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# The Effects of Mutant Gene *hairless* in Chimeric Mice

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**Abstract**—The autosomal recessive gene *hairless* (*hr*) is responsible for the complete hairlessness in mice homozygous for this gene. Hair shedding that begins at the age of 10 days is caused by an abnormal cycle of hair follicle development disturbed at the catagen stage. This results in enhanced programmed cell death (apoptosis) and ultimately leads to the complete hair follicle destruction and shedding of all hairs by the age of three weeks. To study the phenotypic expression of the *hr* gene in a chimeric organism, we have obtained 12 chimeric mice *hr*/*hr* ⟷ +/+ by means of aggregation of early embryos *hr*/*hr* and +/+. In chimeric mice, the hair shedding has begun two days later than in the *hr*/*hr* mice. By day 23 of postnatal development, hairless areas were present on the coat of chimeric mice or the latter were completely hairless depending on the percentage of the *hr*/*hr* mutant component. In four chimeras with high content of the mutant component (68–76%), the hair shedding process was similar to that in the *hr*/*hr* mice, though it was accomplished two days later. In three chimeras with 48–51% of the mutant component, alternating hairless and hair-covered bands were observed. These data suggest that the *hr* gene acts in epidermal cells of a hair follicle, because epidermal cell clones in embryonic skin migrate in the lateral–ventral direction coherently and without mixing. However, some chimeras displayed a pattern which was not so clear-cut: the band borders were illegible and hairs partly covered the hairless areas. In some chimeras, the uniform thinning of the coat was observed. Analysis of the effects of the *hr* mutant gene in chimeric mice differing in the ratio between mutant (*hr*/*hr*) and normal (+/+) components in tissues suggests that the *hr* gene acts in the epidermal cells of the hair follicle. The interactions between cells have an essential effect on the mode and degree of the *hr* gene expression, which leads to distortion of the "ectodermal" coat pattern in chimeras.

## INTRODUCTION

Hair follicles consist of two major components, epidermal and mesenchymal ones. Mesenchymal dermal papilla is much smaller in size than the epidermal component though it plays an important informative role in hair growth and development.

Hair follicle represents a dynamic structure, which forms the hair through interaction of many regulatory and structural proteins synthesized in mesenchymal and epidermal parts. It is still unclear what are the exact nature and a succession of signal molecule effects that regulate the hair follicle cycle, its growth (anagen), regression (catagen), and resting (telogen) [1, 2].

The *hr* gene is one of the genes involved in the regulation of the hair follicle transformation. It was first identified in wild house mice in London. The effects of this gene were described by Brooke in 1926 [3] and later by other authors in detail. In homozygotes, the autosomal recessive gene *hr* causes hairlessness in mice. The *hr*/*hr* mice are indistinguishable from normal sibs until the age of 10 days, but 10 to 14 days after birth, they begin to shed their hair first from lower jaw and above the eyes and afterwards, the process progresses in the cranio-caudal direction to other parts of the body. Almost all hairs except for vibrissa and some hair on fingers and tail tip are lost during 10 days after the onset of the process. Afterward, near the age of six weeks, rare, thin and coiled hairs appear in the *hr*/*hr* mice and this process also progresses in the cranio-caudal direction. These hairs are also shed soon, and the mice remain hairless (except for vibrissa and rare thin hairs) throughout their life. In 14- to 15-day-old mice, hyperplasia and hyperkeratosis of epidermis and hair follicle openings begins. Human ortholog of *hairless*, which is responsible for complete hairlessness and nodular atrichia, has been identified. The other mutant alleles of this gene were also described in mice, as well as the *hairless* orthologs in other mammalian species [4].

The *hairless* mutation in mice is caused by insertion of the endogenous mouse leukemia virus into their genome. The *hr* gene controls synthesis of a protein that consists of 1182 amino acids, including the zinc-finger domain [5].

The wide spectrum of the *hr* gene pleiotropic effects is known. Expression of this gene was found in skin, cartilage, retina, inner ear, brain, colon, and developing teeth [6].

Panteleyev *et al.* [7] have shown that in mice homozygous for the *hr* gene, the proximal part of hair bulb undergoes a premature and massive apoptosis by the end of hair follicle morphogenesis (15th day after birth). This is accompanied by discoordinated cell proliferation in various parts of a hair follicle. The hair bulb and outer root sheath cells break up into individual

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clusters, which impaires the epidermal cell contacts with the dermal papilla cells. The latter form the groups of precursor cells of the dermal cyst, whereas the upper epithelial portion of a follicle is transformed into bundles. In cells of the dermal papilla, the synthesis of adhesive molecules is reduced.

Studying the *hr* mRNA localization by in situ hybridization in C57BL/6 mice has shown the *hr* gene expression in suprabasal cell layer of epidermis. However, no expression of this gene was detected in the basal layer and keratinocytes of high differentiation in the granular layer of epidermis. At the early stages of hair follicle development, *hr* mRNA was detected only in cells of the epidermal component. At the later stages of the hair follicle morphogenesis, the *hr* mRNA has been detected in matrix and inner root sheath, whereas in dermal papilla and outer root sheath, no *hr* mRNA was detectable [8].

Thus, in mice homozygous for the *hr* gene, a loss of the functional product of this locus (transcription zinc-finger factor) initiates severe disorders of catagen regulation deteriorating normal structure of a hair follicle and arresting its developmental cycle.

In experiments with skin grafting (the graft hairs were sheared before transplantation) from the *hr*/*hr* mice onto the back of their normal sibs at the age of three weeks, David [9] showed that three weeks after operation, the donor-type hairs grow on the graft and are shed afterwards. It remains unclear why the disorders of catagen regulation were reversible in these experiments with skin transplantation.

To study specific effects of the mutant genes, chimeric animals bearing two genetically different components are used [10]. The tissues of chimeric animals obtained due to aggregation of early mutant and normal animal embryos represent a mosaic of cells with two parental genotypes, the mutant and normal ones.

In this study we aimed to elucidate features of the phenotypic expression of *hr* in a chimeric organism.

## MATERIALS AND METHODS

The chimeras were obtained using the mutant mouse strains HRS/J and C57BL/6 (genotypes *hr*/*hr c*/*c A*/*A Gpi-1*$^{aa}$ and +/+ *C*/*C a*/*a Gpi-1*$^{bb}$, respectively) differing in pigmentation and glucosophosphate isomerase isomers (GPI). The albino mice HRS/J are characterized by an electrophoretically slow GPI pattern (GPI-1A), whereas C57BL/6 mice have black coat and electrophoretically rapid GPI pattern (GPI-1B). The mutant strain HRS/J was provided by A. M. Malashenko from the Research Laboratory of Experimental Biological Models, Russian Academy of Medical Sciences.

The eight-cell embryos *hr*/*hr*(HRS/J) ⟷ +/+(C57BL/6) (later: *hr*/*hr* ⟷ +/+) taken at a ratio of 1 : 1 were aggregated by the Mintz's method [11] in 0.01% solution of phytohemagglutinin P [12] after removing *zonae pellicidae* in a 0.5% pronase E solution (Serva, 8 DMC-U/mg) in Dulbecco phosphate–saline buffer. The aggregated morulas *hr*/*hr* ⟷ +/+ were cultivated for 1.5 days until the blastocyst stage at 37°C in a drop of modified Whitten medium [13, 14] under paraffin oil (Fluka) in a mixture of three gases (5% $O_2$; 5% $CO_2$; 90% $N_2$). Chimeric blastocysts were transplanted into the uterus of the pseudopregnant females on the third day of pseudopregnancy (the day of copulative plug formation was defined as the first day of pregnancy).

Chimeric mice were identified by mosaic coloration of their coat and GPI patterns in the kidney, liver, and brain.

Chimeric 60-day-old mice were killed by cervical vertebra dislocation and the percentage of their *hr*/*hr* mutant component was determined in kidney, liver, and brain using GPI isozyme electrophoresis on acetate–cellulose plates [15]. Afterwards, the electrophoregrams were stained, dried, and scanned on a densitometer unit of a Gilford spectrophotometer at 560-nm wave length in a quartz cuvette filled with mineral oil [16]. The quantitative ratio of normal and mutant components was determined from the resultant densitograms.

## RESULTS

After aggregation of 72 pairs of eight-cell morulas *hr*/*hr* and +/+, their cultivation until the blastocyst stage, and transplantation into the uterus of the pseudopregnant females, 22 mice were obtained, of which 12 were chimeras *hr*/*hr* ⟷ +/+ and the remaining 10 mice were one-component animals (6 *hr*/*hr* and 4 +/+).

Electrophoretic analysis of GPI isoenzymes revealed the mutant component *hr*/*hr* (GPI-1A) in the kidney, liver, and brain of all chimeras (table). The hairlessness directly depended on the content of the mutant component in the tissues: the higher was the percentage of the mutant components in the kidney, liver, and brain, the larger extent of hairlessness was observed in chimeric mice.

Normal coat (mixed agouti, black, and white hairs) developed in all chimeras until the age of 12 days. The pigmented areas looked like transverse bands and spots and comprise from 15 to 95% of the coat in chimeras. After 12 days, the hair shedding was observed in the cranio-caudal direction similarly to the *hr*/*hr* mice. In conformity with the content of the GPI-1A mutant component determined at the 60-day age by isozyme analysis, the coat of the chimeras varied from separate hairless areas to complete hairlessness by day 23 of postnatal development.

In chimeras 1, 2, 3, and 4, the content of the mutant component ranged on average from 68 to 76% (table). Until the age of 19 days, the main color of coat of the chimeric mice was white, whereas the pigmented areas (spots) on the dorsal and ventral body sides consisted of agouti and black hairs and occupied 15–35% of the

Percentage of the mutant component in the chimeric mice *hr*/*hr* ⟷ +/+ 1–12

| Tissue | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kidney | 73 | 75 | 54 | 83 | 67 | 52 | 62 | 42 | 49 | 49 | 8 | 5 |
| Liver | 80 | 76 | 83 | 70 | 45 | 54 | 53 | 55 | 44 | 48 | 37 | 8 |
| Brain | 76 | 68 | 72 | 52 | 50 | 47 | 36 | 53 | 55 | 46 | 27 | 33 |

Note: GPI-1A (*hr*/*hr*), mutant component; GPI-1B (+/+), normal component.

total coat. By day 23 of postnatal development, complete hairlessness was observed in all chimeric mice and developed in the cranio-caudal direction like in the *hr*/*hr* homozygotes, though the onset and completion of it occurred two days later (Fig. 1).

In chimeras 5–10, the content of the mutant component ranged from 48 to 54% (table). Their pigmented areas looked like transverse bands and separate areas comprising 40 to 85% of the total coat. The main color of the coat was dark gray. The nonpigmented areas were on both dorsal and ventral body sides. By day 23 of their life, the coat thinning was observed in all chimeras; hairless areas with clear-cut borders were present on the dorsal and ventral body sides. In addition, in chimeras 6, 9, and 10, the alternating transverse hairless and hair-covered bands remained until day 60 of their life, i.e., until sacrificing them (Fig. 2). The hair-covered bands contained white, agouti, and black hairs at different ratios.

Chimeric animals 11 and 12 with the least content of the mutant component, from 15 to 24% (table), resembled the normal C57BL/6 mice in their phenotype, because the main color of their coat was black. The chimera 11 had small nonpigmented areas behind the ears, on scapula, and ventral body side, which occupied about 15% of total body (Fig. 3). By day 23, this animal had two clear-cut areas with thinned coat on the dorsal side and two hairless areas on the ventral body side. In chimera 12, a rather large hairless area (~1 cm$^2$) appeared on the caudal region of the back by day 23 of life.

## DISCUSSION

The hair follicle cycle is a repeated morphogenetic process that occurs throughout the life span in mammals. The cycling of this process depends on a complex interactions of the mesodermal and epidermal components of a hair follicle [1, 2, 17].

According to Potter *et al.* [18], the functional product of the *hr* gene represents a corepressor for the thyreoid hormone receptor, which might account for a wide spectrum of pleiotropic effects of the *hr* gene [6].

Panteleyev *et al.* [7] suggested that the normal product of the *hr* locus, which regulates the earliest events associated with normal regression of a hair follicle, is responsible for a balance between proliferation and apoptosis. The lack of the normal gene product of this locus results in unregulated increase in apoptosis within a hair follicle. The authors believe that the *hr* gene locus is not involved in the initiation of hair follicle morphogenesis, because no expression of the *hr* gene was observed at the early stages of the cycle [8].

As shown by hybridization in situ, the *hr* gene is expressed in epidermal cells of a hair follicle [8]. If the *hr* gene effects were confined to only this cell system, the chimeras would have clear-cut alternating hairless and hair-covered bands, like in case of the other mutant genes expressed in epidermal cells of the hair follicles [19, 20]. The alternating bands were indeed observed in some chimeras (6, 9, and 10), which suggests the *hr* gene expression in epidermal cells of hair follicles. However, the other chimeric mice lacked this clear-cut pattern; their hair-covered bands were often interrupted by large hairless areas.

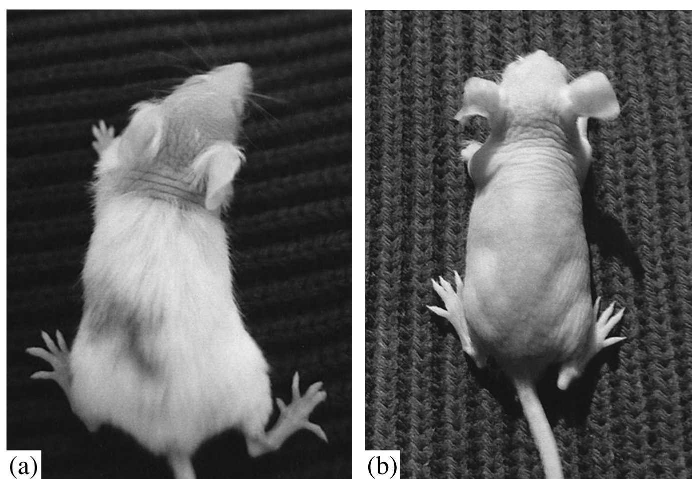


**Fig. 1.** Chimeric mouse 1 (76% of the mutant component) aged 17 days age (a) and 23 days (b).

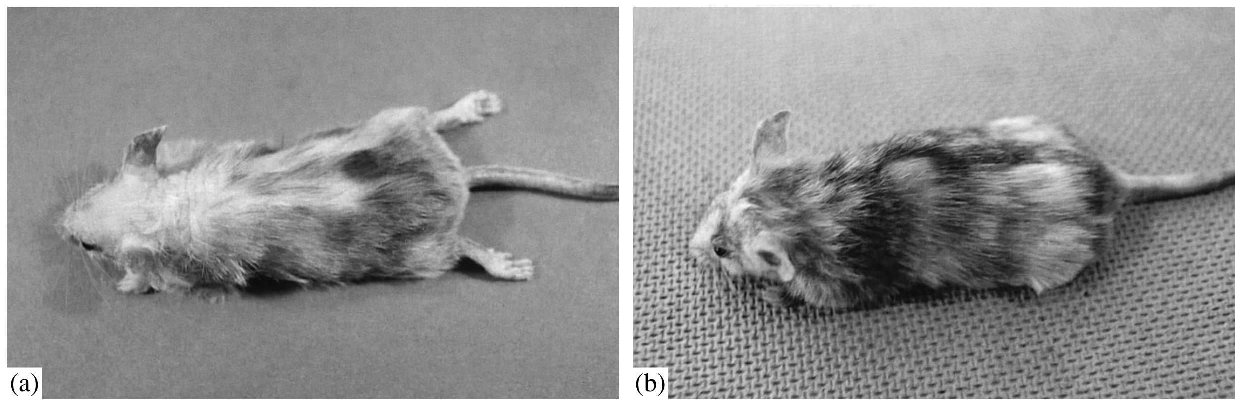

**Fig. 2.** Chimeric mouse 6 (51% of the mutant component) (a) and chimeric mouse 10 (48% of the mutant component) (b) aged 60 days. The alternating hairless and hair-covered transverse bands are observed on the dorsal body region.

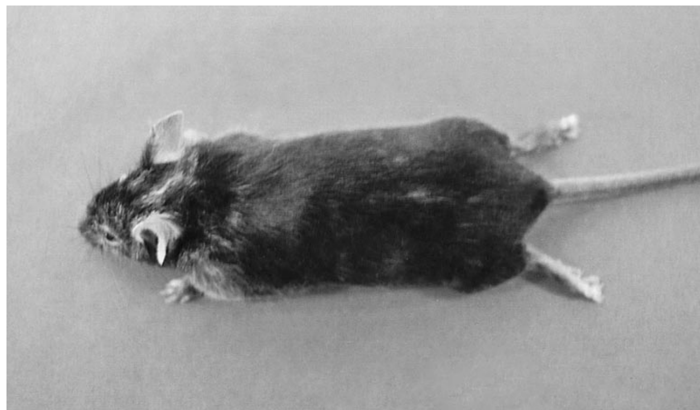

**Fig. 3.** Chimeric mouse 11 (24% of the mutant component) aged 60 days.

The hair formation in the first generation of mutant *hr*/*hr* mice suggest that both mesodermal and epidermal cells of these animals can form normal hair follicles. However, at the stage of catagen during the first generation, the epidermal cells of the *hr*/*hr* hair follicles undergo strong apoptosis, which leads to destruction and degeneration of the transformed follicles. As a result, the first-generation hairs are shed but no normal hair follicles of the second generation are formed.

The results of this study suggest interactions between the epidermal cells of normal and mutant genotypes. At the high content of the mutant component in chimeras 1, 2, 3, and 4 (68–76%), complete hairlessness was observed. Like in *hr*/*hr* mice, hairlessness progressed in cranio-caudal direction but it begun and terminated in chimeras two days later. This testifies to the fact that signals from epidermal cells of the mutant phenotype induce intense apoptosis in the epidermal cells of normal +/+ hair follicles. Nevertheless, the effect of normal follicles (+/+) on mutant (*hr*/*hr*) epidermal cells may account for a two-day delay of hairlessness in chimeras of this group as compared to the *hr*/*hr* mice. A delay may be also caused by a transient attenuation of the apoptosis-inducing signals from the mutant to normal epidermal cells in a mixed population of hair follicles. The apoptosis-inducing signals seems to reach the required intensity two days later than in homozygotes for the *hr* gene, and when it occurs, an intense epidermal cell death begins in hair follicles of this group of chimeras.

In chimeras 11 and 12 with low content of the mutant component (15–24%), small hairless areas appeared in various regions of the coat suggesting that even a small number of the mutant hair follicles may develop within a large population of normal follicles.

In three chimeras (6, 9, and 10) with approximately the same ratio of mutant and normal components (48–54%), the hairless and hair-covered transverse bands with clear-cut border alternated. In other three chimeras of the same group (5, 7, and 8), the borders of the hair-covered bands were illegible; they were interrupted by hairless areas; on the dorsal and ventral body regions, the hairless areas with clear-cut borders were also observed.

Analysis of the results obtained suggest that in chimeric mice *hr*/*hr* ⟷ +/+, the mutant *hr* gene acts in

epidermal cells of the hair follicles. Intercellular interactions have an essential effect on the mode and degree of the *hr* gene expression, which disrupts the "ectodermal" coat pattern in chimeras.

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