Melanization in albino mice transformed by introducing cloned mouse tyrosinase gene

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Summary

We introduced a mouse tyrosinase minigene, mg-Tyrs-J, in which the authentic genomic 5' non-coding flanking sequence was fused to a mouse tyrosinase cDNA, into fertilized eggs of albino mice.

Of the 25 animals that developed from the injected eggs, four mice exhibited pigmented hair and eyes. Histological analysis of the transgenic mice revealed that the melanogenesis was restricted to hair bulbs and eyes.

These results suggest that this minigene encodes active tyrosinase protein and that its 5' flanking region contains the sequences regulating expression of mouse tyrosinase gene. This is the first report of a successful expression of tyrosinase gene and of pigment production in transgenic mice.

Key words: Transgenic mouse, albino, tyrosinase gene, melanization.

Introduction

Tyrosinase (EC 1.14.18.1) is the key enzyme in the production of melanin. The enzyme catalyzes both the oxidation of tyrosine to L-3,4-dihydroxyphenylalanine (L-dopa) and the oxidation of L-dopa to dopaquinone. The structural gene for tyrosinase has been considered to be localized at the c-locus on chromosome 7 in the mouse (Silvers, 1979; Halaban et al. 1988; Muller et al. 1988).

Recently, several cDNA clones for mouse tyrosinase have been isolated independently (Yamamoto et al. 1987, 1989; Kwon et al. 1988; Muller et al. 1988). Amongst those cDNA clones, there are several differences in the deduced amino acid sequences. Muller et al. (1988) reported that one of their cDNA clones, pmcTyr1, possessed the coding capacity for tyrosinase. They demonstrated that pmcTyr1 inserted in the expression vector expressed the enzyme activity in cultured human amelanotic melanoma cells and in cultured human breast cancer cells, although they did not report any pigment production. On the other hand, Yamamoto et al. (1989) constructed mouse tyrosinase minigene, mg-Tyrs-J, in which one of their cDNA, Tyrs-J, was ligated with the authentic genomic 5' non-coding flanking sequence. They then transfected cultured albino melanocytes with the construct. They found that mg-Tyrs-J expressed and directed the production of melanin pigments in the albino melanocytes. Thus it is possible that mg-Tyrs-J contains regulatory elements for expression of mouse tyrosinase gene.

In the present study, we microinjected mg-Tyrs-J into fertilized eggs of albino BALB/c mice and showed that transgenic mice produced melanin pigments. Our result verifies that the mg-Tyrs-J encodes functional tyrosinase protein and contains the regulatory elements of mouse tyrosinase gene. Our result also indicates that mg-Tyrs-J can be a useful marker gene in cotransgenic experiments.

Materials and methods

Preparation of mg-Tyrs-J

Mouse tyrosinase minigene, mg-Tyrs-J, was constructed as described in Yamamoto et al. (1989): the 5' non-coding region of the genomic DNA clone G3L was fused to the cDNA clone Tyrs-J at the XhoI site in the first exon (Fig. 1). The resultant 4.5 kb recombinant DNA contained 2.6 kb 5' non-coding flanking sequence, 1.6 kb coding region, 0.3 kb 3' non-coding flanking sequence and no introns. Then mg-Tyrs-J was cloned into the EcoRI site of a plasmid pUC118. For microinjection, the mg-Tyrs-J insert was isolated free from vector sequences by EcoRI digestion.

Generation of transgenic mice

Transgenic mice were generated by pronuclear microinjection (Hogan et al. 1986). Fertilized eggs of albino BALB/c mice were prepared by in vitro fertilization. The pronuclei were microinjected with approximately 2 pl of 10 mM-Tris-HCl (pH7.5)/0.1 mM-EDTA containing the mg-Tyrs-J DNA at 2 μg ml⁻¹.

The eggs that survived microinjection were transferred into the oviducts of recipient pseudopregnant albino Jcl: MCH (ICR) (CLEA, Japan) females and allowed to develop to term. In some cases, pups were delivered by Caesarian section.
DNA analysis

Tissues of the transgenic mice were frozen in liquid nitrogen and stored at -80°C. DNA extraction and Southern blot analysis were carried out according to Maniatis et al. (1982). Genomic DNA samples were digested with XhoI and PstI. Then 5 μg of digested DNAs were electrophoresed and Southern blotted. The DNA blots were hybridized overnight at 60°C to 32P-labelled probe. As the probe, 1.0 kb XhoI-SphI fragment of mg-Tyrs-J was used. The radioactive probe was prepared by using a Random Primer DNA Labeling Kit (Takara, Japan).

Histology

Tissues were fixed in periodate-lysine-paraformaldehyde (PLP) fixative (McLean and Nakane, 1974) for 15 h at 4°C. They were then soaked for 20 h in 0.1M-sodium phosphate buffer (pH 7.2) prior to dehydration. The dehydration was performed through a graded series of ethanol before being embedded in paraffin (MERCK). 8-10 μm serial sections of the organs were stained with eosin.

Results and Discussion

A 4.5 kb mouse tyrosinase minigene, mg-Tyrs-J (Fig. 1), comprising the authentic genomic 5′ non-coding flanking sequence fused to the cDNA clone, was microinjected into 304 fertilized albino BALB/c mouse eggs. It has been shown that the mg-Tyrs-J expresses and produces melanin pigments in the cultured albino mouse melanocytes (Yamamoto et al. 1989). The BALB/c mice are known to possess both amelanotic melanocytes and normal melanosomes (Rittenhouse, 1968). In addition, it has been indicated that an inactive tyrosinase protein is present in the skin homogenates of BALB/c mice (Takeuchi et al. 1984) and in cultured BALB/c melanocytes (Halaban et al. 1988). Therefore, it was expected that active tyrosinase protein would be produced in the melanocytes of BALB/c mice if the mg-Tyrs-J is tissue-specifically expressed and that melanin pigments would be deposited in melanosomes in melanocytes. On the contrary, if the mg-Tyrs-J is expressed either constitutively or in a non-tissue-specific manner in the transgenic mice, the percentage of transgenic pups generated with the mg-Tyrs-J might decrease because of the cytotoxicity of the intermediate products of melanin pigment (Paweleck and Lerner, 1978).

Of the 142 embryos transferred to recipient females, 25 embryos developed to term. Among them, four individuals produced melanin pigments. These numbers are comparable with the percentages of transgenic mice reported by other investigators. It seems to suggest that the expression of the mg-Tyrs-J is regulated as expected.

The four transgenic mice exhibited the agouti phenotype, though the extent of the pigmentation was different (Table 1). Tg.Tyrs-J 2 was black eyed and grew brown agouti hairs (Fig. 2). Since BALB/c mice carry A/A, bfb, c/c genotype, the phenotype of Tg.Tyrs-J 2 was as expected. Both Tg.Tyrs-J 4 and Tg.Tyrs-J 5 had reduced brown agouti hairs. Tg.Tyrs-J 5 had black eyes, while the eye color of Tg.Tyrs-J 4 was diluted just like that of the pink-eyed dilution mutant. On the other hand, Tg.Tyrs-J 3 exhibited an unexpected phenotype. There was a spot of pigmented hairs on its head, and pigmentation was also observed in the region of its right ear and right eye.

Genomic DNA analysis of Tg.Tyrs-J 2 showed that the mg-Tyrs-J was present in all tissues examined (Fig. 3A). With the probe of XhoI-SphI fragment of Table 1.

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Fig. 1. Construction of the mg-Tyrs-J. The 2.6 kb EcoRI-XhoI fragment of the G3L was fused to the 1.9 kb XhoI-EcoRI fragment of the Tyrs-J. The coding region of the mouse tyrosinase is indicated by solid box.
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Fig. 2. mg-Tyrs-J expression in transgenic Tg.Tyrs-J 2. Left, Tg.Tyrs-J 2 exhibiting black eyes and brown agouti coat color. Right, one of the non-transgenic litter mates of the Tg.Tyrs-J 2. No differences in phenotypes between the transgenic mouse and the control except for the melanization in eyes and hairs. Photograph was taken at 6 weeks of age.

the mg-Tyrs-J (Fig. 3C), band of 7.6 kb was detected in the BALB/c DNA sample (Fig. 3A, lane 1). This band represents the intrinsic tyrosinase gene of albino allele. In the transgenic DNA samples, the transgene was seen as the band of 1.4 kb as expected. In addition, there was a band of 5.2 kb in the transgenic DNA samples (Fig. 3A). One interpretation of these bands is that the mg-Tyrs-J had been integrated into at least two sites of genomic DNA of the hosts and then the rearrangement of the transgene occurred at one site. The presence of extra bands detected in the skin and tail from the transgenic mouse cannot be explained at present.

In order to verify the tissue-specific distribution of the melanin pigments in the transgenic mouse, histological examinations were performed on various organs of Tg.Tyrs-J 2. Melanin pigments were observed only in hair bulbs, hair shafts, choroid and pigment epithelium (Fig. 4A,C). No pigmentation was observed in other organs and tissues. It seems that the mg-Tyrs-J contains the regulatory elements of the mouse tyrosinase gene for expression in melanocytes.

There are variations in the coat and eye color among all transgenic mice generated in our experiments (Table 1). It is not yet known why such differences in the expression arose even when the mg-Tyrs-J DNA was

Fig. 3. Southern analysis of the DNAs from transgenic mice. (A) Genomic DNA analysis of Tg.Tyrs-J 2. Lane 1, placenta DNA of control mouse; lane 2–8, DNAs from Tg.Tyrs-J 2; lane 9, mg-Tyrs-J. DNA sources of lane 2–8 were as follows: lane 2, brain; lane 3, lung; lane 4, liver; lane 5, kidney; lane 6, intestine; lane 7, skin; lane 8, tail. (B) Lane 1, placenta DNA of Tg.Tyrs-J 3. Lane 2 and 3, placenta DNAs of the non-transgenic litter mates of the Tg.Tyrs-J 3. The size of the molecular markers and the hybridizing bands are indicated in kb. (C) A restriction map of the mg-Tyrs-J. The probe used is indicated below the map by solid line.
integrated into chromosomes of all transgenic mice. One possibility is that the differences resulted from the different levels of the tyrosinase activity in each transgenic mouse, probably because of a deletion of a part of the regulatory elements. An alternative possibility is that these variations are due to a position effect. However, the case of Tg.Tyrs-J 3 is more complicated. Tg.Tyrs-J 3 had a spot of pigmented hairs. Southern hybridization analysis of the genomic DNA isolated from the placenta of Tg.Tyrs-J 3 revealed the transgene in placenta DNA (Fig. 3B), which suggests that the integration of the transgene occurred at an early stage of development. In addition, we have obtained a progeny of Tg.Tyrs-J 3, which also exhibited patches of pigmented hairs. Thus it is unlikely that the different phenotypes of Tg.Tyrs-J 3 were the result of cellular mosaicism. Although further analysis is needed to elucidate the phenotypes of transgenic mice, the result obtained should provide a valuable insight into the regulation of expression of the tyrosinase gene.

It should be pointed out here that the mg-Tyrs-J can be fused with any of other genes and microinjected into fertilized eggs so that melanin pigments will be an excellent marker to identify transgenic mice. It is possible to select putative transgenic mice at birth based on pigmentation.

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