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Genetics

A molecular model for the genetic and phenotypic characteristics of the mouse lethal yellow (A^y) mutation

(agouti/*Raly*/physical mapping)

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ABSTRACT **Lethal yellow (A^y) is a mutation at the mouse agouti locus in chromosome 2 that causes a number of dominant pleiotropic effects, including a completely yellow coat color, obesity, an insulin-resistant type II diabetic condition, and an increased propensity to develop a variety of spontaneous and induced tumors. Additionally, homozygosity for A^y results in preimplantation lethality, which terminates development by the blastocyst stage. The A^y mutation is the result of a 170-kb deletion that removes all but the promoter and noncoding first exon of another gene called *Raly*, which lies in the same transcriptional orientation as agouti and maps 280 kb proximal to the 3′ end of the agouti gene. We present a model for the structure of the A^y allele that can explain the dominant pleiotropic effects associated with this mutation, as well as the recessive lethality, which is unrelated to the agouti gene.**

Lethal yellow (A^y) is a dominant mutation at the agouti locus in mouse chromosome 2 that dates back to the mouse fancy and has been studied intensively for decades. Lethal-yellow heterozygotes develop a uniform yellow coat color over their entire body, instead of the wild-type agouti pigmentation in which each hair shaft is black (or brown, or gray, depending on alleles at other loci) with only a subapical band of yellow. In addition to its effect on pigmentation, the A^y allele also causes a number of dominant pleiotropic effects, which include a non-insulin-dependent diabetic-like condition (1), marked obesity (2–6), and an increased propensity to develop a variety of spontaneous and induced tumors (reviewed in refs. 7 and 8). When homozygous, the A^y allele also causes a preimplantation lethality, which was first revealed at the turn of the century as an alteration in normal Mendelian inheritance (9–13). The lethality has subsequently been shown to occur prior to implantation (14–18) and to be associated with abnormalities in both the trophectoderm and the inner cell mass (19–21).

Genetic experiments have demonstrated that recombination can occur between A^y and other alleles at the agouti locus (22, 23). This unusual finding was first revealed in a cross involving A^y and the recessive lethal nonagouti (a^x) allele. In this case, wild-type agouti offspring that arose in crosses between A^y/a^x compound heterozygotes were shown with the aid of flanking markers to result from recombination between these two alleles (22). Recombination also appears to have occurred in crosses involving A^y and the black-and-tan mutation (a^t) and in crosses between A^y and the nonagouti (a) allele (23). Collectively, the results from these recombination events place A^y 0.1 centimorgan (cM) proximal to agouti, which led to the suggestion that A^y is pseudoallelic with agouti (22, 23).

The agouti gene was recently characterized and shown to produce a mRNA of ≈0.8 kb (24, 25). It was further demonstrated that A^y is indeed an allele of agouti and that it gives rise to three distinct size-altered mRNAs, each ≈1.1 kb in length (26). These A^y-specific transcripts are ectopically overexpressed in every tissue examined to date (24, 25) and consist of the normal coding and 3′ untranslated regions of the agouti transcription unit joined to novel sequences at their 5′ ends (24–26). The 5′-most portion of the novel sequence in these A^y transcripts corresponds to the noncoding first exon of a second gene, called *Raly*, which is closely linked to agouti in distal chromosome 2 (26). *Raly* is normally expressed in a ubiquitous manner and codes for one member of a family of RNA-binding proteins implicated in pre-mRNA processing and developmental regulation (26). In mice carrying the A^y allele, the coding region of the agouti gene is apparently under the transcriptional control of the ubiquitous *Raly* promoter. These data led us to propose that the ectopic overexpression of the wild-type agouti gene product is responsible for the suite of dominant pleiotropic effects in A^y heterozygotes (26). Moreover, because wild-type *Raly* is not expressed from the A^y allele, we hypothesized that the lack of the *Raly* gene product in the preimplantation embryo is associated with the recessive lethality of homozygous A^y mice (26).

In an attempt to understand further the molecular basis of the dominant pleiotropic effects, the recessive lethality, and the unusual recombination events associated with the lethal yellow mutation, we have undertaken a more thorough structural characterization of the A^y allele. Here we demonstrate that the 5′ end of the *Raly* gene lies 280 kb proximal to the 3′ end of agouti in wild-type mice and that a 170-kb deletion associated with the A^y mutation removes all of *Raly*, except for the promoter and noncoding first exon. Additionally, we present a model that explains the observed recombination between A^y and other agouti-locus alleles. This model also presents a mechanism through which the novel-sized, ubiquitously expressed, 1.1-kb transcripts can be produced from the A^y allele.

MATERIALS AND METHODS

Mice. All mice originated and were maintained at the Oak Ridge National Laboratory.

Pulsed-Field Gel Analysis. Pulsed-field gel electrophoresis (PFGE) analysis was conducted essentially as described (27). The digested DNAs were electrophoresed in the CHEF-DR II pulsed field electrophoresis system (Bio-Rad) at 200 V, 12°C, 10- to 40-sec ramp, for 25 hr.

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Abbreviations: cM, centimorgan; PFGE, pulsed-field gel electrophoresis.

Southern Blot Analysis. Genomic DNA (10 μg) was digested with restriction enzymes, electrophoresed through agarose gels, and blotted to GeneScreen (DuPont) using standard procedures (28, 29). Radiolabeled hybridization probes were prepared with the random-hexamer labeling technique (30). Posthybridization filter washes were conducted at high stringency (0.2 × SSC/0.1% SDS, 68°C), with the exception of the 83-bp *Raly* second exon and 111-bp agouti probes, which were washed at reduced stringency (0.2 × SSC/0.1% SDS, 50°C).

Probes. Isolation of *Raly* genomic clones was as described (26). Probe A is a 2.0-kb *Xba* I fragment isolated from a *Raly* genomic clone and subcloned into pGEM4 (Promega). Probes B and C were amplified by PCR from plasmid templates and their nucleotide sequences have been published (ref. 26: B, uppercase letters in figure 2; C, 111-bp boxed region in figure 7B).

RESULTS

Long-Range Physical Map of *Raly* and Agouti. Previously, we demonstrated that *Raly* and agouti are tightly linked in mouse chromosome 2 and that the A^y mutation deletes at least the 3′ end of the *Raly* gene but does not affect the genomic DNA structure of the agouti locus (26). We have now compared the DNA structure of the A^y and wild-type alleles using PFGE. DNA samples were digested with the enzymes *Bss*HII, *Eag* I, and *Sma* I, subjected to PFGE, blotted, and hybridized consecutively with probes corresponding to a 5′ segment of the *Raly* first intron (Fig. 1*A*) and the wild-type agouti cDNA (Fig. 1*B*). In wild-type DNA, both the *Raly* and agouti probes hybridize to a 280-kb *Bss*HII fragment. Because the agouti gene contains a *Bss*HII site within its first intron and last exon, the agouti cDNA probe also detects an internal 17-kb *Bss*HII fragment, and a 75-kb fragment that contains the 3′ end and flanking sequences of the agouti gene. In A^y DNA, instead of the 280-kb fragment, both probes detect a smaller *Bss*HII fragment of 110 kb. A similar result was obtained with the enzyme *Sma* I; both the *Raly* and agouti probes hybridize to a 300-kb fragment in wild-type DNA and a smaller 130-kb fragment in A^y DNA (Fig. 1). These data are consistent with the interpretation that the *Raly* and agouti probes are located together on *Bss*HII and *Sma* I fragments of 280 kb and 300 kb, respectively, and that the A^y mutation is associated with a 170-kb deletion (Fig. 1*C*).

The indication (with *Bss*HII) that the *Raly* and agouti genes lie on the same 280 kb of DNA and that the A^y mutation is due to a 170-kb deletion was substantiated by *Eag* I digestions. We first determined that there are *Eag* I sites flanking *Raly* (in the CpG island at the 5′ end of the gene, Fig. 1*C*) and agouti (immediately 3′ of the gene, Fig. 1*C*) that are cut to completion in genomic DNA (data not shown). In wild-type DNA, the probe from the first intron of the *Raly* gene hybridizes with a 110-kb *Eag* I fragment, and the agouti cDNA probe hybridizes with a 170-kb fragment. This result indicates that there is at least one *Eag* I site between the two genes. In the A^y allele, both the *Raly* and agouti probes hybridize to a 110-kb *Eag* I fragment. These data strongly suggest that the *Eag* I site between these loci that yields the 110- and 170-kb fragments in wild-type DNA is encompassed by a 170-kb A^y deletion.

Based on the results from *Bss*HII, *Sma* I, and *Eag* I, and the additional enzymes *Mlu* I, *Ksp* I, and *Not* I (data not shown), a long-range restriction map of wild-type and A^y DNA surrounding the *Raly* and agouti loci was constructed (Fig. 1*C*). Taken together, these data indicate that the 5′ end of *Raly* lies about 280 kb proximal to the 3′ end of agouti, that *Raly* and agouti lie in the same transcriptional orientation, and that the A^y mutation is the result of a 170-kb deletion including most of the *Raly* gene.

FIG. 1. Pulsed-field gel analysis of the chromosome 2 region containing the *Raly* and agouti loci in wild-type and lethal-yellow DNAs. (*A*) A PFGE blot of single and double digests of wild-type (*A*/*A*, FVB/N strain, denoted by A) and heterozygous lethal yellow (A^y/A, denoted by Y) genomic DNAs was hybridized with a ^{32}P-labeled fragment of DNA corresponding to a 5′ segment of the *Raly* first intron (probe A in *C* and Fig. 2*E*). B, *Bss*HII; E, *Eag* I; S, *Sma* I. DNA molecular size standards are shown at left in kb and LM indicates limiting mobility DNA. (*B*) The same filter was stripped and rehybridized with a ^{32}P-labeled wild-type agouti cDNA probe (see figure 2 in ref. 24). Based on *Bss*HII/*Eag* I digestions on other blots, the 280-kb *Bss*HII fragment detected in lane A of the B+E digestion in *A* and *B* is due to incomplete digestion with *Eag* I. (*C*) Long-range restriction map of the *Raly*–agouti region in the *A* and A^y alleles. Shown below the restriction map are expanded views of the 5′ end of the *Raly* gene (the solid box denotes the noncoding first exon) and the 3′ end of the agouti gene (numbered solid boxes indicate exons). Separate scale bars are shown for the long-range restriction map and for each of the expanded regions. The expanded agouti region is shown to scale, except for the four exons, which are enlarged for clarity. The 2.4-kb *Eco*RI (R) fragment shown at the 5′ end of *Raly* contains a CpG island with one or more of each of the following restriction enzyme recognition sites: *Eag* I (E), *Sma* I (S), *Ksp* I (K), *Bss*HII (B), and *Mlu* I (M). All of these enzyme sites are indicated on the long-range map, but only the *Bss*HII and *Mlu* I sites are shown in the expanded region due to space considerations. The probes used to detect the A^y deletion breakpoints (probes A and C) are also shown. The 111-bp probe C is shown larger than scale. Only restriction enzyme sites that are cut in genomic DNA are included on the map. N, *Not* I.

Localization of the A^y Deletion Breakpoints. We previously determined that, in the A^y allele, the noncoding first exon of *Raly* is present, the 3′ end of *Raly* is deleted, and none of the four exons of the agouti gene are deleted or structurally altered (26). To define the location of the A^y 5′ (relative to the transcriptional orientation of *Raly* and agouti) deletion breakpoint more precisely, Southern blots of genomic DNA were hybridized with probes specific to various portions of the 5′ end of the *Raly* gene (Fig. 2). To facilitate these analyses, we

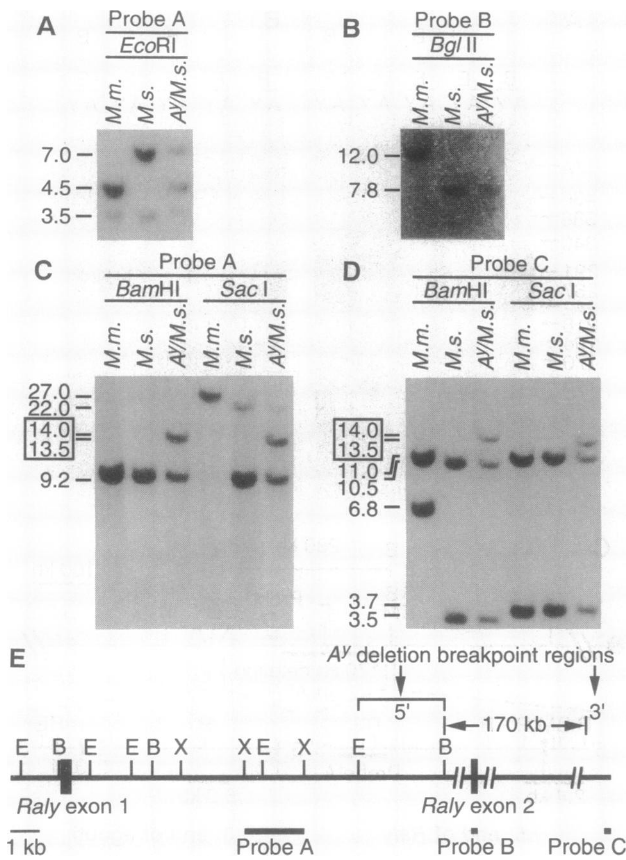


FIG. 2. Detection of the A^y deletion breakpoints by Southern blot analyses. (*A*) *Mus musculus* (*A/A*, FVB/N strain), *Mus spretus* (A^w/A^w), and $A^y/M.s.$ [an F_1 hybrid from the cross *M. musculus* (A^y/a) × *M. spretus* (A^w/A^w)] genomic DNAs were digested with *Eco*RI, blotted, and hybridized with a ^{32}P-labeled fragment of DNA (probe A in *E*) corresponding to a 5′ segment of the *Raly* first intron. (*B*) The same genomic DNAs were digested with *Bgl* II, blotted, and hybridized with an 83-bp ^{32}P-labeled fragment of DNA corresponding to the noncoding second exon of *Raly* (probe B in *E*). (*C*) The same genomic DNAs were digested with *Bam*HI or *Sac* I, blotted, and hybridized with probe A. Probe A detects A^y-specific breakpoint fragments of 14.0-kb with *Bam*HI and 13.5-kb with *Sac* I (highlighted by the boxed region). (*D*) The same filter used in *C* was stripped and rehybridized with the 111-bp probe (probe C in *E*). Probe C detects the same 14.0-kb *Bam*HI and 13.5-kb *Sac* I A^y-specific breakpoint fragments as did probe A, but each of these probes detects unique wild-type *M. musculus* and *M. spretus* fragments. (*E*) Genomic restriction map of the regions surrounding the 170-kb of DNA that is deleted in the A^y allele. The 5′ deletion breakpoint in the A^y allele occurs in the first intron of *Raly*, within the 3-kb *Eco*RI–*Bam*HI interval indicated. The *Raly* second exon (and the remainder of the *Raly* gene) lies within the 170-kb region that is deleted in the A^y allele. Probe C maps to the 3′ end of the 170-kb region and identifies the A^y 3′ deletion breakpoint. The restriction map is shown to scale except for *Raly* exons one and two, which are enlarged for clarity. B, *Bam*HI; E, *Eco*RI; X, *Xba* I.

utilized DNA variants to differentiate the *M. musculus* agouti (*A*) and A^y alleles from the *M. spretus* white-bellied agouti (A^w) allele in an F_1 hybrid (A^y/A^w, referred to as $A^y/M.s.$).

Given that the noncoding first exon of *Raly* is present in the A^y allele (26), a portion of the first intron of *Raly* was used as a probe (probe A in Fig. 2*E*) on Southern blots containing genomic DNA from *M. musculus, M. spretus*, and an F_1 hybrid ($A^y/M.s.$). Probe A detects a 4.5-kb *M. musculus*-specific *Eco*RI fragment, a 7.0-kb *M. spretus*-specific fragment, and both the 4.5- and 7.0-kb fragments in $A^y/M.s.$, indicating that this portion of the *Raly* first intron is present in the A^y allele (Fig. 2*A*). However, a probe specific to the 83-bp second exon of *Raly* (probe B in Fig. 2*E*), which detects 12.0-kb (*M. musculus*) and 7.8-kb (*M. spretus*) *Bgl* II fragments, hybridizes only to the 7.8-kb *M. spretus*-specific fragment in $A^y/M.s.$, indicating that the second exon of *Raly* is deleted in the A^y allele (Fig. 2*B*). In addition to the *Raly* second-exon probe, probes from the first and last coding exons of *Raly* demonstrated that these regions are also deleted in A^y DNA (data not shown, and ref. 26, respectively). Because the *Raly* cDNA probe detects only a 110-kb *Eag* I fragment on the PFGE blot (data not shown), the *Raly* gene does not extend in the 3′ direction past the *Eag* I site that is encompassed by the A^y deletion. Collectively, these data provide compelling evidence that the entire *Raly* gene, except for the promoter and noncoding first exon, is deleted in A^y mice.

To localize further the 5′ deletion breakpoint, probe A was hybridized to DNAs digested with *Bam*HI or *Sac* I. A size-altered fragment unique to the A^y allele was detected with each of these enzymes (Fig. 2*C*). To test whether these size-altered A^y-specific fragments correspond to the deletion breakpoint region, this same blot was also hybridized with a probe mapping 3′ to the deletion breakpoint. This probe corresponds to a 111-bp region (probe C in Fig. 2*E*) that is differentially incorporated into A^y-specific transcripts (26). Probe C is present in A^y DNA and normally hybridizes to a 170-kb *Eag* I fragment in wild-type DNA (data not shown), as does the agouti cDNA probe, which unequivocally places it 3′ to the deleted region in the A^y allele (see above and Fig. 1*C*). As shown in Fig. 2*D*, the same size-altered A^y-specific *Bam*HI and *Sac* I fragments that were detected with the probe A also hybridize with probe C. As expected, probes A and C each detect different wild-type *Bam*HI and *Sac* I fragments because these probes normally lie on opposite sides of the deleted region in the A^y allele. Taken together, these data demonstrate that the 5′ A^y deletion breakpoint occurs ≈12 kb downstream from the *Raly* first exon (Fig. 2*E*) and that the 3′ deletion breakpoint occurs 170 kb downstream from this region, at a position ≈105 kb upstream from the first exon of the agouti gene, as the gene was originally described (Fig. 1*C*).

DISCUSSION

As part of the characterization of the agouti gene, we previously determined that the lethal-yellow mutation expresses three size-altered 1.1-kb mRNAs. In addition to agouti sequences, each of these A^y mRNA species contains the first exon of another gene (*Raly*) that is closely linked to agouti in mouse chromosome 2. We also previously determined that at least a portion of the *Raly* gene is deleted from the A^y allele (26). Here we have utilized PFGE to demonstrate that the 5′ end of the *Raly* gene lies 280 kb proximal to the 3′ end of the agouti gene (see below) and that the deletion associated with the A^y mutation is 170 kb in length. The deletion encompasses a region that starts at a site located ≈12 kb 3′ of the noncoding first exon of *Raly*, extends through the remainder of the *Raly* gene, and terminates at a position estimated to lie 105 kb 5′ of the originally described agouti gene.

The 111-bp probe used to localize the 3′ A^y deletion breakpoint was originally identified by its differential incorporation into three alternately processed A^y-specific transcripts (26). All three A^y transcripts contain the noncoding first exon of *Raly* at their 5′ termini. Two of the three A^y transcripts contain differentially spliced regions, 111 bp and 46 bp in length (labeled A and B, respectively, in Figs. 3 and 4), derived from DNA located between the *Raly* first exon and the agouti coding exons. We originally proposed that these 111- and 46-bp regions might be sequences that arose from cryptic splicing events in a unique A^y pre-mRNA. More recently, however, we have determined that these A^y se-

quences are also found in transcripts derived from other agouti alleles, including A^w and a^t. Therefore, the 111- and 46-bp sequences actually represent alternatively processed exons of the agouti gene (31). In light of these results, the fact that the 111-bp probe maps close to the 3′ A^y deletion breakpoint suggests that a portion of the agouti gene lies very close to, or possibly within, the deleted region, and that the 111-bp agouti probe maps to a site located ≈105 kb upstream of what was previously identified as the first exon of the wild-type agouti gene. Based on the observation that the 111-bp probe does not contain *Bam*HI or *Sac* I enzyme recognition sites, but detects two wild-type fragments with each enzyme (Fig. 2*D*), the 111-bp fragment may actually represent two agouti exons. The nature and functional significance of the agouti transcripts that incorporate the 111- and 46-bp regions are reported elsewhere (31).

Our interpretation of the mapping data is based on the assumption that the 170-kb deletion in A^y is contiguous. We have not, however, mapped the 111-bp probe with respect to the remainder of the agouti gene. For this reason, it is possible that the 170 kb of deleted DNA is actually composed of more than one deletion, with a single deletion of <170 kb occurring between the *Raly* first intron and the downstream 111-bp region and an additional deletion occurring between the 111-bp region and the remainder of the agouti gene. Whether or not the 170-kb A^y deletion is contiguous, these data allow us to propose a model that can explain the recombination between A^y and other agouti alleles and the manner of formation of the novel 1.1-kb transcripts from the A^y allele.

The A^y allele can recombine with a^x (22) and probably also recombines with a and a^t (23). Utilizing the confirmed and probable recombination events, the recombination frequency between A^y and these other agouti locus alleles is estimated to be 0.1% (Table 1), with A^y being proximal to a^x. The fact that A^y can recombine with these other agouti alleles led us to propose previously that the agouti gene is either very large or that A^y is associated with a separate gene that is pseudoallelic with agouti (22, 23). We ruled out the latter possibility as a result of our recent cloning and characterization of the agouti gene (24). To explain the 0.1% recombination frequency between A^y and other agouti alleles, we hypothesized that a recombination hot-spot exists within the first intron of agouti (24). However, based on the unique structure of the A^y allele, the recombination between A^y and other agouti alleles can be reconciled with a model that incorporates a conventional recombination mechanism without the need to invoke a recombination hot-spot. This model is presented in Fig. 3 and is based on the following observations: the 5′ end of the *Raly* gene lies about 280 kb proximal to the 3′ end of the agouti gene; the A^y deletion starts within the first *Raly* intron and continues for 170 kb through the remainder of the *Raly* gene; two of the mutations that A^y probably recombines with, a and a^t, are each associated with the insertion of novel DNA sequences at the same position, located in the intron upstream of the first agouti coding exon (24); and the deletion in A^y, whether it is contiguous or not, would leave ≈105 kb of DNA that normally occurs between the 3′ end of the remaining portion of *Raly* and the region of agouti that is mutated in the a and a^t alleles (2–3 kb 3′ of exon 1). Recombination anywhere within this 105-kb region would give rise to a wild-type agouti allele. The 0.1% recombination frequency observed between A^y and other agouti locus alleles is generally compatible with the 105-kb physical distance between the deletion in A^y and the mutated region associated with the a and a^t alleles.

Table 1. Agouti exceptions from crosses involving lethal yellow (A^y) mice at the Oak Ridge National Laboratory

Cross ♀		Cross ♂	*n*	Agouti exceptions	M*	Recombination frequency (cM)†
A^y/a^t	×	a^t/a	2160	1	2	0.09 (0.005–0.5)
A^y/a	×	a/a	3631‡	2	2	0.11 (0.02–0.4)
A^y/a^x	×	A^y/a^x	4276	5§	1	0.12 (0.05–0.3)

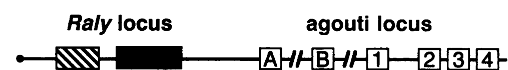
Modified from ref. 23.

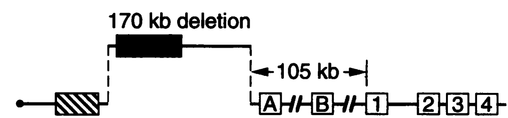
*Multiplier (M). In all crosses, a presumably equivalent number of recombinants would be yellow and, thus, nondetectable. In the third cross, however, recombinants can be generated by both parents.

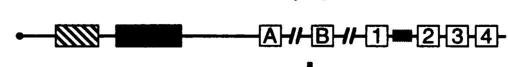
†Assuming exceptions were the result of recombination rather than mutation. Proof for recombination exists for the third cross where flanking markers were present. Parentheses indicate 95% confidence limits.

‡Almost all offspring had a female A^y/a parent. In crosses predominantly a/a females × A^y/a males, made at other laboratories, no agouti exceptions were reported among 6632 offspring (23).

§Four agouti exceptions were observed among 3784 offspring (the remainder were yellow, a^x/a^x being lethal); one A^y/A was identified among 492 tested yellow breeders.







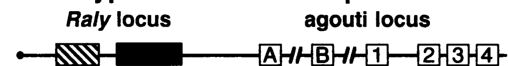


FIG. 3. Simple model for recombination between A^y and other agouti-locus alleles. The 5′ end of the wild-type *Raly* gene is located about 280 kb proximal to the 3′ end of the wild-type agouti gene and both are transcribed in the same orientation (top). The wild-type *Raly* gene is shown as a hatched box (indicating the promoter and untranslated first exon) and a solid box (depicting the remainder of the gene). The four exons of the wild-type agouti gene (as the gene was originally described) are shown as numbered boxes, and the recently identified (31) upstream agouti exons are indicated by the boxes labeled A (corresponding to the 111-bp probe used to identify the 3′ A^y deletion breakpoint) and B (46 bp). The A^y mutation is the result of a 170-kb deletion at the *Raly* locus, with deletion breakpoints occurring in the first intron of *Raly* and immediately upstream of agouti exon A. Approximately 105 kb of DNA remains between the 3′ deletion breakpoint and the first exon of the originally described agouti gene, labeled exon 1. The agouti-locus alleles a and a^t each arose by the insertion of novel DNA sequences (shown by the vertical lines; ref. 24) in the intron that immediately precedes the coding region (exons 2–4) of the gene. Crossing-over within this 105-kb region of DNA between A^y and either a or a^t would result in the generation of a wild-type agouti allele (wild-type recombination product) and the regeneration of a modified A^y allele (not shown) at an expected frequency of about 0.1%. This model also predicts the recombination observed between A^y and a^x; because a^x occurs in a separate complementation group from A^y, the DNA lesion in a^x probably occurs 3′ to the agouti gene.

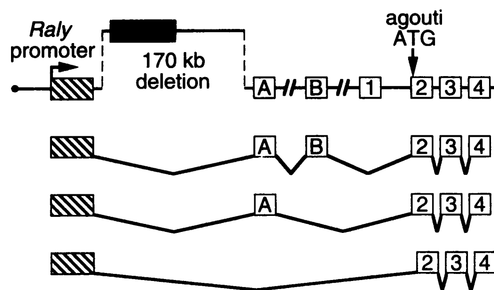


FIG. 4. Model for the production of chimeric *Raly*/agouti transcripts from the A^y allele. Because of the 170-kb deletion in the A^y allele, transcriptional initiation at the *Raly* promoter is proposed to result in the transcription of the noncoding first exon of *Raly* (hatched box), the intergenic sequence, and the downstream agouti gene. The processing of this novel primary transcript could result in the joining of the splice donor of the first *Raly* exon to the downstream acceptor sites of agouti. The result would be A^y transcripts consisting of the noncoding first exon of *Raly* and the three coding exons of the agouti gene (exons 2–4), with alternative splicing of agouti exons A and B, as described (26).

The data regarding the structure of the A^y allele described in this report also suggest a model to account for the production of the different-sized *Raly*/agouti chimeric transcripts that are expressed from the A^y allele (Fig. 4). We propose that transcription initiates normally at the *Raly* promoter and proceeds through the first *Raly* exon; however, because the remainder of the gene, which probably includes any transcription termination signal, is deleted, transcription proceeds into the remaining intergenic DNA and through the downstream exons of the agouti gene. The resulting novel primary transcript could be spliced in a manner whereby the splice donor associated with the first exon of *Raly* connects to the available splice acceptors in the downstream exons of the agouti gene. Additionally, alternative splicing of the primary transcript could occur by differentially incorporating exons from the 111-bp and 46-bp regions that are positioned just 3′ to the A^y deletion breakpoint. Overall, this model predicts that the *Raly* gene is not functional in the A^y allele and that transcripts with the coding potential of the wild-type agouti gene are overexpressed in a ubiquitous manner under the influence of the *Raly* promoter. In accordance with this model, we propose that deletion of the *Raly* gene is responsible for the recessive embryonic lethality of A^y and that the ubiquitous overexpression of the agouti gene, driven by the *Raly* promoter, is directly associated with the dominant pleiotropic effects of A^y.

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1. Hellerström, C. & Hellman, B. (1963) *Metabolism* **12,** 527–536.
2. Dickerson, G. E. & Gowen, J. W. (1947) *Science* **105,** 496–498.
3. Fenton, P. F. & Chase, H. B. (1951) *Proc. Soc. Exp. Biol.* **77,** 420–422.
4. Carpenter, K. J. & Mayer, J. (1958) *Am. J. Physiol.* **193,** 499–504.
5. Plocher, T. A. & Powley, T. L. (1976) *Metabolism* **25,** 593–602.
6. Friedman, J. M. & Leibel, R. L. (1992) *Cell* **69,** 217–220.
7. Wolff, G. L., Roberts, D. W. & Galbraith, D. B. (1986) *J. Hered.* **77,** 151–158.
8. Wolff, G. L. (1987) *Am. J. Clin. Nutr.* **45,** 168–180.
9. Cuènot, L. (1908) *Arch. Zool. Exp. Gen.* **9,** 7–15.
10. Castle, W. E. & Little, C. C. (1910) *Science* **32,** 868–870.
11. Ibsen, H. L. & Steigleder, E. (1917) *Am. Nat.* **51,** 740–752.
12. Kirkham, W. B. (1917) *Anat. Rec.* **11,** 480–481.
13. Kirkham, W. B. (1919) *J. Exp. Zool.* **28,** 125–135.
14. Robertson, G. G. (1942) *J. Exp. Zool.* **89,** 197–231.
15. Eaton, G. J. & Green, M. M. (1963) *Genetica* **34,** 155–161.
16. Pedersen, R. A. (1974) *J. Exp. Zool.* **188,** 307–319.
17. Pedersen, R. A. & Spindle, A. I. (1976) in *Embryogenesis in Mammals*, eds. Elliot, K. & O'Connor, M. (Elsevier, New York), CIBA Foundation Symposium 40, pp. 133–154.
18. Granholm, N. H. & Johnson, P. M. (1978) *J. Exp. Zool.* **205,** 327–333.
19. Papaioannou, V. & Gardner, R. L. (1979) *J. Embryol. Exp. Morphol.* **52,** 153–163.
20. Papaioannou, V. E. (1988) *Dev. Genet.* **9,** 155–165.
21. Barsh, G. S., Lovett, M. & Epstein, C. J. (1990) *Development* **109,** 683–690.
22. Russell, L. B., McDaniel, M. N. C. & Woodiel, F. N. (1963) *Genetics* **48,** 907a (abstr.).
23. Siracusa, L. D., Russell, L. B., Eicher, E. M., Corrow, D. J., Copeland, N. G. & Jenkins, N. A. (1987) *Genetics* **117,** 93–100.
24. Bultman, S. J., Michaud, E. J. & Woychik, R. P. (1992) *Cell* **71,** 1195–1204.
25. Miller, M. W., Duhl, D. M. J., Vrieling, H., Cordes, S. P., Ollmann, M. M., Winkes, B. M. & Barsh, G. S. (1993) *Genes Dev.* **7,** 454–467.
26. Michaud, E. J., Bultman, S. J., Stubbs, L. J. & Woychik, R. P. (1993) *Genes Dev.* **7,** 1203–1213.
27. Klebig, M. L., Kwon, B. S. & Rinchik, E. M. (1992) *Mammal. Gen.* **2,** 51–63.
28. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1988) *Current Protocols in Molecular Biology* (Wiley, New York).
29. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
30. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137,** 266–267.
31. Bultman, S. B., Klebig, M. L., Michaud, E. J., Sweet, H. O., Davisson, M. T. & Woychik, R. P. (1994) *Genes Dev.*, in press.