Insertional mutation of the hairless locus on mouse Chromosome 14

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Abstract. Crosses between heterozygous transgenic mice from line 5053 produced offspring with progressive irreversible hair loss beginning at day 19. With increasing age, the skin of these animals became thicker and plicated in appearance. Histological analysis revealed the complete absence of normal hair follicles and numerous intradermic cystic structures, which enlarged with time and became filled with keratinaceous material. Test crosses demonstrated that the affected animals are homozygous for the transgene insertion. The clinical and histological phenotype of the new mutant closely resembles that of the rhino allele at the hairless locus on Chromosome (Chr) 14. Complementation tests and linkage analysis indicate that the transgene has interrupted the hairless locus. It has been demonstrated previously that mutation at the *hr* locus is accompanied by a variety of immune deficiencies. Many of the older affected transgenic mice developed an impetigo-like skin eruption which responded to antibiotic ointment and which may reflect impaired immune function. The transgenic allele, $hr^{TgN5053Mm}$, will be useful for identification of the transcription unit of the hairless locus.

Introduction

Four independent, spontaneous alleles at the hairless locus on Chr 14 have been described (Green 1989). The first mutation, *hr*, was collected in a British aviary in 1926 (Brooke 1926). In homozygous *hr/hr* mice, the first hair coat grows normally for the first 14 days after birth. Progressive hair loss is initiated around the eyes and progresses caudally, resulting in a completely hairless condition (Crew and Mirskaia 1931). The mice also develop hyperkeratosis of the stratified epithelium and dedifferentiation of the hair canals, resulting in keratinaceous cysts. The *hr* mutation has been

shown to be due to integration of a C-type murine leukemia virus (Stoye et al. 1988). The phenotype of homozygotes for the rhino allele, hr^{rh}, is more severe, resulting in wrinkled skin due to extensive formation of intradermal cysts. Hair loss in these mice also begins above the eyes at about 14 days of age, followed by thinning of hair on the head and shoulders, and progressing to complete hair loss (Howard 1940). Both mutants also demonstrate defective responses to T-cell-dependent antigens and develop autoimmune disease (Takaoki and Kawaji 1980; Kawaji et al. 1980; Morrisey et al. 1980). Homozygotes for the bald allele, hr^{ba}, are intermediate in phenotype between *hr* and hr^{rh} (Garber 1952), while homozygotes for the near naked allele, Hr^n, are completely hairless throughout life (Stelzner 1983).

Mutations at the hairless locus provide a model for human conditions affecting follicular development (Sundberg et al. 1990). We report the generation of a new allele of hairless, $hr^{TgN5053Mm}$ (hr^{rg1}), induced by transgene insertion.

Materials and methods

Mice

Polymerase chain reaction

Transgenic mice were identified by polymerase chain reaction (PCR) amplification of genomic DNA isolated from tails, using prim-

ers complementary to chloramphenicol acetyl transferase (Keller et al. 1990). The Chr 14 marker D14S35 was typed with primers purchased from Research Genetics Inc. (Birmingham, Ala.).

Histology

Full-thickness skin, distal tail, spleen, and lymph nodes underlying skin erosions were fixed in 10% neutral buffered formalin overnight, embedded in paraffin, sectioned at 6 microns, and stained with hematoxylin and eosin (Luna 1968).

Southern blots

Genomic DNA was prepared from spleen by protease digestion and phenol/chloroform extraction. Restriction fragments were separated by electrophoresis on 1% agarose gels and transferred to nylon filters (Zetaprobe, BioRad Laboratories). Blots were hybridized and washed according to the manufacturer's instructions. Probes were labeled by random oligo-primed extension.

Results

Mutant phenotype

In order to identify insertional mutations, heterozygous transgenic mice were crossed, and their offspring were examined for novel phenotypes. In line 5053, the offspring were normal in appearance until 19 days of age, when a progressive irreversible hair loss was observed in some animals. Hair loss began on the head and shoulders and progressed caudally, resulting in complete hair loss by approximately one month of age. With increasing age the skin of affected individuals became thickened and extensively wrinkled (Fig. 1).
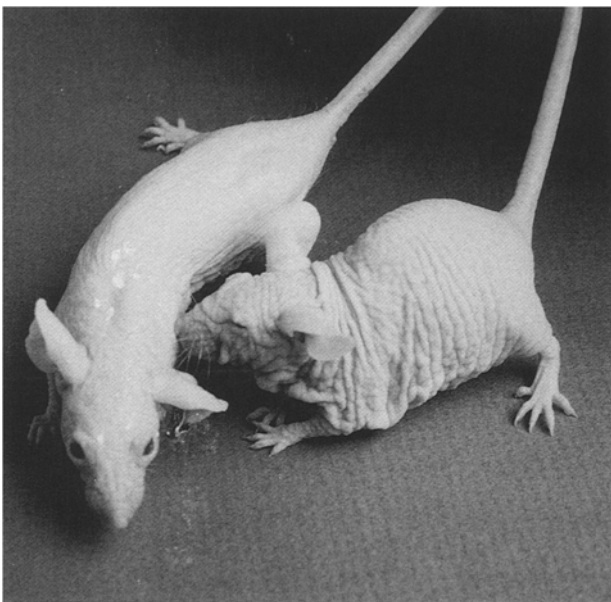
Fig. 1. Appearance of affected mice. The 3-month-old affected transgenic mouse on the right demonstrates the characteristic hair loss with severe thickening and wrinkling of the skin. The mouse at the left is homozygous for the *hr* allele of the hairless locus.

For comparison, a homozygous *hr/hr* animal with smooth skin is also shown.

Several older animals developed crusted erosions between the forelegs with underlying large subcutaneous nodules which on biopsy proved to be lymph nodes (see below). These erosions were accompanied by wasting, increased respiratory rate, and lethargy, all of which responded to daily topical applications of antibiotic ointment.

Skin histology

The skin of unaffected littermates of affected animals demonstrated numerous well-developed hair follicles (Fig. 2A,B). At one month of age, affected animals displayed numerous dilated cystic or cup-shaped structures located immediately below or opening onto the epidermal surface (Fig. 2C,D, open arrows). These structures, histologically reminiscent of comedones in acne, have been termed utriculi (Kligman and Kligman 1979). A smaller number of enclosed dermal cysts were also evident (Fig. 2D, solid arrows). With increasing age, the comedone-like utriculi remained relatively constant in size, while the dermal cysts grew progressively larger; examples are shown at 5 months (Fig. 2E,F) and at 9 months (Fig. 2G,H). Sebaceous glandular structures were found adjacent to both the utriculi and dermal cysts in younger (Figure 2D) and older animals (Figure 2F). Both types of structures contained lamellated keratinaceous debris (Fig. 2F,H).

Biopsies of the crusted erosions in two 9-month-old animals revealed a fibrinopurulent exudate overlying areas of massive cyst formation. Marked expansion of normal mature and immature members of the myeloid series were observed in blood smears and in bone marrow from tail biopsy, consistent with a reactive leukocytosis. No signs of lymphoma or leukemia were evident in the marrow, spleens, or peripheral blood. Enlarged subcutaneous lymph nodes with areas of necrosis and neutrophilic lymphadenitis were evident immediately underlying the crusted skin lesions (data not shown).

Structure of the transgene insert

Genomic DNA from transgenic mice was digested with several restriction endonucleases and probed with a 0.9-kb *Msp*I fragment corresponding to the T-antigen-derived portion of the transgene (Fig. 3). A single hybridizing fragment was observed with a variety of enzymes. None of the digests produced the 1.9-kb fragment predicted for a head-to-tail arrangement of transgene copies. The hybridizing fragments included a 3.8-kb *Pst*I fragment, a 3.0-kb *Pvu*II fragment, a 2.8-kb *Eco*RI fragment, and a 2.2-kb *Nco*I fragment (Fig. 3). The length of these fragments is twice the distance between the restriction site and the 3' end of the transgene, suggesting the presence of two copies of the transgene in a tail-to-tail configuration
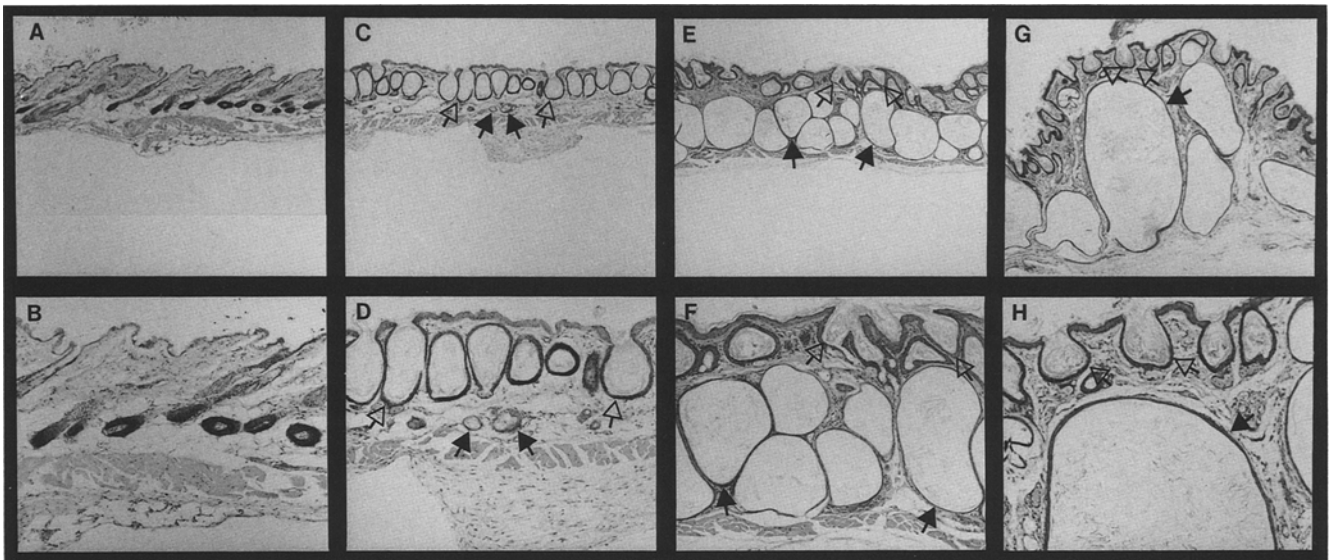
Fig. 2. Progressive degeneration of hair follicles in affected mice. Cross-section of dorsal skin. **A,B:** unaffected littermate at 2 months of age; **C,D:** affected animal at 1 month of age; **E,F:** affected animal at 5 months of age; **G,H:** affected animal at 9 months of age. Open arrows, comedone-like utriculi; solid arrows, enclosed dermal cysts. Sections were stained with hematoxylin and eosin.

(Fig. 3). The hybridization intensity is consistent with this two-copy model.

Autosomal recessive inheritance

In crosses between heterozygous transgenic mice, the frequency of affected offspring was 21% (25/118), consistent with autosomal recessive inheritance of the mutant phenotype. Three affected mice were test-crossed to strain C57BL6/J. The affected mice generated 9, 16, and 24 offspring, all of which carried the transgene. The 100% transmission rate for the transgene is consistent with homozygosity of affected individuals (p < 0.05) and supports the apparent autosomal recessive mode of inheritance of the hairless phenotype.

We have studied a number of transgenic lines carrying constructs similar to the one in this line (Osborn et al. 1988; Keller et al. 1990; Johnson et al. 1993). None of the other lines exhibit the hairless phenotype, indicating that it is related to the insertion site in line 5053.

Noncomplementation of hr

To test the ability of the transgene-induced mutation to complement a mutant allele at the hairless locus, two crosses between line 5053 and strain HRS/J-*hr* were carried out. In the cross (*hr/hr* × Tg/+), 40% of the offspring were transgenic (31/77). All of the transgenic mice exhibited the hairless phenotype, while the non-transgenic mice were unaffected. In the cross (Tg/Tg × *hr*/+), 56% of offspring were hairless (18/32). Hair loss began around the eyes at 13 days, 6 days earlier than in transgenic homozygotes. The results of these crosses demonstrate the inability of the transgenic
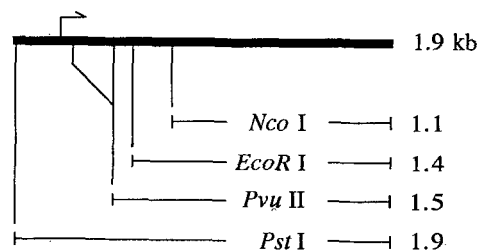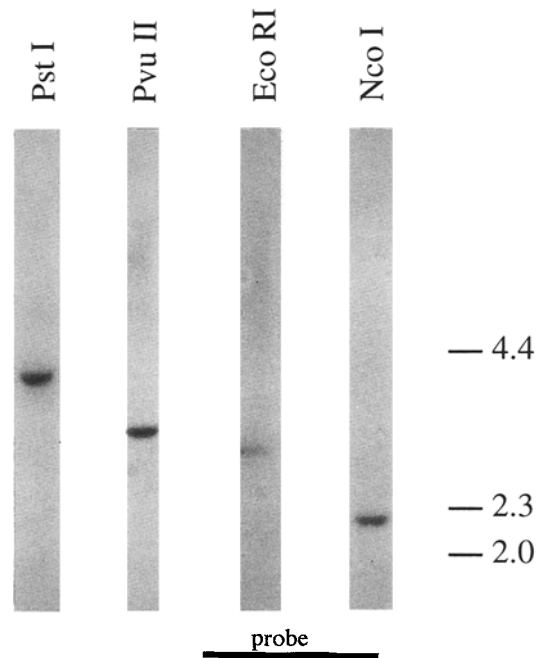
Fig. 3. Structure of the transgene insert. Genomic DNA was digested with a variety of restriction enzymes and hybridized with a transgene-specific probe. The positions of the corresponding restriction sites in the 1.9-kb transgene are shown at the bottom.
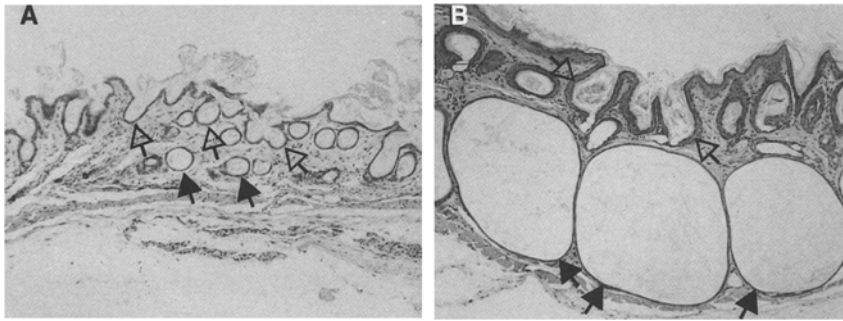
Fig. 4. Partial correction in the compound heterozygote. *Tg/hr*. (**A**) Dorsal skin from a Tg/hr mouse at 6 months of age. (**B**) Dorsal skin from *Tg/Tg* homozygote at 5 months of age. Note the reduced size of intradermal cysts (solid arrows) in the compound heterozygote.

chromosome to complement the *hr* allele, suggesting that the transgene has interrupted the hairless locus.

The level of wrinkling in the compound heterozygotes was intermediate between the transgenic homozygote and the *hr* homozygote. Consistent with this observation, there was a dramatic reduction in the size of the intradermal cysts in the compound heterozygotes (Fig. 4A, solid arrows) compared with the transgenic homozygotes (Figure 4B).

Linkage of the transgene insertion site to a Chr 14 marker

To test the predicted location of the transgene on Chr 14, we crossed transgenic mice with strain CAST/Ei. Transgenic F_1 mice were backcrossed to strain C57BL/6J. Fifty-three mice from the backcross generation were typed for the transgene and for the microsatellite marker *D14Mit35* on Chr 14 (GBASE, 1993). The length of fragments amplified with the *D14Mit35* primers was 226 bp in C57BL/6J, 222 bp in C3H/HeJ, and 244 bp in CAST/Ei. Recombination between *D14Mit35* and the transgene was observed in 5/52 chromosomes (Table 1), giving an estimated distance of 9.6 ± 4.1 cM between the transgene insertion site and *D14Mit35*. The linkage to *D14Mit35* is consistent with insertion of the transgene at the hairless locus.

The PCR product obtained from homozygous transgenic mice was 226 bp in length, suggesting that the transgene insertion site in the F_2 founder animal is derived from the C57BL/6J parent.

Discussion

The observed phenotype of the insertional mutant, the noncomplementation of *hr*, and the linkage to

Table 1. Linkage of the transgene insertion site in line 5053 with the D14Mit35 locus. Tg/+ mice were crossed with strain CAST/Ei, and transgenic offspring were backcrossed to strain C57BL/6J. Genomic DNA from the backcross generation was isolated from tails and typed by PCR for the transgene and for *D14Mit35*.

Genotypes		Number of offspring	Class
Transgene	D14Mit35		
+	BB[a]	24	parental
−	BC	23	parental
+	BC	2	recombinant
−	BB	3	recombinant

[a] B, C57BL/6J; C, CAST/Ei.

D14Mit35 indicate that the transgene in line 5053 has interrupted the hairless locus. The new mutant allele is designated $hr^{TgN5053Mm}$ (hr^{tg1}). The pattern of hair loss and extent of disruption of hair follicles in mice homozygous for hr^{tg1} closely resembles the description of the rhino allele, hr^{rh} (Mann 1971). The hr^{tg1} and hr^{rh} alleles result in more severe histological abnormalities than *hr*. The difference may be accounted for if the retroviral insertion in *hr* permits a low level of gene expression or production of a partially functional product, while the transgene insertion in hr^{tg1} results in complete inactivation of the gene. This interpretation is consistent with the intermediate cutaneous phenotype displayed by the compound heterozygotes hr^{rh}/hr (Mann 1971) and hr^{tg1}/hr (Fig. 4).

The phenotype in *hr/hr* and hr/hr^{rh} mice was shown to result from abnormal coalescence of the internal root sheath of the hair follicle around the terminal portion of the hair shaft during the first catagen phase of the hair cycle, causing the formation of abnormal club hairs (Mann, 1971). As a result, the lower portion of the external root sheath including the hair bulb becomes stranded in the dermis, rather than following the inner root sheath to its normal telogen position near the sebaceous duct. It is now appreciated that the area near the insertion of the arrector pili muscle contains slowly cycling cells which are likely to be the stem cells of the hair follicle (Cotsarelis et al. 1990). The physical separation of the hair bulb from this region in the mutant may be responsible for the failure of the follicles to enter the next anagen cycle. It is interesting that the abnormal structures in the mutant appear to have associated sebaceous gland structures (Fig. 2), because the epidermal stem cell of the hair follicle is thought to reside close to the sebaceous gland (Costarelis et al. 1990). Altered function of the *hr* gene product may be intimately involved with the regulation of hair stem cell function.

In addition to the obvious hair abnormalities, *hr/hr* mice of strain HRS/J display diminished T helper cell responses to alloantigens (Morrisey et al. 1980). The in vitro T-cell response to mitogens is normal in these animals, suggesting that the defect may reside at the level of antigen presentation or co-stimulatory cell function. Rhino mice also have a defective response to T-cell-dependent antigens (Takaoki, and Kawaji 1980) and develop autoimmune disease (Kawaji et al. 1980). The impetigo-like skin eruption in hr^{tg1}/hr^{tg1} mice, with lymphadenitis and reactive leukocytosis, could

be indicative of an immune abnormality. However, these changes could also reflect a secondary interference with skin immunity because of the massive accumulation of cysts in the affected areas. Analysis of T-cell function in these animals will be required to address this question.

The hairless allele described here is one of a growing number of insertional mutations in transgenic mice (reviewed in Meisler 1992). Recent examples include insertional mutation of micropthalmia on Chr 6 (Krakowsky et al. 1993) and a novel situs inversus locus on Chr 4 (Yokoyama et al. 1993). The reported frequency of mutations in transgenic mice is 3 to 5%. Many transgenic insertions, including the one described here, have interrupted genes for which spontaneous mutations had previously been described. The high proportion of re-mutation suggests that between one-third and one-half of the mouse genes capable of generating viable, visible phenotypes have already been identified (Meisler 1992).

References

Brooke, H.C. (1926). Hairless mice. J. Hered. 17, 173–174.

Cotsarelis, G., Sun, T.-T., Lavker, R.M. (1990). Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell 61, 1329–1337.

Crew, F.A.E., Mirskaia, L. (1931). The character "hairless" in the mouse. J. Genet. 25, 17–24.

Garber, E.D. (1952). "Bald," a second allele of hairless. J. Hered. 43, 45–46.

GBASE (1993). The on-line genomic database of the mouse maintained at The Jackson Laboratory by D.P. Doolittle, A.L. Hillyard, L.J. Maltais, J.N. Guidi, M.T. Davisson, T.H. Roderick.

Green, M.C. (1989). Catalog of mutant genes and polymorphic loci. In *Genetic Variants and Strains of the Laboratory Mouse*, 2nd ed., M.F. Lyon, A.G. Searle, eds. (New York: Oxford University Press), pp. 12–403.

Heiniger, H.J., Huebner, R.J., Meier, H. (1976). Effect of allelic substitutions at the hairless locus on endogenous ecotropic murine leukemia virus titers and leukemogenesis. J. Natl. Cancer Inst. 56, 1073–1074.

Howard, A. (1940). "Rhino", an allele of hairless in the house mouse. J. Hered. 31, 467–470.

Johnson, T.M., Rosenberg, M.P., Meisler, M.H. (1993). An insulin-responsive element in the pancreatic enhancer of the amylase gene. J. Biol. Chem. 268, 464–468.

Kawaji, H., Tsukuda, R., Nakaguchi, T. (1980). Immunopathology of rhino mouse, an autosomal recessive mutant with murine lupus-like disease. Acta Pathol. Jpn. 30, 515–530.

Keller, S.A., Liptay, S., Hajra, A., Meisler, M.H. (1990). Transgene-induced mutation of the murine steel locus. Proc. Natl. Acad. Sci. USA 87, 10019–10022.

Kligman, L.H., Kligman, A.M. (1979). The effect on rhino mouse skin of agents which influence keratinization and exfoliation. J. Invest. Dermatol. 73, 354–358.

Krakowsky, J.M., Boissy, R.E., Neumann, J.C., Lingrel, J.B. (1993). Insertional mutation of the mouse microopthalmia gene. Transgenic Res. 2, 14–20.

Luna, L.G. (1968). *Manual of Histological Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed. (New York: McGraw-Hill).

Mann, S.J. (1971). Hair loss and cyst formation in hairless and rhino mutant mice. Anat. Rec. 170, 485–499.

Meisler, M.H. (1992). Insertional mutation of 'classical' and novel genes in transgenic mice. Trends Genet. 8, 341–344.

Morrisey, P.J., Parkinson, D.R., Schwartz, R.S., Waksal, S.D. (1980). Immunologic abnormalities in HRS/J mice. I. Specific deficit in T lymphocyte helper function in a mutant mouse. J. Immunol. 125, 1558–1562.

Osborn, L., Rosenberg, M.P., Keller, S.A., Ting, C.-N., Meisler, M.H. (1988). Insulin response of a hybrid amylase/CAT gene in transgenic mice. J. Biol. Chem. 263, 16519–16522.

Stelzner, K.F. (1983). Four dominant autosomal mutations affecting skin and hair development in the mouse. J. Hered. 74, 193–196.

Stoye, J.P., Fenner, S., Greenoak, G.E., Moran, C., Coffin, J.M. (1988). Role of endogenous retroviruses as mutagens: the hairless mutation of mice. Cell 54, 383–391.

Sundberg J.P., Beamer, W.G., Shultz, L.D., Dunstan, R.W. (1990). Inherited mouse mutations as models of human adnexal, cornification, and papulosquamous dermatoses. J. Invest. Dermatol. 95, 62S–63S.

Takaoki, M., Kawaji, K. (1980). Impaired antibody response against T-dependent antigens in rhino mice. Immunology 40, 27–32.

Yokoyama, T., Copeland, N.G., Jenkins, N.A., Montgomery, C.A., Elder, F.F.B., Overbeek, P.A. (1993). Reversal of left-right asymmetry: a situs inversus mutation. Science 260, 679–682.