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Commentary

Mechanisms for the pleiotropic effects of the agouti gene

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The agouti locus is one of several classic genetic loci that affect the coat color of mice. The wild-type agouti phenotype, exemplified by dorsal coat hairs that are black with a single subapical band of yellow and lighter-colored ventral hairs, is found in many mammals including animals of the genera *Mus* (mice), *Oryctolagus* (rabbits), *Sciurus* (squirrels), and *Canis* (wolves) (reviewed in ref. 1). Much interest has focused on the agouti gene because several dominant mutations result in mice that exhibit adult onset obesity, hyperinsulinemia, and noninsulin-dependent diabetes. In addition, mice carrying dominant agouti mutations show an increased susceptibility to a variety of spontaneous and/or induced solid tumors. These mutations provide potential animal models of human disease, and the mouse provides an organism amenable to molecular genetic, biochemical, physiological, and pharmacological manipulations to investigate the causes and treatments of these diseases. The mechanism of action of the agouti gene in all of these phenotypes is under intense investigation. Several recent papers including two in this issue by Klebig *et al.* (2) and Zemel *et al.* (3) now shed light on some modes of action of the agouti protein as well as provide models for *in vivo* and *in vitro* tests of agouti function.

An Introduction to the Agouti Gene and Its Effects on Coat Color in Mice

The murine agouti protein is composed of 131 amino acids, with a consensus signal peptide, a highly basic central region, and a cysteine-rich carboxyl terminus (4, 5) (Fig. 1*A*). The agouti gene has three coding exons that are alternatively spliced to different 5′ untranslated exons, resulting in four types of mature 0.7- to 0.8-kb transcripts with identical coding regions. Two different isoforms are transiently expressed during hair growth in dorsal and ventral skin; the two hair-cycle-specific exons reside 18 kb upstream of the first coding exon. The remaining two isoforms are expressed specifically in ventral skin; the two ventrum-specific exons reside ≈120 kb upstream of the first coding exon (4, 5, 12, 13). The wild-type light-bellied agouti (A^w) phenotype results from a pulse of agouti expression during the hair-growth cycle in the dorsum (resulting in agouti-colored coat hairs) and continuous expression during the hair-growth cycle in the ventrum (resulting in yellow to white coat hairs). Additional 1.0- to 7.5-kb transcripts were detected in testis, but their significance is unclear (4, 5).

The agouti (*a*) and extension (*e*) loci in the mouse are responsible for the temporal and spatial distribution of phaeomelanin (yellow to red pigment) and eumelanin (black to brown pigment) granules within each hair shaft (reviewed in ref. 14). The melanocyte-stimulating hormone receptor [Mc1r (for melanocortin 1 receptor); formerly called MSHR] encoded by the extension locus is expressed by neural crest-derived melanocytes that reside near the base of the hair follicle. Melanocytes produce pigment and deposit it through their dendritic processes into the growing hair shaft. The agouti protein is expressed by several cell types in the hair follicle but not by melanocytes. The agouti protein is a paracrine signaling factor (since it is expressed by one cell type but acts on a neighboring cell type) that dictates melanocyte pigment production within individual hair follicles (reviewed in ref. 14). The *Mc1r* product transmits an intracellular signal for eumelanin production upon binding to its ligand, α-melanocyte-stimulating hormone (α-MSH) (reviewed in ref. 15), and is a guanine nucleotide-binding protein (G protein)-coupled receptor that, when bound to α-MSH, activates adenylate cyclase, causing an increase in cAMP levels and ultimately resulting in eumelanin production. The agouti protein antagonizes the complex formed by α-MSH and its receptor (*Mc1r* product), preventing the subsequent increase in cAMP levels and shifting melanocyte pigment production from eumelanin to phaeomelanin (16). Thus, constitutive production of agouti protein results in coat hairs with more phaeomelanin, and its absence or reduction results in coat hairs with less phaeomelanin compared to wild type. Absence of a functional agouti protein does not appear to be biologically detrimental to viability or fertility in mice (4, 14).

Problems with Yellow Mice

Several recent studies have shown that yellow mice result when the agouti gene is ectopically overexpressed. In fact, the dominant agouti mutations A^y, A^{vy}, A^{hvy}, A^{iapy}, A^{iy}, and A^{sy} are promoter mutations that result from agouti coding exons being placed under the control of ubiquitous promoters (through either deletion and juxtaposition to a promoter from a neighboring upstream gene or insertion of a transposable element that usurps control of agouti gene expression) (17–22). None of the dominant mutations alter the coding region of the agouti gene. Yellow $A^y/-$ and $A^{vy}/-$ mice exhibit mild hyperphagia, hyperglycemia, non-insulin-dependent diabetes, adult onset obesity, and altered susceptibility to tumorigenesis (reviewed in refs. 14, 23, and 24). A correlation exists between the degree of expressivity of the phenotypes and the level of agouti gene expression (ref. 3; reviewed in refs. 22 and 23). However, these effects are not a secondary result of phaeomelanin production, since mice lacking a functional *Mc1r* gene exhibit only the yellow coat phenotype. Thus, the question remains as to which tissue(s) and receptor(s) are responsive to the agouti protein, enabling it to exert effects on organ systems other than the hair follicle.

Transgenic Models Serve as Tests of Agouti Function *in Vivo*

A formal proof of agouti gene function *in vivo* is whether ubiquitous ectopic overexpression of agouti protein could result in diabetic, obese mice with yellow coats; this phenotype would essentially mimic that seen in $A^y/-$ mice. Several investigators have now made transgenic constructs that place the agouti gene under the transcriptional control of ubiquitous promoters (2, 6, 25). Founder mice usually exhibited yellow stripes or patches on an otherwise nonagouti or agouti coat (unless albino zygotes were used), indicating that they were mosaics carrying a functional transgene. Most transgenic progeny displayed uniformly pigmented fur, although the overall amount of phaeomelanin in coat hairs varied between lines (2, 25). In a subset of lines, germ-line transmission produced progeny with mottled or variegated coats, indicating that transgene expression could be influenced by cis-acting

Abbreviations: α-MSH, α melanocyte-stimulating hormone; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; Mc1r, melanocortin 1 receptor.

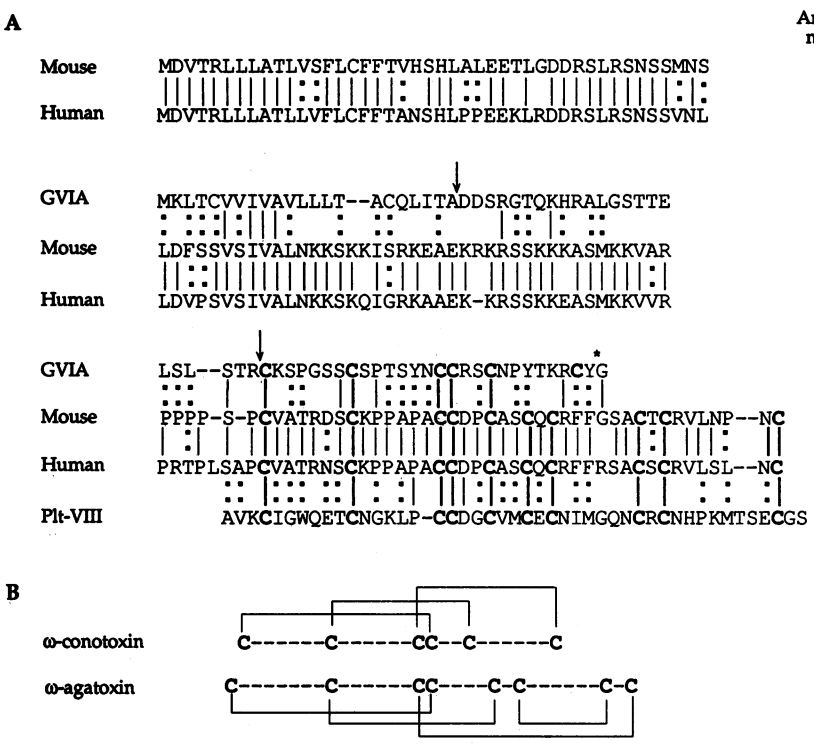

FIG. 1. Comparison of the mouse and human agouti proteins with toxins from the cone snail *Conus geographus* and primitive hunting spider *Plectreurys tristis*. (*A*) The amino acid sequences of the agouti protein in both mouse and human are shown (4–7). The highly basic region of exons 2, 3, and 4 of the agouti protein is separated from the signal sequence (first 22 amino acids) and 5′ end of exon 2 (*A Top*) and the cysteine-rich region of exon 4 (*A Bottom*). Above the agouti sequence is the amino acid sequence of the ω-conotoxin GVIA produced by *C. geographus*. Arrows indicate proteolytic cleavage sites within the GVIA prepropeptide; the first 22 amino acids constitute a signal sequence, the second 23 amino acids compose an intervening region, and the final 27 amino acids constitute the mature toxin (8). The asterisk denotes the carboxyl-terminal glycine that is converted to an amide residue by a posttranslational processing event. Below the human agouti protein is the amino acid sequence of plectoxin VIII (Plt-VIII) produced by *P. tristis* (9). Amino acid identity between adjacent proteins is indicated by straight lines, and conservative amino acid changes are indicated by two dots. Dashes indicate absences of amino acids used to maximize the alignments. (*B*) The disulfide bond structure of the ω-conotoxins and ω-agatoxins is shown by square brackets connecting cysteine residues (reviewed in ref. 10). Dashes indicate the presence of any amino acid residue except cysteine. Because the disulfide structure of the plectoxins is not known, the structure of the ω-agatoxins, which have similar albeit not identical cysteine backbone structures, is shown. The ω-agatoxins are produced by the funnel-web spider (*Agelenopsis*), and like the ω-conotoxins and plectoxins, target presynaptic voltage-activated Ca^{2+} channels. Only specific disulfide bridges result in biologically active peptides (11).

sequences at the integration site and/or unlinked modifier genes but was not a reflection of transgene copy number.

Offspring from different lines were tested for weight gain (25) as well as circulating levels of plasma insulin and glucose (2). Transgenic mice became obese and hyperinsulinemic, but only transgenic males became hyperglycemic. The data also indicated that as the levels of ectopic agouti expression increased, the time of onset of obesity, hyperinsulinemia, and hyperglycemia decreased (2).

These transgenic models now demonstrate that ectopic overexpression of the agouti gene can recapitulate the phenotypes exhibited by mice carrying dominant agouti mutations. In addition, they are useful for examining the role of the agouti protein in influencing susceptibility to spontaneous and/or induced tumors on a variety of genetic backgrounds. By placing the agouti gene under the control of tissue-specific promoters, transgenic mice can provide models to determine which tissues are responsive to overexpression of the agouti protein resulting in disease. In addition, transgenic constructs resulting from site-directed mutagenesis of agouti coding sequences can be tested to establish which amino acids or domains of the agouti protein are necessary for specific functions *in vivo*.

Agouti Gene Appears To Be Related to Toxins from Snails and Spiders

Initial comparisons of agouti coding sequences to various data bases did not identify homology to other known proteins. However, the agouti protein has striking similarities to two classes of toxins produced by snails (marine gastropods) and spiders (arachnids), the ω-conotoxins and the plectoxins, respectively (Fig. 1*A*).

The ω-conotoxins are a family of venoms made by the cone-snail species *Conus* (reviewed in refs. 10, 26, and 27). These marine snails survive by hunting fish, molluscs, and/or polychaete worms. Their sting delivers a venom composed of numerous small peptides (9–30 amino acids long) that paralyzes prey within a few seconds. Individual peptides have specific physiological targets and distinct pharmacological effects. Each toxin targets neurotransmitter receptors [such as acetylcholine or *N*-methyl-D-aspartate (NMDA) receptors] or ion channels (such as voltage-sensitive Ca^{2+} or Na^{2+}) and can discriminate between neuromuscular and neuronal specific targets, binding with high affinity to specific receptor subtypes. The ω-conotoxins target primarily N-type presynaptic neuronal Ca^{2+} channels. Although mammals are not the usual prey, intracranial injection of the ω-conotoxin GVIA elicits shaking symptoms in mice, with increasing dosages causing respiratory distress and death (10).

The primitive hunting spider *Plectreurys tristis* produces a potent toxin to paralyze its arthropod prey. *P. tristis* venom contains at least 50 peptide toxins called plectoxins, several of which have been sequenced and exhibit significant homology to each other (9). These peptides are paralytic and/or lethal to several insect species (9). Components of *P. tristis* toxin are associated with a number of biological activities, including disruption of voltage-dependent Ca^{2+} channels in *Drosophila* (28, 29) and mammals (30). In addition, *P. tristis* venom can inhibit binding of the ω-conotoxin (GVIA) to the ω-CgTX (*C. geographus* toxin) receptors in rat brain synaptic plasma membrane vesicles by a noncompetitive allosteric mechanism (31).

The ω-conotoxins and plectoxins are made from prepropeptides (Fig. 1; D. J. Leisy, personal communication). The amino terminus of the precursor is highly conserved among each class of toxins, as are the number and spacing of cysteine residues in the carboxyl terminus (which is cleaved to produce the mature toxin). The conserved cysteines are crucial to formation of disulfide bonds that establish the core of the peptide; amino acids between the cysteines form loops that presumably face the outer surface of the peptide (Fig. 1*B*). Amino acids present in the loops thus dictate the high level of target specificity exhibited by each toxin. The spacing and number of cysteine residues found in ω-conotoxins and plectoxins are most similar to those found at the carboxyl terminus of the agouti protein. These similarities suggest that the cysteine-rich carboxyl-terminal end of the agouti protein forms a functional three-dimensional structure similar to the multiply disulfide-bonded ω-conotoxins and plectoxins.

Thus, the agouti protein may represent a class of molecules related to the toxins that interact with specific neurotransmitter receptors or ion channels. These findings also suggest that other members of the agouti family exist but have not yet been detected because of divergence at the nucleotide level.

Agouti Protein Can Influence Ca^{2+} Levels

A link between the agouti protein and regulation of intracellular Ca^{2+} concentrations $[Ca^{2+}]_i$ has now been found. Research described by Zemel and colleagues (3) demonstrates a significant difference in $[Ca^{2+}]_i$ levels between insulin-sensitive type I muscle fibers from yellow obese A^{vy}/a mice compared with pseudoagouti lean A^{vy}/a and black nonagouti a/a controls. In addition, incubation of freshly isolated and cultured skeletal muscle myocytes from wild-type mice in agouti-conditioned medium resulted in elevated $[Ca^{2+}]_i$ levels by influencing Ca^{2+} influx but not Ca^{2+} efflux or release from $[Ca^{2+}]_i$ stores. The mechanism by which the agouti protein can affect Ca^{2+} influx is not known, but these findings suggest exciting possibilities for the role of the agouti protein in a variety of cellular functions.

Ca^{2+} plays an important role as second messenger in a wide array of signal-transduction pathways and cell types (reviewed in ref. 32). Changes in $[Ca^{2+}]_i$ levels affect numerous processes, including hormone secretion and neurotransmitter release, enzyme and ion channel activity, and gene expression (reviewed in ref. 32). In addition, these ions are essential for mitosis and meiosis and have been implicated in apoptosis (reviewed in refs. 33–35). Elaborate mechanisms have evolved to maintain $[Ca^{2+}]_i$ that are 4 orders of magnitude lower than those found outside the cell. Many reviews concerning Ca^{2+} signal transduction pathways have been published (32, 36–39). Therefore, the observation that the agouti protein can influence $[Ca^{2+}]_i$ levels provides the first link to internal cellular processes that, when altered, can result in disease phenotypes.

The questions arise as to which tissues are responsive to the agouti protein (other than the hair follicle) and whether these effects are mediated through changes in $[Ca^{2+}]_i$ levels. With respect to obesity and diabetes, the relationship between increased $[Ca^{2+}]_i$ levels and inhibition of insulin-mediated glucose transport remains unclear. One hypothesis involves a direct relationship between the opening of a Ca^{2+} channel and inhibition of insulin-stimulated glucose uptake (as opposed to sustained high $[Ca^{2+}]_i$ levels) and has been implicated in studies of rat adipocytes (40). If this were the case, the role of the agouti protein in insulin resistance would occur at the level of mediating Ca^{2+} influx, presumably in insulin target cells such as adipocytes. An alternative hypothesis suggests that sustained high $[Ca^{2+}]_i$ levels may contribute to insulin resistance by interfering with one or several steps in the signal transduction cascade initiated by insulin binding (reviewed in refs. 41–43). If this were the case, continued (ectopic) overexpression of the agouti protein could lead to sustained high $[Ca^{2+}]_i$ levels, ultimately inhibiting the signal transduction pathway initiated by insulin binding. Zemel and colleagues (3) favor the latter hypothesis and suggest that the hyperinsulinemia/insulin resistance observed in yellow obese mice causes increased $[Ca^{2+}]_i$ levels, which induce increased activity of inhibitor 1, a protein involved in insulin-stimulated glucose transport (reviewed in ref. 41). However, the initial trigger for the hyperinsulinemic/insulin-resistant state must occur in another tissue (such as adipocytes or pancreatic β-cells) and could still be the direct result of overproduction of the agouti protein (7). If the agouti protein can affect adipose cells resulting in hypertrophy, obesity may be the primary factor that leads to insulin resistance and non-insulin-dependent diabetes (7).

It has been suggested that the agouti protein may work through related melanocortin receptors expressed in tissues other than the hair follicle (3, 6, 16). Thus, agouti may represent a new class of molecules that have target specificity for the melanocortin receptor family and act specifically as antagonists to these receptors. The conservation of the cysteine backbone structure of the agouti protein with distantly related toxins, coupled with the fact that related toxins target other members of the melanotropin family (acetylcholine and NMDA receptors), are suggestive of this hypothesis. However, we favor the possibility that the primary function of the agouti protein is to directly target specific subtypes of Ca^{2+} channels.

Another effect of increased $[Ca^{2+}]_i$ levels resulting from sustained overexpression of the agouti protein may be the initiation of gene expression leading to effects on cell proliferation, invasion, and tumor metastasis (reviewed in refs. 33 and 44). Effects such as mild hyperplasia could be responsible for observed increases in overall body mass as well as increased susceptibility to tumorigenesis. Alternatively, increased $[Ca^{2+}]_i$ levels may result in secretion of hormones or growth factors that can act systemically to induce cell proliferation or other effects. Thus, the ability of the agouti protein to influence $[Ca^{2+}]_i$ levels may be the key to understanding its effects on different tissues resulting in the various phenotypes seen in dominant agouti mutants.

Implications for Mechanisms of Human Disease

The human homolog of the agouti gene was recently cloned (6, 7) and shown to influence coat color in transgenic mice (6). The open reading frames of the mouse and human agouti genes are 85% identical at the nucleotide level and 80% identical at the amino acid level (Fig. 1*A*). Expression of the human agouti gene was detected in adipose tissue, heart, ovary, and testis and to a lesser extent in foreskin, kidney, and liver (6, 7). The exon/intron organization of the mouse and human agouti genes is similar, with identically positioned introns separating the three coding exons. However, the promoter regions and/or 5′ untranslated exons are likely to be different than in the mouse, given that higher primates do not exhibit banded hairs and that expression of the human agouti gene was detected in more tissues than in the mouse. The presence of agouti transcripts in skin, heart, and adipose tissue implicates a role for the agouti gene in a variety of processes including skin pigmentation, cardiovascular function, and energy metabolism. In addition, the observation of agouti expression in premenopausal but not postmenopausal ovaries suggests a role for the agouti protein in fertility as well.

An intriguing and potential link to agouti protein function is the observation of elevated $[Ca^{2+}]_i$ levels in tissues from hypertensive, obese, and diabetic patients (reviewed in refs. 41, 42 and 45). It has been proposed that hyperinsulinemia, insulin resistance, non-insulin-dependent diabetes mellitus, obesity, hypertension, and coronary artery disease are the result of common abnormalities in cellular ion metabolism (reviewed in refs. 41, 42, and 45). These diseases may present clinically as parts of a spectrum of metabolic defects ultimately dependent on genetic, dietary, and/or environmental factors (reviewed in refs. 42 and 45). The finding of agouti gene expression in normal human heart as well as adipose tissue (6, 7), coupled with the finding that the agouti protein can influence $[Ca^{2+}]_i$ levels (3), suggests that the agouti protein is one common link between these diseases. The level of expression of the agouti gene may be dictated by a series of alleles in the human population that can predispose individuals to obesity, cardiovascular disease, and/or diabetes.

Mutations or alterations in factors responsible for agouti gene expression that result in the agouti protein being present in the wrong place at the wrong time or at higher levels than normal are likely to be responsible for disease phenotypes in humans. The tools are now in hand to decipher whether the agouti gene acts as a paracrine (or other) factor in each tissue to elicit specific cellular responses and to

determine which effects of the agouti protein are mediated through melanocortin receptors, ion channels, or other factors. The agouti gene may ultimately prove useful for diagnostic and therapeutic intervention in human disease.

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1. Searle, A. G. (1968) *Comparative Genetics of Coat Colour in Mammals* (Academic, New York).
2. Klebig, M. L., Wilkinson, J. E., Geisler, J. G. & Woychik, R. P. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4728–4732.
3. Zemel, M. B., Kim, J. H., Woychik, R. P., Michaud, E. J., Kadwell, S. H., Patel, I. R. & Wilkison, W. O. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4733–4737.
4. Bultman, S. J., Michaud, E. J. & Woychik, R. P. (1992) *Cell* **71,** 1195–1204.
5. Miller, M. W., Duhl, D. M. J., Vrieling, H., Cordes, S. P., Ollmann, M. M., Winkes, B. M. & Barsh, G. S. (1993) *Genes Dev.* **7,** 454–467.
6. Wilson, B. D., Ollmann, M. M., Kang, L., Stoffel, M., Bell, G. I. & Barsh, G. S. (1995) *Hum. Mol. Genet.* **4,** 223–230.
7. Kwon, H. Y., Bultman, S. J., Loffler, C., Chen, W. J., Furdon, P. J., Powell, J. G., Usala, A. L., Wilkison, W., Hansmann, I. & Woychik, R. P. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 9760–9764.
8. Colledge, C. J., Hunsperger, J. P., Imperial, J. S. & Hillyard, D. R. (1992) *Toxicon* **30,** 1111–1116.
9. Quistad, G. B. & Skinner, W. S. (1994) *J. Biol. Chem.* **269,** 11098–1110.
10. Olivera, B. M., Miljanich, G. P., Ramachandran, J. & Adams, M. E. (1994) *Annu. Rev. Biochem.* **63,** 823–867.
11. Woodward, S. R., Curz, L. J., Olivera, M. & Hillyard, D. R. (1990) *EMBO J.* **9,** 1015–1020.
12. Bultman, S. J., Klebig, M. L., Michaud, E. J., Sweet, O. H., Davisson, M. T. & Woychik, R. P. (1994) *Genes Dev.* **8,** 481–490.
13. Vrieling, H., Duhl, D. M. J., Millar, S. E., Miller, K. A. & Barsh, G. S. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5667–5671.
14. Silvers, W. K. (1979) *The Coat Colors of Mice* (Springer, New York).
15. Jackson, I. J. (1993) *Curr. Biol.* **3,** 518–521.
16. Lu, D., Willard, D., Patel, I. R., Kadwell, S., Overton, L., Kost, T., Luther, M., Chen, W., Woychik, R. P., Wilkison, W. O. & Kone, R. D. (1994) *Nature (London)* **371,** 799–802.
17. Michaud, E. J., Bultman, S. J., Stubbs, L. J. & Woychik, R. P. (1993) *Genes Dev.* **7,** 1203–1213.
18. Duhl, D. M. J., Stevens, M. E., Vrieling, H., Saxon, P. J., Miller, M. W., Epstein, C. J. & Barsh, G. S. (1994) *Development (Cambridge, U.K.)* **120,** 1695–1708.
19. Duhl, D. M. J., Vrieling, H., Miller, K. A., Wolff, G. L. & Barsh, G. S. (1994) *Nat. Genet.* **8,** 59–65.
20. Michaud, E. J., Bultman, S. J., Klebig, M. L., van Vugt, M. J., Stubbs, L. J., Russell, L. B. & Woychik, R. P. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2562–2566.
21. Michaud, E. J., van Vugt, M. J., Bultman, S. J., Sweet, H. O., Davisson, M. T. & Woychik, R. P. (1994) *Genes Dev.* **8,** 1463–1472.
22. Siracusa, L. D., Washburn, L. L., Swing, D. A., Argeson, A. C., Jenkins, N. A. & Copeland, N. G. (1995) *J. Hered.*, in press.
23. Yen, T. T., Gill, A. M., Frigeri, L. G., Barsh, G. S. & Wolff, G. L. (1994) *FASEB J.* **8,** 479–488.
24. Siracusa, L. D. (1994) *Trends Genet.* **10,** 423–427.
25. Perry, W. L., Hustad, C. M., Swing, D. A., Jenkins, N. A. & Copeland, N. G. (1995) *Genetics*, in press.
26. Olivera, B. M., Rivier, J., Clark, C., Ramilo, C. A., Corpuz, G. P., Abogadie, C. F., Mena, E. E., Woodward, S. R., Hillyard, D. R. & Cruz, J. L. (1990) *Science* **249,** 257–263.
27. Olivera, M. B., Rivier, J., Scott, K. J., Hillyard, D. R. & Curz, J. L. (1991) *J. Biol. Chem.* **266,** 22067–22070.
28. Branton, W. D., Kolton, L., Jan, Y. N. & Jan, L. Y. (1987) *J. Neurosci.* **7,** 4195–4200.
29. Leung, H.-T., Branton, W. D., Phillips, H. S., Jan, L. & Byerly, L. (1989) *Neuron* **3,** 767–772.
30. Lundy, P. M. & Frew, R. (1993) *Toxicon* **31,** 1249–1256.
31. Feigenbaum, P., Garcia, M. L. & Kaczorowski, G. J. (1988) *Biochem. Biophys. Res. Commun.* **154,** 298–305.
32. Clapham, D. E. (1995) *Cell* **80,** 259–268.
33. Means, A. R. (1994) *FEBS Lett.* **347,** 1–4.
34. Nicotera, P., Zhivotovsky, B. & Orrenius, S. (1994) *Cell Calcium* **16,** 279–288.
35. Stewart, B. W. (1994) *J. Natl. Cancer Inst.* **86,** 1286–1296.
36. Berridge, M. J. (1993) *Nature (London)* **361,** 315–325.
37. Clapham, D. E. & Neer, E. J. (1993) *Nature (London)* **365,** 403–406.
38. Pozzan, T., Rizzuto, R., Volpe, P. & Meldolesi, J. (1994) *Physiol. Rev.* **74,** 595–636.
39. Tsien, R. W. & Tsien, R. Y. (1990) *Annu. Rev. Cell Biol.* **6,** 715–760.
40. Kelly, K. L., Deeney, J. T. & Corkey, B. E. (1989) *J. Biol. Chem.* **264,** 12754–12757.
41. Draznin, B. (1993) *Am. J. Kidney Dis.* **6,** Suppl. 3, 32–38.
42. Levy, J., Gavin, J. R. & Sowers, J. R. (1994) *Am. J. Med.* **96,** 260–273.
43. Roth, R. A., Liu, F. & Chin, J. E. (1994) *Horm. Res.* **41,** Suppl. 2, 51–55.
44. Cole, K. & Kohn, E. (1994) *Cancer Metastasis Rev.* **13,** 31–44.
45. Resnick, M. L. (1993) *Am. J. Hypertens.* **6,** 125S–134S.