day 1, wild-type vole neonates display a variable pattern of dark pigmentation on the back and head (Nadeau 1985). This pattern was absent in blond neonates.

Microscopic examination of the fur from wild-type and blond voles showed that the blond coloration was the result of at least two major differences in the type and distribution of pigmentation. The fur of wild-type voles contained black pigment that was distributed throughout the length of each hair shaft. In contrast, hair from blond voles contained brown pigment and the pigment was not present in the distal portion of the hair.

Discussion

Color mutations have been identified in several small rodent species. Mutations similar to that found in meadow voles in the present study have been observed in *Peromyscus* (Hance 1969; Roth and Dawson 1996), *Microtus* (Gaines 1985; Owen and Shackleford 1942), and *Mus* (Silvers 1979). In most cases these mutations have been monogenic and autosomal. The gene responsible for the blond color mutation in our voles also appears to be autosomal, since the blond phenotype was expressed in individuals of both sexes. Further, the results of testcrosses suggest that a single gene is responsible for coat coloration in meadow voles. Heterozygous individuals were indistinguishable from homozygous dominant individuals.

The original pair from which the mother of the blond line descended had previously produced in excess of 30 pups, all of which displayed wild-type phenotypes. Since a mating involving the male from this pair and a daughter produced pups with the blond phenotype, the most likely explanation is that the original male was heterozygous for the color gene while the original female was homozygous wild type and that wild type is dominant. The origin of the blond mutation is unknown. The blond mutation may have arisen de novo within our colony. Our breeding colony has produced several hundred individuals, none of which displayed the blond coloration. Crosses of randomly chosen wild-type males from our colony with blond females produced only wild-type pups, suggesting that the blond allele is not common.

Alternatively, the blond allele may be present in the general population, but at such a low frequency that it is rarely found in the homozygous condition. Descriptions of meadow voles variously described as "yellow," "cream," and "buffy" occur in the literature (Clark 1935; Gaines 1985; Owen and Shackleford 1942). However, in most cases, enough differences in phenotype (i.e., fur color, eye color, pigment color and distribution, etc.) exist to suggest that the various yellow voles are the result of different mutations. The color variation most similar to that found in the present report was a description by Clark (1935) of meadow voles captured in southern Michigan. It is possible that the voles found by Clark and the voles in the present report are from the same breeding population. Although separated by several hundred miles, these voles are considered to be of the same subspecies (Hoffman and Koeppi 1985), and it is possible that the mutation may spread longitudinally through a contiguous population. The possibility of longitudinal spread is enhanced by the facts that vole littermates often disperse as a group (Hilborn 1975), both male and female voles are likely to disperse (Lidicker 1985), and individuals heterozygous for the blond mutation are indistinguishable from wild-type voles (present report).

From the Neuroscience Program, Department of Psychology, Florida State University, 209 Copeland Ave., Tallahassee, FL 32306. I would like to thank Dr. Z. X. Wang for generously allowing access to his vole colony. This work was supported by NIH grants NIMH 58616 (to Z.X.W.) and HD 40722 (to J.T.C.). Address correspondence to J. Thomas Curtis at the address above or e-mail: tcurtis@psy.fsu.edu.

References

Carlton MD, 1985. Macroanatomy. In: Biology of New World *Microtus* (Tamarin RH, ed). Special publication no. 8. Lawrence, KS: American Society of Mammalogists; 116–175.

Clark FH, 1935. Coat color in the meadow vole. J Hered 29:265–266.

Gaines MS, 1985. Genetics. In: Biology of New World *Microtus* (Tamarin RH, ed). Special publication no. 8. Lawrence, KS: American Society of Mammalogists; 845–883.

Hance J, 1969. Pseudobrown, a mutant in the oldfield mouse, *Peromyscus polionotus.* Bull S C Acad Sci 31:42–43.

Hilborn R, 1975. Similarities in dispersal tendency among siblings in four species of voles (*Microtus*). Ecology 56:1221–1225.

Hoffman RS and Koeppi JW, 1985. Zoogeography. In: Biology of New World *Microtus* (Tamarin RH, ed). Special publication no. 8. Lawrence, KS: American Society of Mammalogists; 84–115.

Lidicker WZ Jr, 1985. Dispersal. In: Biology of New World *Microtus* (Tamarin RH, ed). Special publication no. 8. Lawrence, KS: American Society of Mammalogists; 420–454.

Nadeau JH, 1985. Ontogeny. In: Biology of New World *Microtus* (Tamarin RH, ed). Special publication no. 8. Lawrence, KS: American Society of Mammalogists; 254–285.

Owen RD and Shackleford RM, 1942. Color aberrations in *Microtus* and *Pitymys.* J Mamm 23:306–314.

Roth VL and Dawson WD, 1996. Coat color genetics of *Peromyscus*: V. California Blond, a new recessive mutation in the deer mouse. J Hered 87:403–406.

Silvers WK, 1979. The coat colors of mice. New York: Springer-Verlag.

Received March 2, 2001
Accepted March 29, 2002

Corresponding Editor: Roger H. Reeves

The Charles River "Hairless" Rat Mutation Maps to Chromosome 1: Allelic With *Fuzzy* and a Likely Orthologue of Mouse *Frizzy*

K. Ahearn, G. Akkouris, P. R. Berry, R. R. Chrissluis, I. M. Crooks, A. K. Dull, S. Grable, J. Jeruzal, J. Lanza, C. Lavoie, R. A. Maloney, M. Pitruzzello, R. Sharma, T. A. Stoklasek, J. Tweeddale, and T. R. King

Recent evidence has indicated that the recessive mutation affecting hypotrichosis in the Charles River (CR) "hairless" rat does not involve the *hairless* gene (*hr*) on rat chromosome 15. To determine if this mutation might be allelic (or orthologous) with any other previously mapped hypotrichosis-generating mutation in mammals, we have produced a panel of backcross rats segregating for the CR hairless rat mutation as well as numerous other markers from throughout the rat genome. Analysis of this panel has located the CR hairless rat's hypotrichosis-generating mutation on chromosome 1, near *Myl2,* where only the *fuzzy* mutation in rat (*fz*) and the *frizzy* mutation in mouse (*fr*) have been previously localized. Intercrossing *fz*/*fz* and CR hairless rats produced hybrid offspring with abnormal hair, showing that these two rat mutations are allelic. We suggest that the CR hairless rat mutation and *fuzzy* be renamed *frizzy*-Charles River (fr^{CR}) and *frizzy*-Harlan (fr^{H}), respectively, to reflect their likely orthology with the mouse *fr* mutation.

More than a dozen mutations have been reported that generate recessive hypotrichosis in rats (reviewed by Ferguson et al. 1979; Hanada et al. 1988; Hedrich 1990; Moemeka et al. 1998; Robinson 1979). Among these, a molecular defect has been identified only for the Rowett and New Zealand *nude* mutations, which have been

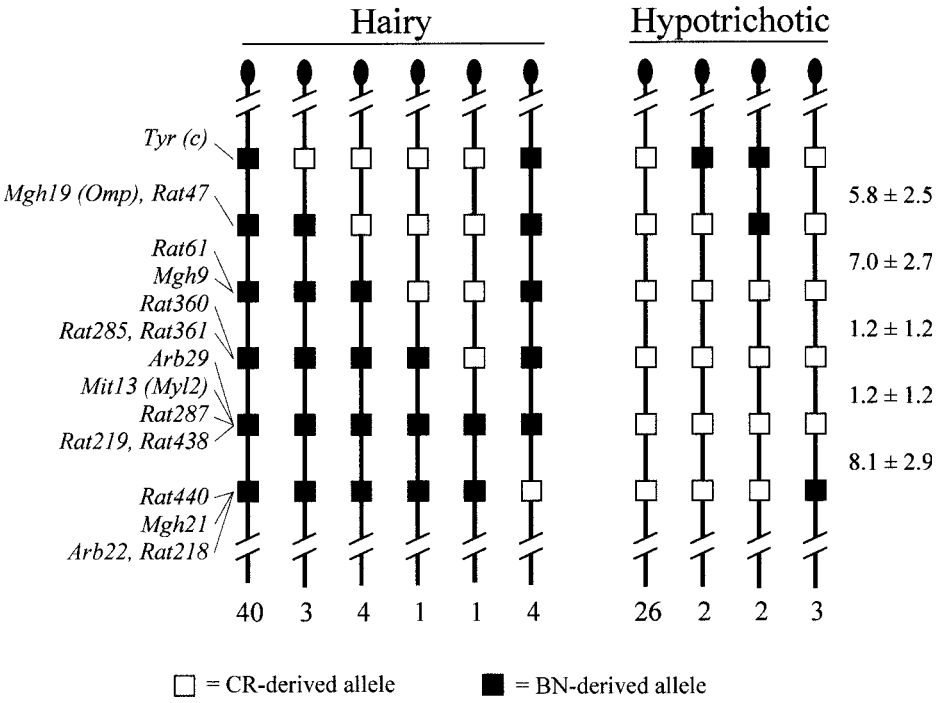

Figure 1. Segregation of 2 visible and 16 DNA markers on rat chromosome 1 among 86 (CR × BN) F_1 × CR backcross progeny. Fifty-three nonmutant (hairy) progeny (which inherited a wild-type CR "hairless" allele from the F_1 parent) are shown on the left; 33 mutant (hypotrichotic) progeny (which inherited a mutant CR hairless allele from the F_1 parent) are shown on the right. Loci typed in this study are listed down the left side of the diagram. The haplotype depicted is that inherited from the F_1 parent. Open boxes indicate CR-derived alleles; filled boxes indicate BN-derived alleles. The number of backcross progeny that inherited each haplotype is shown below it. The centromere is indicated by a knob at the top. Genetic distances, shown at the right, are the percentage recombination ± 1 standard error. Markers assigned to the same box were not genetically separated in this panel. Such markers are listed in order, if known; markers shown on the same line remain unordered (to our knowledge). *D1Mit13* is an alias for *Myl2* (myosin, light polypeptide 2, alkaline); *D1Mgh19* is an alias for *Omp* (olfactory marker protein); *Tyr* (tyrosinase) is the current symbol for the albino locus (*c*).

found to be the result of a defect in the winged-helix protein-encoding gene, *whn* (Nehls et al. 1994). Several other hypotrichosis-generating rat mutations have been genetically mapped (e.g., Greaves and Ayres 1985; Hall et al. 2000) or histologically characterized (e.g., Ferguson et al. 1979; Hanada et al. 1988; Palm and Ferguson 1976), but for most, genetic and histologic data are limited or absent. Indeed, several named hypotrichotic rat mutants are likely to be extinct. Thus it has been difficult to determine for many of the named hypotrichosis-generating rat mutations if any might be alleles of one another, or whether any are potential orthologues of hypotrichotic mutations identified in other mammals.

One of the more widely used hypotrichotic rat models is the Charles River (CR) "hairless" rat, which is commercially available from Charles River Laboratories (Wilmington, MA). While the skin morphologic features of mutant CR hairless rats have recently been described (Panteleyev and Christiano 2001), the genetic basis of this variant remains unknown. Indeed, recent molecular analyses (Panteleyev and Christiano 2001) have shown definitively that the CR hairless rat mutation, in spite of its designation in the literature as *hr,* is not an allele of the cloned and characterized *hairless* gene (*hr*; Thompson 1996; GenBank accession no. U71293). While disruption of the *hr* gene in both mice and humans results in hypotrichosis (Cachon-Gonzalez et al. 1999; Panteleyev et al. 1998), no mutation in this gene on rat chromosome 15 has yet been reported.

To determine if the CR hairless mutation might identify a novel genetic locus with a critical function in the normal development of a hairy mammalian coat, we have produced a panel of 86 backcross rats segregating for the CR hairless mutation as well as numerous polymorphic microsatellite markers from throughout the rat genome (Brown et al. 1998; Jacob et al. 1995; Steen et al. 1999). Analysis of this backcross panel has allowed us to genetically map the CR hairless mutation to rat chromosome 1, in the region where *fuzzy* has been previously mapped in the Norway rat (Ferguson et al. 1979; Palm and Ferguson 1976). Complementation testing has further revealed that the *fuzzy* and CR hairless mutations do not complement, but are instead allelic.

Materials and Methods

Outbred CR hairless rats [Crl:CD(SD)-*hr*BR, designated herein as CR] were obtained from Charles River Laboratories (Wilmington, MA). Inbred Brown Norway (BN/SsNHsd, designated herein as BN) and outbred Fuzzy rats (Hsd:FUZZY-*fz*) were obtained from Harlan (Indianapolis, IN).

For outcrossing, a single, pigmented BN (*C/C*) male was crossed with a single, albino CR hairless rat female (*c/c*). Three hybrid (CR × BN) F_1 females were then backcrossed to three CR hairless males to produce 31, 24, and 10 offspring. In addition, two hybrid (CR × BN) F_1 males were backcrossed to two CR hairless females to produce 11 and 10 offspring. Backcross rats were scored visually for sex, pigmentation, and hair morphology. All parents and progeny were then killed, and organs (spleen, liver, and kidney) were harvested and stored at −70°C.

Genomic DNA was extracted from the frozen spleen of individual rats according to the method of Jenkins et al. (1982). Dinucleotide repeat DNA markers were typed in 13 μl amplification reactions using 75 ng genomic DNA as a template, 0.2 μM forward and reverse primers (MapPairs from Research Genetics, Huntsville, AL), 0.2 μM dNTPs, Titanium *Taq* DNA polymerase, and reaction buffer as supplied by the enzyme manufacturer (Clontech Laboratories, Palo Alto, CA). Parameters for amplification reactions were 95°C for 25 s, 60–68°C for 20 s, 68°C for 10 s for 35 cycles, followed by 68°C for 3 min. To visualize products, 10–13 μl aliquots were electrophoresed through 3–4% NuSieve 3:1 agarose gel (BioWhittaker Molecular Applications; Rockland, ME) in TBE buffer with 0.1 μg/ml ethidium bromide. Genetic distances based on backcross data are presented as percentage recombination ± 1 standard error.

Results

Backcrossing (CR × BN) F_1 rats to CR hairless rats produced 86 progeny; 33 were hypotrichotic and 53 with normal hairy coats (deviation from the expected 1 bald:1 hairy ratio is significant; $\chi^2 = 4.65$; $P \approx 0.03$). A similar non-Mendelian ratio has also been reported by Panteleyev and Christiano (2001) in a CR hairless rat testcross (which produced only 39% mu-

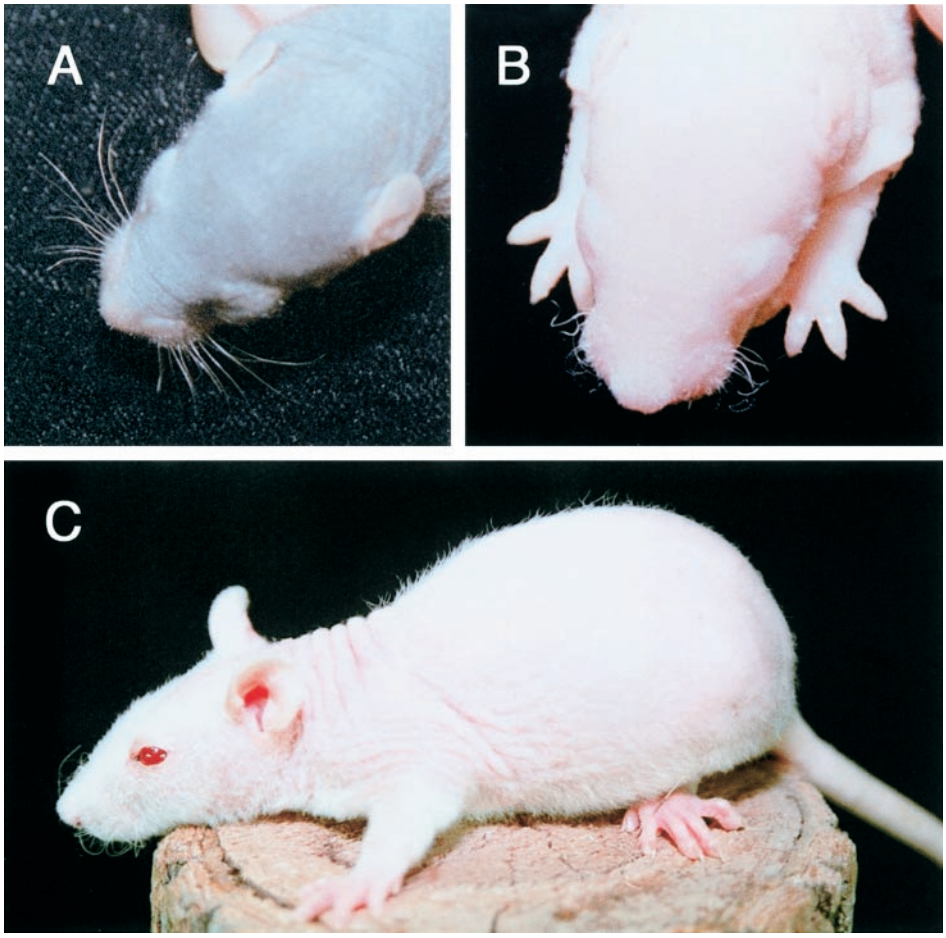

Figure 2. The CR hairless mutation and fuzzy (*fz*) do not complement. (**A**) A 10-day-old wild-type (hooded) CR hairless heterozygote (genotypically $fr^{CR}/+$; see Discussion for an explanation of allele symbols), produced in a (CR × BN) F_1 female × CR hairless male backcross. (**B**) An age-matched hybrid rat (genotypically fr^{H}/fr^{CR}; see Discussion) produced by crossing a *fuzzy* female (Hsd:FUZZY-*fz*) with a CR hairless male (Crl:CD(SD)-*hr*BR). The severity of the hypotrichotic phenotype in these fr^{H}/fr^{CR} hybrids appears intermediate between that of CR hairless (more severe hypotrichosis; see Panteleyev and Christiano 2001) and *fuzzy* rats (less severe hypotrichosis, see Palm and Ferguson 1976). (**C**) A hybrid (fr^{H}/fr^{CR}) female at 40 days of age.

tants), and apparently reflects the poor viability of the mutant class. In addition, mutant mothers—while initially producing litters of normal size (9.1 ± 4.6 born versus 12.2 ± 2.1 born from F_1 mothers)—raised only small numbers of progeny to weaning (2.0 ± 2.4 weaned versus 10.8 ± 1.8 weaned from F_1 mothers; based on 10 and 6 litters, respectively).

Immediate scoring of visible markers in this 86-member backcross panel showed that inheritance patterns for the CR hairless mutation and albino—73 parentals (hairy and pigmented, or hypotrichotic and albino) and 13 recombinants (hairy and albino, or hypotrichotic and pigmented)—deviated dramatically from the 1 parental:1 recombinant ratio predicted by independent assortment ($\chi^2 = 41.9$; $P < .0001$). This suggested a location for the CR hairless mutation on chromosome 1, 15.1 ± 3.9% recombination from the albino locus.

To refine the map location for the CR hairless mutation, DNA was isolated from these 86 backcross rats and typed for polymerase chain reaction (PCR)-scorable DNA markers known to reside on rat chromosome 1. (Numerous microsatellite markers known to map in our region of interest, including all 35 microsatellite markers listed between *D1Rat360* and *D1Arb22* in the [SHRSP × BN] F_2 panel [Rat Genome Data 2001] were tested for polymorphism, but the majority were found to be monomorphic in our cross.) Segregation patterns for all polymorphic markers typed among the 86-member backcross panel are shown in Figure 1. This analysis placed the CR hairless mutation between *D1Rat285, −361* (on the centromeric flank) and *D1Rat440* (on the telomeric flank). The CR hairless mutation was not separated from *D1Arb29, D1Mit13* (alias for myosin, light polypeptide 2, alkali; *Myl2*), *D1Rat219, −287,* or *−438* in this backcross panel.

This location suggested possible allelism of the CR hairless mutation and the rat *fuzzy* mutation (*fz*; Ferguson et al. 1979; Palm and Ferguson 1976), since *fz* has been mapped close to warfarin resistance (*Rw*) in rats (0.0% recombination; Greaves and Ayres 1985), and warfarin resistance in mice (*War*) has not been separated from sialophorin (*Spn*; Mouse Genome Database 2001), which has not been separated from *Myl2* in rat (Jacob et al. 1995). Therefore crosses between fuzzy females and CR hairless males were conducted to assess complementation. The 27 resulting hybrid progeny (from two litters) displayed curly whiskers at birth and failed to develop normal hair (see Figure 2), indicating that these mutations do not complement. Thus the CR hairless mutation is allelic with the rat *fuzzy* mutation. It is perhaps noteworthy that these two fuzzy females successfully reared 25 of their 27 hybrid (mutant) progeny, in contrast with the routinely poor survival seen among litters raised by mutant CR hairless mothers.

Discussion

The CR hairless mutation maps to rat chromosome 1 near *D1Arb29, Myl2, D1Rat219, −287,* and *−438* (see Figure 3), and is an allele of rat *fz*. It would seem that mutations at this site have been identified numerous times in the rat, yielding recessive hypotrichosis of differing degrees (Ferguson et al. 1979; Palm and Ferguson 1976; Panteleyev and Christiano 2001). This region of rat chromosome 1 shares homology with chromosome 7 in mouse (Mouse Genome Database 2001; see Figure 3), where the recessive coat-morphology mutation *frizzy* (*fr*; Snell 1951) has previously been mapped (Falconer and Snell 1952; MacSwiney and Wallace 1978; Wallace and MacSwiney 1976). Thus rat *fz* and mouse *fr* are likely orthologues, as has already been suggested by Greaves and Ayres (1985). Unfortunately another—clearly distinct—hypotrichosis-generating mutation mapping to mouse chromosome 1 is also named *fuzzy* (*fz*). For the sake of clarity, we suggest that both the CR hairless and the rat *fz* mutations be renamed *frizzy*-Charles River (fr^{CR}) and *frizzy*-Harlan (fr^{H}), respectively, to reflect their likely orthology with (and priority of) the mouse *fr* mutation, as well as to distinguish this locus from that identified by mouse *fz*. We recommend distinct allele designations for these commercially available rat *fr* mutations, especially because they control

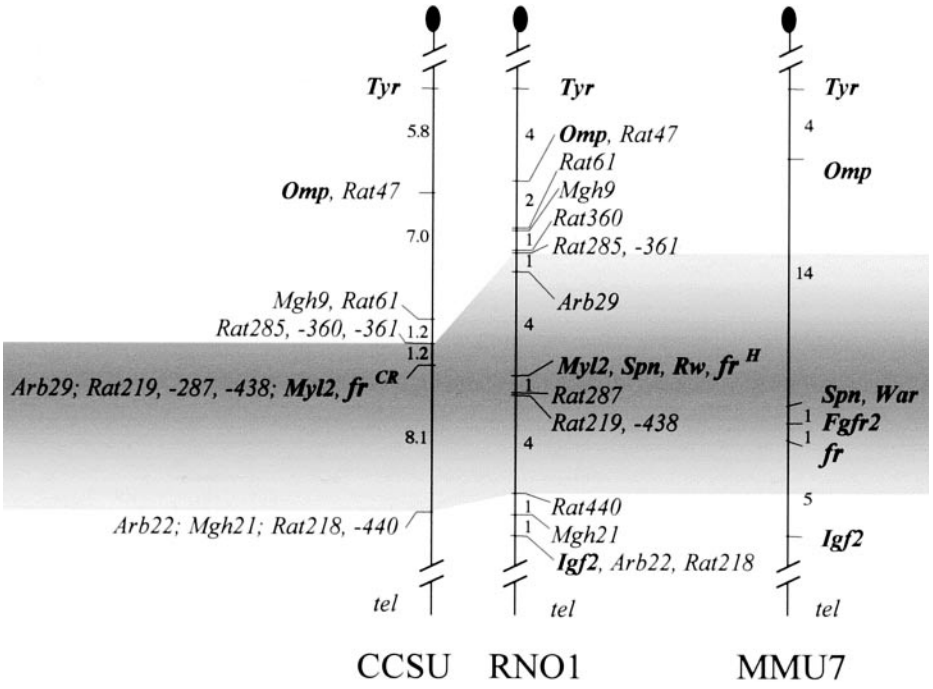

Figure 3. Genetic maps of distal rat chromosome 1 and the homologous region of mouse chromosome 7. Positions for markers shown on the left-most rat map (marked CCSU) are based on data described here; the middle map (labeled RNO1) was roughly composited from several different databases (ARBRat Genome Database 2001; Rat Genome Database 2001) and published reports (Greaves and Aryes 1985; Jacob et al. 1995; Steen et al. 1999). Data from this report allow the CR hairless mutation to be positioned between *D1Rat285, −361* and *D1Rat440* (as indicated by the shading); very near *D1Arb29, Myl2, D1Rat219, −287,* and *−438.* Positions for loci on the mouse map (labeled MMU7) were retrieved from the Mouse Genome Database (2001), which was compiled from numerous distinct crosses. All maps are drawn in centiMorgans, with shared flanking loci (*Tyr,* and *Igf2* or *D1Mgh21*) aligned. Symbols: fr^{CR}, *frizzy*-Charles River (i.e., the CR hairless mutation); fr^{H}, *frizzy*-Harlan (i.e., the rat *fuzzy* mutation); *fr,* the mouse *frizzy* mutation. These and all other gene symbols are defined in the text. On all diagrams, a knob at the top depicts the centromere and *tel* marks the distal telomere.

very distinct phenotypes (in terms of coat morphology, postnatal mutant viability, and mutant maternal success) and are therefore likely to have distinct mutational bases. Nomenclature revision for other rat "*fuzzy*" alleles (see, e.g., Ferguson et al. 1979), if such lines remain extant, is similarly advised.

A molecular assignment for *fr* is now needed to allow a detailed functional analysis of its role in both the normal and disrupted development of the mammalian integument. The map location for *fr* in both rat and mouse suggests a number of potential candidates for the gene affected by these mutations (see Mouse Genome Database 2001). Perhaps the most compelling among these candidates is the fibroblast growth factor receptor 2 gene (*Fgfr2*), which has been shown to be expressed in hair follicles (see Figure 4F in Makarenkova et al. 2000). Evaluation of this and any other colocalizing *fr* candidates should be facilitated by the availability of at least three mutant alleles of the *fr* gene in rodents.

From the Department of Biological Sciences, Central Connecticut State University, 1615 Stanley Street, New Britain, CT 06050. The authors thank Drs. K. A. Martin-Troy and J. P. Mulrooney for critical review of the manuscript. This work was supported by a research grant from Connecticut State University. Address correspondence to T. R. King at the above address.

References

ARB Rat Genetic Database, 2001. National Institute of Arthritis and Musculoskeletal and Skin Diseases. Bethesda Maryland: The National Institutes of Health. Available at http://www.niams.nih.gov/rtbc/ratgbase/ratgbase.htm.

Brown DM, Matise TC, Koike G, Simon JS, Winer ES, Zangen S, McLaughlin MG, Shiozawa M, Atkinson OS, Hudson JR II, Chakravarti A, Lander ES, and Jacob HJ, 1998. An integrated genetic linkage map of the laboratory rat. Mamm Genome 9:521–530.

Cachon-Gonzalez MB, San-Jose I, Cano A, Vega JA, Garcia N, Freeman T, Schimmang T, and Stoye JP, 1999. The hairless gene of the mouse: relationship of phenotypic effects with expression profile and genotype. Dev Dyn 216:113–126.

Falconer DS and Snell GD, 1952. Two new hair mutants, rough and frizzy in the house mouse. J Hered 43:53–57.

Ferguson FG, Irving GW III, and Stedham MA, 1979. Three variations of hairlessness associated with albinism in the laboratory rat. Lab Anim Sci 29:459–465.

Greaves JH and Ayers PB, 1985. Fuzzy: a genetic marker for warfarin resistance in the Norway rat. Lab Anim 19: 145–147.

Hall EH, Lathrop JA, Medina B, Mutcherson RJ II, and King TR, 2000. The hypotrichosis-generating shorn (*shn*) mutation maps to distal chromosome 7 in the Norway rat. J Hered 91:345–347.

Hanada K, Chiyoya S, and Suzuki K, 1988. Study of the skin of a new hairless rat mutant. J Dermatol Surg Oncol 15:257–262.

Hedrich HJ, 1990. Mutant genes and polymorphic loci of the laboratory rat. In: Genetic monitoring of inbred strains of rats (Hedrich HJ, ed). New York: Gustav Fischer Verlag; 289–409.

Jacob HJ, Brown DM, and Bunker RK, 1995. A genetic linkage map of the laboratory rat, *Rattus norvegicus.* Nat Genet 9:63–69.

Jenkins NA, Copeland NG, Taylor BA, and Lee BK, 1982. Organization, distribution and stability of endogenous ecotropic murine leukemia virus DNA sequences in chromosomes of *Mus musculus.* J Virol 43:2226–2236.

Makarenkova HP, Ito M, Govindarajan V, Faber SC, McMahon G, Overbeek PA, and Lang RA, 2000. FGF10 is an inducer and Pax 6 a competence factor for lacrimal gland development. Development 127:2563–2572.

MacSwiney FJ and Wallace ME, 1978. Genetics of warfarin-resistance in house mice of three separate localities. J Hyg (Lond) 80:69–75.

Mouse Genome Database, 2001. Mouse genome informatics. Bar Harbor, ME: The Jackson Laboratory. Available at http://www.informatics.jax.org/.

Moemeka AN, Hildebrandt AL, Radaskiewicz P, and King TR, 1998. Shorn (*shn*): a new mutation causing hypotrichosis in the Norway rat. J Hered 89:257–260.

Nehls M, Pfeifer D, Schorpp M, Hedrich H, and Boehm T, 1994. New member of the winged-helix protein family disrupted in mouse and rat nude mutations. Nature 372:103–107.

Panteleyev AA and Christiano AM, 2001. The Charles River "hairless" rat mutation is distinct from the *hairless* mouse alleles. Comp Med 51:49–55.

Panteleyev AA, Paus R, Ahmad W, Sundberg JP, and Christiano AM, 1998. Molecular and functional aspects of the hairless (h) gene in laboratory rodents and humans. Exp Dermatol 7:249–267.

Palm J and Ferguson FG, 1976. Fuzzy, a hypotrichotic mutant in linkage group 1 of the Norway rat. J Hered 67:284–288.

Rat Genome Database, 2001. Mouse genome informatics. Bar Harbor, ME: The Jackson Laboratory. Available at http://www.informatics.jax.org/rat.

Robinson R, 1979. Taxonomy and genetics. In: The laboratory rat (Baker HJ, Lindsey JR, and Weisbroth SH, eds). New York: Academic Press; 37–54.

Snell GD, 1951. *fr*-frizzy. Mouse Newslett 5:36.

Steen RG, Kwitek-Black AE, Glenn C, Gullings-Handley J, Van Etten W, Atkinson OS, Appel D, Twigger S, Muir M, Mull T, Granados M, Kissebah M, Russo K, Crane R, Popp M, Peden M, Matise T, Brown DM, Lu J, Kingsmore S, Tonellato PJ, Rozen S, Slonim D, Young P, Knoblauch M, Provoost A, Ganten D, Colman SD, Rothberg J, Lander ES, and Jacob HJ, 1999. A high density integrated genetic and radiation hybrid map of the laboratory rat. Genome Res 9:AP1–8.

Thompson CC, 1996. Thyroid hormone-responsive genes in developing cerebellum include a novel synaptotagmin and a hairless homolog. J Neurosci 16: 7832–7840.

Wallace ME and MacSwiney FJ, 1976. A major gene controlling warfarin-resistance in the house mouse. J Hyg (Lond) 76:173–181.

Received January 28, 2002
Accepted March 29, 2002

Corresponding Editor: Muriel T. Davisson