Physiological consequences of ectopic agouti gene expression: the yellow obese mouse syndrome

GEORGE L. WOLFF,1,2,3 DEAN W. ROBERTS,1,3 AND KATHLEEN G. MOUNTJOY 4
1Division of Biochemical Toxicology, National Center for Toxilogical Research/ Food and Drug Administration, Jefferson 72079; Departments of 2Biochemistry/ Molecular Biology and 3Pharmacology/ Interdisciplinary Toxicology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205; and 4Research Centre for Developmental Medicine and Biology and Department of Molecular Medicine, University of Auckland, Auckland 1, New Zealand

Wolff, George L., Dean W. Roberts, and Kathleen G. Mountjoy. Physiological consequences of ectopic agouti gene expression: the yellow obese mouse syndrome. Physiol. Genomics 1: 151–163, 1999.—This review summarizes primary and downstream phenotypic manifestations, with emphasis on altered responsiveness to environmental stimuli, of dominant yellow mutations at the mouse agouti locus. Obvious effects include hyperinsulinemia, obesity, stimulation of somatic growth and tumorigenesis, and coat color. Downstream influences of hyperinsulinemia and obesity on the individual’s physiology determine important components of the obese yellow agouti mouse syndrome. Collectively, the phenotypic aberrations described support the concept that identical genomes are expressed in a spectrum of physiological phenotypes that reflect the complex interdependence of gene-regulated physiological pathways and processes in the organism throughout extended, but temporally ordered, periods of fetal and neonatal development and aging. This summary identifies important areas for additional research and provides integrated information required for a systematic approach to the development of interventions for common adult human health problems.

background genome; melanocortin; pseudoagouti mice; response to environmental stimuli; tumorigenesis

PHYSIOLOGICAL GENOMICS was foreshadowed more than 65 years ago by Sewall Wright’s statement (46) that “All characters are affected by many genes and each gene affects many characters.” In those days, the study of the influence of genes on physiological parameters was called mammalian physiological genetics and emphasized the significance of interactions among genes, as well as the generally underappreciated influence of the background genome on individual gene expression (47).

Molecular genetics has revealed that the majority of these interactions actually occur at the physiological level rather than at the level of the gene. In this review, we shall use the terms “gene interactions” and “background genome effects” as shorthand for the following concepts. 1) Each gene specifies a protein or RNA that modulates a specific step in one or more physiological pathways or cellular processes. The apparent interactions among such genes actually represent the effect of each gene-specified protein or RNA on a different step in the same physiological pathway or cellular process. 2) The apparent effects of the background genome on expression of particular genes actually represent the effects of the qualitative and quantitative differences in the physiological pathways and cellular processes, resulting from the presence of different alleles at many loci and different patterns of expressed genes in each inbred strain and in each individual in noninbred populations.

The ontogeny of each individual mouse and human involves the development of functional physiological, immunologic, and cognitive systems through complex interactions, programmed sequential cell differentiation and migration, and subsequent organization of diverse cell types throughout an extended, but temporally ordered, period of fetal and neonatal development.

Today, the combination of physiological and molecular biological concepts and techniques facilitates the investigation of the reciprocal relations of gene function and physiology at all levels of biological organization. It is now possible to 1) define gene product (polypeptide) actions and interactions that, in many cases, modulate cellular signaling pathways and 2) trace in detail the
intra- and intercellular molecular paths from gene to in vivo phenotype.

An illuminating example of the power of molecular techniques and concepts to address problems of physiological genetics is the “yellow agouti obese mouse syndrome.” This syndrome, induced by alternative dominant alleles at the agouti locus on mouse chromosome 2, is characterized by yellowish hair, adult-onset obesity, hyperinsulinemia, somewhat increased lean body mass, and heightened susceptibility to endogenously and exogenously induced tumors (reviewed in Refs. 38, 44, 48). These phenotypic effects result, directly or indirectly, from the binding of agouti protein, specified by the agouti gene, to melanocortin receptors, the proteins specified by the melanocortin receptor genes. Thus the binding of agouti protein modulates the signaling pathways associated with the melanocortin receptors. The interaction occurs not between the agouti and melanocortin receptor genes per se but rather between the proteins specified by these genes. Therefore, the “interaction between the genes” occurs downstream at the level of the products of DNA transcription and translation. Each gene merely serves as a template for synthesis of a protein, which is a part of signaling pathways and/or cellular processes. In short, physiological genomics deals with the effects of the interactions of gene products in the complex network of homeostatic and cellular processes that constitute the living organism.

The human homologue of this locus, agouti signaling protein (ASIP), located in a syntenic section on human chromosome 20q, is regularly expressed at a low level in several tissues. Because the murine agouti gene is normally transcribed only in the skin and then only transiently during formation of the yellow subapical band in the hair, the usual expression of ASIP in multiple tissues suggests that its transcription is regulated quite differently from that of agouti. The function of the ASIP protein in Homo sapiens is unknown (33).

**LETHAL (A\(^v\)) AND Viable (A\(^v\)) YELLOW MUTATIONS AT THE MOUSE AGOUTI LOCUS**

Two of the dominant agouti mutations have been studied extensively, namely, “lethal yellow” (A\(^v\)), first described by Cuénot (5), and “viable yellow” (A\(^v\)), first described by Dickie (6). The A\(^v\) mutation was propagated by the mouse fancy in Europe at least as early as the 1800s, whereas the A\(^v\) mutation was discovered at The Jackson Laboratory (Bar Harbor, ME) in 1960. In addition to the phenotypic traits common to the other dominant “yellow” mutants, the lethal yellow mutation is characterized by prenatal lethality of the homozygous A\(^v\)/A\(^v\) genotype. The viable yellow A\(^v\)/– phenotype differs from that of the clear yellow A\(^v\)/– phenotype by exhibiting eumelanin motting, i.e., irregular areas or small spots of agouti/black hair on a yellow background.

Molecular definition of these mutations was made possible by identification and cloning of the agouti locus at the Oak Ridge National Laboratories (2). Briefly, A\(^v\) is characterized by a large deletion that includes the coding regions of the Raly (heterogeneous nuclear ribonucleo-protein associated with lethal yellow) gene, a member of the hnrNp gene family. The hnrNp proteins are involved in pre-mRNA packaging and processing. Although the precise function of Raly is unknown, the lethal yellow mutation suggests that it is important for early embryonic development.

Raly is constitutively expressed in all somatic cells. Therefore, fusion of the Raly promoter with the agouti gene results in ectopic overexpression of agouti (17). In short, the Raly promoter overrides the agouti gene’s own promoter in regulating its transcription and thus induces uninterrupted formation of yellow pigment by the hair follicle melanocytes. The embryonic lethality, hitherto associated with the A\(^v\)/A\(^v\) genotype, may actually result from the absence of Raly expression in the developing embryo.

Ectopic overexpression of the agouti gene induced by a heterologous promoter is also basic to the syndrome expressed by A\(^v\)/a mice. In this case, however, it is due to insertion of an intracisternal A particle (IAP) into noncoding exon 1A (reviewed in Ref. 48).

**MATERNAL EFFECTS ON METHYLATION OF IAP LONG TERMINAL REPEATS**

When methylated, the long terminal repeats (LTRs) of the IAP inserted in the noncoding region of agouti cease to induce agouti transcription (18). When such methylation occurs in progenitor cells of some A\(^v\)/a or A\(^v\)/a (IAP yellow)/a melanocyte clones, the agouti gene’s own promoter regulates its transcription in these clones so that phaeomelanin is synthesized only during formation of the subapical yellow band in the hair. This results in eumelanic islands (motting) on a yellow background.

Prenatal exposure to methyl-supplemented diets, maternally administered, increases such areas of eumelanin motting, most likely due to increased IAP LTR methylation (42). In the most extreme case, with or without dietary methyl supplementation, a lean mouse, called “pseudoagouti,” is produced with a coat color pattern that closely resembles that of the wild type.

Maternal factors strongly influence the genomic imprinting observed in the A\(^v\) mutants (reviewed in Ref. 42). The relatively low proportions of the pseudoagouti phenotype produced on most inbred and F1 hybrid backgrounds have made it economically impractical to elucidate the specific maternal factors involved in regulation of the genomic imprinting of the mutant agouti gene. An exception is strain AKR females mated with A\(^v\)/a bearing males, inasmuch as their A\(^v\)/a offspring segregate into approximately equal proportions of mottled yellow and pseudoagouti mice (Ref. 35; K. Gärtnert and G. L. Wolff, unpublished observations). Therefore, populations of mottled yellow, pseudoagouti, and black (AKR × C57BL/6-A\(^v\))/F1 hybrid mice would provide an economically justifiable experimental system for defining the maternal physiological and molecular factors involved in the regulation of genomic imprinting.

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FUNCTIONS OF AGOUTI PROTEIN

Agouti protein inhibits binding of α-melanocyte-stimulating hormone (α-MSH) to melanocortin receptor 1 (MC1-R) in hair follicle melanocytes. This prevents activation of adenyl cyclase and a subsequent increase in intracellular cAMP concentration (reviewed in Ref. 4). As a result, eumelanin synthesis is prevented, and the default synthesis of pheomelanin proceeds, resulting in completely yellow hairs.

Ectopic agouti protein also binds to another melanocortin receptor, MC4-R, and inhibits its function in the hypothalamus (reviewed in Ref. 4). Disruption of the gene, Mc4r, results in obese mice that, however, do not have a yellow coat color (12). These mice are slightly longer than the normal control mice, indicating an effect on somatic growth; however, whether tumor formation is also increased has not been determined yet.

Besides inhibiting binding of α-MSH to MC1-R and MC4-R, agouti protein also inhibits the activity of the promoter of microphthalmia-associated transcription factor (Mitf; chromosome 6) and “markedly reduces the expression of the protein” (1). Agouti protein antagonism of α-MSH binding to MC1-R and MC4-R is likely to have numerous downstream effects, including effects on gene transcription. However, it is also not unreasonable to assume that agouti protein may interact directly with additional, as yet unidentified, gene loci, especially when ectopically expressed.

The only direct effect of agouti protein that has been demonstrated so far is binding to melanocortin receptors. The phenotypic expression of the agouti protein is altered by a protein specified by a recessive mutant, mahogany (mg), at the Mahogany locus. Because less pheomelanin is synthesized, the coat color of mg/mg Avy/− mice is darker than that of Mg/Mg Avy/− mice. Although the specific mode of action of the mahogany protein, either wild-type or mutant, is unknown, it appears, at the very least, to modulate the binding of agouti protein to MC1-R and MC4-R and may even bind agouti protein directly (20).

Agouti protein also enhances the influx of Ca²⁺ into skeletal muscle cells and thus increases their intracellular Ca²⁺ concentration ([Ca²⁺]). The mode of action of the agouti protein in this case is unknown (47). Spider and snail venoms that attack Ca²⁺ channels (14) exhibit spatial homology with the agouti protein’s COOH-terminal region in number and spacing of cysteine residues. This homology is particularly striking because this region of the agouti protein has functional activity similar to the intact protein (30); therefore, agouti may act on a specific Ca²⁺ channel.

OBESITY AND HYPERINSULINEMIA

A current working hypothesis postulates that hyperphagia in yellow agouti mice is induced by the inhibition, by ectopic agouti protein, of MC4-R, which are normally regulated by the agouti gene-related protein (Agrp) (22) in the hypothalamus. In addition, it is postulated that excess lipid synthesis and deposition results from the effects of agouti protein on Ca²⁺ influx and [Ca²⁺], in adipocytes (reviewed in Ref. 49).

Yellow A¹⁰/a mice were more sensitive than black a/a siblings to the neurochemical effects of early postnatal monosodium glutamate (MSG) administration. Food intake was decreased, as were the hypothalamic contents of dopamine, pro-opiomelanocortin (POMC), and β-endorphin (3). In addition to decreasing POMC expression in the hypothalamus, it is likely that MSG treatment also decreases the expression of Mc3r and Agrp genes. Reduced expression of these genes in the arcuate nucleus may contribute to the delay in weight gain of the yellow agouti mice observed following neonatal MSG treatment.

Disruption, by adrenalectomy, of the ACTH feedback loop, which includes melanocortin receptors and POMC peptides, also slowed the weight gain of both yellow A¹⁰/a and black a/a mice, with the reduction being greater in the yellow agouti mice. Corticosterone replacement in adrenalectomized yellow agouti mice resulted in a dose-dependent increase in body weight, including the adipose component (28).

Potentially contributing to the tendency toward obesity, 21-day-old A¹⁰/A (BALB/c × VY) F₁ mice have significantly (P < 0.001) more pancreatic β-cells than their A/a siblings (30). If these extra β-cells are secreting insulin, this may be sufficient to initiate increased lipid deposition and weight gain in yellow agouti mice, as suggested by the findings of Mynatt et al. (19). These workers found that the expression of agouti in adipose tissue of transgenic FVB/N mice, combined with an elevated circulating insulin level induced by daily subcutaneous insulin injections, caused more weight gain in the transgenic mice than in wild-type FVB/N mice. Peripheral insulin resistance would follow and result in the persistent hyperinsulinemia characteristic of obese mottled yellow agouti mice.

EFFICIENCY OF FEED UTILIZATION

In a study of the effects of 30% caloric restriction on (BALB/c×CrlF344/Nctr×W/Wic3H/F1 hybrid mottled yellow A¹⁰/A and agouti A/a mice (41), the body weights of ad libitum-fed A¹⁰/A mice increased considerably more relative to their feed consumption than did those of the ad libitum-fed A/a mice. Ad libitum-fed yellow agouti mice consumed only ~8.7 g feed per gram body weight gain during an active growth period between 16 and 44 wk. In contrast, the ad libitum-fed agouti mice consumed ~30.5 g feed per gram body weight gain, i.e., the ratio among agouti mice was 3.5-fold greater than among the yellow agouti mice. These data confirm the earlier conclusion that the A¹⁰ genotype affects the efficiency of food utilization (9).

Previously, we reported that A¹⁰/A females gained ~14.0 g/kcal, whereas A/a females gained only ~3.5 g/kcal, a fourfold greater increase among yellow agouti mice than among agouti mice (9). This greater efficiency of calorie utilization by yellow agouti mice was abolished by caloric restriction, with a feed consumption-to-body weight gain ratio for both calorie-restricted A¹⁰/a and calorie-restricted A/a mice of ~28.

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Although the binding of ectopic agouti protein to MC4-R induces some hyperphagia, this does not appear to be the major cause of the obesity. In a study to determine possibly differential effects of the tumor promoter lindane (γ-hexachlorocyclohexane) on yellow and pseudoagouti A<sup>v</sup>/a mice (45), food consumption of the yellow agouti (VY × YS)F<sub>1</sub> hybrid female mice was only ~10% greater than that of the pseudoagouti and black siblings. For three different diets (control, high fat, and high sucrose) in a different study (9), the weekly food intakes of yellow A<sup>v</sup>/A (BALB/c × VY)F<sub>1</sub> mice were 32%, 16%, and 4% greater, respectively, than those of the A/a siblings. Corresponding body weight gains of the yellow agouti mice were 432%, 238%, and 142% greater than those of the agouti mice. The greater body weight gain in the yellow agouti mice fed the high-fat and high-sucrose diets (~27 g) rather than the control diet (~21 g) was due to increased efficiency of feed utilization, since the absolute caloric intake per mouse with these diets was actually decreased.

Results of the above studies bolster the conclusion that ectopic agouti protein “alters the response to changes in dietary composition and affects the efficiency of food utilization more than the total caloric intake” (9). No published data appear to be available on energy expenditure during rest or activity, on core body temperature in yellow agouti mice, or on relative activities in yellow agouti mice of uncoupling protein (UCP) genes, which specify uncoupling proteins that may be involved in modulating metabolic rates (27). Accordingly, identification of the cellular and physiological processes that determine the efficiency of nutrient utilization and are modulated, directly or indirectly, by the ectopic agouti protein requires considerable further work.

**IMMUNITY AND HOST RESISTANCE TO MALARIAL PARASITE INFECTION**

Obese mottled yellow A<sup>v</sup>/a (YS × VY)F<sub>1</sub> hybrid mice differ from their lean pseudoagouti A<sup>v</sup>/a and lean black a/a siblings in certain immune responses such as decreased antibody response to the T-dependent immunogen tetanus toxoid, enhanced antibody response to the T-independent immunogen type III pneumococcal polysaccharide, decreased rates of carbon clearance, and increased levels of IgA (26). The fact that the obese yellow agouti mice differ from both the lean pseudoagouti and lean black mice in these immune responses suggests that these alterations are probably associated, directly or indirectly, with the obese phenotype rather than with ectopic agouti protein per se; however, the possibility that ectopic agouti protein may directly affect immune responses cannot be excluded and requires further investigation.

Obese mottled yellow A<sup>v</sup>/a (YS × VY)F<sub>1</sub> hybrid mice also differ from their lean pseudoagouti A<sup>v</sup>/a and lean black a/a siblings in their response to infection with the murine malarial parasite *Plasmodium yoelii*. These differences include lower peak parasitemia and an altered survival pattern (24). Because erythrocytes are the targets of malaria, differences in hematological responses (31) may contribute to the atypical response to *P. yoelii* infection observed in yellow agouti obese mice (44). Notably, iron overload, induced by feeding AIN-76A diet fortified with 1,500, 3,500, 5,000, or 10,000 mg/kg carboxyl iron for 12 wk to mottled yellow A<sup>v</sup>/a and black a/a (C57BL/6NNctr × YS/WHiC3Hf/ Nctr-A<sup>v</sup>F<sub>1</sub>) hybrid male mice (31), significantly decreased (P < 0.001) the number of erythrocytes in the yellow agouti mice but not in the black mice (unpublished observations). Erythrocytes from the yellow agouti mice were also more fragile, exhibiting increased susceptibility to osmotic stress (P < 0.01) (44), which may be associated with hyperinsulinemia as postulated by Engström et al. (7) with respect to similar observations in obese Lep<sup>ob</sup>/Lep<sup>ob</sup> mice.

Altered resistance of yellow agouti mice to *P. yoelii* may also be related to decreased T cell competence. Infection of agouti A/a (BALB/c × VY)F<sub>1</sub> hybrid mice and their mottled yellow A<sup>v</sup>/A siblings with *P. yoelii* resulted in an increase in relative spleen-to-body weight ratio that was significantly greater in agouti mice compared with their yellow agouti siblings (P < 0.001). This is consistent with the report that splenomegaly is a T cell-dependent, protective response to *P. yoelii* infection (23) and the observation that yellow agouti mice have a decreased capacity (P < 0.005) to mount a T cell-dependent antibody response to tetanus toxoid (26).

Although leptin, agouti protein, and melanocortin receptors are all associated with immune function, their precise interrelationships require additional definition. Compared with their lean littermates, obese yellow agouti mice have increased leptin levels (14). Leptin differentially regulates the proliferation of naive and memory T cells, directly linking immune function with nutritional status (13). MC1-R, antagonized by agouti protein, is expressed on macrophages (29). Mahogany associates agouti protein with immune functions, since it is a homologue of attractin, produced by activated T cells (10), and also necessary for agouti antagonism of melanocortin receptors (20).

**DIFFERENTIAL MORTALITY**

Differential mortality, unrelated to treatment, was observed between the mottled yellow and pseudoagouti A<sup>v</sup>/a phenotypes between 17 and 24 mo of age (Table 1).

<table>
<thead>
<tr>
<th>Phenotype (Genotype)</th>
<th>Obese Yellow (A&lt;sup&gt;v&lt;/sup&gt;/a)</th>
<th>Lean Pseudoagouti (A&lt;sup&gt;v&lt;/sup&gt;/a)</th>
<th>Lean Black (a/a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>46/96 (48%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>22/96 (23%)</td>
<td>24/96 (25%)</td>
</tr>
<tr>
<td>Lindane fed</td>
<td>50/95 (52%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>25/95 (26%)</td>
<td>20/96 (21%)</td>
</tr>
<tr>
<td>Overall</td>
<td>96/192 (50%)</td>
<td>47/192 (24%)</td>
<td>44/192 (23%)</td>
</tr>
</tbody>
</table>

Mortality results are from previously unpublished data from study reported in Ref. 45. *P < 0.001 compared with pseudoagouti and black mice.

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in the lindane study (45). The mortality rate for yellow agouti mice was approximately double ($P < 0.001$) that of the pseudoagouti and black phenotypes. Whether this was a consequence of the obesity and its effects on the physiology of the animal or was due to more direct interference with homeostatic processes by the ectopic agouti protein is unknown. The body weight of yellow agouti mice normally begins to decline, for unknown reasons, toward that of their nonyellow siblings beginning around 18 mo of age; thus this decline in body weight seems to coincide with the increased mortality.

**STIMULATION OF SOMATIC GROWTH AND TUMORIGENESIS**

Probably the most significant and challenging “yellow agouti mouse” problem remaining to be addressed is the physiological/metabolic basis of the stimulatory effect on somatic growth and tumorogenesis associated with ectopic expression of the agouti protein. The lean body mass of $A^{vy}/a$ and $A^{a}/a$ mice has long been known to be somewhat greater than that of their non-$A^{vy}$ or non-$A^{a}$ littermates. Likewise, lean pseudoagouti $A^{vy}/a$ mice are slightly (−5%), but significantly ($P < 0.01$), heavier than their lean black $a/a$ siblings (37). This strongly suggests that even a low level of ectopic agouti protein affects one or more cellular processes involved in somatic growth.

It has been known, at least since the 1940s, that yellow agouti mice exhibit a higher frequency of tumor formation whether induced endogenously or exogenously (reviewed in Ref. 36). The most obvious factor to explain this growth effect has been the hyperinsulinemia associated with obesity. This possibility seemed to be supported by the observation that allogeneic ascites tumor cells formed larger subcutaneous solid tumors when implanted in yellow $A^{vy}/a$ and $A^{a}/a$ mice than in black $a/a$ mice (reviewed in Ref. 44).

Doubt regarding hyperinsulinemia as the primary promoter of tumorogenesis in yellow agouti mutant mice was generated by the appearance of benign lung tumors in lindane-treated lean pseudoagouti $A^{vy}/a$ and obese mottled yellow $A^{vy}/a$ (YS $\times$ YY)F₁, female mice with the same frequency, whereas no such tumors developed in the sibling black $a/a$ mice (45). Because the pseudoagouti mice are normoinsulinemic, hyperinsulinemia could not have played a role in the growth of these tumors.

That the somatic and neoplastic growth effects associated with ectopic agouti protein are probably related is suggested by the enhancement of hyperplastic mammary, bladder, and lung lesions in yellow agouti mice (reviewed in Ref. 44). Hyperplasia results in a larger population of cells at risk for neoplastic transformation and therefore, on a stochastic basis, would be the source of more tumors than the analogous normal cell population. Thus the earlier and greater mammary tumor prevalence among virgin yellow $A^{vy}/A$ (C3H/HeNlcrrWF $\times$ YY/Wf-A$^{vy}$)F₁ hybrid female mice was, most likely, a consequence of the greater number of hyperplastic alveolar nodules (HAN) that appeared earlier in yellow agouti females than in the agouti females. Thus, in yellow agouti mice, the pool of cells at risk was earlier and larger than that of the agouti mice.

Consistent with this tendency toward increased relative hyperplasia, the grade of bladder hyperplasia induced by feeding $N$-acetylaminofluorene in the diet was higher in yellow $A^{vy}/A$ (BALB/cStCr/HfC3Hf/Nctr $\times$ YY/WfC3Hf/Nctr-A$^{vy}$)F₁, hybrid mice at each dose level than in their agouti $A/a$ siblings (reviewed in Ref. 44). Similarly, after 6 mo of lindane feeding, pulmonary Clara cell hyperplasia was present in 77% of the yellow agouti (YS/ChWfC3Hf/Nctr $\times$ YY/WfC3Hf/Nctr-A$^{vy}$)F₁ hybrid mice but in only 50% of the pseudoagouti and 56% of the black mice (45). After 24 mo of treatment, the prevalence of this lesion had equalized among the three phenotypes. Here again, lesion formation occurred earlier and more rapidly among the yellow agouti mice.

Lindane feeding to another set of female yellow agouti and black mice was stopped at 6 mo of age to determine whether continuous treatment was necessary to maintain hyperplasia or tumorogenesis (45). At 13 mo of age, the prevalence of Clara cell hyperplasia among these yellow agouti mice had regressed by only 18% in comparison with the continuously fed mice; in contrast, among black mice the prevalence had decreased by 48%. Apparently, the presence of ectopic agouti protein was conducive to maintenance of Clara cell hyperplasia.

Formation of benign lung tumors was promoted by lindane treatment in yellow and pseudoagouti females but not in black females. Among yellow agouti females, tumor prevalence was 15%, among pseudoagouti mice it was 7%, but among black mice it was only 1% above the respective background prevalences. Among the yellow agouti mice fed the lindane-supplemented diet to 25 mo of age, terminal lung tumor prevalence was 19% compared with 10% among the mice fed the diet to only 6 mo of age but held to 25 mo of age.

**HEPATOCellular ADENOMAS**

The incidence (12%) of hepatocellular adenomas in lean normoinsulinemic pseudoagouti $A^{a}/a$ mice fed lindane for 24 mo was greater ($P = 0.03$) than that observed in normoinsulinemic black $a/a$ mice (3%) but less ($P = 0.0001$) than the incidence (35%) in hyperinsulinemic yellow agouti mice. Presumably, agouti protein is synthesized in fewer hepatocytes in pseudoagouti mice because of increased IAP LTR methylation in the hepatocyte population compared with mottled yellow $A^{vy}/a$ mice. If the expression of agouti protein in hepatocytes is a factor in hepatic tumorogenesis, this could account for the lower frequency of hepatocellular adenomas in this phenotype compared with the mottled yellow phenotype. These observations imply that the ectopic expression of agouti protein per se in at least some hepatocytes in the pseudoagouti mice may either facilitate initiation or itself exert an initiating effect whereby some cells are transformed and subsequently promoted by the lindane (45).

The carcinoma incidence among the yellow agouti mice fed the control diet was 13% (12/93) and in the
lindane-fed group it was 17% (16/94); in contrast, carcinoma prevalence among pseudoagouti mice was only 2% in the control group and 5% in the treated group, whereas among the black mice it was only 3% in the control group and 1% in the treated group. Thus the progression of some adenomas to carcinomas was facilitated or stimulated by some endogenous factor in the yellow agouti mice that was not present in either the pseudoagouti or black mice. Hyperinsulinemia and DNA fragility are two possible candidates. This suggestion is supported by the finding of E. J. Michaud and A. Kuklin (personal communication) in which, following initiation with diethylnitrosamine, transgenic FVB/N mice bearing an expression vector, containing the agouti gene, targeted to hepatocytes develop significantly more hepatocellular tumors than nontransgenic littermates.

**LIVER WEIGHT**

An increase in liver weight ($P < 0.05$) in response to lindane, detectable after 6 mo of treatment in the yellow and pseudoagouti $A^{v}/a$ mice, was another indication of the presence of ectopic agouti protein in the livers of pseudoagouti mice, since such an increase was not detected in black $a/a$ mice until after 24 mo of treatment (45). As shown in Table 2, liver weights of both phenotypes increased in response to lindane treatment; however, the increase was greater among the livers from yellow agouti mice than among those from pseudoagouti mice. This suggests that the difference in response may have been due to the lower concentration of ectopic agouti protein in the livers and/or the lower insulin levels in the pseudoagouti mice.

**HEPATIC BENZO(a)PYRENE MONOOXYGENASE ACTIVITY**

After 12 mo of lindane treatment, hepatic benzo(a)pyrene monooxygenase activity exhibited significant differences between treated and control pseudoagouti as well as black mice; however, such differences were not observed between treated and control obese yellow agouti mice. Treated pseudoagouti and black mice exhibited greater enzyme activity than their respective controls. In contrast, treated and control yellow agouti mice had lower enzyme activities than either treated pseudoagouti or black mice (45).

<table>
<thead>
<tr>
<th>Treatment Duration, mo</th>
<th>Proportional Increase in Liver Weight, %</th>
<th>Yellow mice</th>
<th>Pseudoagouti mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td></td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>31</td>
<td>17</td>
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</table>

Data were calculated from Ref. 45.

**TUMOR LATENCY**

The first sarcomas induced by the subcutaneous implantation of plastic films were detected at 16 mo postimplantation in mottled yellow $A^{v}/a$ $YS/ChWffC3Hf/Nctr-A^{v}$ mice compared with 22 mo postimplantation in their black $a/a$ siblings. Among $A^{v}/a$ $VY/WffC3Hf/Nctr-A^{v}$ mice, however, the first sarcomas were already detected at 11 mo postimplantation compared with 14 mo postimplantation in the black $a/a$ $VY/Wf$ mice (44). Thus, although the tumors appeared 21–27% earlier in yellow agouti mice than in their black siblings regardless of strain, the background genome determined the basic length of time required from implantation to tumor detection. This is a vivid example of the importance of the interaction of a mutant gene with different background genomes in determination of a physiological endpoint.

Another example of the shorter latency/more rapid growth of tumors in yellow agouti mice are mammary carcinomas, induced by 7,12-dimethylbenz(a)anthracene (DMBA) in yellow $A^{v}/A$ (BALB/c $x$ $VV$) $F_1$ hybrid female mice. These appeared about 28% earlier and with a 29% greater frequency compared with their agouti $A/a$ sisters (reviewed in Ref. 44).

**DNA STRAND BREAKAGE**

A clue to at least one aspect of the basis for the liver tumorigenesis effect may be provided by a recent observation on hepatocytes in a chronic iron overload study. Frequencies of DNA strand breaks in hepatocytes from mottled yellow $A^{v}/a$, pseudoagouti $A^{v}/a$, and black $a/a$ mice (C57BL/6N $x$ $YS/WffC3Hf/Nctr-A^{v}$) $F_1$ hybrid mice were determined by a previously described method (23) (B. Miller and S. J. James, personal communication). The mice had been fed iron-free AIN-93M diet fortified with 35 mg/kg, 1,500 mg/kg, 3,500 mg/kg, or 5,000 mg/kg carbonyl iron for 12 mo. Iron overload had no effect on either DNA strand breakage or hepatocellular tumor incidence at any dose level in any of the mouse phenotypes. However, obese yellow $A^{v}/a$ mice exhibited a significantly higher ($P < 0.01$, adjusted for multiple comparisons) frequency of DNA strand breakage [3,392 ± 74 strand breaks ($n = 11$)] than lean pseudoagouti $A^{v}/a$ mice [2,911 ± 104 strand breaks ($n = 6$)] or black $a/a$ mice [2,790 ± 74 strand breaks ($n = 12$)].

These data suggest that ectopic agouti protein increases chromosomal fragility, at least in hepatocytes, although it is difficult to imagine the mechanism involved. However, if confirmed, such fragility might constitute a mutational event facilitating initiation of neoplastic transformation and thus could contribute to the increased liver tumorigenesis characteristic of yellow $A^{v}/a$ mice.

The association of agouti protein with increased DNA strand breakage may not be limited to hepatocytes. Fibroblast cell lines from yellow $A^{v}/a$ mice exhibited a significant degree of spontaneous transformation (11). In contrast, no fibroblast cell lines established from black $a/a$ mice exhibited spontaneous transformation.
When the transformed cell lines were analyzed for a restriction fragment length variant (RFLV), which is informative for A\textsuperscript{\textalpha}-bearing and \textalpha-bearing chromosomes, most of the transformed cells had lost the \textalpha-associated RFLV, and one transformant exhibited amplification of the A\textsuperscript{\textalpha}-associated RFLV. The increase in gene dosage of the A\textsuperscript{\textalpha} allele and loss of the \textalpha allele may have involved increased chromosomal fragility initiated by agouti protein.

GROWTH HORMONE, INSULIN, AND INSULIN-LIKE GROWTH FACTOR I

Because yellow agouti mice exhibit increased somatic growth, it seems paradoxical that growth hormone levels in yellow A\textsuperscript{\textalpha}/a mice “were low throughout most of the day with little indication of a diurnal rhythm” (16). In contrast, the black a/a siblings showed a large number of diurnal fluctuations in growth hormone concentration.

Insulin levels increased with age in yellow agouti mice but not in black mice, whereas growth hormone levels decreased in both genotypes. Thus the insulin-to-growth hormone ratio increased ~3.5 times as fast in yellow agouti mice compared with that in black mice between 30 and 90 days of age.

A marked biphasic increase in serum insulin-like growth factor I (IGF-I) levels in both black a/a and yellow A\textsuperscript{\textalpha}/a VY/WHC3HF/Nctr-A\textsuperscript{\textalpha} mice of both sexes has been observed (41). The first increase, between 2–3 wk and 3–4 wk of age, was about twice as great in the yellow agouti mice (312% in females, 322% in males) compared with that in the black mice (167% in females, 154% in males). Between 3–4 wk and 4–5 wk of age, serum IGF-I concentration decreased 33% among the yellow agouti females and 42% among the black females. The second increase in IGF-I levels occurred between 4–5 and 6–7 wk of age, namely 121% among the yellow agouti females and 288% among the black females. The serum IGF-I concentrations then remained similar in both genotypes to at least 12–13 wk of age.

The first phase of the increase was twice as great among the yellow agouti mice compared with that in the black mice. Conversely, the second increase was greater among the black than among the yellow agouti mice. Thus the total increase in circulating IGF-I between the ages of 2–3 and 6–7 wk, 553% among yellow agouti females and 504% among the black females, was similar in both genotypes. However, the first, presumptively mitogenic, stimulus was stronger and occurred about 2 wk earlier in yellow agouti mice than in black mice.

Transiently higher circulating IGF-I levels in yellow agouti mice may alter their normal metabolic programming and contribute to the greater lean body mass observed in A\textsuperscript{\textalpha}/– mice; it may also be a factor in the greater metabolic efficiency observed in yellow agouti mice. This difference in timing of a major increase in serum IGF-I concentration between yellow A\textsuperscript{\textalpha}/a and black a/a strain VY mice reflects altered developmentally induced, directly or indirectly, by ectopic agouti protein with implied effects on growth.

The background strain genome plays a major role in determining the response of plasma insulin and glucose concentrations to a glucose load. This is indicated by the markedly different responses to glucose tolerance tests of lean black a/a mice of the inbred YS and VY strains (Fig. 1). These data suggest that the YS mice have an inherent genome-associated insulin resistance on which, in the obese yellow A\textsuperscript{\textalpha}/a YS mice, the insulin...
resistance associated with their obesity is superimposed.

CASTRATION: RESPONSE OF CIRCULATING PLASMA CORTICOSTERONE

Castration of VY/Wf males increased plasma corticosterone concentration 90% in black \(a/a\) males but only 47% in yellow \(A^c/a\) males; however, no differences between the genotypes were observed in sham-operated controls (39). In contrast, among sham-operated YS/ChWf mice, black \(a/a\) males had significantly higher plasma corticosterone levels than did the yellow \(A^c/a\) males. Castration of YS/ChWf males increased corticosterone levels ~190% in black \(a/a\) males and ~280% in yellow \(A^c/a\) males (Table 3).

The effect of castration on circulating corticosterone levels was about twice as great among black YS/ChWf mice as among black VY/Wf mice. Among yellow \(A^c/a\) YS/ChWf mice, however, this effect was almost six times greater than in yellow \(A^c/a\) VY/Wf mice.

These observations suggest markedly differential effects of the strain genomes, as well as a direct or indirect effect of ectopic agouti protein, on hormonal balance and on the hormonal response to castration; however, the mechanisms involved are unknown. Their identification and definition would provide valuable new insights into the homeostatic regulation of hormonal balance and response to stress.

CASTRATION: RESPONSE OF LEAN BODY AND LIVER MASS AND HEPATOTUMORIGENESIS

Castration at 28 days of age resulted in decreased fat-free dry body weights in 16 wk-old black \(a/a\) YS/ChWf males compared with sham-operated controls but had no effect on lean body mass in similarly aged yellow \(A^c/a\) males. Conversely, fat-free dry liver weight in yellow agouti males was increased by castration but not in black males (34).

Castration increased the mean number of hepatocellular tumors in yellow \(A^c/A\) (C3H × YBR)F1 hybrid males by 120%; however, there was no effect on tumor multiplicity in the sibling agouti \(A/a\) mice (34).

<table>
<thead>
<tr>
<th>Table 3. Plasma corticosterone concentration at ~3 mo of age in sham-operated and castrated YS/ChWf and VY/Wf male mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma Corticosterone Concentration, (\mu g/100) ml</strong></td>
</tr>
<tr>
<td><strong>Strain YS/ChWf</strong></td>
</tr>
<tr>
<td>Yellow (A^c/a)</td>
</tr>
<tr>
<td>Black (a/a)</td>
</tr>
<tr>
<td><strong>Strain VY/Wf</strong></td>
</tr>
<tr>
<td>Mottled yellow (A^c/a)</td>
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<tr>
<td>Black (a/a)</td>
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Values are means \(\pm SE\); \(n = no.\) of assays, each on a pooled sample of 3–5 mice. Data are from Ref. 39.

VARIATION IN RESPONSE TO TOXICANTS

Studies to elucidate the mechanistic bases of the main phenotypic effects of ectopic agouti protein and the relations among them have been reviewed (36, 44, 48). The data emphasize that single gene mutations can result in a multiplicity of altered responses to environmental stimuli. There are also marked phenotypic differences within these responses based on unexplained variations in gene expression among genetically identical individuals (38).

This important aspect of physiological genomics, namely the molecular basis for the considerable variations in degree of responsiveness to low levels of toxicants among genetically homogeneous individuals, has not been addressed experimentally so far. Two examples of this phenomenon observed in yellow \(A^c/A\) F1 hybrid male mice are summarized here. Although these data probably do not reflect differences directly attributable to the function of the agouti protein, they are analogous to the phenotypic variability of the viable yellow mice, which is induced by variable methylation of the IAP LTR inserted in the agouti locus. These data illustrate the variability in the expression of many, if not most, genes even among same sex, same age, and genetically homogeneous, if not identical, individuals. They emphasize that identical genomes can be, and are, expressed in a spectrum of physiological phenotypes, which reflect the complex interdependence of physiological pathways and processes in the organism.

SODIUM PHENOBARBITAL

Beginning ~5 mo after being placed on NIH-31 diet containing 0.05% sodium phenobarbital (PB), male yellow \(A^c/A\) and agouti \(A/a\) (C3H- MTV -HeN × VY/WfC3Hf/ Nctr-A\(^c\)V)F1 hybrid mice bearing multiple treatment-associated hepatocellular adenomas had a higher rate of body weight gain than those that had only one tumor or had failed to develop any tumors (reviewed in Ref. 38).

Did these subgroups, which differed in the response to PB with respect to tumorigenesis and body weight gain, also differ in the inducibility of PB-dependent enzymes by PB? Differential inducibility would reflect differential transcription of the genes coding for the enzymes and would suggest that, although the DNA coding sequence of a gene might be identical in all individuals, its transcription might be differentially modulated in different individuals. To test this hypothesis, male yellow \(A^c/A\) mice of the same F1 hybrid were fed the same PB-containing diet for 7 mo beginning at 7–8 wk of age. At the end of that period, a battery of phase I and phase II isozyme activities was assayed in the livers of the heaviest [control 51.0 \(\pm 0.7\) g \((n = 5)\), treated 56.8 \(\pm 0.8\) g \((n = 7)\)] and lightest [control 43.2 \(\pm 0.6\) g \((n = 6)\), treated 43.0 \(\pm 1.3\) g \((6)\)] mice (reviewed in Ref. 38).

Differences in responsiveness to PB between the heavier and lighter mice were demonstrated by PB-inducible \(P-450\,11B\)-selective 7-pentoxyresorufin-O-dealkylase activity. A sixfold increase in the treated “heavy” mice compared with the control heavy mice was...
observed. In contrast, in the “light” mice there was only a threefold increase in activity. In comparison, the non-PB-inducible P-450I A-selective 7-ethoxyresorufin-
O-deethylase activity was increased only 70% and 84%, respectively, in the treated heavy and light mice.

Other constitutive isozyme activities, not P-450IIB-dependent, likewise differed between the heavy and light mice, e.g., testosterone-6b-hydroxylase (P-450IIIA) and 7-ethoxyresorufin-O-deethylase (P-450I A).

Increased responsive ness to PB induction of glutathi-
one-S-transferase isozymes of the N1:1 gene family was also associated with greater body and carcass (body minus liver) weights, as was repression of cytochrome P-450IIE isozymes. In contrast, associated with decreased susceptibility to PB promotion of body and carcass weight was decreased constitutive expression but increased PB inducibility of cytochrome P-450 isozymes of the IIIA gene family and of 17-hydroxyster-
odid UDP-glucuronyltransferase. Thus the patterns of constituent levels of isozyme activities as well as the patterns of their inducibilities differed between the light and heavy phenotypic subgroups, even though the mice were genetically homogeneous.

Retrospectively, we found that those mice that were heavy and those that were light at the end of the study could have been identified by body weight at least as early as 3–4 wk of age. Therefore, it seems likely that the potential for increased body weight gain and responsiveness to PB was acquired some time during their development before weaning, i.e., before exposure to the toxicant. The reader may wish to refer to the earlier discussion of serum IGF-1 (see GROWTH HORMONE, INSULIN, AND INSULIN-LIKE GROWTH FACTOR) in regard to possible differences in metabolic programming during development.

STREPTOZOTOCIN

Physiological responses other than tumor formation, e.g., diabetogenic response to streptozotocin, are also quite variable among genetically identical individuals. When 150 mg/kg or 200 mg/kg streptozotocin was admin-
istered intraperitoneally to 4-wk-old yellow A/a and agouti A/a (BALB/cStCrlIC3Hf/Nctr × VY/WflC3Hf/
Nctr-A(v))F1, hybrid female mice, there were low and high responders within both genotypes during the subsequent 22-wk observation period (40). In the high-
dose groups, 80% of the yellow agouti mice but only 55% of the agouti mice became grossly hyperglycemic as well as hypoinsulinemic and gained almost no body weight. At the low dose, 25% of the A/v/A mice exhib-
ited decreased insulin and elevated glucose levels as well as reduced body weight gain; in contrast, however, no A/a mice treated with the low dose (150 mg/kg) of streptozotocin showed such diabetic responses.

DISCUSSION

The spectrum of phenotypic effects associated with ectopic agouti protein reflects not only the responses of diverse cell types but also the downstream effects of agouti protein interactions with melanocortin receptors and other polypeptides. Patterns of gene expression specifying particular processes and pathways will differ developmentally and temporally not only among different cell and tissue types but also among individual cells of the same type.

Thus the interaction of ectopic agouti protein with different gene products in diverse cellular processes and signaling pathways in different cells and tissues can result in a multiplicity of major and minor phenotypic effects.

Many, if not most, of the differences between the obese mottled yellow and lean pseudoagouti A(v)/a phenotypes undoubtedly result from the physiological alterations associated with hyperinsulinemia and obesity. However, some of the physiological differences between obese yellow agouti and lean pseudoagouti A(v)/a mice, such as their differential responses to lindane treatment, may be due to the ubiquitous pres-
ence of agouti protein in the tissues and cells of the obese yellow agouti mice compared with its limited presence, due to widespread methylation of IAP LTRs in cell clones, in lean pseudoagouti mice. In some cases, agouti protein in ectopic sites may modulate, directly or indirectly, homeostatic cellular processes affecting, for example, DNA fragility.

Background genome. Four diverse examples illustrate the importance of the background genome to the physiological expression of the mutant A(v)/– phenotype, namely, 1) circulating plasma corticosterone level and its response to castration, 2) effect of maternal methyl-supplemented diet on the degree of eumelanic mottling induced in the A(v)/a offspring, 3) response to a glucose load, and 4) tumor latency and prevalence.

In the VY/WflC3Hf/Nctr (VY) strain, the circulating corticosterone levels were similar in the A(v)/a and a/a sham-operated males; in contrast, strain YS/ChWflC3Hf/Nctr-A(v) (YS) A(v)/a males had lower hormone levels than their a/a siblings. Castration in-
creased hormone levels only half as much (47%) in yellow agouti VY mice compared with that in black siblings (90%); in castrated YS males, however, the corticosterone level increased 280% in yellow agouti mice but only 190% in black males. Thus the regulation of constituent hormone levels in the YS mice was influenced by an interaction of the ectopic agouti pro-
tein and unknown component(s) of the strain genome. In addition, components of both strain genomes inter-
acted, directly or indirectly, with the ectopic agouti protein in regulating the response of the hormone level to castration in both mouse genotypes.

On the maternal NIH-31 control diet, 66% of the A(v)/a strain YS offspring exhibited high “eumelanic mottling” (EM) compared with only 43% of A(v)/a strain VY offspring. The methyl-supplemented diet had no effect on the proportion of high EM among A(v)/a strain YS offspring but increased the proportion of high EM to 60% of the A(v)/a strain VY offspring.

The markedly different responses of the lean black a/a mice of the inbred YS and VY strains to glucose tolerance tests suggest that the YS mice have an inherent genome-associated insulin resistance on which,
in the obese yellow A\textsuperscript{\textit{\textalpha}}/\textit{\textalpha} mice, the insulin resistance associated with their obesity is superimposed.

YS mice, both yellow agouti and black, tend to be somewhat lighter in body and liver weight and tend to have a lower incidence of hepatocellular tumors than comparable VY mice (43). The latent period for detection of foreign body-induced subcutaneous sarcomas was longer in YS mice than in VY mice (YS-\textit{\textalpha}/\textit{\textalpha} = 22 mo, YS-A\textsuperscript{\textit{\textalpha}}/\textit{\textalpha} = 16 mo; VY-\textit{\textalpha}/\textit{\textalpha} = 14 mo, VY-A\textsuperscript{\textit{\textalpha}}/\textit{\textalpha} = 11 mo) (44).

These examples of divergent effects of different strain genomes on the physiological effects of ectopic agouti protein highlight not only the desirability of using a genetically uniform strain background, i.e., inbred or \textit{F}_1 hybrid, for physiological genomic studies, but also the necessity of incorporating the influence of the background genome as a variable in data interpretation.

\textbf{Downstream consequences.} Many of the phenotypic effects associated with ectopic agouti protein are indirect, i.e., downstream, effects of the binding of the protein to melanocortin receptors.

The abnormal response to infection with the malarial parasite \textit{P. yoelii} of obese yellow A\textsuperscript{\textit{\textalpha}}/\textit{\textalpha} mice combined with their increased erythrocyte fragility illustrate the complexity of downstream effects of a single mutation. Engström et al. (7) observed decreased erythrocyte resistance to osmotic stress, as well as enhanced erythropoiesis, in obese Lep\textsuperscript{\textit{\textalpha}}/Lep\textsuperscript{\textit{\textalpha}} mice and suggested the hyperinsulinemia characteristic of these mice as the inducer of these symptoms. They postulated that elevated insulin levels directly stimulate erythroid colony-forming cells via their IGF receptors. In view of the similarity of our observations on obese yellow A\textsuperscript{\textit{\textalpha}}/\textit{\textalpha} mice and those on obese Lep\textsuperscript{\textit{\textalpha}}/Lep\textsuperscript{\textit{\textalpha}} mice, it seems likely that similar mechanisms, associated with hyperinsulinemia/obesity, are involved in both obese mouse models.

In turn, this downstream physiological effect of mutant gene expression also appears to alter host resistance to \textit{P. yoelii} and may affect the outcome of other parasitic infections in which a stage of the parasite lifecycle infects erythrocytes.

\textbf{Growth effects.} The time differentials between A\textsuperscript{\textit{\textalpha}}/\textit{\textalpha} and non-A\textsuperscript{\textit{\textalpha}}/\textit{\textalpha} mice with respect to the appearance of mammary tumors following DMBA treatment, and of sarcomas following subcutaneous plastic film implantation, suggest that, regardless of background genome, tumor formation in yellow agouti mice is 21–28% faster than in their non-yellow agouti littermates.

The increased somatic growth exhibited by yellow agouti mice appears not to be due to growth hormone. In contrast to black a/a mice, mottled yellow A\textsuperscript{\textit{\textalpha}}/\textit{\textalpha} mice exhibit low growth hormone levels throughout most of the day with little indication of pulsatility or diurnal rhythm (16). In this respect, the A\textsuperscript{\textit{\textalpha}}/\textit{\textalpha} mice are no different from other obese rodents or humans (8). The physiological relevance of suppressed growth hormone secretion in obesity is unknown; however, because growth hormone decreases lipogenesis and stimulates lipolysis, decreased growth hormone secretion may play a role in the maintenance of an obese phenotype.

When total serum IGF-I levels were assayed in yellow A\textsuperscript{\textit{\textalpha}}/\textit{\textalpha} and black a/a mice from 2 wk to 3 mo of age, yellow agouti mice had higher total IGF-I levels than black mice at 3–4 wk, but at later time points there were no significant differences.

The IGF-I levels measured at this time probably largely reflected alterations in IGF-I binding proteins, which, like IGF-I, are under the regulation of growth hormone. It is conceivable, therefore, that yellow agouti mice have a transiently higher circulating growth hormone concentration than black mice at 3–4 wk of age and that this alters their metabolic programming relating to lean body mass and obesity (41). Interestingly, inactivation of inducible growth hormone transgenes in mice, following periods of elevated growth hormone levels, was recently shown to lead to obesity (21).

Our observations suggest the following working hypothesis. Ectopic agouti protein can exert a mitogenic effect that results in hyperplasia at least in bladder, mammary gland, and lung, depending on the background genome. The increased pool of cells thus at risk, e.g., HAN in mammary glands, together with increased DNA fragility, at least in hepatocytes, results in increased frequency of tumorigenesis. Whether ectopic agouti protein actually induces an initiating effect in individual hyperplastically transformed cells may depend not only on the specific tissue and cell type but also on the specific pattern of gene expression characteristic of the particular cell (38). Hyperinsulinemia, which accompanies the obesity among yellow agouti mice, then may act as a promoting agent via increased binding of insulin to the IGF-I receptors and thus speed proliferation of the transformed cells to form tumors.

\textbf{Pseudoagouti A\textsuperscript{\textit{\textalpha}}/a mice.} Physiologically, lean pseudoagouti A\textsuperscript{\textit{\textalpha}}/a mice resemble their black a/a siblings quite closely but differ significantly from their obese mottled yellow A\textsuperscript{\textit{\textalpha}}/a siblings. However, they also exhibit some marked differences from their black a/a siblings (37), which, presumably, reflect low levels of expression of the ectopic agouti gene. For example, pseudoagouti mice are ~5% heavier than their black a/a siblings (\textit{P} < 0.01), suggesting that a low level of ectopic agouti protein affects somatic growth even in the absence of hyperinsulinemia.

Observed differences between black a/a and pseudoagouti A\textsuperscript{\textit{\textalpha}}/a mice, such as the 12% incidence of hepatocellular adenomas in pseudoagouti mice fed lindane for 24 mo compared with the 3% incidence observed in similarly fed black a/a mice (\textit{P} = 0.03), might result from expression of agouti protein in fewer hepatocyte clones in pseudoagouti A\textsuperscript{\textit{\textalpha}}/a mice than in mottled yellow A\textsuperscript{\textit{\textalpha}}/a mice due to methylation of the IAP LTRs in most pseudoagouti hepatocytes. Resolution of the cellular and molecular basis for this phenotypic difference should provide valuable clues for the physiological genomic aspects of agouti protein. In the case of lung tumors observed in mottled yellow and pseudoagouti A\textsuperscript{\textit{\textalpha}}/a mice but not in the black a/a littermates (45),
ectopic agouti protein may have exerted an initiating effect on Clara cells, which were then promoted to form tumors by lindane exposure.

There is a possible additional factor that may be involved in the physiological differences between the obese mottled yellow and lean pseudoagouti phenotypes, namely differential methylation of genic sites other than just the IAP LTR inserted in the agouti locus. The relative extent of methylation of 5′-CG-3′ di-nucleotide (CpG) islands at loci other than the agouti locus in these two mouse phenotypes is undetermined as yet. However, it seems unlikely that this differential methylation would be limited to a single IAP inserted in a single locus, whatever the mechanism modulating methylation may be, whether maternal imprinting or other as yet unknown factor. Therefore, assessment of the relative states of global methylation in both phenotypes would be a major contribution to reaching an understanding of the many physiological differences between them.

The lower frequency of hepatocellular adenomas among pseudoagouti $A^{vy}/a$ compared with yellow $A^{vy}/a$ mice may reflect the absence of hyperinsulinemia. In pseudoagouti mice, only one tumor-promoting agent was active, namely lindane, whereas in yellow agouti mice two tumor-promoting agents were active, namely hyperinsulinemia and lindane.

CONCLUSIONS

The diversity of phenotypic effects found in physiological genomic studies of the $A^{vy}$ and $A'$ mouse mutants probably reflects the response of diverse cell types in different tissues to ectopic agouti protein. In addition, it may reflect downstream effects of the binding, or other modification, of melanocortin receptors and/or modulation of $[\text{Ca}^{2+}]$ by agouti protein. Such downstream and indirect effects of mutant genes are expected but may be overtly expressed only in response to environmental stimuli. Therefore, the importance of comparing physiological responses between mutant and nonmutant siblings with identical genomic background cannot be overemphasized. However, even with identical genomic backgrounds and treatment regimens, it is noteworthy that some responses, e.g., development of hyperplastic and neoplastic lesions, do not occur in all members of the genetically homogeneous and similar treated mouse populations. This phenomenon may reflect differentially modulated gene transcription among genetically identical individuals (38).

Clearly, the understanding of the basis of the yellow agouti obese mouse syndrome has been greatly increased as a result of the cloning of the agouti and melanocortin receptor genes. Nevertheless, recent work indicates that the effects of the proteins specified by these genes on the physiology and metabolism of the mouse are not only widespread but also very complex.

A major problem to be solved is whether the effect of the agouti protein on $\text{Ca}^{2+}$ channels and agouti protein binding to melanocortin receptors are distinct and separate or interrelated.

Some puzzling relationships among components of the yellow agouti obese mouse syndrome remain to be resolved by future research. For example, are the high efficiency of nutrient utilization and the increased somatic growth of yellow agouti obese mice directly related? Is increased tumorigenesis part of the syndrome associated withMC4-R-deficient mice as is increased body length? The anomalous effects of castration on corticosterone, somatic growth, and hepatocarcinogenesis in yellow agouti mice also remain unexplained.

Another aspect of the yellow agouti obese mouse syndrome, which has remained almost completely unexplored, is the role of the proteins specified by the agouti and melanocortin receptor genes in embryonic and postnatal development. The considerable prenatal variability in phenotypic expression of the $agouti$ gene, which precedes and determines adult development of the yellow agouti obese mouse syndrome, suggests that these genes play important roles in regulating and directing prenatal and postnatal physiological development. Studies to unravel the interrelationships involved should provide valuable insights into the basis of physiological changes during development, as well as on the effects of gene-specified proteins on these changes. Such studies also should be designed to provide information on the specific nature of maternal influences on prenatal variability and physiological development. This is quite practical because of the known maternal effect on the frequency of production of lean pseudoagouti $A^{vy}/a$ offspring.

Health and physiology are the integrated end results of multifactorial interactions among the transcription products of the genes comprising the functional mammalian genome. Patterns of gene expression that specify and regulate cellular processes in each cell and tissue can now be defined; however, the developmental, temporarily changing, and regional aspects of gene expression and regulation remain elusive. Identification of the genes involved and resolution of the complex interactions of their products will be greatly aided by recent developments, including high throughput methods utilizing DNA arrays and automated miniature assays to quantify gene products. However, these methodologies have limitations associated with sampling, such as the inability of DNA from peripheral blood to adequately represent expression patterns that show critical temporal and regional (organ) differences and the difficulty of interpreting functional measures taken at a single time point during the life cycle.

The ontogeny of each individual organism, whether mouse or human, and the development of functional metabolic, immunologic, and cognitive systems involve complex interactions, directed and sequential cell migration, and subsequent organization of diverse cell types throughout extended, but temporally ordered, periods of fetal and neonatal development and aging. These considerations dictate the need for coordinated, multidisciplinary efforts to assess adverse health effects, i.e., physiological dysregulations in humans in the context of physiological genomics. Inclusion of the concepts of physiological genomics in graduate education is a sine qua non for such endeavors.
References


