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Genetics

Tissue-specific genetic variation in the level of mouse alcohol dehydrogenase is controlled transcriptionally in kidney and posttranscriptionally in liver

(mRNA/transcription rates/androgenic induction)

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ABSTRACT **Tissue-specific genetic variation in expression of the alcohol dehydrogenase, encoded by the *Adh-1* gene, is found between C57BL/6J (B6) mice and B6.S congenic mice. B6.S mice contain a variant *Adh-1* allele derived from a wild Danish strain in a B6 genetic background. B6 mice have nearly twice the alcohol dehydrogenase activity in liver but less than half the activity in kidney as B6.S mice. These tissue-specific genetic changes in alcohol dehydrogenase expression are manifest at the level of *Adh-1*-encoded mRNA. The regulatory site(s) involved act cis in both kidney and liver. These strains also differ in the extent to which androgen induces mRNA encoded by kidney *Adh-1*, with androgen increasing these levels 17-fold and 7.4-fold in the B6 and B6.S kidney, respectively. To identify the regulatory mechanism(s) underlying this strain variation in *Adh-1* expression, estimates were obtained for the relative rate of *Adh-1* transcription in the B6 and B6.S kidney, liver, and androgen-induced kidney. For both uninduced and induced kidney, a difference in the transcription rate alone accounts for the strain difference in mRNA concentration. In contrast, because the *Adh-1* transcription rate in liver does not differ significantly between B6 and B6.S mice, strain-specific variation in posttranscriptional regulation must be operative. Taken together these results indicate that the variation in *Adh-1* expression between B6 and B6.S mice results from changes in both transcriptional and posttranscriptional control, and these controls are differentially operative in kidney and liver.**

Inbred mouse strains have been a rich source of genetic variants useful for the study of gene regulation. Such variants, which often exhibit tissue- or stage-specific differences in gene expression, have allowed regulatory loci to be identified and studied on a biochemical basis (1–12). For many of these loci, however, the regulatory mechanism on a molecular level is not known. We here identify tissue-specific genetic variation in the expression of a murine *Adh* gene and find that these changes derive from tissue-specific changes in either transcriptional or posttranscriptional control.

Alcohol dehydrogenase (ADH) catalyzes the reversible conversion of a number of alcohols with their corresponding aldehydes and ketones. In the mouse the enzyme exists as three dimeric isozymes, ADH-A_2, ADH-B_2, and ADH-C_2 (the subscript indicates the active dimeric form of the enzyme), which differ in both tissue and substrate specificity. ADH-A_2 is the major ADH activity found in liver but is also found in kidney, adrenal gland, lung, and, to a lesser extent, seminal vesicle and testis (13). ADH-B_2 is widely distributed among mouse tissues, whereas ADH-C_2 is primarily found in stomach, lung, and reproductive tissues (13). ADH-A_2, ADH-B_2, and ADH-C_2 are encoded by the *Adh-1*, *Adh-2*, and *Adh-3* genes, respectively.

Adh-1 is subject to both developmental and hormonal control (10, 14, 15). Developmental variation in liver ADH-A_2 expression among inbred strains is due to variation at a single locus designated *Adh-1t*, which acts by controlling the rate of ADH-A_2 synthesis (10). In the kidney, androgen increases the rate of ADH-A_2 synthesis and ADH-1 mRNA concentration (14, 16). Mechanistically, the response involves an increase in the relative rate of transcription as well as changes in posttranscriptional control (16). Little significant strain variation, however, has been seen in the magnitude of this induction response.

Characterization of *Adh-1* expression in the Danish strain, Skive, has now revealed additional regulatory variation for this gene. Detailed analysis of the regulatory properties of *Adh-1* from Skive mice was facilitated by the use of the congenic mouse strain B6.S. This strain was constructed by transferring the *Adh-1* gene from Skive mice to the C57BL/6J (B6) genetic background by repeated backcrossing (see *Materials and Methods*). The resulting strain is then genetically identical to B6, except for the region of chromosome 3 containing *Adh-1*; this technique allows a comparison of *Adh-1* regulation between the two strains without complication by extraneous, unlinked genetic differences.

We find that, relative to B6 mice, B6.S mice have two to three times the ADH-A_2 activity and corresponding *Adh-1*-encoded mRNA in kidney but only 60% the ADH-A_2 activity and *Adh-1*-encoded mRNA in liver. Estimates of the relative transcription rate in the B6 and the B6.S kidney indicate a difference in transcription rate between the two strains sufficient to account for the different mRNA concentrations. In contrast to the kidney, the relative rate of *Adh-1* transcription in liver did not differ between the two strains, suggesting that the strain difference in liver mRNA results from a change in posttranscriptional control.

In addition to the tissue-specific variation in ADH expression, the magnitude of the androgenic induction response in kidney differs between B6 and B6.S mice. While androgen induces ADH-1 mRNA levels 17-fold in the B6 kidney, ADH-1 mRNA concentration increases only 7.4-fold in the B6.S kidney. A comparison of the relative transcription rate in kidney before and after androgen administration between the two strains indicates that the difference in the extent of induction results from a difference in the transcriptional component of *Adh-1* induction.

Finally, differences in *Adh-1*-encoded mRNA levels between the two strains are also seen in the lung, seminal vesicle, testis, and adrenal gland. There is no apparent tissue pattern, however, to the strain differences in mRNA levels—

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Abbreviation: ADH, alcohol dehydrogenase.

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the B6-to-B6.S ratio is <1 in testes and adrenal gland and >1 in seminal vesicle and lung.

MATERIALS AND METHODS

Mice. C57BL/6J and A/J mice were obtained from The Jackson Laboratory. Skive breeding pairs were the gift of Verne Chapman (Roswell Park Memorial Institute). The *Adh-1*^{a} allele carried in B6 mice and the *Adh-1*^{b} allele found in Skive mice (17) encode electrophoretically different ADH-A^1 and ADH-A^2 polypeptides (polymorphic forms of a polypeptide that are encoded by allelic genes are designated by superscripts), respectively (18). The B6.S congenic strain was constructed by crossing a B6 female to a Skive male. F_1 females were backcrossed to B6 males and heterozygous *Adh-1*$^{a/b}$ female mice were identified (10, 18) and mated to B6 males. After 10 generations of backcrossing, heterozygous animals were mated to obtain the B6.S congenic line that is homozygous for the *Adh-1*^{b} allele. The mice used in these experiments have been subsequently inbred for four to six generations.

For the androgenic induction of *Adh-1*, a pellet containing 30 mg of testosterone was implanted s.c. at the nape of the neck in female mice.

Enzyme Activity Assays and Starch Gel Electrophoresis. ADH activity was measured in the high-speed supernatant of 20% kidney and 10% liver homogenates as described (10). Starch gel electrophoresis was performed as described (10), except the Tris citrate buffer was adjusted to pH 7.2 and the gel contained 1 mM NAD. Gels were stained for ADH activity as described (10) by using ethanol as substrate.

RNA. Total RNA was isolated using the guanidine hydrochloride method of Cox (19). RNA preparations were routinely analyzed for quality by agarose gel electrophoresis as well as absorbance ratios at 260 and 280 nm. Tissue levels of *Adh-1*-encoded mRNA were estimated by hybridization to slot blots as described (14) except that radioactivity was detected by scintillation counting. Different amounts of total RNA were spotted, depending on the relative concentration of ADH-1 mRNA in that tissue, to ensure that the measurements were in the range of linearity.

Relative Rates of Transcription. Nuclei were prepared according to the method of Lamers *et al.* (20) and stored as described (16). Transcription run-on reactions were performed as described (16) with the following modifications. The amount of [^{32}P]UTP (800 Ci/mmol; 1 Ci = 37 GBq) per reaction was increased to 500 μCi, and the total reaction volume was increased to 140 μl with the final concentration of the reaction components being held constant. The reaction time at 26°C was for 40 min—well within the linear range for incorporation of labeled nucleotide (16). Labeled transcripts were analyzed by hybridization to plasmids, either pGEM-3Z or pGEM-ADH-1, immobilized on nitrocellulose circles (6-mm diameter, made with a hole punch). The plasmid pGEM-ADH-1 contains the 1050-base-pair cDNA insert from pADHm16 (16) inserted into the *Pst* I site of pGEM-3Z (Promega). The pGEM-3Z plasmid, lacking insert, was used to determine background levels for each hybridization. Plasmid DNA (4 μg) was denatured and spotted on nitrocellulose, as described by McKnight and Palmiter (21). Filters were baked at 80°C for 2 hr, washed as described (21), and placed in 5-ml polypropylene tubes (Falcon), one pGEM-3Z-containing filter and one pGEM-ADH-1-containing filter per tube. Prehybridizations were performed for 2 hr at 65°C in 1 ml of 10 mM Tris·HCl, pH 7.4/0.2% SDS/10 mM EDTA/0.3 M NaCl/1× Denhardt's solution (0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin) containing tRNA at 0.1 mg/ml and heparin at 0.25 mg/ml. The prehybridization solution was then discarded and replaced with 200 μl of the same solution containing 5 × 10^7–2 × 10^8 cpm of ^{32}P-labeled transcripts. In addition to the ^{32}P-labeled transcripts, hybridization reactions contained small amounts (5 × 10^3–10^4 cpm) of ^{3}H-labeled sense-strand RNA (synthesized by SP6 polymerase transcription of *Bam*HI-cut pGEM-ADH-1) to allow the efficiency of hybridization and recovery to be estimated. The hybridization solution in each tube was overlaid with light paraffin oil to prevent evaporation.

After 40 hr at 65°C, the hybridization solution was removed, with 20 μl being saved to determine the actual input of ^{32}P and ^{3}H. Filters were washed as described (16) and placed in 4-ml scintillation vials. The RNA was released, and the radioactivity was determined as described by McKnight and Palmiter (21). Data were corrected for spillover of 6% of the ^{32}P counts into the ^{3}H channel. Background was taken to be the radioactivity recovered from the filter containing pGEM-3Z DNA and was typically 90 cpm for liver and 70 cpm for kidney. Counts were usually 150–300 cpm above background for the uninduced kidney, 300–600 cpm above background for the androgen-treated kidney, and 1500–3000 cpm above background for the liver. The relative rate of transcription was calculated as follows: (^{3}H cpm input/^{3}H cpm recovered minus background) × (^{32}P cpm recovered minus background/^{32}P cpm input).

RESULTS

ADH-A$_2$ Enzyme Activity. ADH-A$_2$ activity levels were measured in the kidneys and livers from five B6 and B6.S female mice and expressed as units per g of tissue. The values for B6 mice are 46.3 ± 6.3 in liver and 1.25 ± 0.04 in kidney. Values for the B6.S liver and kidney are 28.6 ± 3.5 and 3.13 ± 0.26, respectively. The B6-to-B6.S activity ratio is then 1.62 in liver and 0.40 in kidney. The catalytic properties of ADH-A$_2^2$ and ADH-A$_2^1$, expressed here in B6.S and B6 mice, respectively, have previously been compared and found to be indistinguishable (22).

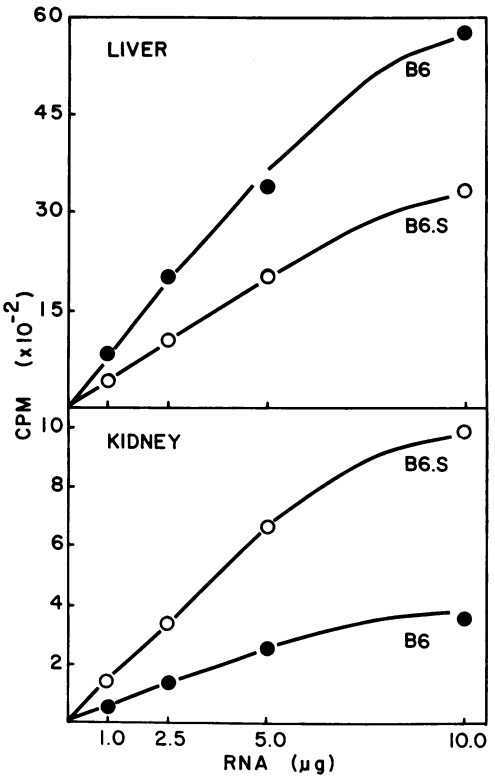

FIG. 1. ADH-1 mRNA levels in liver (*Upper*) and kidney (*Lower*) of B6 (●) and B6.S (○) mice.

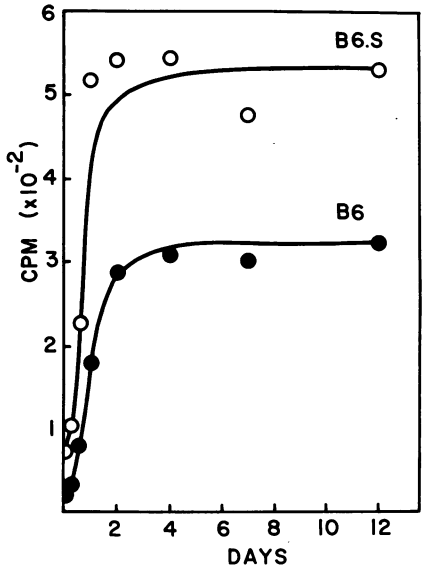

FIG. 2. Androgenic induction of kidney *Adh-1*-encoded mRNA in B6 (●) and B6.S (○) mice. Total kidney RNA was isolated at the indicated times after androgen treatment. The data are expressed as cpm/μg of RNA.

***Adh-1*-Encoded mRNA.** ADH-1 mRNA levels in the B6 and B6.S kidney and liver were compared by hybridization (Fig. 1). When different absolute amounts of kidney and liver RNA are spotted, we find the mean ratio of B6-to-B6.S *Adh-1*-encoded mRNA content to be 1.8 in liver and 0.36 in kidney. The tissue-specific strain differences in ADH-A_2 activity are therefore reflected at the level of *Adh-1*-encoded mRNA.

It should also be noted that ADH-1 mRNA levels differ 13-fold between the B6 liver and kidney and 3-fold between the B6.S liver and kidney; therefore, a substantial portion of the tissue difference in ADH activity with a mouse is due to differences in mRNA concentration.

The ratio of B6-to-B6.S *Adh-1*-encoded mRNA concentration in kidney rises steadily during androgenic induction and by day 12 is 0.58 (Fig. 2). At full induction ADH-1 mRNA levels have increased 17-fold in B6 mice and 7.4-fold in B6.S mice. These data indicate that B6.S mice are less responsive to androgen than B6 mice.

Relative Transcription Rates. Estimates of the relative rate of *Adh-1* transcription in kidney, liver, and androgen-induced kidney of both B6 and B6.S mice are shown in Table 1. A mean B6-to-B6.S ratio of 0.37 was observed for the relative transcription rate in the uninduced kidney, which accounts for the strain difference in kidney mRNA levels. In contrast to the kidney, no strain difference was seen for the relative rate of *Adh-1* transcription in liver. Furthermore, the difference in mRNA levels between the B6 liver and kidney and the B6.S liver and kidney is also seen at the level of transcription.

Adh-1 transcription rates increase ≈3-fold in the B6 kidney after 5 days of androgen treatment and only 1.7-fold in the B6.S kidney over the same time period. For both B6 and B6.S mice the increase in transcription rate was less in extent than the increase in mRNA concentration. This is consistent with the recent finding of Felder *et al.* (16) that the androgenic induction of *Adh-1*-encoded mRNA results from changes in both transcriptional and posttranscriptional control. However, the increase in transcription rates reported here is not as large as that reported earlier. This is probably attributable to differences in experimental procedure (see *Materials and Methods*). Even though both transcriptional and posttranscriptional controls are apparently involved in the induction of ADH-1 mRNA, the B6-to-B6.S ratio for induced transcription rates is comparable to that for induced mRNA levels, suggesting that the strain difference in the extent of induction is due to a difference in the transcriptional response to androgen. However, even though B6 mice have a greater transcriptional response to androgen, at full induction they still exhibit less *Adh-1* transcriptional activity than B6.S mice, suggesting that some differences (mechanisms) operating in the uninduced state also operate after induction.

Taken together these data indicate that the strain variation in both basal and androgen-induced kidney mRNA levels is controlled transcriptionally. However, the strain difference in liver mRNA levels is controlled posttranscriptionally.

Cis or Trans Regulation. When considering mechanisms of regulation, it is informative to know whether the regulatory site acts cis or trans. Cis-acting sites serve as regulators of the structural gene on their own chromosome, whereas trans-acting sites act on the structural gene of both chromosomes—presumably through a diffusible regulatory molecule. The electrophoretic variation between ADH-A_2^1 and ADH-A_2^2 allowed a measure of the relative amounts of enzyme derived from each chromosome in appropriate heterozygotes (Fig. 3). In the kidney of progeny from a B6.S × B6 cross there is a strong skewing toward the ADH-A_2^2 homodimer, suggesting cis regulation. There is also a skewed distribution of isozymes in the liver of (B6.S × B6)F_1 mice but in an opposite direction from kidney. In liver the ADH-A_2^1 homodimer is increased in amount relative to ADH-A_2^2, also suggestive of a cis effect. As a control, distribution of the three ADH-A_2 isozymes in the liver of (B6.S × A/J)F_1 mice was determined and shown to have no skewing (Fig. 3). Activity levels in liver are comparable between A/J and B6.S strains of mice (10).

Table 1. Estimates of the relative rate of *Adh-1* transcription

	Relative transcription rate* × 10^6						B6:B6.S ratio	
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	$\bar{x}$ ± SE	Transcription	mRNA
B6 kidney	4.36	3.96	5.60	6.29	5.19	5.08 ± 0.47	0.37	0.36 ± 0.02
B6.S kidney	16.1	7.49	20.0	14.4	10.2	13.6 ± 2.5		
B6 kidney (+ 1dT)	16.4	10.0				13.2 ± 2.3	0.59	0.39
B6.S kidney (+ 1dT)	23.8	20.8				22.3 ± 1.2		
B6 kidney (+ 5dT)	14.1	16.0	14.4			14.8 ± 0.52	0.63	0.58
B6.S kidney (+ 5dT)	25.0	24.0	21.6			23.5 ± 0.87		
B6 liver			39.6	61.7 88.2	46.2	58.9 ± 10.8	1.1	1.78 ± 0.03
B6.S liver			37.7	63.2 75.2	47.0	55.8 ± 8.4		

1dT and 5dT, 1- and 5-day treatments with testosterone. Exp., experiment.

*Liver and kidney transcription rate determinations on uninduced animals represent experiments using three to four different nuclear preparations. All kidney nuclei preparations were done using pooled data from three mice. Some liver nuclear preparations were on pooled data from three livers, whereas some were from a single liver.

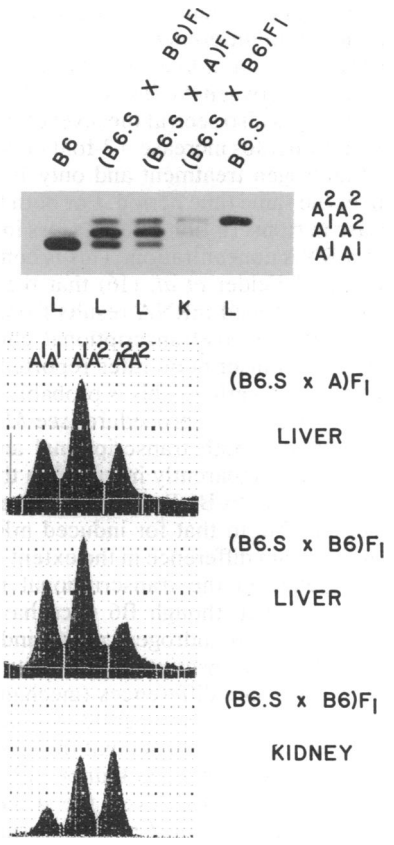

FIG. 3. Cis-acting nature of the regulatory differences expressed in liver and kidney between B6 and B6.S mice as revealed by the phenotype in F_1 hybrids. A representative starch gel stained for ADH-A_2 activity is shown at top (L, liver; K, kidney). Polypeptide composition of each active form of the enzyme is shown at right of the gel. The lower three panels show representative densitometric scans of the isozymic forms found in liver and kidney of informative F_1 hybrid animals. Five (B6.S × B6)F_1 hybrid animals were examined for liver and kidney isozymic content, and all exhibited the substantial skewed distribution shown here in the bottom two panels. The (B6.S × A)F_1 panel shows a scan with the 1:2:1 distribution expected in a hybrid between two parents not differing in enzyme activity.

***Adh-1*-Encoded mRNA in Other Tissues.** ADH-1 mRNA levels are compared between the B6 and B6.S lung, adrenal gland, seminal vesicle, and testis in Table 2. The mean B6-to-B6.S ratio is 1.9 for lung and seminal vesicle, 0.82 for adrenal gland, and 0.69 for testis. These tissue-specific changes in RNA concentration were also seen when these RNAs were subjected to Northern (RNA) blot analyses (data not shown). Strain comparisons of enzyme activity in these tissues were also consistent with the RNA data (data not shown). In regards to the relative concentration of *Adh-1*-encoded mRNA among tissues within a mouse, the concentration in lung is comparable to that in female kidney (unpublished work and ref. 16). ADH-1 mRNA levels in seminal vesicle and testis are lower, by as much as 10-fold depending on the strain, than those in kidney, and adrenal tissue has a higher ADH-1 mRNA concentration than does liver (unpublished work and ref. 16). In tissues where *Adh-1*-encoded mRNA can be accurately quantitated then, we observe tissue-specific changes in *Adh-1* expression that vary between the two strains.

DISCUSSION

We find tissue-specific differences in ADH expression between B6 and B6.S mice, with B6 mice having nearly twice the ADH-A_2 activity in liver but less than half the activity in kidney as B6.S mice. These genetically determined, tissue-specific changes in ADH expression are manifest at the level of mRNA. The regulation of *Adh-1*-encoded mRNA is also variant in other tissues between these strains, with the B6-to-B6.S ratio for mRNA being >1 in some cell types and <1 in others. Finally, the androgenic induction of ADH-1 mRNA in kidney is ≈2-fold greater for B6 mice than B6.S mice.

Table 2. *Adh-1*-encoded mRNA levels in B6 versus B6.S tissues

	B6:B6.S ratio of hybridized counts			
	Blot 1	Blot 2	Blot 3	Blot 4
Lung	1.8	1.6	2.4	1.9
Seminal vesicle	1.9	1.7	2.0	1.9
Testis	0.76	0.62		0.69
Adrenal		0.89	0.76	0.82

ADH-1 mRNA levels were estimated using slot blot analyses. For blots 1, 3, and 4 an average number of counts per spot was taken from a single RNA preparation spotted in triplicate and compared between strains. For blot 2 the average for each tissue was taken from two to three independent RNA preparations spotted in duplicate. Tissues from three mice were pooled for each RNA preparation. Counts were 100–1000 cpm above background depending on the tissue.

Estimates of the relative rate of *Adh-1* transcription indicate that the genetic variation of kidney mRNA is due to a change in transcriptional control, whereas the variation in liver mRNA is due to a change in posttranscriptional control. The strain difference in the extent of the androgenic induction response is due, in large part, to a change in the gene's transcriptional response to effector.

We do not know whether the genetic change(s) affecting transcriptional control, posttranscriptional control, and the gene's response to effector involve the same or separate DNA sequences. However, in each case the DNA sequences are closely linked to *Adh-1* and for the kidney and liver the regulatory sequences act cis.

DNA sequences involved in the tissue-specific regulation of transcription, such as with *Adh-1* in the kidney, generally fall into four categories: (*i*) upstream promoter elements (23–27), (*ii*) upstream enhancers (23, 25, 28–30), (*iii*) intronic enhancers (30–34); and (*iv*) the promoter (29, 35). Several investigators have proposed that tissue-specific transcription of a gene may be achieved in a given cell type through the interaction of such cis-acting elements with differentially expressed trans-acting factors (for review, see ref. 36). The interaction of cell-specific factors with a given cis-acting element could obviously be altered by a genetic change in that element. Such a mechanism would readily explain the strain-specific differences in uninduced and androgen-induced kidney *Adh-1* transcription rates. Southern blot analyses have revealed that an *Eco*RI fragment at the 5′ end of the *Adh-1* gene is larger in B6 mice than in other inbred strains (37, 38) including B6.S (data not shown), perhaps owing to an insertion. A change of this nature could disrupt a kidney-specific regulatory element, thereby affecting the initiation of transcription. Intronic insertions that reduce (39) or totally abolish (40) transcription have been reported.

In liver, posttranscriptional regulation of steady-state mRNA levels is suggested by the lack of a measurable difference in transcription rates. Several possibilities for the mechanism remain, and therefore the kinds of DNA changes involved because after transcription there are multiple points in a transcript's lifetime at which regulation may occur. To begin with, a series of processing steps are required to convert the precursor RNA into a functional mRNA; these steps include 5′ capping, methylation, splicing, and processing of the 3′ end. This series suggests at least two possible mechanisms for the difference in B6 and B6.S liver mRNA levels. The efficiency of splicing could differ for the $Adh\text{-}1^a$ and the $Adh\text{-}1^b$ transcripts. Splicing efficiency has been

correlated not only with splice-site sequence (41–44) but also with intron length (44–46). Or the efficiency of 3′ end formation could differ for the two transcripts. Processing of the 3′ end, for most eukaryotic mRNAs, involves endonucleolytic cleavage and polyadenylylation. Two sequence elements are required for efficient cleavage and polyadenylylation, one upstream (for review, see ref. 47) and one downstream (48–51) of the poly(A) site. The upstream element is highly conserved; however, because the exact sequence of the downstream element varies, it has been suggested that this element is used to regulate 3′ end formation (48, 50–52). Although, to our knowledge, no reports directly link 3′ end formation with the regulation of mRNA levels, there are precedents for mRNA levels being affected by 3′ end sequences. For instance, the posttranscriptional regulation of dihydrofolate reductase mRNA is affected by sequences in the 3′ noncoding portion of the gene (53), and the 3′ terminal part of the histone *H4* gene has been shown to regulate mRNA levels during the cell cycle (54).

Alternatively, the cytoplasmic stability of the *Adh-1*^{a} and *Adh-1*^{b} transcripts could differ in liver cells. While we know of no reports where a genetic change alters the stability of a mRNA in a cell-specific manner, differential stability among mRNAs within a given cell type has been reported (55). Therefore different mRNAs can be differentially regulated within the same cell type at the level of turnover. Precedent for cell-specific changes in the stability of a given mRNA lies in the many instances where the stability of a specific mRNA is affected by a developmental or a regulatory signal (56–60).

In the androgen-treated kidney, the increase in transcription rates relative to the increase in mRNA is proportionally the same for both the B6 and B6.S responses, suggesting that transcription initiation is the component of induction that differs between the two strains. It is widely held that steroid hormones, complexed with their receptors, increase the efficiency of transcription initiation by binding to specific DNA sequences (or receptor elements) located within or nearby the regulated gene (61). Multiple copies of these receptor elements, which act as enhancers of transcription, may be associated with a gene (61). The difference in the extent of the B6 and B6.S induction responses could involve either a qualitative or a quantitative change in such elements.

Genetic variation in *Adh-1* expression between B6 and B6.S mice will ultimately be useful in identifying the DNA sequences, as well as the kinds of changes in these sequences, that are important in the regulation of transcription, both in a cell-specific manner and as a response to effector, and in the posttranscriptional, cell-specific regulation of steady-state mRNA levels.

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