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Genetics

Ectopic expression of the agouti gene in transgenic mice causes obesity, features of type II diabetes, and yellow fur

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ABSTRACT **Mice that carry the lethal yellow (A^y) or viable yellow (A^{vy}) mutation, two dominant mutations of the agouti (*a*) gene in mouse chromosome 2, exhibit a phenotype that includes yellow fur, marked obesity, a form of type II diabetes associated with insulin resistance, and an increased susceptibility to tumor development. Molecular analyses of these and several other dominant "obese yellow" *a*-locus mutations suggested that ectopic expression of the normal agouti protein gives rise to this complex pleiotropic phenotype. We have now tested this hypothesis directly by generating transgenic mice that ectopically express an agouti cDNA clone encoding the normal agouti protein in all tissues examined. Transgenic mice of both sexes have yellow fur, become obese, and develop hyperinsulinemia. In addition, male transgenic mice develop hyperglycemia by 12–20 weeks of age. These results demonstrate conclusively that the ectopic agouti expression is responsible for most, if not all, of the phenotypic traits of the dominant, obese yellow mutants.**

Several dominant mutations at the agouti (*a*) locus confer a phenotype of obesity and yellow fur in mice (1). The most extensively analyzed dominant mutations, lethal yellow (A^y) and viable yellow (A^{vy}), also cause a form of type II diabetes that is characterized by insulin resistance (2–4), pancreatic islet hypertrophy and hyperplasia (5, 6), hyperinsulinemia (2–4, 7–9), and impaired glucose tolerance (2–4, 10). Moderate nonfasted hyperglycemia has also been observed in males, but seldom in females (2, 4, 5, 9, 11). In addition to obesity and diabetes, $A^y/-$ and $A^{vy}/-$ mice have greater-than-normal muscular and skeletal growth and an increased risk of developing hyperplasia or neoplasia in a variety of tissues (reviewed in ref. 12). We will refer to mice that carry these dominant *a*-locus mutations and exhibit the pleiotropic effects as obese yellow mutants.

The only known function of the agouti gene in wild-type mice is to regulate hair-pigment production by the melanocyte in a manner that results in the production of the agouti coat color (1). Cloning and characterization of the wild-type agouti gene revealed that the gene has at least two different promoters that utilize three common coding exons (13–15). All forms of agouti mRNA have the potential to encode a 131-amino acid protein that has a consensus signal peptide (13–15). The predicted agouti protein contains a presumed structural motif composed of a series of regularly spaced cysteine residues near its carboxyl terminus (13). A similar motif is also present in several of the insecticidal peptides of the primitive hunting spider that function by inhibiting neuronal Ca^{2+} channels (16).

Skin transplantation experiments revealed that the production of agouti protein is not cell-autonomous to the melanocyte, but instead occurs within the cells in the follicular environment (reviewed in ref. 1). These results, coupled with our molecular evidence that agouti is a secreted protein (13), suggest that agouti is a paracrine factor that signals the melanocyte to switch from the production of eumelanin (black) to phaeomelanin (yellow) hair pigment granules. Recent experiments with recombinant agouti protein indicated that the protein may accomplish this function by acting as a competitive antagonist for the binding of α melanocyte-stimulating hormone (α-MSH) to its receptor (MSH-R) on the melanocyte (17). Normally, α-MSH binding activates the MSH-R and initiates a signal-transduction pathway for eumelanin synthesis that is mediated by the activation of adenylate cyclase and the elevation of intracellular cAMP levels (18, 19). By competing with α-MSH for the MSH-R, the agouti protein prevents the increase in the level of cAMP (17), thereby allowing only the pathway of phaeomelanin synthesis to proceed.

In adult wild-type mice (A/A and A^w/A), agouti gene expression has been detected thus far in the skin during the hair growth cycle and not in liver, muscle, fat, or numerous other tissues (13, 14). In contrast, in each of the dominant obese yellow mutants analyzed, agouti gene expression has been altered in a manner that results in the expression of agouti mRNAs in numerous, if not all, tissues (13, 20–22). Each of these different mRNA forms has the potential to encode a normal agouti protein (13, 20–22). These observations suggested that the action of an ectopic agouti protein is responsible for the obesity, diabetes, and other dominant pleiotropic effects in these mutant mice (13, 20, 23). However, since each of the dominant mutant alleles analyzed contains structural changes in or near the agouti locus, including a 170-kb deletion of 5′ flanking DNA (23) and the insertion of retrotransposable elements within the locus (21, 22), it was unclear whether the widespread expression of agouti *per se* causes the pleiotropic effects or whether effects of these mutations on an additional gene located in the vicinity of the agouti locus contribute to the complex phenotype. We now demonstrate that transgenic mice that ectopically express a wild-type agouti cDNA in numerous tissues develop the obesity, hyperinsulinemia, hyperglycemia, and yellow fur commonly observed in the spontaneous obese yellow mutants.

MATERIALS AND METHODS

Mice. All mice were maintained at the Oak Ridge National Laboratory. The FVB/N (A/A) mice were obtained from our partially inbred stock, and C57BL/6J (a/a; nonagouti black) mice were purchased from The Jackson Laboratory. The A^y/A mice are F_1-hybrids resulting from a cross between mice of our A^y/a stock (originally on the C57BL/6 background) with

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Abbreviations: α-MSH, α melanocyte-stimulating hormone; MSH-R, α-MSH receptor.

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FVB/N mice. All mice were provided with a high-fat diet (Mouse Diet 5015, PMI Feeds, ≥11% fat) and water *ad libitum*, except those used in FVB/N line maintenance, which were fed a normal diet (Lab Diet, PMI Feeds, 4.5% fat).

Agouti Expression Constructs. The portion of the agouti cDNA included in the expression constructs extends from nucleotide 8 to nucleotide 662 in the previously reported agouti cDNA sequence (13). It was generated by PCR amplification (15) from the agouti cDNA clone (13) by using the oligonucleotide primers 5′-ACAGGAAAGACATTCTGGC-CTGGC-3′ (forward) and 5′-TTTAGCTTCCACTAGGTT-TCC-3′ (reverse). The amplified product was cloned directly into the pCRII vector (Invitrogen) and subcloned into pBluescript II (Stratagene) as a 676-bp *Eco*RI fragment (clone pa-E.68). To generate the initial expression construct, designated BAPa (Fig. 1*A*), the cDNA segment was isolated from pa-E.68 as a *Hin*dIII–*Bam*HI fragment and cloned into the corresponding sites of pBAP.2 (25). To make the second construct, designated PGKPa (Fig. 1*B*), the neomycin-resistance gene of the PGK-neo expression vector (24) was removed by *Pst* I digestion and replaced with the 676-bp *Eco*RI agouti cDNA fragment after filling in the *Eco*RI ends with the Klenow fragment and introducing *Pst* I ends by linker ligation (26). The nucleotide sequences of the agouti cDNA and immediate flanking regions were determined (26) to verify the integrity of the expression constructs.

Transgenic Mice. One-cell FVB/N embryos were microinjected with either the BAPa (5.3-kb *Cla* I fragment) or PGKPa (1.7-kb *Eco*RI–*Hin*dIII fragment) construct (3 μg/ml in 10 mM Tris·HCl, pH 7.5/0.1 mM EDTA), and transgenic mice were derived as described (27).

DNA (Southern) and RNA (Northern) Blot Analysis. Southern- and Northern-blot hybridization analyses were performed as described (13, 26). The Raly cDNA probe has been described (20); the agouti cDNA probe was the 676-bp *Eco*RI cDNA fragment in clone pa-E.68.

Weight Gain and Blood Analysis. From 4 to 24 weeks of age, body weights of mice were measured every 2 weeks (±3 days), after which weights were taken every 4 weeks. Blood was obtained by retroorbital sinus puncture from nonfasted mice of various ages. Plasma insulin levels were measured by RIA (ICN) with porcine insulin as a standard, and glucose levels were determined by glucose oxidase (Trinder reagent; Sigma) or hexokinase/glucose-6-phosphate dehydrogenase (Abbott) assays. All data are reported as the mean ± SEM for four categories of mice: transgenic females, control females, transgenic males, and control males. Statistical comparison between transgenic mice and controls was performed by using an unpaired two-group *t* test (STATVIEW II; Abacus Concepts, Berkeley, CA).

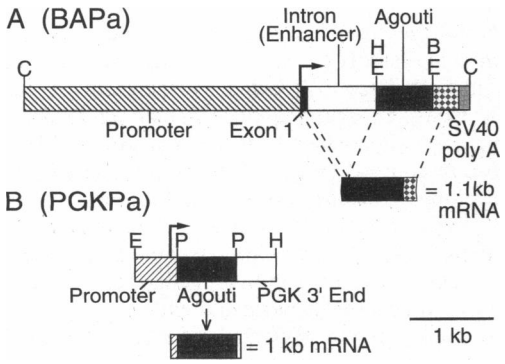

FIG. 1. β-actin promoter–agouti (BAPa) and phosphoglycerate kinase promoter–agouti (PGKPa) transgene expression constructs. (*A*) The components of the 5.3-kb BAPa construct are indicated. All of the components are from the human β-actin gene except the agouti cDNA and the simian virus 40 polyadenylylation signals. The first β-actin exon (78 bp) is untranslated, and the β-actin intron contains the endogenous enhancer and splice acceptor and donor sites. (*B*) The 1.7-kb PGKPa construct consists of the agouti cDNA under the transcriptional control of the promoter/enhancer region of the mouse *Pgk-1* gene from base pair −437 to +65 (24). The polyadenylylation signals are provided by the *Pgk-1* 3′ flanking region. For both constructs, "promoter" refers to the upstream region of the gene that contains the promoter and additional 5′ flanking DNA. The mRNAs expected to be expressed from these constructs are indicated below them. Arrows indicate sites of transcription initiation. C, *Cla* I; H, *Hin*dIII; E, *Eco*RI; B, *Bam*HI; P, *Pst* I.

RESULTS

Analysis of Transgene-Directed Agouti Gene Expression. The objective of this work was to generate and study transgenic mice that ectopically express wild-type agouti transgenes in as many tissues as possible. To accomplish this, the wild-type agouti cDNA was placed under the transcriptional control of either the human β-actin or mouse *Pgk-1* gene promoter and enhancer (expression constructs BAPa and PGKPa, respectively; Fig. 1). β-actin and *Pgk-1* promoters have been reported to direct widespread gene expression in transgenic mice (25, 28, 29). Several BAPa and PGKPa transgenic founder mice were generated by pronuclear microinjection of the expression constructs. Transgenic lines were established from two BAPa founder mice and one PGKPa founder (lines TgN(BAPa)20Rpw, TgN(BAPa)52Rpw and TgN(PGKPa)8Rpw, abbreviated here BAPa20, BAPa52, and PGKPa8, respectively) and maintained in the FVB/N (albino) strain (data not shown).

Adult mice that were hemizygous for the transgene (Tg/−) were examined for the levels of ectopic agouti gene expression. The levels of transgene-derived agouti mRNA in numerous tissues of Tg/− mice from lines BAPa20, BAPa52, and PGKPa8 are shown in Fig. 2. Agouti mRNA levels in A^y/A mice are also shown for comparison. Tg/− mice from lines BAPa20 and PGKPa8 (i.e., BAPa20/− and PGKPa8/− mice) express the agouti transgenes at high levels in all of the 14 tissues examined (pancreas not shown) and produce mRNAs of the expected sizes (1.1 kb and 0.95 kb in BAPa20/− and PGKPa8/−, respectively). In contrast, BAPa52/− mice express very low or undetectable levels of agouti mRNA in liver, pancreas (not shown), small intestine, kidneys, and salivary gland, and high

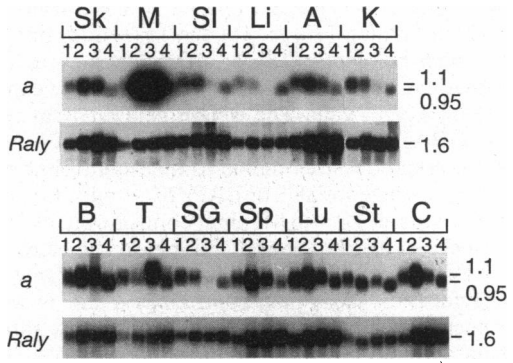

FIG. 2. Expression of agouti transgene-derived mRNAs in several tissues of adult transgenic (Tg/−) mice. Northern blots containing poly(A)$^+$ RNA (≈2.5 μg per lane) from tissues of Tg/− and A^y/A mice were hybridized with a radiolabeled agouti cDNA probe and subsequently with a cDNA probe for the *Raly* gene, which was used as a loading control because it is expressed at comparable levels in numerous tissues of mice (20). Lanes 1–4 refer to A^y/A, BAPa20/−, BAPa52/−, and PGKPa8/− mice, respectively. Sk, skin; M, skeletal muscle; SI, small intestine; Li, liver; A, white adipose tissue; K, kidney; B, brain; T, testis; SG, salivary gland; Sp, spleen; Lu, lung; St, stomach; C, colon. Numbers refer to average sizes (kb) of the mRNAs. All of the mRNAs detected in Tg/− mice with the agouti probe were derived from transgene expression, since agouti is not expressed in any adult tissues of wild-type mice (13).

levels in the remaining tissues. Unexpectedly, BAPa20/− and BAPa52/− animals express agouti at extremely high levels in skeletal muscle. BAPa20/− mice express agouti at higher levels than A^y/A mice in every tissue examined except liver, pancreas (not shown), kidney, testis, and salivary gland. In contrast, the levels of agouti mRNA in PGKPa8/− mice are less than those in A^y/A mice in most tissues, whereas the levels are roughly comparable in skin, muscle, stomach, and brain. In the livers of PGKPa8/− mice, the level of agouti expression is considerably greater than in A^y/A mice.

Transgenic Mice Ectopically Expressing the Agouti Gene Develop Yellow Fur, Obesity, Hyperinsulinemia, and Hyperglycemia. Since the BAPa20/− and PGKPa8/− mice expressed the agouti transgenes in all of the tissues examined, these mice were analyzed for several of the phenotypic traits of the obese yellow mutants. In view of the fact that BAPa52/− mice did not express agouti in all of their tissues, they were not analyzed further in this study. Because several previous studies reported that $A^y/-$ mice become much more obese on F_1 hybrid backgrounds than on inbred backgrounds (reviewed in refs. 30 and 31), Tg/− mice on the wild-type FVB/N genetic background (A/A) were mated with non-agouti-black C57BL/6J (a/a) mice, and the (C57BL/6J × FVB/N)F_1 [i.e., (B×F)F_1] Tg/− progeny were analyzed for several of the dominant pleiotropic effects. In addition, many of the $A^y/-$ and $A^{vy}/-$ mice studied previously carried the a allele or had genetic backgrounds that included contributions from the C57BL or C57BL/6 strains (7, 12, 30).

Although FVB/N mice have the agouti (A/A) genotype, it was not possible to observe the effects of ectopic agouti gene expression on coat color in Tg/− mice on the FVB/N genetic background because FVB/N mice are albino (c/c). Therefore, the availability of the (B×F)F_1 Tg/− offspring (Tg/−; A/a; C/c), which are pigmented, allowed us to evaluate these effects. The (B×F)F_1 BAPa20/− and PGKPa8/− progeny have solid yellow or mottled yellow fur, respectively, as opposed to the agouti coat of the nontransgenic littermates (+/+; A/a; C/c). This was the first available evidence indicating that the agouti transgenes are capable of giving rise to functional protein that can alter the phenotype of the animals.

The weights of yellow Tg/− mice and nontransgenic littermate controls were analyzed as they matured, beginning at 4 weeks of age. In view of reports that the weight gain of $A^y/-$ and $A^{vy}/-$ mice is accelerated relative to control littermates when fed high-fat diets (10, 11, 31), the Tg/− and control mice were fed a high-fat diet (≥11% fat) during their entire lifespan to accentuate any effect that the agouti transgene expression may have on weight gain. The weight gain analyses revealed that yellow BAPa20/− and PGKPa8/− mice of both sexes develop a marked obesity relative to their control littermates (Fig. 3). The average weights of BAPa20/− mice first became significantly greater ($P < 0.01$) than control weights by about 4 and 6 weeks of age in females and males, respectively (Fig. 3 *Upper*). PGKPa8/− females and males first became consistently heavier ($P < 0.01$) than controls by 8 and 14 weeks of age, respectively (Fig. 3 *Lower*). Tg/− females ultimately developed a greater obesity relative to controls (1.7-fold increase in line BAPa20 at 24 weeks of age and 1.6-fold increase in line PGKPa8 at 32 weeks of age) than did Tg/− males (1.4-fold increase in line BAPa20 at 16 weeks of age and 1.3-fold increase in line PGKPa8 at 26 weeks of age) (Fig. 3). At similar ages, BAPa20/− mice were heavier than PGKPa8/− mice (e.g., BAPa20/− mice are 1.2-fold heavier than PGKPa8/− mice at 16 weeks of age; Fig. 3).

The plasma insulin and glucose levels of the Tg/− mice and littermate controls were also analyzed as they aged. By 20 weeks of age, yellow BAPa20/− and PGKPa8/− mice of both sexes had developed significantly higher ($P < 0.01$) levels of insulin in their blood than control littermates, but the hyperinsulinemia was more severe in males than in females (Fig. 4).

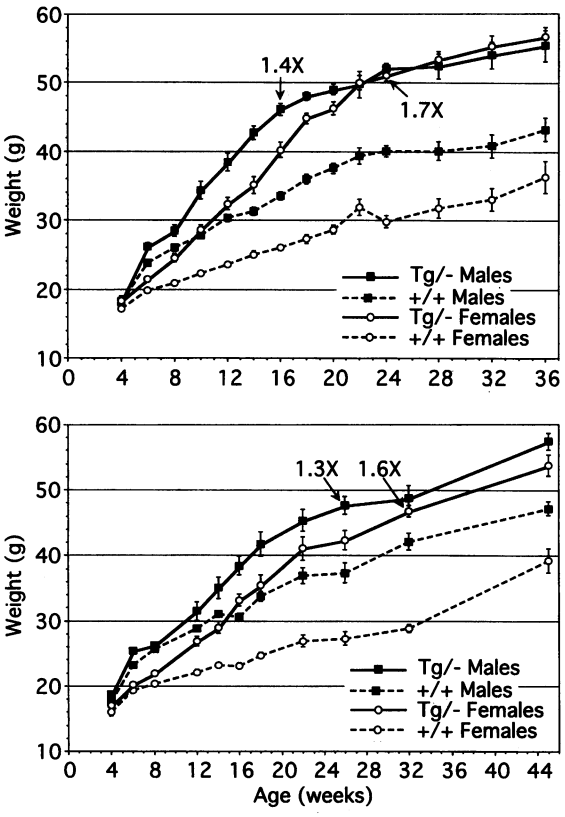

FIG. 3. Weight gain of transgenic (Tg/−) and normal control nontransgenic (+/+) mice from lines BAPa20 (*Upper*) and PGKPa8 (*Lower*). Average weights in grams are plotted with respect to age in weeks. Separate curves are shown for each genotype and sex. Bars represent 1 SE from the mean. The mean weights of Tg/− mice were significantly greater than those of nontransgenic +/+ littermates ($P < 0.01$) at every age point except at 4 weeks for male BAPa20/− mice, 4 and 6 weeks for female PGKPa8/− mice, and 4, 8, and 12 weeks for male PGKPa8/− mice. Arrows indicate time points at which weights of Tg/− mice reach their greatest difference from controls (indicated as fold increase of Tg/− mice over +/+ controls).

Not only did the ectopic expression of agouti lead to hyperinsulinemia in these Tg/− mice, but it also caused male Tg/− mice from both lines to become hyperglycemic (Fig. 5). By about 20 weeks of age, the plasma glucose levels of male BAPa20/− mice increased to levels that were ≈70% greater than those of controls (Fig. 5 *Upper*), while male PGKPa8/− mice developed glucose levels 40% greater than those of controls (Fig. 5 *Lower*). In contrast to males, female Tg/− mice did not exhibit significant elevations in their plasma glucose levels at any of the individual ages examined (Fig. 5), even though they were hyperinsulinemic (Fig. 4) and obese (Fig. 3). As was found for the extent of obesity, the BAPa20/− mice were more hyperinsulinemic and hyperglycemic than PGKPa8/− mice at similar ages.

DISCUSSION

To test the hypothesis that the ectopic expression of the agouti gene is the only primary molecular abnormality responsible for the obesity and the form of type II diabetes exhibited by the spontaneous obese yellow mice, we generated transgenic mice that express the wild-type agouti cDNA in all 14 tissues analyzed. Tg/− mice of both sexes developed yellow fur, obesity, and hyperinsulinemia. Tg/− males also became hyperglycemic by 12–20 weeks of age; in contrast, Tg/− females did not develop hyperglycemia despite becoming considerably hyperinsulinemic. This effect is consistent with previous ob-

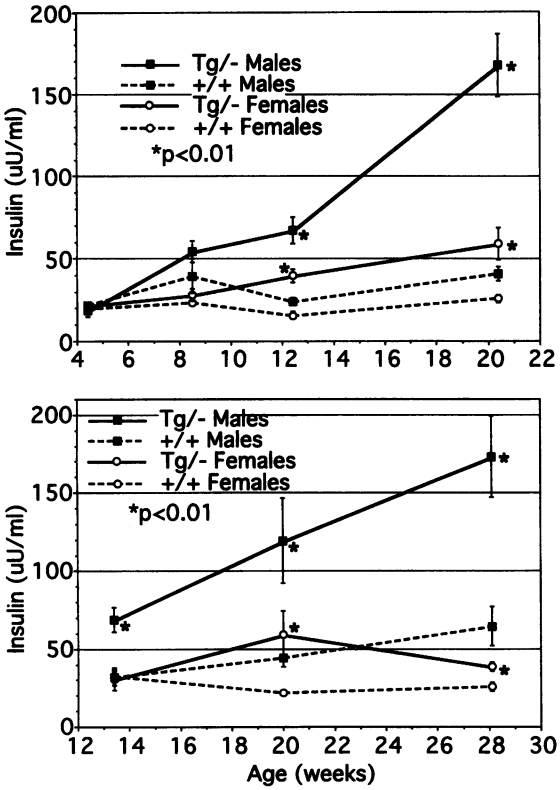

FIG. 4. Mean plasma insulin levels in microunits (μU) of insulin per ml plotted as a function of age in weeks for transgenic lines BAPa20 (*Upper*) and PGKPa8 (*Lower*). Separate curves are shown for each genotype and sex. Bars represent 1 SE from the mean. The mean insulin levels of Tg/− mice that are significantly greater ($P < 0.01$) than those of nontransgenic +/+ controls are marked with an asterisk.

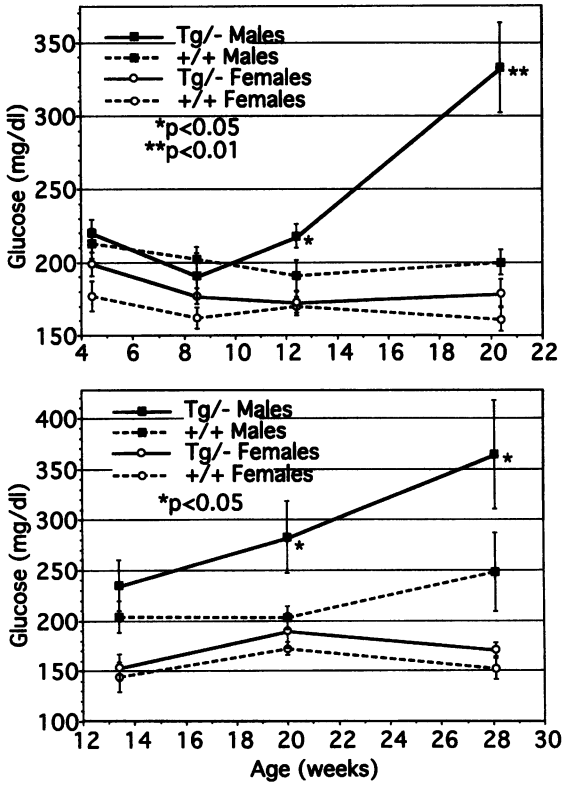

FIG. 5. Mean plasma glucose levels in mg of glucose per dl plotted as a function of age in weeks for transgenic lines BAPa20 (*Upper*) and PGKPa8 (*Lower*). Separate curves are shown for each genotype and sex. Bars represent 1 SE from the mean. The mean glucose levels of Tg/− mice that are significantly greater than those of nontransgenic +/+ controls are marked with one or two asterisks as indicated.

servations of hyperglycemia in male but not female obese yellow mice (2, 4, 5, 9, 11) and with the reported antihyperglycemic effects of estrogens in genetically obese–diabetic (*db/db*) mice (32). These results demonstrate unequivocally that ectopic expression of a normal agouti protein alone is sufficient to cause yellow fur, obesity, and a form of type II diabetes in mice. In view of the close association between obesity and the increased susceptibility to cancer in the $A^y/-$ and $A^{vy}/-$ mice (reviewed in ref. 12), we predict that the Tg/− mice will also be more tumor-prone than their +/+ littermates.

Close examination of the data in Figs. 2–5 reveals that the level of ectopic agouti gene expression may influence the time of onset and severity of the obesity, hyperinsulinemia, and hyperglycemia. The level of agouti expression in most tissues of PGKPa8/− mice is less than the level observed in the same tissues of A^y/A mice, while the level of agouti mRNA is greater in most tissues of BAPa20/− mice than in A^y/A mice (Fig. 2). These differences in agouti expression between the different transgenic lines correlate with the findings that solid yellow BAPa20/− mice are more severely obese, hyperinsulinemic, and hyperglycemic than mottled yellow PGKPa8/− animals at similar ages (Figs. 3–5). Therefore, it may be that increasing the average overall level of ectopic agouti gene expression may actually increase the rate of progression of these disorders. Additionally, the fact that PGKPa8/− mice do eventually develop the obese yellow traits whereas "pseudoagouti" A^{iapy}/a and A^{vy}/a mice do not (8, 21, 33), despite having very low levels of ectopic agouti gene expression (21, 22, 34), suggests that a threshold level of expression, between the levels found in the pseudoagouti and PGKPa8/− mice, is required for the development of yellow fur, obesity, and diabetes.

The finding that solid yellow BAPa20/− mice develop obesity and diabetes faster than mottled yellow PGKPa8/− mice is in contrast to the previously reported observation that solid yellow and mottled yellow A^{vy}/a mice, which do show differences in the level of agouti expression (34), do not show any consistent difference in the development of obesity (33). The reason for the discrepancy between our observations and these previously reported results is at present unknown. One possibility for the unexpected finding is that the difference between the levels of agouti expression in BAPa20/− and PGKPa8/− mice may be greater than that between the levels of expression in solid yellow and mottled yellow A^{vy}/a mice.

It has been demonstrated that although the agouti protein is secreted, it appears to function in a localized manner (1) and is probably not present at high levels in the general circulation (35). We have now shown that widespread ectopic expression of the normal agouti protein induces the obesity and diabetes of the obese yellow mutants. However, we predict that the ectopic expression of the agouti protein in a specific tissue(s) is directly responsible for the development of the dominant phenotypic effects in these animals. For example, it may be that the ectopic expression of the protein in muscle is solely responsible for the insulin resistance, obesity, and eventual diabetes and that its expression elsewhere in the animal does not alter the phenotype. In the accompanying paper, Zemel *et al.* (34) report that A^{vy}/a mice have elevated levels of intracellular free calcium ($[Ca^{2+}]_i$) in their soleus muscle and that recombinant agouti protein induces elevated $[Ca^{2+}]_i$ levels in skeletal muscle myocytes *in vitro*. This finding is provocative because skeletal muscle is the primary site of peripheral glucose disposal (36), and increased $[Ca^{2+}]_i$ can lead to insulin resistance and hyperinsulinemia (37–38). In fact, the finding that hyperinsulinemia is known to lead to obesity by stimulat-

ing lipogenesis and decreasing lipolysis (36) suggests that the obesity of dominant yellow agouti-locus mutants may be a secondary effect of the insulin resistance and hyperinsulinemia.

Conversely, it is possible that the obesity of dominant yellow mice is a consequence of the ectopic expression of agouti in adipocytes. Adipocytes of A^{vy}/a mice have depressed basal lipolytic rates, which may be due to an agouti-mediated defect in the signal transduction pathway for lipolysis at the level of the production or maintenance of intracellular cAMP (reviewed in ref. 39). This is compatible with the fact that the agouti protein appears to affect hair pigmentation by modulating the intracellular levels of cAMP in the melanocyte. While this effect appears to occur in the melanocyte through the antagonism of α-MSH activation of its receptor (17), it may occur in adipocytes by agouti antagonism of the MSH-R, if the MSH-R is normally in adipocytes, or of another as yet unidentified receptor that could be a member of the melanocortin receptor family. Obesity-promoting effects of the agouti protein in adipocytes could be independent of its possible hyperinsulinemia-inducing effects in muscle. Alternatively, the hyperinsulinemia of the dominant yellow mice could be a secondary consequence of obesity (36) induced by the action of ectopic agouti protein on adipocytes. It is also possible that the obesity and hyperinsulinemia could result from synergistic effects of the agouti protein on both muscle and fat.

In addition to possible primary effects of the agouti protein in muscle or fat or both, it is also conceivable that the protein could lead to hyperinsulinemia by exerting direct effects on pancreatic β-islet cells. β-Cell hyperplasia has been detected in viable yellow mice before the onset of hyperinsulinemia (6). Whatever the mechanism of the β-cell proliferation may be, it is possible that it may be responsible for the chronic hyperinsulinemia, which could lead to obesity, insulin resistance, and, eventually, type II diabetes in the obese yellow mutants.

Another possibility is that the ectopic agouti protein is causing the obesity and even hyperinsulinemia indirectly as a result of a primary effect on areas of the brain that control weight, body fat, and insulin production. Consistent with this possibility is the recent demonstration that recombinant agouti protein antagonizes α-MSH activation of the melanocortin receptor MC4 (17), which is expressed in brain nuclei involved in neuroendocrine and sympathetic control (17, 40). It is conceivable that this action in the brain may trigger the hyperphagia, increased efficiency of food utilization, and reduced sympathetic tone observed in obese yellow mice (10, 39, 41). Decreased adrenergic tone is known to lead to decreased lipolysis and increased lipogenesis and insulin production (reviewed in ref. 39).

Additional experiments involving transgenic mice that ectopically express the normal agouti protein specifically in muscle, fat, pancreas, brain, or other tissues should help to resolve the issue regarding which tissues are responding to the ectopic expression of agouti in mice. Experiments directed at solving how the agouti gene is functioning at the cellular level to cause the obesity and insulin resistance may provide some insight into the general molecular mechanisms of these disorders. In light of our recent cloning and characterization of the human agouti gene (42), these types of experiments may also advance our knowledge of some forms of obesity and type II diabetes in humans.

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