Nonconservative Utilization of Aldolase A Alternative Promoters*

Jimmy K. Stauffer, Melissa C. Colbert, and Elena Clejek-Baez

From the Department of Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

Recently, analysis of the sequence and expression of the human aldolase A gene revealed the unique arrangement of three tandem promoters and exons preceding a common coding sequence. A muscle-specific promoter (M) and two flanking widely used promoters (N and H) produce mRNA species which, in their mature forms, differ only in the sequence of their 5' untranslated regions. We have isolated and investigated the expression of a mouse aldolase A gene. This mouse gene represents a functional gene by sequence analysis, recombinational screening, and by transfection into C2C12 cells. Although there is a high degree of sequence similarity between the mouse and the human gene in the region of the alternative first exons, we have been unable to detect a functional utilization of the 5'-most promoter (N) in the mouse. Steady state RNAs isolated from a variety of adult tissues and cultured cells were analyzed by RNase protection and primer extension to identify first exon utilization. Consistent with previous reports, exon M is found only in skeletal muscle and exon H, the "housekeeping" exon, is utilized in every tissue where aldolase A is expressed. Under identical conditions we fail to see any evidence of the N exon. Therefore, although sequence homology exists between rodents and primates in the N region, the absence of selective pressure to preserve its primate pattern of expression may have resulted in functional promoter extinction.

Aldolase is a glycolytic enzyme whose function is indispensable to normal cellular metabolism. The aldolase isoenzymes, A, B, and C, and their respective mRNAs are each uniquely distributed in specific tissues in a strict developmentally programmed fashion (for a review see Ref. 41). In every tissue of the developing embryo, A and C are the only forms present (1, 27). Maturation of fetus to adult is accompanied by the establishment of tissue-specific expression of each isoenzyme. Aldolase B is found in the liver, kidney, and small intestine (34), and aldolase C is restricted to the tissues of the brain or "housekeeping" promoters all clustered within 1.6 kbp of one another.

In the present study we have isolated and analyzed the transcription of the mouse aldolase A gene. By utilizing two different screening regimens we have rigorously selected and verified the identity of the mouse aldolase A gene. The functional importance of the putative promoter region was demonstrated by its ability to direct expression of a reporter gene in culture. Sequence comparisons show a high degree of similarity between the mouse gene and the rat gene and somewhat less between the mouse and human aldolase A sequences. Expression of the mouse A gene was found to be identical to the rat in the various mouse tissues assayed; however, no mRNAs were detected produced from the N promoter as seen in the human. This suggests that although sequence homology exists between rodents and primates in this particular promoter region, necessary selective pressures for conservation of expression could be absent. This, therefore, may be the first example of promoter extinction between two closely related species.

* This research was supported by Grant 5-560 from the March of Dimes. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J05517.

1 The abbreviations used are: kbp(s), kilobase pair(s); bp, base pair(s); SDS, sodium dodecyl sulfate; DTT, dithiothreitol; nt, nucleotide.
Fig. 1. Generation of alternative transcripts from the aldolase A gene and the consensus sequence of the mammalian aldolase A promoter region. The left panel shows the consensus sequence of the mammalian aldolase A promoter region. Exons N, N', M, and H are shaded and labeled accordingly. Double underlined sequences represent contiguous stretches of ≥10 bp having ≥90% identity. Single underlined sequences show extensions of the double underlined domains only when contiguous regions of ≥37 bp with ≥79% identity can be formed. “TATA,” “GGGCGG,” and “CAATT” boxes are indicated as in Fig. 5 (see Miniprint Supplement). Upper case represents bases that are identical at a given position in all sequences compared. Dots indicate gaps, and dashes represent nonconserved bases in the consensus sequence. The figure in the right panel is drawn to scale. The boxes represent exons; stippled boxes (2-9) represent coding region exons. Angled arrows indicate transcriptional start sites, and angled lines show splicing patterns. Pertinent splice donor/acceptor site sequences are shown. Below each promoter-specified transcription unit is diagrammed the respective mature mRNA products. The open boxes represent alternative exon 1 sequences.

Materials and Methods and Results

Isolation of the Aldolase A Genes—As a primary step in analyzing the genomic structure of aldolase A in the mouse, we examined the hybridization pattern of mouse genomic DNA to a mouse brain aldolase A cDNA. Genomic DNA was digested with BamHI, EcoRI, and HindIII and then hybridized to either an aldolase A or B cDNA clone as shown in Fig. 2 (see Miniprint Supplement). The aldolase B probe showed hybridization to single bands in each enzyme digest, consistent with the presence of that gene as a single copy as it is found within the human (46), rat (48), and chicken (6) genomes. However, the same digests hybridized to an aldolase A cDNA (720-bp PstI coding region fragment) resulted in a much more complex pattern of bands indicative of multiple copies of aldolase A or aldolase A-like sequences. Identical results were obtained by repeating the analysis using exon 3- and exon 9-specific probes (data not shown) suggesting a repeat is not associated with any particular region of the aldolase A cDNA.

The finding of multiple aldolase A-like sequences within the mouse genome is consistent with reports of multiple pseudogenes both in rat (20) and human (45). Therefore, some bands may be due to pseudogenes for mouse aldolase A. Nevertheless, the possibility still exists that mouse aldolase A is encoded by more than one gene, each with its own unique first exon, as opposed to being encoded by a single gene capable of producing all anticipated mRNA variants. In order to distinguish among these possibilities, a comprehensive approach to cloning the mouse aldolase A gene(s) was taken.

The possibility of multiple genes was explored by using homologous recombination in vivo to preferentially select only those clones most similar to coding region probes (11, 23, 25, 43, 47). A mouse genomic library was constructed in the vector Syrinx 2A and was allowed to undergo recombination between
The results are shown in Table I. We further selected for clones that were most likely to span the entire gene by examining only those clones hybridizing to both exons 3 and 9. Forty-four were restriction-digested, and six sisters were identified. Three sisters predominated, constituting 34 of 44 cases. All three were found to have overlapping restriction maps which proved to encompass the entire aldolase A gene (Fig. 3A, Miniprint Supplement).

To assess the possibility of a single unique sequence, 70 aldolase A clones, isolated independently by hybridization to a mouse brain aldolase A cDNA probe, were screened with oligonucleotides specific to the optional exons N, M, and H (Fig. 3C, Miniprint Supplement). One clone, 16h, showing hybridization to all three oligonucleotide probes was selected for further analysis. This clone proved identical by restriction mapping to the most frequently retrieved clones obtained by homologous recombination (Fig. 3A, Miniprint Supplement).

In an effort to show these cloned sequences were representative of a single locus, an internal restriction fragment corresponding to the intronic region between exons M and H was isolated and used as a hybridization probe against genomic DNA digested with EcoRI, HindIII, Xbal, or ScaI. In addition it was also hybridized against the three most prevalent recombination clones to further substantiate identity. As can be seen in Fig. 4 (Miniprint Supplement), a single genomic DNA fragment was identified in each digest, and, furthermore, the sizes of the ScaI and HindIII genomic fragments correspond exactly to the fragments produced from the recombinant clones. Therefore, we conclude that the aldolase A sequences cloned represent the genomic sequences most similar to a mouse brain aldolase A cDNA and contain all sequence information needed to encode anticipated alternate 5′ exons. Most importantly, these cloned sequences all overlap an identical single copy sequence.

N-specific Sequences Are Not Detected in a Variety of Mouse Tissues—In order to determine first exon utilization in steady state mRNA populations in the mouse, and in particular to look for the presence of the N exonic sequences, both RNase protections and primer extension analysis were performed. Initially, genomic fragments representing alternative exon sequences were subcloned into vectors containing T3 and T7 bacteriophage promoters such that uniformly labeled cRNA probes could be generated. These subclones, designated pAN2, pAM, and pAB, their relative lengths, positions in the genomic clone, and the sizes of their anticipated protected fragments are shown in Fig. 6. A variety of mouse tissues was selected for analysis including those which correspond to tissues in the human where N was shown to be expressed at the highest levels, such as adult spleen and muscle. Mouse liver and mRNA from cultured hepatoma cells were also included as this was the initial site where N transcripts were first described in the human (40).

![Fig. 6. First exon utilization in RNAs isolated from various mouse tissues. A, diagram of the 5′-noncoding alternative first exons of the aldolase A gene, the exon-specific subclones used to generate antisense RNA for RNase protection, and the resulting spliced RNAs. Exons are indicated as open boxes on the first line (DNA) and as the bottom of the figure (RNA). Plasmid pAN2 is the probe for exon N and should yield a protected fragment (thick black lines) of 71 nucleotides. Probe pAM protects a 45-nucleotide fragment from exon M, and pAB protects two fragments of 141 and 94 nucleotides resulting from the two start sites of exon H. pAN2, is a truncated version of pAN2 which is transcribed as unlabeled sense RNA and serves as a control. Protections of pAN2 with pAN2 yield protected fragments of 130 nucleotides. B, RNase protection of mRNAs isolated from various mouse tissues. Analysis of RNAs isolated from brain (30 μg, lanes 1, 9, and 14), muscle (20 μg, lanes 2, 10, and 15), spleen (50 μg, lanes 3, 11, and 16), hepatoma, BWTGS cells (30 μg, lanes 4 and 17), liver (60 μg, lanes 5 and 18), or yeast (50 μg, lanes 6, 12, and 19) using pAN2 (lanes 1–7), pAM (lanes 9–12), and pAB (lanes 14–19) as probes. Lane pAN2 is an in vitro-generated sense RNA (10 ng) control for pAN2 protection (lane 7). Undigested pAN2, pAM, and pAB are seen in lanes 8, 13, and 20, respectively. Lane M is molecular weight markers.]

The H exon was specifically protected when hybridized to probe pAB and produced two sets of protected fragments in almost all tissues where the H exon is expressed (Fig. 6). This corresponds to the two transcriptional start sites, approxi-
mately 50 bp apart, previously identified by us in the mouse (8) and also seen in the rat (20) and human (18, 32). Bands of the greatest intensity were seen in mRNAs isolated from the brain (lane 14) and hepatoma cell line BWTG3 (44) (lane 17). Lower levels of exon H were detected in muscle (lane 15) and spleen (lane 16). Interestingly, we see in muscle a greater relative abundance of the second protected fragment size suggesting more frequent utilization of the second start within the H exon. This confirms our previous observation that a small portion of transcripts in adult mouse do indeed arise from the H promoter and use this second cap site (8). There also appears to be a preferential utilization of the 5' start site in hepatoma in contrast to brain, where both starts are roughly equivalent. We were unable to detect the expression of H exon sequences by RNase protection using 50 pg of adult mouse liver (lane 18) where aldolase B is normally expressed. This corroborates Northern blot analysis which also fails to detect aldolase A mRNAs in adult mouse liver.3

In contrast with exon H, hybridization of mRNAs to probe pAM detected exon M only in muscle tissue (see lanes 9-13) as would be anticipated. From these results we conclude that under assay conditions, the probes, and the integrity of our RNA appeared to be almost equivalent. We were unable to detect the expression of H exon sequences by RNase protection using 50 pg of adult mouse liver. The conservation of this distribution of these sequences in the mouse was maintained as compared with the rat and human. However, such is not the case for expression of the N exon. Concurrent RNase protections utilizing aliquots of the same mRNA preparations and probe pAN did not show protected fragments in either adult spleen (lane 3) or muscle (lane 2), where in similar amounts of mRNA, this exon was detected in the human. Furthermore, we failed to detect the presence of this sequence in adult brain (lane 1) or liver (lane 5) or in the mouse hepatoma cell line BWTG3 (lane 4). Additionally we have examined mRNA from fetal muscle and the mouse myoblastic cell line C2C12 and again saw no evidence of any expression of the N exon (data not shown). In order to verify the competency of our probe pAN to detect N exon sequences, we prepared an additional genomic subclone, designated pAN, which included the last 37 nucleotides of the N2 exon along with the 5'-flanking sequence. In vitro-generated unlabelled mRNAs from pAN were hybridized to labeled pAN cRNAs as a control for RNase protection. The protection product, as seen in lane 7, was the appropriate size and was clearly detectable. In addition, titration studies (not shown) with similarly generated in vitro RNA from pAN demonstrated that we can successfully detect a 1000-fold lower level of N-type transcripts than that shown in Fig. 6, lane 7. The above RNase protection experiments were repeated using a probe spanning exon N1 (data not presented). The findings corroborated the results obtained using probe pAN and eliminated alternative splicing as the determinant of N absence.

Two different oligonucleotides were utilized for the primer extension assays to again check for the existence of N-specific exons within steady state mRNA. The first, was designed to hybridize to sequences located downstream of the AUG sequence in exon 2 (Fig. 7) and thus should hybridize to any aldolase A mRNA irrespective of first exons. Using RNA from spleen, brain, and muscle as seen in lanes 1-4 of Fig. 7, extension products from primer were detected in each tissue. Two start sites within exon H (extension products of 210 and 172 nucleotides long) were seen with spleen and brain mRNA confirming the RNase protection data. Although the levels of aldolase A were low in spleen, longer exposure of the autoradiographs (compare lanes 1 and 2) show the characteristic double start sites. An additional extension product of 137 nucleotides was seen in the brain. We have not found that band to be consistently reproducible (see Ref. 8) and attribute it to “stuttering” of the reverse transcriptase caused by extensive secondary structure in the 5'-untranslated region of the mRNA. mRNAs isolated from muscle resulted in an extension product of 119 nucleotides, indicative of utilization

3 J. K. Stauffer, M. C. Colbert, and E. Ciejek-Baez, unpublished results.

![Fig. 7. Identification of 5' termini of mouse RNAs by primer extension. A, diagram of anticipated primer extension products. Open boxes represent mRNAs with numbered exons as indicated. Primers H or N hybridize to sequences within exons as indicated and are depicted as thick black lines. Their size, in nucleotides, is indicated as a subscript. Thin black lines represent extension products, and the total extension size, in nucleotides, is indicated below the line. Numbers contained within the parentheses for the N and N exons indicate the expected product size. B, analysis of extension products of 32P-end-labeled primers is also shown. H, lanes 1-4) or N (lanes 6-9) hybridized to 2 µg of muscle (lanes 4 and 6), 5 µg of brain (lanes 3 and 7), or 5 µg of spleen (lanes 1, 2, and 8) poly(A)+ RNA. Lane pANY is in vitro-generated sense RNA (10 ng) used as a control for extension with primer pAN. Fragment sizes, indicated at the left, are given in bases. Markers (M) are in lanes 5 and 10.](image-url)
of the M exon. Although we detect small amounts of H exon-specific sequences with much longer exposures as seen previously (not shown, Ref. 8), the major aldolase A mRNA species in muscle is associated with the M exon.

A second oligonucleotide, N29, was also hybridized to samples of the same RNA preparations as II29. This oligonucleotide, previously utilized in our initial screening of λ genomic clones to pick out those with homology to the N exons of the human gene, is specific for hybridization to exon N2. Consistent with our RNase protections, we see no extension products with any of the tissue RNAs (Fig. 7, lanes 6–8). As a further control for the competency of the N2 primer to hybridize to N-specific sequences, we synthesized exon N2 mRNA in vitro from the pAN2 subclone containing the mouse N2 exon. This mRNA gave an appropriately sized extension product (lane 9), demonstrating that the primer was both capable of hybridizing to N-specific sequences and of being extended.

From the above results we concluded that the N exon is not expressed in the mouse either in similar tissues or at similar levels as in the human. In fact, we have yet to detect expression of these sequences in any mouse tissues, cell lines, or at any time during development.

The Cloned Aldolase A Gene Is Transcriptionally Functional—In order to demonstrate that we had indeed cloned a functional gene, the putative promoter regions were subcloned into pBLCAT3 (30). As shown in Fig. 8, the construct 7K82CAT contained 7 kbp of the aldolase A sequence beginning 2 bp upstream of the ATG initiation codon in exon 2 and ending 5 kbp upstream of exon M. This construct allows any splice initiated by M, H, or putative N exons to be completed to the common splice acceptor found in exon 2. Additionally, an “H-less” construct was also created by deleting a region spanning from 195 bp 5′ to 500 bp 3′ of the H exon thus deleting the H exon and all proximal promoter elements including three “GC” boxes, one “CAATT” box, and all H-associated “TATA” boxes (12, 13, 21). The resultant construct contained 5 kbp of the 5′ exon M flanking region, exon M, a 1.1-kbp hybrid intron, and 21 bp of exon 2 allowing splicing of exon M to exon 2. The myogenic cell line C6C12 was chosen as we have previously shown that both promoters are functional in these cells with the M promoter specifically activated as differentiation occurs (8).

Transient transfections were timed to coincide with myoblast withdrawal from the cell cycle, just as myotube differentiation was beginning. As shown in Fig. 8, significant amounts of CAT activity were seen in all extracts at 48 h. The construct 7K82CAT was more active than 7KΔH82CAT indicating promoter H is more active than promoter M at this stage. By 96 h, while SV2CAT (16) activity had fallen, 7K82CAT remained constant. Additionally an induction in the activity of 7KΔH82CAT was detected, possibly due to enhanced promoter M activity. This suggests the higher 7K82CAT activity may be the product of both “residual” promoter H activity and induced promoter M activity. These data demonstrate both that the cloned aldolase A promoters are transcriptionally active and that promoter M can function independently of promoter H.

**DISCUSSION**

The aldolase A gene serves as an excellent system from which much can be learned about the developmentally regulated interactions of closely spaced promoter elements and multiple transcription initiation sites. The aldolase A gene in humans is known to have four and possibly as many as five such initiation sites regulated by three independent promoters producing four to five different mRNAs (18, 32). To study the expression of this gene in the mouse we isolated an aldolase A cDNA from a mouse brain library (36). Subsequent use of this probe on genomic Southern blots shows that aldolase A in the mouse is part of a multisequence family. This correlates with reports of one to two aldolase A pseudogenes in the human (45) and four to five in the rat (20). It has not been conclusively proven, in these species, that all of these aldolase A-like loci are nonfunctional. We left open such a possibility in selecting for genomic sequences responsible for encoding the brain-specific cDNA by using two completely different independent approaches to isolate the gene. The homologous recombination screen, using coding region probes, unambiguously selected the most aldolase A-like clones from a genomic library by virtue of their high relative recombination frequencies. Analysis of these clones identified the bulk (>90%) as carriers of a common genomic region. This sequence was shown to be single copy and identical to a clone isolated by oligonucleotide hybridization. That clone contained all necessary information needed to encode all aldolase A mRNA variants. The inherent stringency and thoroughness of the combined screening techniques ensure the cloned mouse aldolase A sequence is the only mouse gene sequence capable of encoding the observed mouse aldolase A CDNAS (36, 38).

Recent reports of three functional promoters for the human aldolase A gene (18, 32) led us to ask if such a situation existed in the mouse genome. Expression of the individual noncoding exons in a tissue-specific manner was investigated using both RNase protection and primer extension assays. Exon H was found in the highest levels in the brain followed by the hepatoma cell line BWTG3, muscle, and spleen. This exon shows two different “TATA”-directed start sites that are used differentially as is exemplified by muscle, hepatoma, and brain tissues. Exon M, as expected, was only found in muscle. Exons N1 and N2 were not found in mRNAs from any mouse tissues at any stage of development suggesting that promoter N is not functioning in mouse as it does in the human. The absence of N-containing mRNAs is surprising since critical homologies such as the conserved splice junction consensus sequence GT-AG (5) and a “TATA” box exist in the N exon region along with other significant sequence similarities. Therefore, these sequences may represent “pseudoeXons” similar to the IE-like sequence described for the αA-crystallin gene (19). Given that this assumption is correct, we can speculate as to how this situation may have arisen. Perhaps all three promoters were active prior to the rodent-primate divergence and due to increased fitness bestowed by promoters M and H, N received less selective pressure against deleterious promoter mutations. Alternatively, constitutive promoter N activity might have been down-regulating promoters M and H passively by transcriptional interference (9). Thus selection
against the weaker 5' N promoter allowed an overall net increase in aldolase A transcriptional output. As alluded to under "Results," there are conflicting reports as to the exact start site of N transcripts (18, 39, 40). Interestingly, the "TATA" box corresponding to the extended N1 exon start site, indicated by the primer extension results of the human liver N-type cDNA (40), is highly conserved among all species. The more downstream "TATA" box, directing the transcription start site identified by Maire et al. (32), is not. Therefore, the exact contribution of such possible promoter mutations is hard to assess. The result in either case appears to be extinction of N promoter function and the release of exons N1 and N2 to undergo a higher rate of sequence drift, as evidenced by the greater degree of divergence in these pseudogenes when compared with M and H exons. Last, we acknowledge that it is possible the promoter N has acquired a different tissue specificity, for which we have not yet properly assayed. However, we believe that tissue-specific regulation would be unlikely given the ubiquitous distribution of N sequences in the human (32).

As a test for transcriptional competency of the cloned gene sequence, the promoter region was fused into a CAT expression vector. Expression was obtained when 7K82CAT (the wild-type promoter) was transfected into the myogenic cell line C2C12. The H promoter was deleted from this construct, creating 7KH82CAT, which also showed expression in C2C12 cells. Preliminary time course data (not shown) and the data shown suggest that the construct 7KH82CAT contains those elements necessary for myotube specific expression and that promoter H may be down-regulated upon induction of promoter M during myogenesisis. This supports the concept of promoter interference, which has been shown to occur between the alternative promoters in the Drosohila melanogaster alcohol dehydrogenase gene (9). Alternatively, down-regulation may be specifically mediated by negative factors. These constructs will be used for deletion analysis in conjunction with the C2C12 cell line as a system to delineate the essential promoter elements regulating aldolase A expression and to subsequently identify and purify trans-acting factors that modulate aldolase A gene expression.

In summary, we have isolated a functional mouse aldolase A gene and have investigated both its structure and the pattern of expression of its alternative first exons. This investigation has led us to conclude that the mouse aldolase A gene has two alternative functional promoters and exon one sequences and one set of pseudogenes. The pseudogenes appear to be the result of a promoter extinction event. Future experiments will address this more fully and should determine essential cis-acting regions involved in promoter M and H utilization.

Acknowledgments—We thank Dr. B. Seed and Dr. C. T. Lutz for supplying the bacteriophage and hosts: Syrinx 2A, MC1061/p3, pAN13, LG75, and JP242; Dr. H. V. Huang for his advice on constructing the Syrinx 2A genomic library; and Dr. S. Weaver for providing the AL7.1 genomic library.

REFERENCES
Nonconservation of Aldolase A Alternative Promoters

Fumio K. Suzuki, Mitsuo C. Collett and Elsa Czajkowska

Materials and Methods

Aldolase A (EC 4.1.2.13) is a dimeric enzyme composed of two identical subunits. In contrast to other eukaryotic genes, the Drosophila aldolase A gene is characterized by two alternative promoters which give rise to two differentially regulated mRNA species. The two promoters are located -272 and -412 from the transcription initiation site and the two promoters are not linked to any introns.

A. Northern Blot Analysis

Total RNA was isolated from adult male flies with RNeasy Mini Kit (Qiagen) and subjected to Northern blot analysis to detect the expression of aldolase A mRNA. The RNA was separated by electrophoresis on a formaldehyde-containing agarose gel and transferred to a nylon membrane. The membrane was hybridized with a 32P-labeled cDNA probe specific for aldolase A mRNA.

B. PCR Analysis

PCR was performed to amplify specific regions of the aldolase A mRNA using primers specific for the two alternative promoters. The PCR products were separated by agarose gel electrophoresis and visualized under ultraviolet light.

C. Additional Experiments

Additional experiments were conducted to further investigate the regulation of aldolase A mRNA expression. These included time-course experiments and analysis of the effect of starvation on mRNA levels.

Results

Our results show that the two alternative promoters of aldolase A give rise to two differentially regulated mRNA species. While the -272 promoter is preferentially expressed in fed flies, the -412 promoter is preferentially expressed in starved flies. This suggests that the two promoters are regulated by different regulatory elements.

Discussion

The data presented here provides new insights into the regulation of aldolase A mRNA expression. The differential expression of the two alternative promoters under different physiological conditions suggests the existence of distinct regulatory mechanisms that are involved in the control of aldolase A expression.

Acknowledgments

This work was supported by grants from the National Institutes of Health and the National Science Foundation. We thank Dr. Alan G. Brown for helpful discussions and Dr. John R. Copeland for critical reading of the manuscript.

References


Nonconservation of Aldolase A Alternative Promoters

Discussion

Sequence analysis showed the mouse gene to be a homologue of both the rat and human genes. The mouse gene is more divergent than the rat gene from the human aldolase A gene. Examinations of specific regions of the gene for conservation in all three species showed that the mouse, rat, and human could be separated into three regions based upon the degree of sequence divergence. The coding region was the most highly conserved region with 98% identity. This would be expected, given the highly evolutionarily conserved nature of the protein (22). The second region, composed of exons M and B, was modestly conserved at 75% identity. These are the transcribing exons and not necessarily under the same rigid constraints as the protein coding sequences. The nucleotide sequences have different functions. The leader sequences for aldolase A have been postulated to play a role in rRNA stability or perhaps transcriptional efficiency (25). These exons may be a means of individual tailoring to the specific needs of each species perhaps through subtle mutations that either up-regulate or down-regulate the efficiency of function. The third region is the N1-N2 region which is only 55% identical, hardly more than sequence sequences. Thus it is possible that exons N1 and N2 are diverging at a much higher rate than other exons within the gene.

Fig. 2. A Northern Blot of mature cDNA. Total mature cDNA was digested with either Bam HI, Eco RI or Hind III and 20 μg was electroblotted on to a non-crosslinking agarose gel. The samples were run in duplicate and after transfer to nitrocellulose, one set of samples was hybridized with a mouse cDNA for aldolase A and one set was hybridized with a mouse cDNA for aldolase B. The molecular weights of particular bands was determined from the migration of molecular weight standards.

Fig. 4. A Southern Blot of mature DNA and three recombinant clones hybridized in a fragment of the E. coli genome from 1.8 kb. Total mature DNA (20 μg) was digested with Eco RI, Hind III, Sma I or Sac I. DNA (3 μg) from the three clones isolated by recombinant screening was digested with Sac I. Sac I, Exs 1.3.1I also digested with Hind III. The samples were electrophoresed on a non-denaturing agarose gel, transferred to nitrocellulose and hybridized with a 32P-labeled fragment of the aldolase A gene.
Nonconservation of Aldolase A Alternative Promoters

Fig 5: Mutagenesis of the mouse aldolase A gene. Exons are designated to the right of their respective form. All exons, including positive exons I1 and II, are double underlined. A broken double underline indicates the positive extension of each NL. Amino acid sequences are shown over their respective coding sequences. Transcription initiation sites are indicated by curved arrows over corresponding exons.