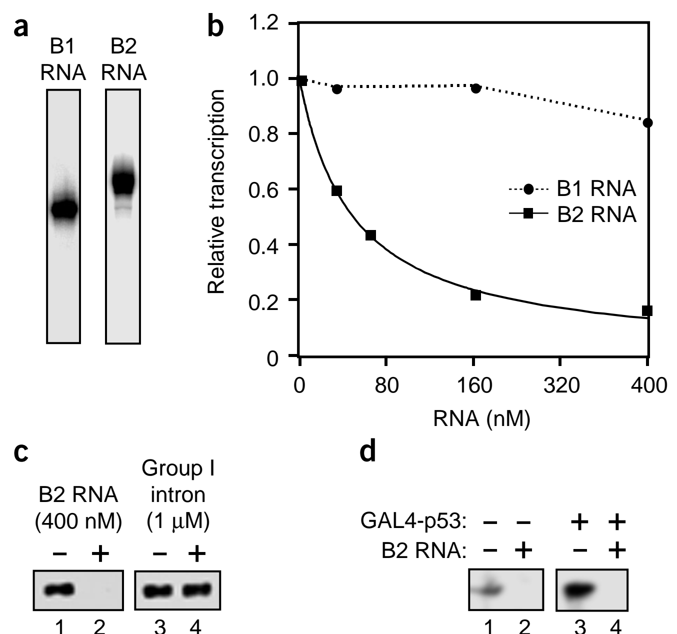

Figure 3 B2 RNA inhibits Pol II transcription *in vitro*. (**a**) B1 RNA (136 nt) and B2 RNA (178 nt) were produced by *in vitro* transcription and gel-purified. The RNAs were resolved by denaturing PAGE. (**b**) B2 RNA, but not B1 RNA, inhibited Pol II transcription when added to NIH 3T3 nuclear extract. B1 RNA and B2 RNA were titrated into transcription reactions and GAL4-p53 activated transcription was analyzed by primer extension. The IC_{50} for inhibition by B2 RNA was 60 nM. (**c**) A control RNA, the group I intron, did not inhibit GAL4-p53 activated Pol II transcription when added to NIH 3T3 nuclear extract. (**d**) B2 RNA inhibited basal transcription when added to NIH 3T3 nuclear extract. B2 RNA (400 nM) was added to basal and GAL4-p53 activated transcription reactions and products were analyzed by primer extension.

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~60 nM; by contrast, B1 RNA had little effect at concentrations as high as 400 nM (**Fig. 3b**). As a control, we tested another structured RNA, the 158-nt P4–P6 region of the Dunaliella group I intron[29]. This control RNA had no effect on Pol II transcription when added to reactions at a final concentration of 1 μM (**Fig. 3c**). These experiments show that B2 RNA inhibits Pol II transcription with specificity. Moreover, of the two Pol III transcripts that increase upon heat shock in mouse cells, B2 RNA can inhibit Pol II transcription *in vitro* and B1 RNA cannot.

B2 RNA could act as a general repressor of transcription or as an inhibitor of transcriptional activation. To distinguish between these possibilities, we asked whether B2 RNA could inhibit basal (unactivated) transcription *in vitro*. In reactions in which GAL4-p53 was omitted, we found that B2 RNA inhibited basal transcription by Pol II (**Fig. 3d**). Therefore, B2 RNA is likely to function as a general repressor of transcription. Together, the data in **Figure 3** show that B2 RNA directly and specifically inhibits Pol II transcription *in vitro*.

B2 RNA blocks mRNA synthesis upon heat shock in cells

We next determined whether B2 RNA represses mRNA transcription in mouse cells after heat shock. To do so, we introduced an antisense oligonucleotide directed against B2 RNA into mouse cells and monitored changes in actin and hexokinase II transcription in response to heat shock. We used an antisense oligonucleotide that annealed to native B2 RNA *in vitro*, as determined by RNase H degradation (data not shown). The antisense oligonucleotide was coupled to a membrane permeant peptide (Penetratin) that can transduce cargo across cell membranes. Control experiments with a fluorescently labeled oligonucleotide coupled to Penetratin showed that transduction occurred with >80% efficiency in NIH 3T3 cells (data not shown).

The antisense oligonucleotide, or a control oligonucleotide of scrambled sequence, was transduced into NIH 3T3 cells, the cells were heat shocked, and after 1 h of recovery, nuclear RNA was isolated. Notably, in cells treated with the antisense oligonucleotide nuclear actin and hexokinase II, mRNA levels remained constant in response to heat shock (**Fig. 4**, lanes 3 and 4), whereas their levels decreased in response to heat shock in cells treated with the control oligonucleotide (lanes 1 and 2). Treatment with the antisense oligonucleotide did not disrupt hsp70 induction in response to heat shock nor did it affect the levels of 7SK RNA. Nuclear B2 RNA levels (monitored by northern blot to avoid interference of the antisense oligonucleotide with RT-PCR) were reduced by treatment with the antisense oligonucleotide both before and after heat shock. It is possible that this decrease alone is responsible for attenuating the repression of actin and hexokinase II transcription that normally occurs upon heat shock, although the antisense oligonucleotide could function through other mechanisms. The results in **Figure 4** show that B2 RNA is required for the repression of actin and hexokinase II transcription after heat shock in mouse cells.

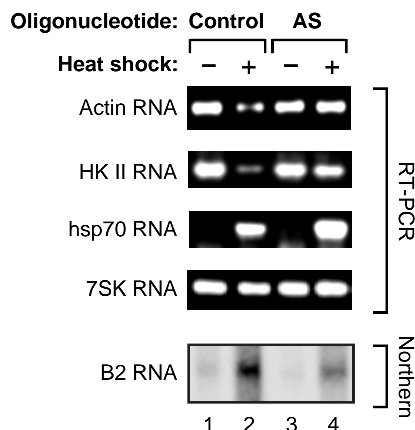

Figure 4 B2 RNA is required for repression of mRNA transcription upon heat shock in mouse cells. Repression of actin and hexokinase II mRNA levels in response to heat shock was attenuated by a B2 RNA antisense oligonucleotide (AS), but not by a control oligonucleotide (control). RNAs were monitored by RT-PCR or northern blotting as indicated.

B2 RNA associates with Pol II after heat shock

Our initial hypothesis that a natural RNA would repress mRNA transcription in mammalian cells was based on previous studies in which we found that a single-stranded polyguanosine RNA oligonucleotide inhibits transcription *in vitro* by binding tightly to Pol II[6]. Accordingly, we hypothesized that transcriptional inhibition by B2 RNA after heat shock would involve its association with Pol II. As an initial test of this hypothesis, we immunoprecipitated Pol II from nuclear extracts prepared from NIH 3T3 cells before and after heat shock and probed for B2 RNA. B2 RNA coimmunoprecipitated with Pol II exclusively after heat shock, whereas 7SK RNA did not, as detected by northern blot (**Fig. 5**). Thus, B2 RNA is in a complex with Pol II specifically after heat shock. This association was not highly sensitive to salt; the nuclear

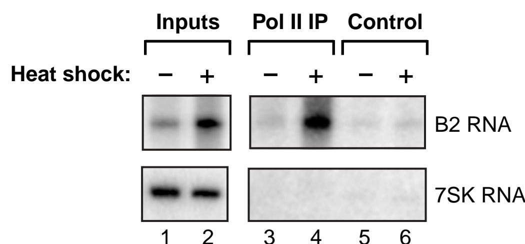

Figure 5 B2 RNA associates with Pol II in response to heat shock. Nuclear extracts prepared from NIH 3T3 cells before and after heat shock were incubated in the presence (Pol II IP) or absence (Control) of an antibody against Pol II. B2 RNA and 7SK RNA in the immunoprecipitate were detected by northern blotting.

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extracts and buffers used in the coimmunoprecipitation contained 300 mM KCl. Western blots showed that the levels of immunoprecipitated Pol II did not change with heat shock (data not shown). By comparison with known amounts of recombinant B2 RNA and purified Pol II, we estimated that there was a two- to three-fold excess of B2 RNA over Pol II in nuclear extracts after heat shock using primer extension and western blots, respectively. In addition, ~25% of the immunoprecipitated Pol II had B2 RNA associated with it. These results suggest that B2 RNA inhibits mRNA transcription in mouse cells after heat shock via association with Pol II.

DISCUSSION

Here we show that B2 RNA represses Pol II transcription after heat shock in mouse cells. Our results lead to an understanding of the mechanism by which mRNA transcription is repressed in response to heat shock, and demonstrate the first function for the SINE-encoded mouse B2 RNA. We found that transcriptional repression of the actin and hexokinase II genes after heat shock is rapid and concurrent with the induced expression of B2 RNA by Pol III. Inhibiting ongoing Pol III transcription alleviates the repression of mRNA transcription, thereby establishing a link between the two polymerases after heat shock. B2 RNA specifically represses transcription when added to *in vitro* transcription reactions. Moreover, an antisense oligonucleotide that targets B2 RNA blocks transcriptional repression of the actin and hexokinase II genes after heat shock in mouse cells. Finally, B2 RNA associates with Pol II specifically after heat shock. Together our results support a model (**Fig. 6**) for mRNA transcriptional repression upon heat shock. Transcription of B2 RNA by Pol III increases upon heat shock, resulting in increased levels of nuclear B2 RNA. The abundant B2 RNA associates with Pol II and inhibits mRNA transcription.

Coimmunoprecipitation experiments (**Fig. 5**) demonstrate a specific association of B2 RNA with Pol II after heat shock. In related work, we found that recombinant B2 RNA binds directly to an RNA docking site on core Pol II with high affinity and specificity[30]. We also found that B2 RNA assembles into preinitiation complexes *in vitro* along with Pol II to block transcript synthesis[30]. It is likely that upon heat shock in mouse cells, B2 RNA binds the RNA docking site on Pol II and associates with the polymerase at the promoters of repressed genes to inhibit transcription. Future analysis of the molecular composition of the immunoprecipitated Pol II complex after heat shock could reveal other factors involved in the repression of mRNA transcription upon heat shock in mouse cells.

A mechanism must exist to allow the transcription of genes such as *hsp70* despite the presence of nuclear B2 RNA during the heat shock response. In addition, a mechanism must exist to relieve repression by B2 RNA at genes such as those encoding actin and hexokinase II as cells recover from heat shock over the course of hours. Studies characterizing changes in the transcription of all genes in the mouse genome after heat shock could provide insight into the mechanism by which specific genes bypass and/or recover from repression by B2 RNA. Understanding the interplay of B2 RNA, the upregulation of heat shock genes, and the return to homeostasis will be the goal of future research.

In the past several years, RNA molecules have begun to be recognized as mediators of several cellular processes. A limited number of previous studies have identified small eukaryotic RNAs capable of regulating mRNA transcription. The 7SK RNA binds to the human positive transcription elongation factor (P-TEFb) and inhibits its kinase activity, thereby repressing mRNA transcription[3,4]. The steroid receptor coactivator (SRA) functions as an RNA in a ribonucleoprotein complex to activate transcription[1]. U1 snRNA associates with TFIIH and regulates transcription initiation[2]. Small interfering RNAs have recently been found to mediate the formation of transcriptionally silent chromatin domains[31]. Unlike B2 RNA, the aforementioned RNAs do not directly bind to Pol II to modulate its activity. The action of the mouse B2 RNA is most closely related to that of the bacterial 6S RNA, a natural small RNA that binds *Escherichia coli* RNA polymerase in *trans* to repress transcription[5]. Structural and phylogenetic studies of 6S and B2 RNAs will be needed to determine if they are evolutionarily related.

Much research has focused on the abundance, mobility and evolutionary characteristics of SINEs; however, their functions have remained largely unknown. Our finding that B2 RNA represses Pol II transcription in response to heat shock provides the first experimental evidence supporting a biological function for the SINE-encoded B2 RNA. B2 RNA also has the potential to control mRNA transcription in response to a plethora of other cellular signals. B2 RNA levels change during development[32], in cancer cells[33,34], in response to UV irradiation[35] and upon viral infection[36], all of which also involve changes in Pol II transcription. It remains to be determined whether B2 RNA participates in regulating Pol II transcription under any of these cellular conditions.

The repression of mRNA transcription during heat shock in human cells is likely to involve a small noncoding RNA. A human RNA with sequence similarity to B2 RNA has not been identified[23]; however, the human Alu RNA shares many characteristics with B2 RNA and is a potential candidate for regulating mRNA synthesis during heat shock. Alu RNA is transcribed by Pol III from SINEs that are abundant in the human genome[23]. Moreover, Alu RNA is upregulated in response to heat shock[18] as well as in response to stresses other than heat shock,

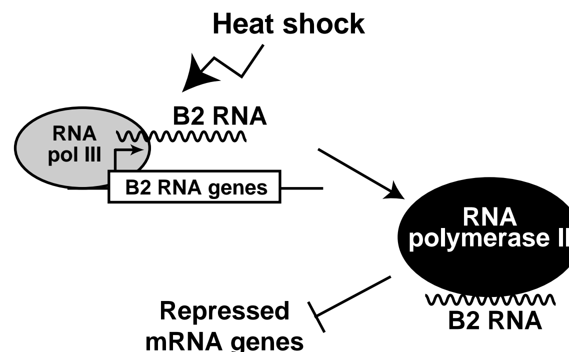

Figure 6 Model illustrating the mechanism of transcriptional repression after heat shock in mouse cells. Upon heat shock, B2 RNA is synthesized by Pol III, binds to Pol II and inhibits mRNA transcription.

such as UV irradiation and viral infection[35,37–41]. Previously, Alu RNA has been found to stimulate translation in response to cell stress[42]; it remains to be determined whether Alu RNA is involved in inhibiting Pol II transcription under similar conditions. The upregulation of specific Pol III transcripts in response to stress is not unique to mammalian cells. A heat shock–induced Pol III transcript exists in *Tetrahymena thermophila*[43]. In addition, the silkworm Bm1 SINE RNA has been found to increase in response to stress[26]. Our studies showing that B2 RNA has a function provide a rationale for the evolutionary maintenance of SINEs in genomes.

METHODS

Heat shock and isolation of nuclear RNAs. NIH 3T3 cells were heat shocked for 15–25 min at 45 °C and recovered at 37 °C for 1 h unless otherwise indicated. Nuclei were isolated by resuspending cells in 80 µl of buffer A (2 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, and 0.5% (v/v) NP-40) per 1 million cells and incubating for 5 min on ice. Nuclei were harvested by centrifugation and washed once in an equal volume of Buffer A. Nuclear RNA was isolated using either Trizol reagent (Invitrogen) or cell lysis buffer (Ambion, Cells-to-cDNA II) per manufacturer's instructions.

Detection of nuclear RNAs. For RT-PCR, RNA samples isolated as described above were treated with DNaseI (1–4 Units) at 37 °C for 30 min, which was then heat-inactivated. The RNA was titrated into reactions containing 25 pmol of the reverse primers described below, 12 Units of RNA guard (Amersham Pharmacia), and Moloney murine leukemia virus reverse transcriptase in 40 µl buffer B (25 mM KCl, 50 mM Tris, pH 7.5, 10 mM DTT, 3.5 mM $MgCl_2$, 100 µg ml^{-1} BSA, and 0.5 mM of each dNTP). Reactions were incubated at 42 °C for 1 h, then heated at 95 °C for 3 min. Parallel reactions were carried out in the absence of reverse transcriptase. cDNA was then titrated into PCR reactions containing 25 pmol of the primers described below. Titrations of the RNA into the reverse transcriptase reactions and the cDNA into the PCR reactions were carried out to ensure that signals were within the linear response range. The following primers were used (5′→3′): hexokinase II forward, CCCTGTGAAGATGTTGCCCAC; hexokinase II reverse, TGCCCATGTACTCAAGGAAGT; actin forward, TGGTGGGTATGGGTCAGAAG; actin reverse, GGTCATCTTTTCACGGTTGG; hsp70 forward, ACGTGGCCTTCACCGACACC; hsp70 reverse, CGATCTCCTTCATCTTCGTC; 7SK forward, ATTGATCGCCAGGGTTGATT; 7SK reverse, CGGGGAAGGTCGTCCTCTTC; B1 forward, TGGTGGTGCATGCCTTTAAT; B1 reverse, CCTGGTGTCCTGGAACTCACT; B2 forward, GGCTGGTGAGATGGCTCAGT; B2 reverse, TACACTGTAGCTGTCTTCAGACA.

For primer extension, RNA samples were incubated with 6 pmol ^{32}P-labeled primer (B2 primer, 5′-TACACTGTAGCTGTCTTCAGACA; B1 primer, 5′-GAACTCACTCTGAAGACCAG; 7SK primer, 5′-CGGGGAAGGTCGTCCTCTTC) in 10 µl of 250 mM KCl, 10 mM Tris, pH 7.5, and 1 mM EDTA for 1 h at 56 °C. Buffer B (40 µl) and 0.5 µl reverse transcriptase were added and incubated at 37 °C for 1 h. Products were ethanol-precipitated and resolved by 8% (w/v) denaturing PAGE.

For northern blotting, RNAs were resolved by 6% (w/v) denaturing PAGE and subsequently transferred to Hybond N^+ membrane (Amersham Pharmacia). ^{32}P-labeled probes (1×10^7 c.p.m.) were hybridized to the membrane overnight at 55 °C (B2 probe, 5′-GATGGTTGTGAGCCACCATGTGGTTGCTGGCA; 7SK probe, 5′-CGGGGAAGGTCGTCCTCTTC). Blots were washed in 0.1× SSC.

Tagetitoxin and antisense experiments. For experiments involving tagetitoxin, NIH 3T3 cells were incubated with 45 µM tagetitoxin (Epicentre Technologies, Tagetin) for 60 min at 37 °C before heat shock. For antisense experiments, oligonucleotides (B2 antisense, 5′-TCAGATCTCGTTACGGATGGTTGTGA; B2 control, 5′-TTGGTACGCATACGTGTTGACTGTGA) were purchased with a 3′-thiol group (thiol modifier C3 S–S) and phosphorothioate linkages at three positions on both ends (Integrated DNA Technologies). Oligonucleotides were coupled to activated Penetratin 1 peptide (Q-Biogene) per manufacturer's instructions. NIH 3T3 cells were incubated with 200 nM Penetratin-oligonucleotide fusion overnight in DMEM containing 0.5% (v/v) serum before heat shock.

Immunoprecipitation. NIH 3T3 cells were heat shocked for 15 min at 45 °C and recovered 45 min at 37 °C. Nuclei were isolated as described above. Nuclei from 8 million cells were resuspended in 250 µl of ice cold buffer C (10% (v/v) glycerol, 300 mM KCl, 10 mM HEPES, pH 7.6, 10 mM Tris, pH 7.5, 0.1% (v/v) NP-40, 4 mM $MgCl_2$, 2 mM DTT, 40 Units ml^{-1} apyrase (Sigma, grade III), 160 Units ml^{-1} Superasin (Ambion), 1 mM PMSF, and 1× protease inhibitors (Complete cocktail tablets, Roche)). Samples were frozen and thawed three times using liquid nitrogen and then spun at 16,000*g* for 15 min at 4 °C. Supernatants were nutated for 2 h at 4 °C with paramagnetic protein A beads (Dynal Biotech, 75 µl of slurry per sample) containing immobilized α-Pol II (8WG16). Beads were washed four times with Buffer C. RNAs were eluted in formamide.

Recombinant RNAs. A DNA fragment that contained a consensus mouse B1 RNA gene[44] was assembled via annealing of oligonucleotides and fill-in PCR. A second DNA fragment was assembled via ligation of oligonucleotides that contained a mouse B2 RNA gene (region 121488–121665 of *Mus musculus* chromosome 18, clone RP23-6p18, GenBank accession number AC020972). Both DNA fragments contained upstream T7 ϕ10 promoters, downstream *Dra*I restriction sites, and *Bam*HI and *Kpn*I overhangs at the upstream and downstream ends of the fragments, respectively. Plasmids pUC-T7-B1 and pUC-T7-B2 were generated by ligation of the respective DNA fragments into pUC18 digested with *Bam*HI and *Kpn*I.

B1 RNA and B2 RNA were produced via runoff transcription. DNA templates for the transcription reactions were prepared by digesting pUC-T7-B1 and pUC-T7-B2 with *Bam*HI and *Dra*I and gel-purifying the released fragment. The transcription reactions (100 µl) included 40 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 1 mM spermidine, 25 mM NaCl, 10 mM DTT, 1mM UTP, 1 mM GTP, 1mM ATP, 1 mM [α-^{32}P]CTP (1 µCi per reaction), 100 ng of template DNA, and 200 Units of T7 RNA polymerase (Gibco). Reactions were incubated at 37 °C for 1 h and ethanol-precipitated. RNAs were purified using 6% (w/v) denaturing PAGE and a crush and soak elution procedure.

***In vitro* transcription.** Reactions were carried out as described[45], using 100 ng of p(GAL4)$_5$-E1b-CAT template DNA[46], containing five GAL4-binding sites and the adenovirus E1b TATA box upstream of the CAT gene. Nuclear extract (10 µg total protein) from NIH 3T3 cells was added to reactions. Nuclear extracts[47] and GAL4-p53 (ref. 48) were prepared as described. B1, B2 and control RNAs were added prior to the nuclear extract.

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COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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