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# Phenotypic expression of the fused (*Fu*) gene in chimaeric mice

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**Summary**

The dominant gene Fused (*Fu*) produces skeletal abnormalities during embryonic development. It was previously shown that C57BL/6 mice contain a suppressor of *Fu*, which acts after fertilization. Chimaeras were used to study whether this gene would suppress the *Fu* phenotype after the 8-cell stage of embryo development. We found no effect of the suppressor gene on *Fu* phenotype (its degree and frequency of expression) in chimaeric mice. We conclude that either the suppressor gene from C57BL/6 mice can only influence *Fu* expression at the intracellular level or *Fu* expression is determined before the 8-cell embryonic stage.

## 1. Introduction

Chimaeric animals help us to understand the mechanisms of gene cooperation and the patterns of phenotypic development (McLaren, 1976). In the present work we used chimaeric mice to study the phenotypic expression of the Fused (*Fu*) gene. The semidominant gene *Fu* is located on mouse chromosome 17. It produces shortened and kinked tail in both homozygotes and heterozygotes (Reed, 1937; Dunn & Gluecksohn-Waelsch, 1954). Both the frequency and the degree of Fused expression vary strongly. Some of the most frequent expressions of Fused consist in various types of asymmetrical fusions of vertebrae, lack of the whole tail or a part of it, and ribs fused at their proximal ends but divided into component ribs at any place along their length (Reed, 1937).

The *Fu* penetrance is dependent on the genetic background (Reed, 1937; Ruvinsky & Agulnik, 1990) but modifiers do not determine the degree of its expression (Reed, 1937). *Fu* is subject to gametic imprinting (Ruvinsky & Agulnik, 1990) and it is prone to inherited inactivation at low frequency (Belyaev *et al.* 1979).

It was previously shown that C57BL/6 mice contain a suppressor gene sharply lowering the frequency of *Fu* expression in $F_1$ hybrids. This suppressor gene influenced *Fu* expression after fertilization (Ruvinsky & Agulnik, 1990). The question was whether this suppressor gene could affect *Fu* expression after the 8-cell stage of embryonic development or only before this stage.

## 2. Material and methods

The mouse strains were as follows: DD/HeIcgn (hereafter DD), albino (*c*, *c*); C57BL/6JYIcgn (B6), black; TFN, homozygous for tufted (*tf*/*tf*), black, showing characteristic baldness from the age of 6 wk; *Rb1 Fu tf*/*Rb1 Fu tf* (homozygous for *Fu*, *tf* and *Rb1* (Rb(8,17)1Iem) from our own stock, and also albino (*c*, *c*).

Hormones, 5 i.u. PMSG (Pokrovsky Plant of Biopreparations, Russia) and 48 h later 5 i.u. HCG (Moscow Plant of Endocrine Preparations, Russia) were administered to induce superovulation in females both in control and in experimental crosses.

To obtain 8-cell embryos for the albino component of chimaeras, we used ♀♀ +/+ (DD) × ♂♂ *Rb1 Fu tf*/*Rb1 Fu tf* cross. The same cross was used as a control.

Embryos from this cross were aggregated 2 d after mating in pairs with 8-cell embryos from B6 × B6 matings, and cultured under standard conditions overnight according to described procedures (Mintz, 1971*a*). Chimaeric blastocysts were implanted into foster mothers made pseudopregnant by mating with sterile males. The percentage of coat colour

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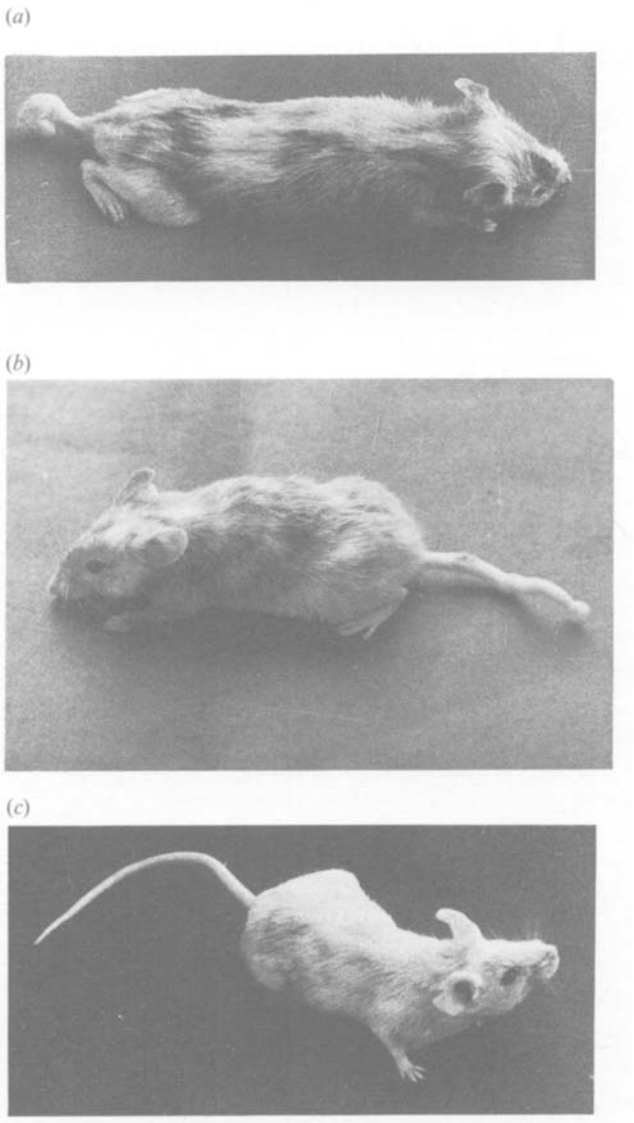

Fig. 1. Chimaeric mouse *Rb1 Fu tf*/+ + + ↔ +/+ (B6). (*a*) With the strong *Fu* phenotype. (*b*) With the mid *Fu* phenotype. (*c*) With the Normal tail.

chimaerism and the Fused phenotype in mice were identified visually. One-colour mice were considered to be single-component. According to above crosses, albino single-component mice and all cells of albino component in chimaeras were of *Rb1 Fu tf*/+ + + genotype; black single-component mice and all cells of black component in chimaeras were of +/+ genotype. The degree of Fu expression was scored as strong (mice lacked more than half the tail, which was kinked very strongly – Fig. 1 *a*), mid (mice had about half the normal tail length and more than two places of vertebral fusion along the tail – Fig. 1 *b*) or weak (mice had tails of almost normal length and only one or two places of vertebral fusion along the tail). Chimaeras and single-component mice with normal tails were scored as mice with Normal phenotype (Fig. 1 *c*). Karyotype analysis of one chimaeric male (No. 39) was carried out on bone marrow biopsy (Udalova, 1971). Air-dried chromosome preparations were C-band stained (Sumner, 1972). Student's *t*-test was used (Pearson & Hartley, 1956).

## 3. Results

Twenty-six animals were produced in the experiments. Of these, 20 were true chimaeras, with chimaerism percentage ranging from 1 to 95%, and six mice were single-component: 5 albino (*Rb1 Fu tf*/+ + +) and 1 black (+/+) (Table 1).

### (i) *The frequency of Fused expression in chimaeras*

*Fu* was expressed in 13 out of 20 chimaeric mice. The frequency of the *Fu* phenotype (the *Fu* penetrance) in chimaeras was slightly higher but did not differ significantly from the *Fu* penetrance in the control cross (Table 2).

No interdependence between the percentage of albino (or black) component and the *Fu* penetrance was found in chimaeras (Table 1). The *Fu* phenotype was registered in mice which were almost entirely black (nos. 11, 26, 39, 22). We analysed the bone marrow karyotype and germ-line genotype of the blackest chimaeric male, No. 39. Only about 1% of his coat melanocytes were unpigmented and, therefore, had genotype *Rb1 Fu tf*/+ + + (Fig. 2). Karyotyping of this male showed that only five among 294 metaphase plates contained the chromosome with *Rb1* used as the cytological marker of albino (*Fu*)-component cells. Both cell types (with *Rb1* and without *Rb1*) contained the Y chromosome. Consequently, the bone marrow of this chimaera consisted of 1·5–2% cells with *Rb1 Fu tf*/+ + + genotype. The genotype of this male was XY/XY. Mated with females of the TFN strain, it produced 30 offspring, all black. Therefore, germinative epithelium in the gonads of this male consisted mainly of cells derived from B6. These data showed that bone marrow, germ-line and coat of the chimaeric male No. 39 had the same percentage of mutant cells (1–2% of *Rb1 Fu tf*/+ + + cells). This very low percentage of the *Fu*-component was sufficient for the Fused phenotypic expression.

### (ii) *The degree of Fused expression in chimaeras*

A variable degree of phenotypic expression (the expressivity) is typical for the Fused gene (Reed, 1937; Dunn & Gluecksohn-Waelsch, 1954). In our experiment the expressivity of the *Fu* gene also varied from weak Fused to strong Fused. We found no correlation between the expressivity of the *Fu* gene and the percentage of white (or black) component in chimaeras (Table 1).

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Table 1. *Phenotypes of chimaeras and single-component mice*

| Males | | | Females | | |
|---|---|---|---|---|---|
| Mouse no. | % of albino colour | *Fu* phenotype | Mouse no. | % of albino colour | *Fu* phenotype |
| 27* | 100 | *Fu* strong | 30* | 100 | Normal |
| 28 | 95 | *Fu* mid | 31* | 100 | Normal |
| 24 | 90 | Normal | 36* | 100 | Normal |
| 17 | 70 | *Fu* strong | 37* | 100 | *Fu* weak |
| 16 | 50 | *Fu* strong | 12 | 95 | *Fu* mid |
| 21 | 50 | *Fu* mid | 13 | 90 | Normal |
| 10 | 50 | *Fu* mid | 23 | 90 | Normal |
| 42 | 30 | *Fu* mid | 41 | 60 | *Fu* weak |
| 11 | 15 | Normal | 25 | 50 | Normal |
| 26 | 5 | *Fu* mid | 29 | 50 | *Fu* strong |
| 39 | 1 | *Fu* mid | 14 | 50 | *Fu* mid |
| | | | 22 | 10 | *Fu* strong |
| | | | 15 | 10 | Normal |
| | | | 16 | 5 | Normal |
| | | | 40** | 0 | Normal |

Single-component mice: * albino (*Rb1 Fu tf*/+ + + genotype); ** black (+/+ genotype).

Table 2. *The Fu penetrance in control cross and in chimaeras*

| | Offspring phenotype | | *Fu* penetrance (%) |
|---|---|---|---|
| | *Fu* | Normal | |
| Control cross | | | |
| ♀♀ DD × ♂♂ $\frac{Rb1\ Fu\ tf}{Rb1\ Fu\ tf}$ | 100 | 87 | 53·5 |
| Chimaeras | | | |
| $\left(\text{♀♀ DD} \times \text{♂♂} \frac{Rb1\ Fu\ tf}{Rb1\ Fu\ tf}\right) \leftrightarrow$ B6 | 13 | 7 | 65·0* |

* The *Fu* penetrance does not differ significantly in control cross and in chimaeras ($t_a = 1{\cdot}1$; $P > 0{\cdot}05$).

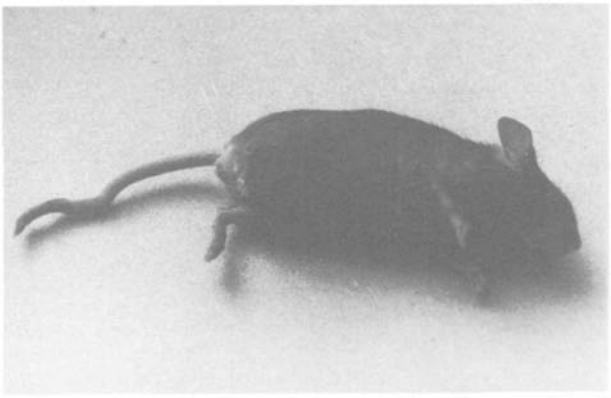

Fig. 2. Chimaeric male, No. 39 (genotype *Rb1 Fu tf*/+ + + ↔ +/+ (B6)).

## 4. Discussion

The previous study demonstrated that B6 mice contained the dominant suppressor gene and it decreased the penetrance of *Fu* in $F_1$ hybrids (Agulnik & Ruvinsky, 1990). However, in chimaeric mice containing the B6 suppressor gene, we found no decrease of *Fu* penetrance.

Some chimaeric mice with 90% of albino cells in their coat had normal tails. In our experiment the penetrance of the *Fu* gene in control crosses and in the group of chimaeric mice did not differ significantly. Apparently, chimaeras with normal tails and a high proportion of albino cells in their coat appeared due to incomplete penetrance of *Fu* rather than to the effect of the B6 suppressor gene.

We found that some almost entirely black chimaeras had the *Fu* phenotype. The blackest one (No. 39) had only 1–2% of mutant cells in its coat, bone marrow and germinative epithelium. A thorough mixing of cells during development of the chimaeric embryo (Mystkowska *et al.* 1979) often leads to similarities in the quantitative composition of various tissues in



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chimaeric mice (Wegmann & Gilman, 1970; Mystkowska *et al.* 1979). Thus, we may suppose the same percentage (1–2%) of mutant cells was present in the vertebral column of this chimaera (No. 39) but not the predominance of these mutant cells. This fact suggests that the *Fu* gene may impose its phenotype in the normal cells, as it was found in chimaeras involving semi-dominant spotting mutants (Mintz, 1971*b*; Stephenson, Glenister & Hornby, 1985). In the cases cited, non-mutant melanocytes behave as though they carried the spotting gene and chimaeras displayed phenotypes identical to heterozygotes.

The absence of correlation between the percentage of the mutant cells and the *Fu* phenotype and the facts mentioned above suggest that the B6 suppressor gene is unable to affect *Fu* phenotypic expression in (♀♀ + / + (DD) × ♂♂ *Rb1 Fu tf*/ *Rb1 Fu tf*) < —— > B6 chimaeras.

It is well known that genes which cause morphological defects can display different patterns of interaction in chimaeras. These may produce normal (Mintz, 1964; Bennett, 1978; Malinina *et al.* 1984) or abnormal phenotype (McLaren & Bowman, 1969; Kupriyanov & Konyukhov, 1984; Kindyakov & Konyukhov, 1986) or may have a definite quantitative effect on the development of the skeletal characteristics (Forsthoefel *et al.* 1983). The distribution of the two cell types in chimaeras is also important. An analysis of skeletal development in chimaeric mice C57BL/6 ↔ C3H revealed a tendency for C57BL/6 skeletal morphology to be in the head region, with C3H components being localized in the lumbosacral region (Moore & Mintz, 1972). On this basis, we would expect the same character of C57BL/6 cell distribution in our experiment.

In conclusion, we can suggest two possible explanations of the absence of interaction between *Fu* and its suppressor in chimaeras: (1) Fused expression is determined before the 8-cell stage of embryo development and cannot be modified by a suppressor gene acting after this stage; (2) peculiarities of the interaction or distribution of the two cell types prevent the B6 suppressor gene having any effect in the chimaeras.

The degree of the *Fu* phenotypic expression has been studied in some previous works (Reed, 1937; Dunn & Gluecksohn-Waelsch, 1954) but the reasons for its variety were not explained. The fact that *Fu* expressivity varied independently of the relative numbers of *Rb1 Fu tf*/ + + + and B6 cells present in the chimaera suggests that the degree of the *Fu* phenotypic expression was not affected by the B6 suppressor gene component in chimaeras. Consequently, neither intracellular interaction with normal alleles (Reed, 1937; Dunn & Gluecksohn-Waelsch, 1954) nor intercellular interaction with normal genotype in chimaeras were able to affect the degree of the *Fu* phenotypic expression.

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