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STIMULATION AND INHIBITION OF GROWTH OF MICE BEARING THE HUMAN GROWTH HORMONE GENE

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Microinjection of cloned genes into the male pronucleus (nucleus) of a fertilized mouse oocyte [6] is an effective method of introducing foreign genes into the genome [3, 6], where they not only are integrated and transmitted to the progeny [7, 15], but in some cases they may also be expressed [2, 5, 10]. It has been shown that in mice, in whose genome the rat [11] or human [12] growth hormone gene has been integrated with mouse metallothionein I gene promotor, the size and body weight were considerably increased, and a high concentraton of foreign growth hormones was determined in the blood. Meanwhile, after introduction of human growth hormone gene, with its own promotor, into the mouse genome this gene was not expressed, but in some mice signs of a mutagenic action of the foreign DNA, built into the genome [15], were found. Thus the question of the phenotypic action of the foreign growth hormone gene requires further study.

The investigation described below is part of a series devoted to the introduction of foreign genes through the oocyte into the mammalian genome [1, 2]. It gives the results of experiments with human growth hormone gene, under mouse metallothionein I gene promotor, showing that expression of this gene can induce not only stimulation of growth, but also a paradoxical effect: drastic inhibition of growth of the transformed mice.

EXPERIMENTAL METHOD

Plasmid pMThGH 137 [12] was obtained from R. Palmiter (USA). Its EcoRI fragment with human growth hormone gene were isolated by electrophoresis in agarose gel and electroelution. Experiments were carried out on (CBZ × C57BL)F_1 hybrid mice.

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TABLE 1. Results of Experiments with Microinjection of Human Growth Hormone Gene

Plasmid or its fragment	Number of oocytes in experiments	Number of newborn mice	Human growth hormone gene in mice			
			integration	presence of human growth hormone in the blood	growth of transgenic mice (expression)	
					stimulation	inhibition
pMThGH	89	22	14	10	9	2
MThGH	64	17	10	9	0	8

Legend. 200 copies of plasmid pMThGH 137 or of its EcoRI fragment, containing human growth hormone gene, were injected into the male pronucleus of each fertilized mouse ooctye.

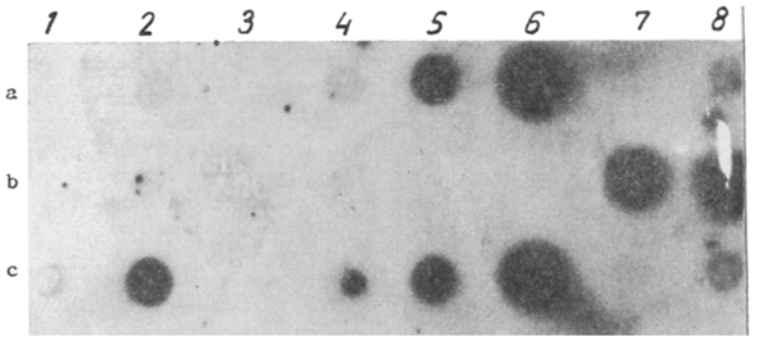

Fig. 1. Analysis of DNA from progeny of mice obtained after microinjection of human growth hormone gene, by DNA dot hybridization. a: 1, 2) 2 and 10 μg DNA of control mouse respecgively; 3, 4) DNA of mouse No. 8; 5, 6) DNA of mouse No. 6; 7, 8) DNA of mouse No. 23; c: 1, 2) DNA of mouse No. 44; 4, 5, 6) DNA of plasmid pMThGH (5, 20, and 100 pg respectively); 7, 8) DNA of control mouse.

Microinjections into the male pronucleus of fertilized oocytes of (CBA × C57BL)F_1 hybrid mice, transplantation of the embryos into the oviduct of recipient females, isolation of DNA from the mouse tissues, and hybridization analysis of DNA were carried out as described previously [1]. As the probe in the hybridization experiment, the ^{32}P-labeled BamH I-EcoRI fragment of DNA of plasmid pMThGH 137 was used. The concentration of human growth hormone in the blood serum or tissue extract of the mice was measured by radioimmunoassay using kits from "Behringwerke" (West Germany). The mouse blood serum proteins were transferred to a nitrocellulose membrane by the method in [14]. The immobilized proteins were treated with antibodies to human growth hormone and labeled with ^{125}I-protein A from Staphylococcus aureus.

EXPERIMENTAL RESULTS

Table 1 gives the results of experiments involving microinjection of the recombinant plasmid or its fragment, containing human growth hormone gene, into mouse oocytes. Altogether in the two series of experiments 41 mice were obtained, of which 24 had DNA sequences of the human growth hormone gene in their genome, i.e., integration of this gene took place with a frequency of close to 60% (Fig. 1). In this group of transgenic mice nine animals

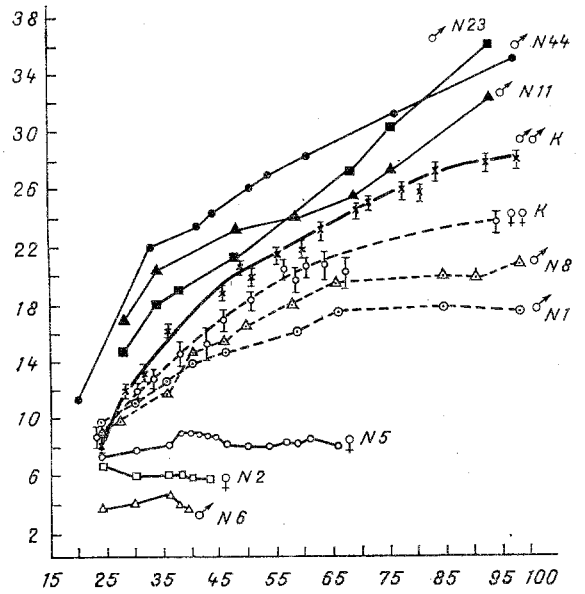

Fig. 2. Comparison of rates of growth of transgenic and control mice. Abscissa, age (in days); ordinate, body weight (in g).

grew much faster than the controls in the postnatal period and reached sexual maturity more quickly: at the age of 1 month they weighed 17-20 g, or 1.2-1.7 times (average 1.5 times) more than normally (Fig. 2). These mice had normal fertility and, as preliminary observations showed, they transmitted the human growth hormone gene to their progeny, among which mice of large size, with accelerated growth ability and earlier attainment of sexual maturity, also were found.

At the same time, in these experiments the weight of the largest genetically transformed mice at the age of 100 days did not exceed 35 g (control mice of this age weighed 27-28 g), whereas in [12] the weight of individual mice of this age, with an expressed human growth hormone gene, amounted to 60 g. These differences are probably attributable to the fact that our experiments were carried out on (CBA × C57BL)F_1 hybrids. We know that it is possible, by genetic selection, to breed mice of large size, and capable of transmitting this feature to subsequent generations [8]. It can be tentatively suggested that the investigations cited [11, 12] were conducted on such a colony of mice. Thus the degree of increase of growth under the influence of the foreign growth hormone gene may perhaps depend on the genetic characteristics of the mice and may be strongly expressed in anaimals which are genetically predisposed to more rapid growth.

In some mice containing immunoreactive human growth hormone, inhibition of growth and not acceleration was observed. For instance, among 22 mice obtained after injection of a circular plasmid into the oocyte, one male at the age of 1-3 months was 1.6-2 times less in weight than control males of the same age, and was sterile. This paradoxical action of human growth hormone gene was found particularly clearly in experiments with a linear fragment of the plasmid. For instance, in this series of experiments eight of the 17 mice were significantly retarded in growth compared with the control animals. The body weight of five of these mice was 1.2-1.5 times less (at the age of 2 to 5 months), and two of them (males) were sterile. Growth of three animals was very considerably inhibited (by 2.4-3 times). These dwarf mice died at the age of 40-70 days after birth (Fig. 2).

Radioimmunoassay showed that human growth hormone was produced by 11 transgenic mice, i.e., the introduced gene was expressed. Meanwhile the blood level of immunoreactive human growth hormone varied considerably in the transgenic mice from 5 to 1700 ng/ml. In the control, no immunoreactive protein of human growth hormone type was found.

RNA dot hybridization, and also direct measurement of the concentration of immunoreactive human growth hormone in the liver, brain, and skeletal muscles of the experimental mice showed that this hormone is synthesized mainly in the liver. The results thus confirms

that the tissue-specificity of expression of foreign genes introduced into the mammalian genome is determined by the structural features of the region of DNA adjacent to the promotor, and largely depends on the promotors [10], although it may also be connected with the specificity of regions of transcription intensifiers [5].

The observed inhibition of growth of the mice may be due to mutagenic action of the integrated DNA [7, 15] and inhibition of hormone synthesis in them, as has been described for an inherited growth hormone gene defect [4, 13]. However, radioimmunoassay showed that hormone synthesis is determined in animals with both acceleration and inhibition of growth and does not depend essentially on its intensity, at least over the range from 20 to 1700 ng/ml serum. Moreover, in a transgenic mouse with inhibition of growth (No. 1) the serum hormone level was almost 20 times higher than in mouse No. 23 with the maximal rate of growth. The suggestion arose that an abnormal hormone is synthesized in the mice with inhibition of growth. To test this hypothesis, immunoblotting of human growth hormone, present in the blood of transgenic mice, was carried out.

The results showed that whereas normal human growth hormone has a molecular weight of 22,000 daltons, in mice with accelerated growth an immunoreactive protein with mol. wt. of 25,000 daltons was found, corresponding evidently to human growth prehormone, before removal of the signal sequence [9]. In dwarf mice, immunoreactive proteins were found with molecular weights somewhat lower than normal, namely 21,000, 24,000, and 34,000 daltons. These observations are preliminary in character, and before a final conclusion can be drawn further investigations are needed. It can only be postulated as yet that an incomplete protein is formed in mice with inhibition of growth, and while preserving the antigenic determinant of human growth hormone, at the same time it cannot stimulate growth of mice. This abnormal protein can compete for receptors with mouse growth hormone, preventing its action. It can either inhibit production of its own growth hormone in the pituitary gland of the mouse, or it may itself possess the properties of an active "antigrowth factor." The mechanisms of action of the growth hormone and of the system controlling it are very complex and multistaged [8], and further investigations are needed to reveal which of the hypotheses mentioned above corresponds to reality.

In conclusion, it must be emphasized that the paradoxical effect of expression of human growth hormone gene which we found, namely inhibition of growth of the animals and not stimulation, indicates that after introduction of foreign genes into the mammalian genome, the results may be not only insertion mutagenesis [7, 15], but also other unexpected events.

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RESISTANCE OF THE ALIMENTARY CANAL OF Daphnia magna STRAUS TO ENTEROPATHOGENIC NAG VIBRIOS

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At the present time daphnias are being used on an ever-increasing scale as bioindicators of exposure to various exogenous pathogens. In the study of mechanisms of susceptibility and resistance of animals of different classes to bacteria enteropathogenic for man it has been shown that lower crustaceans (daphnias), even after direct contact with enormous numbers of NAG vibrios, do not develop the disease, but utilize the vibrios as food [1]. This fact can be partly explained by the peculiarities of the structure of the alimentary tract of daphnias, as established in the course of evolution.

This paper describes a study of the normal structure of the alimentary tract of daphnias and its response to the action of NAG vibrios.

EXPERIMENTAL METHOD

The investigation was conducted on 30 daphnias (Daphnia magna Straus), infected by the scheme described previously [1]. To obtain semithin sections the daphnias were fixed whole in 2.5% glutaraldehyde solution in 0.1M phosphate buffer (pH 7.3) for 1 h. After rinsing in several portions of the same buffer, the daphnias were tested with 1% OsO_4 solution in the same buffer and dehydrated in acetone solutions of increasing concentration. Semithin sections 1 μ thick were cut from blocks embedded in epoxide resins on the LKB-III ultrotome, and these were then stained with methylene blue, azure II, and fuchsine. The daphnias were investigated by a luminescence serologic method at different times after contact with NAG vibrios. For this purpose the daphnias were fixed whole in a 4% solution of neutral formalin, buffered according to Lillie, and embedded in paraffin wax. The resulting serial sections were subjected to parallel histologic investigation by the indirect Coons' method [5]. Specific sera to a culture of NAG vibrios (agglutination titer 1:800) were used to identify the bacteria by this method. Necessary controls for specificity of fluorescence were set up.

EXPERIMENTAL RESULTS

The study of semithin sections shows the precise structure of the epithelial layer of the alimentary tract of the daphnias. In particular, the proximal portion of the intestine was found to be lined with simple cubical epithelium, the cells of which were 11.3 ± 0.56 μ in height (Fig. 1a). The apical surface of the epitheliocytes was covered with a dense fuchsine-positive chitin membrane 2.18 ± 0.31 μ thick. The cell nuclei were large, oval in shape, and contained one or two dark nucleoli. The nuclei as a rule were located in the central part of the cell. The terminal zone was clearly distinguishable and contained tiny vacuoles. Sometimes epitheliocytes with a holocrine type of secretion were observed: their cytoplasm was highly translucent on account of numerous large, optically empty vacuoles (Fig. 1a).

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