Overlapping roles and asymmetrical cross-regulation of the USF proteins in mice

MARIO SIRITO, QUN LIN, JIAN MIN DENG, RICHARD R. BEHRINGER, AND MICHELE SAWADOGO*

Department of Molecular Genetics, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030

Communicated by Steven L. McKnight, University of Texas Southwestern, Dallas, TX, January 22, 1998 (received for review October 29, 1997)

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ABSTRACT USF1 and USF2 are ubiquitously expressed transcription factors implicated as antagonists of the c-Myc protooncoprotein in the control of cellular proliferation. To determine the biological role of the USF proteins, mutant mice were generated by homologous recombination in embryonic stem cells. USF1-null mice were viable and fertile, with only slight behavioral abnormalities. However, these mice contained elevated levels of USF2, which may compensate for the absence of USF1. In contrast, USF2-null mice contained reduced levels of USF1 and displayed an obvious growth defect: they were 20–40% smaller at birth than their wild-type or heterozygous littermates and maintained a smaller size with proportionate features throughout postnatal development. Some of the USF-deficient mice, especially among the females, were prone to spontaneous epileptic seizures, suggesting that USF is important in normal brain function. Among the double mutants, an embryonic lethal phenotype was observed for mice that were homozygous for the Usf2 mutation and either heterozygous or homozygous for the Usf1 mutation, demonstrating that the USF proteins are essential in embryonic development.

USF was initially characterized as a transcription factor implicated in the regulation of the adenovirus major late promoter (1–4). In mammals, two different genes, Usf1 and Usf2, are ubiquitously expressed (5, 6). The USF1 and USF2 polypeptides assemble in both homo- and heterodimers and interact specifically at DNA sites containing a CACGTG or CACATG core sequence (7, 8). Many of these USF binding sites are also potential targets for regulation by other transcription factors, including the various members of the Myc (9, 10) and TF3 (11–13) families, which greatly complicates the identification of genes that are under USF control. Besides a common DNA-binding specificity, the Myc-related proteins also share with USF a strong structural similarity in their dimerization and DNA-binding domains, which contain a highly conserved basic helix-loop-helix leucine-zipper motif (14–16).

The relationship and extent of overlap between the functions of the Usf1 and Usf2 genes have been difficult to ascertain. The USF1 and USF2 polypeptides display a very high degree of homology in their C-terminal DNA-binding domains, with >70% identical residues (6). They also share a small but extremely conserved domain called USF-specific region, located just upstream of the basic region, that is implicated both in nuclear localization and in the specific activation of promoters containing an initiator element (17). USF1 and USF2 are otherwise quite different in their N-terminal regions, which include in each case at least one additional transcriptional activation domain (17, 18). Thus, although the different USF dimers display identical DNA-binding properties, they may well control different sets of genes by establishing specific interactions with other transcription factors. The major form of USF present in most cell lines and tissues is the USF1-USF2 heterodimer. USF1 homodimers are less abundant and USF2 homodimers are usually quite scarce (7). There are also minor USF species that contain other products of the Usf1 and Usf2 genes generated by alternative splicing (5, 7, 19).

The important role of Myc in the control of cellular proliferation, differentiation, and apoptosis is well established (20, 21). Also, Myc overexpression is a common event in cancer progression that leads to uncontrolled growth (21). In contrast, the biological function of the USF proteins remains poorly understood. In vitro experiments suggest antagonistic roles of USF and Myc in growth control, because USF overexpression is sufficient to abolish Myc-mediated cellular transformation in primary rat embryo fibroblasts (22). Other properties of USF also point toward a role of this transcription factor in growth control. These include for instance the ability of both USF1 and USF2 to interact with proteins of the Fos family (23, 24) and to inhibit proliferation in several transformed cell lines (22). Also, recent findings in our laboratory indicate a cell cycle regulation of USF expression and activity (T. Lu and M.S., unpublished results) and a complete loss of USF function in several cancer cell lines (Y. Oyang, P. M. Ismail, X. Luo, T. Lu, and M.S., unpublished results).

To investigate the biological function of the USF proteins in vivo, we generated USF-deficient mice by using gene targeting in embryonic stem (ES) cells. Herein, we report the phenotype of individual and combined null-mutations in the Usf1 and Usf2 genes. These animals, as well as the cell lines that can be derived from them, should constitute a powerful model system to investigate the specialized and overlapping functions of the USF transcription factors and identify some of their specific target genes.

MATERIALS AND METHODS

Mutagenesis of the Usf1 Gene in Murine ES Cells. A 129/SvEv mouse genomic library (Stratagene) was screened with a mouse Usf1 cDNA clone. Among 11 positive A-phage clones isolated, 3 were identified by PCR analysis as originating from the Usf1 locus, whereas the others contained Usf1 pseudogenes (25). The former were further characterized by restriction mapping and DNA sequencing to determine the genomic organization of the Usf1 locus. A detailed description of the Usf1 gene has been reported by others (19). A 3.5-kb SacI–SpeI fragment and a 3.3-kb XhoI–EcoRI fragment from the Usf1 locus were used to construct a replacement gene targeting vector (Fig. 1A). A PGK-neopA resistance expression cassette (26) was inserted in the same orientation as the Usf1 transcription between the two Usf1 homologous regions.

Abbreviations: MEF, mouse embryo fibroblast; ES cells, embryonic stem cells; EMSA, electrophoretic mobility shift assay.

*To whom reprint requests should be addressed. e-mail: msawadog@notes.mdacc.tmc.edu.
The Structure of the wild-type probes in wild-type DNA are shown above. E, exons. The sizes of the restriction fragments detected by the indicated enzymes are shown above. The targeting vector was linearized at a unique EcoRI and EcoRV restriction sites; tk, the MC1-tk expression cassette used for negative selection. The arrows beneath neo indicate the direction of transcription of each cassette. (Bottom) Structure of the targeted locus, with the sizes of the restriction fragments detected by the Southern probes shown above. (B) Southern blot showing genotypes of newborn mice. EcoRI-digested tail DNA was hybridized with the 3′ probe, and EcoRV-digested tail DNA was hybridized with the 5′ probe. The wild-type and mutant bands are shown in each case. Lanes: +/+; wild type; −/−, homozygous mutant; −/+, heterozygous mutant.

An MC1tkpA herpes simplex virus thymidine kinase expression cassette (27) was added onto the long arm of homology to enrich for homologous recombinants by negative selection. The targeting vector was linearized at a unique NotI site outside of the Usf1 homology region. Fifty micrograms of linearized vector was electroporated into 2 × 10^7 AB-1 ES cells that were subsequently cultured in the presence of G418 and I2, I5, and I9. Among 192 G418-resistant clones that were initially screened by EcoRV digestion and hybridization with a unique 3′ probe external to the vector homology region, 64 were correctly targeted. Of those, 36 were expanded for further Southern blot analysis by EcoRI digestion and hybridization with a unique 5′ probe and with a neo probe; 34 correctly targeted clones were identified.

Mutagenesis of the Usf2 Gene in Murine ES Cells. The organization of the Usf2 locus has been reported (29). A replacement gene targeting vector was constructed that contained a PGKneoBP A cassette, a 2.3-kb KpnI-NotI 5′ fragment, a 2.5-kb EcoRV-KpnI 3′ fragment, and an MC1tkpA cassette downstream of the 3′ fragment (Fig. 2A). This targeting vector (50 μg) was linearized at a unique KpnI site outside of the homology region and electroporated into 2 × 10^7 ES cells. A targeted ES cell clone was injected into a C57BL/6 female embryo to generate mutant ES cell clones. Both of the mutant ES cell clones contributed to the germ line of chimeric mice.

Generation of Mutant Mice. Two of the Usf1 mutant ES cell clones and four of the Usf2 mutant ES cell clones were injected into C57BL/6J or C57BL/6J albino blastocysts, and the resulting chimeric embryos were transferred to the uterine horns of 2.5 pseudopregnant foster mothers (30). Chimeras were identified among the resulting progeny by their ES-cell-derived agouti fur. Male chimeras were bred to C57BL/6 or C57BL/6 albino females to produce the first generation of heterozygous mutants. Both of the Usf1 mutant clones and one of the Usf2 mutant clones contributed to the germ line of chimeric mice.

For the Usf1 mutation, the first generation heterozygotes were interbred to generate mice of all three genotypes. For the Usf2 mutation, the low number of first generation heterozygotes was expanded by breeding these animals with C57BL/6 mice, and the second generation heterozygotes were used to generate mice of all three genotypes. USF2-null pups were very small and so were weaned 2–3 weeks later than their wild-type and heterozygous siblings. To generate the double mutants, Usf1(−/−) mice were first bred with Usf2(+/−) mice to generate animals that were heterozygous for both mutations. These double heterozygotes were interbred to generate mutants with all genotype combinations at both loci.

Analysis of USF Expression in Mutant Embryos. At 13.5 days, embryos generated by interbreeding either Usf1(+/−) or Usf2(+/−) mice were collected and used to establish mouse embryonic fibroblast (MEF) cell lines. For each MEF cell line, one cell of cells was used for preparation of genomic DNA and Southern blot analysis. Another plate was used for mini nuclear extract preparation as described by Schreiber et al. (31). The presence of different USF dimers in these extracts was identified by electrophoretic mobility shift assay (EMSA), using a radiolabeled 150-bp probe containing a USF-specific binding site. DNA-binding reactions (10 μl) contained 100 mM Tris-HCl (pH 7.9), 60 mM KCl, 10 mM DTT, (dI-C) (20 μg/ml), 0.1% Triton X-100, and 1.5 μl of nuclear extract (~3.4 μg of protein). After a 20-min incubation at room temperature, the reaction mixtures were supplemented with 2 μl of 30% glycerol and analyzed by electrophoresis as described (6).
For Western blot analysis, 16 μg of protein from each nuclear extract was resolved by SDS/PAGE on a 10% polyacrylamide gel and transferred to nitrocellulose. The blot was probed successively with USF2- and USF1-specific peptide antibodies (Santa Cruz Biotechnology). In each case, the horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies were detected by the ECL chemiluminescence method (Amersham).

RESULTS

Generation of Usf1 and Usf2 Mutant Alleles in the Mouse Germ Line. To mutate the Usf1 gene in mouse ES cells, we generated a targeting vector that deleted about 3.4 kb of the Usf1 locus, including most of the coding exons, by replacing them with a neomycin-resistance expression cassette (Fig. 1A). When the vector recombined with the endogenous gene, novel EcoRI and EcoRV sites were introduced (Fig. 1A). Correctly targeted clones could be detected by the presence of an additional 4-kb EcoRI band when hybridized with a 5′ probe external to the vector homology region and an additional 9-kb EcoRV band when hybridized with a 3′ probe external to the vector homology region (Fig. 1B). Correct targeting deleted the DNA region encoding amino acids 27–301 from the total 310 amino acids of USF1, which was predicted to functionally inactivate the locus. Two independent correctly targeted ES clones successfully contributed to the germ line of chimeric mice generated by blastocyst injection. The phenotypes of the Usf1 mutants from these two clones in the C57BL/6 × 129/SvEv hybrid genetic background were identical.

A similar strategy was used to generate a Usf2 mutant allele in the mouse germ line. In this case, the targeting vector deleted about 11 kb of the Usf2 locus, including most of the 5′ untranslated region and all of the coding exons, thus creating a null allele. Recombination of the vector with the endogenous gene deleted a cluster of four EcoRI sites normally present in the seventh intron of the Usf2 gene and introduced a novel site (Fig. 2A). In correctly targeted clones, hybridization with 5′ or 3′ probes external to the vector homology region detected novel 6.5-kb and 7-kb EcoRI bands, respectively (Fig. 2B).

Expression of the Different USF Dimers in Mutant Mice. The expression of USF in the different mutants was analyzed in embryos produced by interbreeding heterozygous mice (Fig. 3). Timed pregnancies were interrupted at day 13.5, and MEF cell lines were established from each embryo. Genomic DNA was prepared from each cell line for genotype determination, and nuclear extracts were used to examine by EMSA the absolute and relative levels of the USF DNA-binding complexes present in the different embryos (Fig. 3A and B).

As previously observed in many other cell types, the major USF DNA-binding species detected by EMSA in nuclear extracts from wild-type MEFs was the heterodimer containing full-length USF1 and USF2 polypeptides. The USF1 homodimers were present at a lower level and the USF2 homodimers were hardly detectable (Fig. 3A, lanes 1 and 6). As predicted for null mutants, nuclear extracts from MEFs homozygous for the Usf1 mutation contained only USF2 homodimers (Fig. 3A, lanes 2 and 10). The abundance of these USF2 homodimers was similar to that of the heterodimers in wild-type MEFs, indicating an increase in USF2 polypeptides as a result of the Usf1 mutation. Nuclear extracts from heterozygous embryos displayed a USF DNA-binding pattern that was intermediate between that observed in wild-type and homozygous embryos, suggesting a gene dosage effect. The abundance of USF polypeptides in the Usf1 mutants was also examined by Western blotting using USF1- and USF2-specific antibodies (Fig. 3C). This analysis confirmed the complete absence of USF1 in Usf1(−/−) embryos, whereas the abundance of USF1 in the heterozygotes was about half of that present in wild-type embryos. As predicted from the EMSA results, the abundance of the USF2 polypeptide increased stepwise as one or both copies of the Usf1 gene were mutated. Quantitation revealed an increase in USF2 of 24% in Usf1(+/−) embryos and 66% in Usf1(−/−) embryos. From this result, we concluded that the phenotype of the Usf1 mutants may be attenuated by the compensatory increase in USF2 if the two Usf genes performed partially redundant functions.

A similar experiment was carried out with MEFs derived from embryos produced by Usf2 heterozygous mice (Fig. 3B). When analyzed by EMSA, Usf2(+/−) MEFs displayed a USF
DNA-binding patterns vary very similar to that of the wild-type embryos. In contrast, Usf2(−/−) MEFs contained, as expected, USF1 homodimers exclusively (Fig. 3B, lanes 5, 6, and 8). However, the total level of DNA binding was in this case significantly reduced, indicating a decreased USF1 expression in these mutants. Indeed, the concentration of USF1 homodimers was no greater in the Usf2(−/−) embryos than it was in the corresponding heterozygous and wild-type embryos. Yet a significant increase in USF1 homodimers would have been expected if all of the USF1 polypeptides normally present as heterodimers were redistributed into homodimers in the absence of USF2. To exclude the possibility that additional USF1 polypeptides were present in Usf2(−/−) embryos in a form that was unable to bind DNA, further analysis was carried out by Western blotting. As shown in Fig. 3C, the complete absence of the USF2 polypeptide in Usf2(−/−) embryos was accompanied by a drastic decrease in the level of USF1, in perfect agreement with the EMSA results. Quantitation revealed that the level of USF1 in Usf2(−/−) embryos was 20% of the level present in wild-type embryos, indicating that the Usf2 gene normally stimulates the expression of USF1.

From these analyses, we concluded that each Usf gene was capable of influencing the level of expression of the other gene. However, this cross-regulation was asymmetrical. USF2 expression decreased in a dose-dependent fashion in the presence of wild-type USF1. The absence of USF2 polypeptide was accompanied by a drastic decrease in the level of USF1, in perfect agreement with the EMSA results. Quantitation revealed that the level of USF1 in Usf2(−/−) embryos was 20% of the level present in wild-type embryos, indicating that the Usf2 gene normally stimulates the expression of USF1.

Phenotype of the Usf Mutants. Mutant mice homozygous for the Usf mutation were produced at the expected frequency from heterozygote matings. The pups were completely indistinguishable from their heterozygous and wild-type siblings. Anatomical and histological analyses in adults up to 3 months of age did not reveal any physical abnormality resulting from the Usf1 mutation. USF1-null mice were also fertile and took good care of their young. However, some aspects of their behavior, especially among the females, were unusual, and they were also prone to spontaneous epileptic seizures.

First, we noticed that nearly all cages containing two or more USF1-null females had all or all but one animal lacking both whiskers and nasal fur. Whisker trimming and hair nipping are known behavioral traits of mice, possibly related to social ranking, that are occasionally observed in animals sharing the same cage for extended periods of time (32, 33). In our own colony, this practice was observed in about 20% of all cages in which animals were maintained for several months and at the same frequency for the males and the females. In contrast, this practice was observed in more than 90% of the cages containing USF1-null mice as young as 2 months of age.

Another characteristic of the USF1-null mice was their behavior under conditions of moderate stress. In particular, epileptic seizures were occasionally observed when the animals were handled just after awakening. The actual series of events observed during these seizures varied in different episodes. Most noticeable were cases where one or several of the following events were noted: presence of foamy saliva around the mouth, the animal squeaking before falling on their side, a brief period of intense convulsions, abnormal posturing (head bent backwards, curled up tail, folding back of limbs if picked up by the tail), complete immobility lasting up to 5 min, and abundant urination. Progressive recovery required several minutes. Most of the USF1-null mice known to have had one or more seizures were female, although a few males were also affected. Similar behaviors were never observed in any of the wild-type or heterozygous control mice. From these observations, we concluded that the Usf1 gene was important for normal behavior in response to stress.

Phenotype of the Usf2 Mutants. USF2-null mice are viable. The numbers of wild-type, heterozygous, and homozygous pups born from heterozygote matings did not significantly differ from those expected for a Mendelian distribution (Table 1). However, the USF2-null mice displayed an obvious growth defect (Fig. 4). At birth, these animals could readily be distinguished from their siblings because they were noticeably smaller (Fig. 4A and B). They had in addition a distinct bend in the middle of the tail that disappeared within a few days (Fig. 4B). A significant percentage of USF2-null pups died shortly after birth (Table 1). It is unclear at this point whether this increased perinatal mortality rate had other causes besides the decreased ability of these animals to compete for their mother’s milk. Indeed, many of them were still without milk several hours after birth. However, most of the USF2-null pups that survived this critical period (nearly 50%) subsequently developed in an apparently normal fashion.

At birth, the weight of the Usf2 homozygotes was 20–40% less than the weight of their wild-type and heterozygous siblings. This size difference remained unchanged during postnatal development (Fig. 4A and C), and adult USF2-null mice displayed proportionate body features (Fig. 4D). About 10% of these animals had one or two spots of unpigmented fur on the belly. Fertility was also clearly affected by the Usf2 mutation, because only one out of three males that mated at the age of 2.5 months with wild-type females eventually produced a litter. As for the Usf1 mutants, occasional epileptic seizures were observed in some of the USF2-null females. Finally, although the cause of death is unclear at this point, USF2-null males had drastically decreased lifespans. Most of them died between the ages of 2.5 and 4.5 months, and no male so far has survived past 6 months of age. In contrast, all of the females, currently 4–10 months old, are still alive. From these results, we concluded that the Usf2 gene was indispensable for normal embryonic development and also played an essential role in adult mice, with pleiotropic effects on at least brain function, fertility, and male lifespan.

Embryonic Lethality of the Double Usf1/Usf2 Mutation. The viability of double Usf1/Usf2 mutants was evaluated by using Usf1(+/−)/Usf2(+/−) mice. These double heterozygotes, which appeared normal, were obtained at the expected 50% frequency from crosses between Usf1(−/−) and Usf2(+/−) mice. The double heterozygotes were interbred to generate mice with all possible genotypes at the Usf1 and Usf2 loci. As illustrated in Table 2, their progeny was characterized by a complete absence of double-null mutants (0 instead of the expected 18) and Usf2(+/−)/Usf2(+/−) pups (0 instead of the expected 36). In addition, two genotypes were underrepresented, Usf1(−/−)/Usf2(−/−) and, as expected from the previous results, Usf1(+/−)/Usf2(−/−) (Table 2). From this analysis, we concluded that a minimum level of USF activity, whether contributed by products of the Usf1 or Usf2 genes, was...
absolutely necessary for embryonic development. These results also indicate that the two Usf genes serve functions that are at least partially redundant.

**DISCUSSION**

We have analyzed the phenotypes resulting from mutations introduced either individually or in combination in the murine Usf1 and Usf2 loci. The targeting strategies used were expected to result in a null (Usf2) or essentially null mutation (Usf1). These predictions were confirmed by the complete absence of the corresponding USF polypeptides as observed both by EMSA and Western blotting.

The patterns of USF complexes detected by EMSA of nuclear extracts prepared from MEFs of different genotypes confirmed our interpretation of the wild-type pattern of USF1-USF2 complexes, with prominent bands corresponding to complexes containing USF1-USF2 heterodimers and USF1 homodimers and a minor band corresponding to the USF2 homodimers. These assignments were originally made by comparison with the patterns obtained by using recombinant USF polypeptides (6) and subsequently verified by antibody supershift assay. Because the USF1-USF2 heterodimers are normally the major USF species, their absence in both USF1- and USF2-null mice may be responsible for phenotypic traits that are common to both mutants. These include a propensity for spontaneous epileptic seizures, which is in both cases more common among the females and may reflect a decreased ability of these animals to adapt to stressful situations. Preliminary examination of brain sections from several USF1-null mice did not reveal obvious histological abnormalities. More detailed analyses will thus be necessary to establish whether there is a structural basis for the observed neurologic defect.

Other aspects of the phenotype of USF-deficient mice were specific to the Usf1 or Usf2 mutants. For instance, the increased frequency of whisker-trimming behavior among USF1-null females was not observed in the Usf2 mutants. More significantly, a distinct growth defect was found only in the USF2-null mice. Note that this particular phenotype is reminiscent of that of the insulin-like growth factor (IGF) II and, to a lesser extent, IGF-I null mice, which are also born smaller with an increased perinatal lethality (34, 35). On the other hand, a kinky tail similar of that of the USF2-null pups was one of the characteristics of the IGF-II receptor mutant embryos (36). It will therefore be of interest to investigate the status of the various components of the IGF receptors/IGF receptors pathway in the different USF-deficient mutant mice. Note also that in the case of the Usf2 mutants, different abnormalities may be caused either by the absence of USF2 homodimers or by the general decrease in total USF activity, which is much more pronounced than in the Usf1 mutants. To ascertain which of these differences is directly responsible for the observed phenotype, construction of Usf2 mutants with enhanced USF1 expression may be necessary.

One clear difference between the Usf1 and Usf2 genes evidenced by the consequences of the individual mutations was

![Fig. 4](https://example.com/f4.png)

**Fig. 4.** Growth defect in Usf2(−/−) mutant mice. (A) All pups in one litter produced by a Usf2 heterozygous breeding pair were weighed at various times of postnatal development. Subsequent genotyping by tail DNA analysis revealed that the two smaller animals were homozygous mutants and that the wild-type and heterozygous pups all displayed similar weights. (B–D) Photographs of Usf2(−/−) mutants next to one of their siblings at birth (B) and at 6 days (C) or 1 month of age (D). Note the smaller size and proportionate body features of the mutants and the characteristic kinky tail of the newborn USF2-null pup.

### Table 2. Progeny of the double heterozygotes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of pups</th>
<th>Observed frequency</th>
<th>Expected number</th>
<th>Expected frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usf1(+/+)/Usf2(+/+)</td>
<td>19</td>
<td>0.067</td>
<td>18</td>
<td>0.062</td>
</tr>
<tr>
<td>Usf1(+/+)/Usf2(+/-)</td>
<td>61</td>
<td>0.214</td>
<td>36</td>
<td>0.125</td>
</tr>
<tr>
<td>Usf1(+/+)/Usf2(-/-)</td>
<td>12</td>
<td>0.042</td>
<td>18</td>
<td>0.062</td>
</tr>
<tr>
<td>Usf1(+/-)/Usf2(+/-)</td>
<td>47</td>
<td>0.164</td>
<td>36</td>
<td>0.125</td>
</tr>
<tr>
<td>Usf1(+/-)/Usf2(-/-)</td>
<td>100</td>
<td>0.351</td>
<td>71</td>
<td>0.250</td>
</tr>
<tr>
<td>Usf1(-/-)/Usf2(+/+)</td>
<td>0</td>
<td>0</td>
<td>36</td>
<td>0.125</td>
</tr>
<tr>
<td>Usf1(-/-)/Usf2(+-)</td>
<td>22</td>
<td>0.077</td>
<td>28</td>
<td>0.062</td>
</tr>
<tr>
<td>Usf1(-/-)/Usf2(-/-)</td>
<td>24</td>
<td>0.084</td>
<td>36</td>
<td>0.125</td>
</tr>
<tr>
<td>Usf1(-/-)/Usf2(−/-)</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>0.062</td>
</tr>
</tbody>
</table>

All 285 pups from 56 litters produced by 19 breeding pairs of Usf1(+−/−)/Usf2(+−/−) mice were genotyped by tail DNA analysis. There were no sex differences among the pups of the various genotypes.
the influence of each gene on the expression of the other. Indeed, USF1-null mice displayed an overall USF DNA-binding activity similar to that found in wild-type mice due to an enhanced USF2 expression and a drastic increase in the levels of USF2 homodimers. In contrast, USF2-null mice displayed a significant decrease in total DNA-binding activity and depressed USF1 expression. These results indicate that, in wild-type cells, the Usf1 gene normally represses the level of the USF2 homodimers, whereas the Usf2 gene increases expression of the USF1 polypeptides. The cross-regulation between the two Usf genes is thus clearly asymmetrical. This regulation may involve transcriptional mechanisms, as suggested by the presence of USF binding sites within the promoter of the Usf2 gene (29), as well as posttranscriptional mechanisms requiring for instance the extremely conserved 5' and 3' untranslated regions of the Usf1 and Usf2 mRNAs (7,19). Further analysis will be necessary to determine how each gene influences the expression of the other and whether the changes in USF expression observed in mutant embryos are reflected by similar changes in different adult tissues.

An embryonic-lethal phenotype was observed for the double Usf1/Usf2 mutants, as well as for Usf2(+/–) mutants containing a single wild-type Usf1 allele. Given that the latter probably contained even less USF1 activity than mice lacking only the Usf2 gene, these results indicate that a minimum level of USF activity is absolutely required for embryonic development. Interestingly, a sea urchin transcription factor with strong homologies to the mammalian USF proteins has also been implicated in the regulation of gene expression during the development of Lytechinus embryos (37). In mice, this essential role of USF during embryonic development can be performed by the normal complement of USF species, which includes mostly heterodimers and USF1 homodimers. However, USF2 homodimers alone can serve the same function, as evidenced by the normal development of the USF1-null mice. Therefore, it is clear that the activities of the Usf1 and Usf2 genes are partially redundant. Because the phenotypes of the different mouse mutants also indicate specialized functions for the USF1 and USF2 proteins, it is most likely that these transcription factors regulate sets of genes that overlap but are not identical.

A major benefit provided by the various USF-deficient mice will be the availability of animal tissues and cell lines with different levels of expression of the USF1 and USF2 polypeptides. These should be particularly useful for investigating the role of USF in various phases of the cell cycle as well as in cellular immortalization and transformation. These cell lines and animal tissues should also allow unambiguous determination of genes that are specific targets of USF1, USF2, or both.

We thank Allan Bradley for the AB-1 ES cell line and Hui-Xin Yang for excellent technical assistance. This work was supported in part by a grant from the M. D. Anderson Cancer Center Physician Referral Service (M. Sawadogo) and by a postdoctoral fellowship from the Italian National Research Council (M. Sirito). The animal core facility at the M. D. Anderson Cancer Center is supported by Grant CA16672 from the National Institutes of Health.