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On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus

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Abstract We have previously shown that, when administered orally to mice, bacteriophage M13 DNA, as a paradigm foreign DNA without homology to the mouse genome, can persist in fragmented form in the gastrointestinal tract, penetrate the intestinal wall, and reach the nuclei of leukocytes, spleen and liver cells. Similar results were obtained when a plasmid containing the gene for the green fluorescent protein (pEGFP-C1) was fed to mice. In spleen, the foreign DNA was detected in covalent linkage to DNA with a high degree of homology to mouse genes, perhaps pseudogenes, or to authentic *E. coli* DNA. We have now extended these studies to the offspring of mice that were fed regularly during pregnancy with a daily dose of 50 μg of M13 or pEGFP-C1 DNA. Using the polymerase chain reaction (PCR) or the fluorescent in situ hybridization (FISH) method, foreign DNA, orally ingested by pregnant mice, can be discovered in various organs of fetuses and of newborn animals. The M13 DNA fragments have a length of about 830 bp. In various organs of the mouse fetus, clusters of cells contain foreign DNA as revealed by FISH. The foreign DNA is invariably located in the nuclei. We have never found all cells of the fetus to be transgenic for the foreign DNA. This distribution pattern argues for a transplacental pathway rather than for germline transmission which might be expected only after long-time feeding regimens. In rare cells of three different fetuses, whose mothers have been fed with M13 DNA during gestation, the foreign DNA was detected by FISH in association with both chromatids. Is maternally ingested foreign DNA a potential mutagen for the developing fetus?

Key words Foreign DNA · Gastrointestinal and transplacental uptake · FISH · PCR

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Introduction

The uptake and genomic fixation of foreign DNA in mammalian cells is a frequently observed event in oncogenic virus transformation, reverse genetics, and in the generation of transgenic organisms, and will be a goal in long-term somatic gene therapy in humans. While these situations arise from experimental procedures in molecular biology, the most frequent site of natural exposure of mammalian and other organisms to foreign DNA is the gastrointestinal tract following the ingestion of nutrients. A very sizeable multifaceted surface of resorptive epithelial cells, including one of the largest components of the immune system of the organism facilitates the entry not only of nutrients but also of macromolecules. We have demonstrated earlier that 1–2% of orally ingested M13 DNA resist the gastrointestinal passage and persist as fragments. These DNA fragments have been detected by FISH – exclusively in the nuclei – in intestinal epithelia, in cells in Peyer's patches in the submucosal intestinal wall stratum, in peripheral white blood cells, and in cells in spleen and liver (Schubbert et al. 1994, 1997). There is evidence that in spleen cells this foreign DNA can, in rare instances, be linked covalently to DNA with a high degree of homology to mouse DNA (Schubbert et al. 1997). Recently, our results have been confirmed by demonstrating the uptake of pCMV/β-gal plasmid DNA through the gastrointestinal tract of rats in experiments using polyanhydride copolymer microspheres as an artificial delivery system (Mathiowitz et al. 1997). However in our work we used naked M13 DNA without synthetic additives (Schubbert et al. 1994, 1997).

In the present report, we demonstrate that M13 DNA, if fed* daily to pregnant animals for 1 to 2 weeks, can be detected in fetuses or in newborn animals by PCR and by FISH. The analyses with the FISH technique reveal the foreign, ingested DNA exclusively in the nuclei of a few cells that are frequently clustered in different organs of the animals. M13 DNA has never been found in all cells of the offspring. Thus, transmission of the foreign DNA is likely to occur transplacentally and not via the germ line. The latter mode of transfer seems unlikely under the feeding schemes employed. In rare instances, the foreign DNA is associated with both chromatids in metaphase spreads of fetal cells.

Materials and methods

General procedures

Most techniques employed in this study, such as the recloning of M13 and adjacent DNA sequences from spleen DNA (Schubbert et al. 1997), the polymerase chain reaction (PCR) (Saiki et al. 1988), fluorescence in situ hybridization (FISH) experiments (Lichter et al. 1990; Heller et al. 1995), and Southern blot hybridization experiments (Southern 1975) were described earlier (Schubbert et al. 1994, 1997). Nucleotide sequences were determined by the dideoxy chain termination procedure (Sanger et al. 1977) using an Applied Biosystems Model 377 automated DNA sequencer. Appropriate synthetic oligodeoxyribonucleotide primers were chosen from the authentic M13 DNA sequence (Messing and Vieira 1982) by first hybridizing ^{32}P-labeled cloned DNA from mouse spleen to *Rsa*I- or *Ssp*I-cleaved M13 DNA. In this way, the M13 DNA segment present in the clone reisolated from spleen DNA was determined. The cloned DNA was also hybridized to total mouse DNA to ascertain whether mouse DNA was present in the clone. Nucleotide sequence searches and comparisons to the EMBL and NCBI data banks were performed by using the program BLASTN.

Feeding of M13 or pEGFP-C1 DNA to pregnant mice

Double-stranded circular or *Eco*RI-linearized M13mp18 DNA (termed M13 DNA in this report) or plasmid pEGFP-C1 DNA (Clontech) was fed to 3- to 6-month-old C57BL/6 pregnant mice. A daily dose of 50 µg of M13 DNA in TE (10 mM Tris-HCl pH 7.5, 1 mM EDTA) was administered orally by pipette to pregnant mice from day 0 to 14 or from day 6 to 14 of gestation (see Table 3). Day 0 was defined as the time of mating. Fetuses were sacrificed on day 10–14 of gestation and sectioned after embedding in paraffin according to standard methods. Newborn animals were analyzed 3 days to 3 months after birth.

Preparation of fetuses and newborn animals for histology and FISH

Mice were anaesthetized in a CO_2 atmosphere or by injecting 15 µg of avectin (2,2,2-tribromoethanol dissolved in 2-methyl-2-butanol). Fetuses were excised from the uterus and washed in sterile phosphate-buffered saline without Mg^{2+} and Ca^{2+} (PBS-d).

Some of the fetuses or individual organs from newborn animals were fixed in 4% formalin in PBS and embedded in Paraplast. Subsequently, the preparations were sectioned using a MICROM rotating microtome. Sections were stained with propidium iodide and subjected to FISH reactions using biotinylated M13 or pEGFP-C1 DNA as hybridization probes as described elsewhere (Schubbert et al. 1997).

A second group of the fetuses were homogenized by forcing them through a 200-µm mesh filter. The cells were then propagated for 4 to 10 days in RPMI medium supplemented with 20% fetal calf serum at 37° C in an air atmosphere containing 5% CO_2 at 86% humidity. These cells were used for chromosomal preparations and for analyses by the FISH method.

A third set of fetuses was homogenized by repeatedly forcing the tissue through a 0.9 × 40 mm gauge needle using a 2-ml syringe. The preparations were then incubated overnight at 37° C in 10 mM TRIS-HCl pH 8.0, 0.2 mM EDTA, 10 mM NaCl, 1% sodium dodecyl sulfate (SDS) and 1.33 mg of proteinase K per ml. The DNA was extracted by repeated phenol-chloroform extractions followed by ethanol precipitation. The DNA was then analyzed by PCR as described (Schubbert et al. 1997).

Results and discussion

Feeding of pEGFP-C1 DNA confirms results obtained with M13 DNA

The 4.7-kb pEGFP-C1 plasmid carrying the gene for the *Aequorea victoria* green fluorescent protein under control of the early cytomegalovirus (CMV) promoter (Clontech) was orally administered to mice as described for M13 DNA (Schubbert et al. 1994, 1997). The pEGFP-C1 DNA lacks homology to mouse DNA. The double-

Table 1 Detection of pEGFP-C1 plasmid DNA in internal organs (intestine, liver, spleen and kidney)

Methods[a]	Fragment lengths	Organs	Time after feeding	Number of animals fed	
				pEGFP	TE[b]
Southern blotting	200–900 bp	Intestinal contents	3–8 h	5/9	0/4
PCR	1277 bp	Liver	3 h	1/9	0/4
	”	Spleen	3–8 h	3/9	0/4
	”	Kidney	3–8 h	3/9	0/4
FISH[c]	–	Intestinal wall	3–8 h	3/6	0/2
	–	Liver	3–8 h	2/5	0/3
	–	Spleen	3–8 h	2/5	0/3
	–	Kidney	3 h	1/5	0/3

[a] Methods were detailed earlier (Schubbert et al. 1997)
[b] In control experiments, 25 µl of 10 mM Tris-HCl, pH 7.5, 1 mM EDTA (TE) was fed to mice
[c] An organ was scored positive when at least three, in most cases many more cells, showed hybridization signals in consecutive serial sections

stranded circular plasmid was fed to 6- to 10-week-old C57BL/6 mice in a single dose of 50 μg. The data summarized in Table 1 demonstrate that the foreign pEGFP-C1 DNA could be detected by Southern blot hybridization in the intestinal contents up to 8 h after feeding, by PCR in DNA from liver (3 h), spleen and kidney up to 8 h after feeding, and by FISH in cell nuclei in the intestinal wall, spleen and liver 3–8 h after feeding. By FISH and histological analysis, the foreign DNA was localized to the nuclei of organ-specific cells. We presume that the test DNA has been transported to the different organs by white blood cells. Organs from a total of 18 animals were analyzed. In control mice, which had never received the pEGFP-C1 DNA, the foreign DNA was not present. The data on the uptake of ingested foreign DNA by the mouse organism are thus independent of the test DNA – M13 or pEGFP-C1 DNA – used.

The intact cytomegalovirus promoter-GFP sequence can be retrieved 3 to 8 h after feeding

At 3–8 h after feeding the pEGFP-C1 test DNA to mice, the persistence of the intact cytomegalovirus promoter-GFP sequence (map in Fig. 1b) was documented by PCR in the DNAs from spleen and kidney, and up to 3 h in DNA from liver (Fig. 1a). The locations of the primer pairs chosen for these analyses are shown in the map in Fig. 1b, and their nucleotide sequences are given in the legend to Fig. 1. In the retrieved molecules, at least the test promoter-gene ensemble was still intact, but we were unable to demonstrate GFP expression in the organs analyzed.

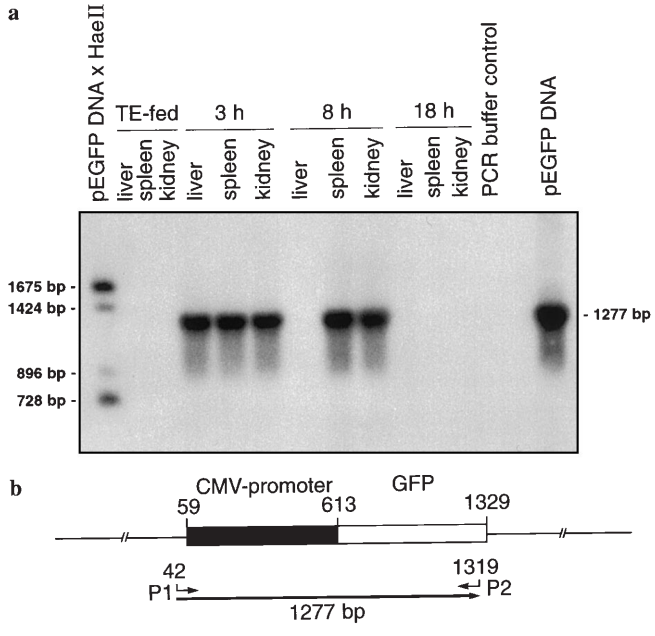

Fig. 1a The intact CMV promoter-GFP gene construct can still be detected by PCR as a 1277-bp product in DNA from liver, spleen and kidney of mice that had been fed with the construct 3–8 h previously. **b** The map of the construct and the locations of primer oligodeoxyribonucleotides used for PCR are indicated. The nucleotide sequences of the primers were P1,5′-GCCCATATATGGAGTTCCGCG-3′; P2,5′-CGTCCATGCCGAGAGTGATCC-3′

Orally ingested M13 DNA sequences can be recloned from spleen DNA in covalent linkage to *E. coli* DNA or to DNA with up to 80% homology with authentic mouse DNA

The recloning of the M13 test DNA and verification of its persistence in mouse spleen DNA by nucleotide sequence determination of recloned segments confirmed the intestinal uptake of the foreign DNA into the mouse organism (Fig. 5 in Schubbert et al. 1997; Table 2 in this report). The following aspects of these data are of interest. (i) The M13 test DNA was covalently linked to authentic DNA sequences from *E. coli* or to DNA sequences that show a high degree of homology to known mouse DNA sequences. Thus, it appears very likely that the ingested M13 test DNA had become integrated into the mouse genome, probably in the vicinity of pseudogenes. The presence of *E. coli* DNA suggests that there is a steady stream of foreign DNA from the intestinal or other external surfaces of the organism to the spleen and possibly to other organs of the animals. (ii) Different sections of the M13 test DNA molecule were found in the recloned segments. These segments did not include the sequence between nucleotide coordinates 1800 to 2400 of the M13 DNA sequence (see below and Vassart et al. 1987).

Control experiments

The results discussed above were corroborated by several different control experiments (Schubbert et al. 1994, 1997). (i) Amounts of between 10 and 70 μg of genomic DNA obtained from mouse liver or spleen were cleaved with *Eco*RI and analyzed by Southern blot hybridization to ^{32}P-labeled M13 DNA or pEGFP-C1 DNA. Hybridization signals were not found even upon extended exposure of the autoradiograms. (ii) Among several times 10^8 plaques of recombinant λ phage carrying spleen DNA from mice previously fed with M13 DNA, only very few M13 DNA-positive plaques were obtained (Table 2). (iii) FISH analyses of tissue sections from control mice that had never received M13 DNA or pEGFP-C1 DNA did not reveal signals specific for these test DNAs. (iv) An older report in the literature (Vassart et al. 1987) had claimed that a segment between nucleotide coordinates ~1800 to 2400 in the M13 DNA molecule allegedly hybridized to human and animal DNAs on Southern blots under certain conditions. Although we never saw any hybridization to mouse DNA in our experiments, we systematically excluded this segment of M13 DNA from our selection of PCR primers in all our analyses as a precautionary measure.

Table 2 M13 DNA recovered from the spleen of mice fed 24–72 h previously with 50 μg of M13 DNA and its covalently linked flanking nucleotide sequences

Left flanking nucleotide sequence[a]	Nucleotides of unknown orgin between M13 DNA and adjacent sequence[b]	Nucleotide coordinates in the M13 sequence	Right flanking nucleotide sequence[a]
100% homology to *E. coli* Clp protease gene[c] (175 nucleotides M31045)	–	4640–5433	90% homology to lambda EMBL
Unknown nucleotide sequence[c]	–	2660–3300	Unknown nucleotide sequence
70% homology to mouse IgE receptor gene[c] (80 nucleotides) (M59317)	5′-GATATAT-3′	4736–6034	Unknown nucleotide sequence
100% homology to *E. coli* minute 9 to 12 DNA[d] (120 nucleotides) (82598)	–	2726–5521	Unknown nucleotide sequence
81% homology to mouse beta 2 thyroid hormone receptor gene[d] (138 nucleotides) (U15542)	5′-GATTTAAAAGGTGGA-3′	3355–5030	Unknown nucleotide sequence

[a] The experimental procedures for these recloning analyses using the lambda DASHII vector were detailed previously (Schubbert et al. 1997). All nucleotide sequences were determined by the chain termination method (Sanger et al. 1977) using a Model 377 Applied Biosystems DNA Sequencer. The nucleotide sequences were compared to the EMBL and NCBI GenBank data sources online. The accession number of the sequences identified are listed in parentheses

[b] Similar cases of a few nucleotides of unknown origin being inserted in between integrated foreign and recipient DNA were described for non-homologously recombined SV40 and pBR322 DNA (Wilson et al. 1982) and for adenovirus type 2 DNA integrated in hamster cells (Gahlmann and Doerfler 1983)

[c] These data were reported earlier. See Fig. 5 in Schubbert et al. (1997)

[d] These experiments were not previously published

The results of the types of analyses described preclude the existence of homologies between M13 DNA and mouse DNA that can be detected by the methods applied in this project.

Transfer of orally ingested foreign DNA to mouse fetuses and newborn animals

The obvious question arises whether the ingested foreign DNA can be transmitted via the germ line or transplacentally to the next generation during gestation. Here we present data demonstrating that, following prolonged feeding of M13 or plasmid pEGFP-C1 DNA to mice during pregnancy, the orally ingested foreign DNA can be detected by FISH in cell clusters in several organs, both in fetuses and in newborn mice. The distribution of the foreign M13 or plasmid pEGFP-C1 DNA was assessed at the single-cell level by preparing histological sections of various organs from newborn mice or from fetuses and by demonstrating the presence of the foreign DNA by the FISH method. The fluorescence photographs in Fig. 2 comprise a representative selection from several hundred sections in which the nuclear localization of M13 or pEGFP-C1 DNA was documented. Since FISH analyses on tissue sections constituted a very important component of the proof that DNA was present in the progeny of mice fed DNA, several different examples were presented (Fig. 2). Using FISH, M13 or pEGFP-C1 DNA was found in between 3- and 84-day-old mice and in various tissues of fetuses at a gestational age of 10–14 days. In several hundred sections of various organ derivations, the fluorescent foreign DNA signals were always located in the nucleus, never in the cytoplasm. The patchy distribution of M13 or pEGFP-C1 plasmid DNA-containing cells in various organ systems suggested transplacental rather than germ line transmission of foreign DNA ingested by the maternal animal during gestation.

For the FISH experiments, four critical control experiments were performed using serial sections from both directly fed animals and their offspring. From animals, that had been fed pEGFP-C1 plasmid DNA, one section was hybridized to biotinylated pEGFP-C1 DNA to document the nuclear presence of this signal. The following section from this series was hybridized to biotinylated M13 DNA as a negative control. No signals were detected. Reciprocal controls were carried out with serial sections from mice fed M13 DNA or from their fetuses. The presence of M13 DNA in nuclei could be

Fig. 2a–g Detection by the FISH technique of the M13 (**a**, **c**, **d**, **e**, **f**) or pEGFP-C1 (**b**, **g**) test DNA in various tissues and organs of fetuses (**a**, **b**, **g**) or newborn mice (**c**, **d**, **e**, **f**) whose mothers had been fed daily with 50 μg of test DNA during pregnancy. **a–f** In sections of 10-(**a**) or 14 (**b**)-day-old fetuses, signals of plasmid DNA are detectable in brain (**c**) or eye tissue (**d**) as yellow-green dots in nuclei of clusters of cells. In sections of liver tissue (**e**), cells in the endothelium of blood vessels are positive for foreign DNA, in sections of testis tissue (**f**) epithelial cells are positive. (**g**) Both chromatids of one chromosome in a fetal mouse cell in culture carry pEGFP-C1 DNA ingested by pregnant mice. The chromosome spread analyzed was derived from a cell preparation that had been cultured from a 16-day mouse fetus. Its mother had received 50 μg of pEGFP-C1 DNA daily for 14 days during pregnancy. Metaphase arrest with colchicine and the FISH reaction were performed as described earlier (Schubbert et al. 1997). Magnification 1200×; additional magnification for reproduction: 2 × (**a–f**) and 3 × (**g**)

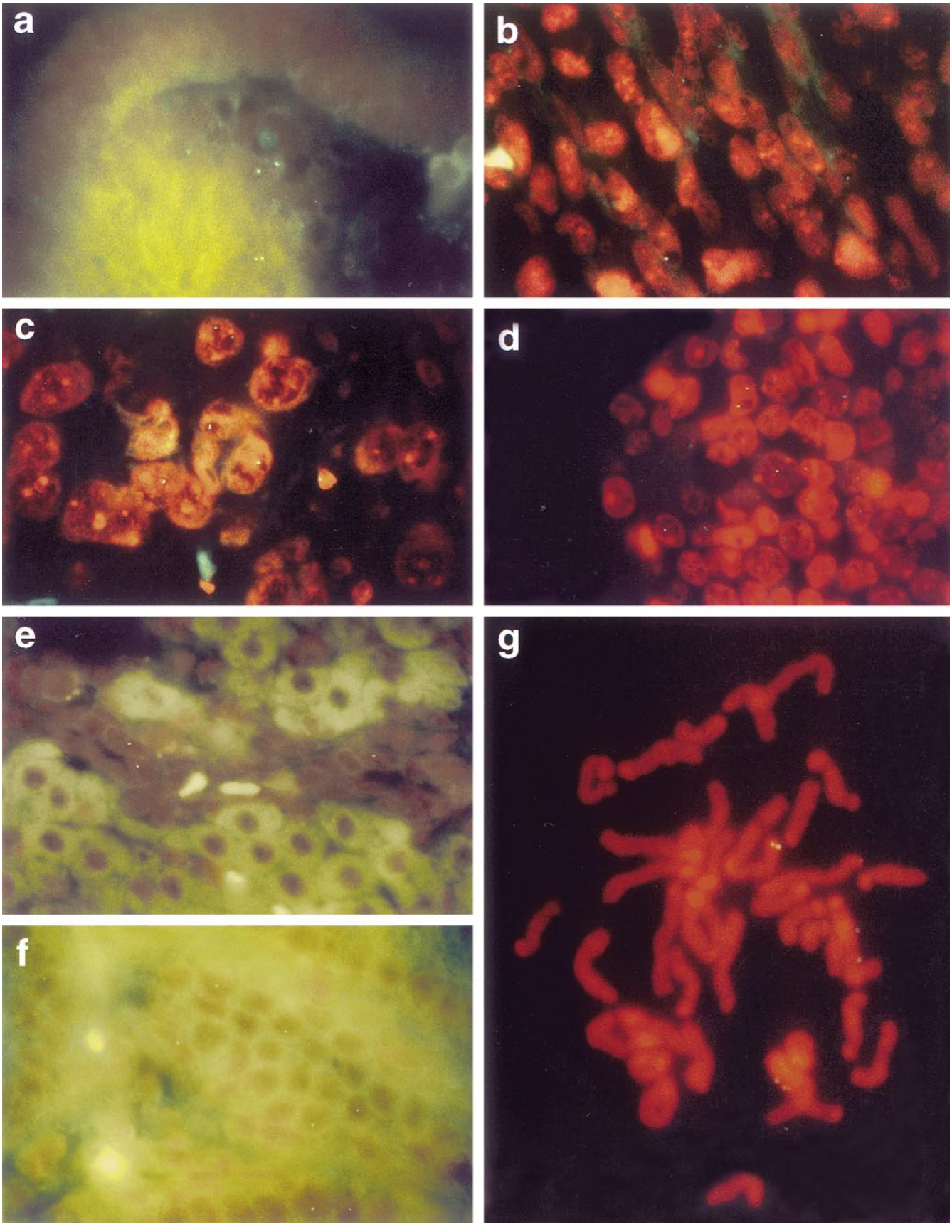
a
b
c
d
e
g
f

Table 3 Feeding of foreign DNA to pregnant mice and detection by FISH of the food ingested DNA in various tissues of fetuses or newborn animals

Pregnant mouse number	Test DNA fed	Period of feeding[a]	Size of litter	Age[b] of newborn mice or fetuses[e] when tested	Negative animals[c]	Positive animals[c]	Positive tissues (analyses by FISH)
1	pEGFP-C1	Day 0–14	2	18 days after birth	2	0	
2	M13	Day 0–14	4	18 days after birth	2	1	Brain
						1	Liver (blood vessel)
3	M13	Day 0–14	2	18 days after birth	0	1	Brain,
						1	Eye, liver
4	M13	Day 0–14	6	3 days after birth	6	0	
5	M13	Day 0–14	5	3 days after birth	1	1	Brain, eye
					3 n.i.		
6	pEGFP-C1	Day 0–5	4	16 days after fertilization	4	0	
7	M13	Day 0–14	5	3 days after birth	1	1	Connective tissue
					3 n.i.		
8	M13	Day 0–14	3	3 days after birth	1	1	Eye
					1 n.i.		
9	M13	Day 0–14	9	16 days after fertilization	8	1	Unknown tissue
10	pEGFP-C1	Day 0–5	9	16 days after fertilization	8	1	Eye
11	M13	Day 6–14	5	3 days after birth	4	1	Brain, heart, thymus, intestine
12	M13	Day 0–14	9	14 days after fertilization	8	1	Unknown tissue
13	pEGFP-C1	Day 0–14	11	14 days after fertilization	8	2	Chromosome
						1	preparation of unknown tissue
14	M13	Day 0–14	2	3 months after birth	1	1	Testes
15	M13	Day 0–14	8	14 days after fertilization	8	0	
16	M13	Day 0–14	8	14 days after fertilization	8	0	
17	pEGFP-C1	Day 0–12	8	16 days after fertilization	8	0	
18	pEGFP-C1	Day 0–12	8	16 days after fertilization	7	1	Chromosome preparation
			Total 108		Total 85; 7 n.i.	Total 16	

[a] Day 0 was defined as the day male and female animals were mated. During the feeding period, the pregnant females received doses of 50 μg test DNA daily

[b] Age was estimated from the state of development of fetuses or counted in days after birth

[c] For the experimental design, see Materials and methods. Fetuses were scored positive when several cells in several organs showed hybridization signals for the M13 or pEGFP-C1 DNA probes in consecutive serial sections. n.i. = not investigated

demonstrated. The following section from the same series did not hybridize to pEGFP-C1 DNA.

Table 3 presents a summary of all the feeding experiments with pregnant mice performed to date. We have so far fed M13 or pEGFP-C1 DNA to 18 different pregnant C57BL/6 mice and obtained positive results by FISH (16 animals) or by PCR (4 animals) in a total of 74 fetuses and of 34 newborn animals at the ages detailed in Table 3. Similar experiments were performed with 15 animals that had not received M13 or pEGFP-C1 DNA. FISH or PCR analyses did not yield any positive signals.

We isolated DNA from spleen and liver of M13-fed mice at different times after feeding. Moreover, DNA was prepared from 14 day old fetuses or from several organs of newborn animals, whose mothers had been fed M13 daily during pregnancy. Feeding of pregnant mice was discontinued 48–72 h prior to the preparation of the fetuses for FISH or PCR analyses, to guard against contamination with M13 DNA from maternal sources. The DNA samples were examined for the presence of M13 DNA by PCR. The locations of the primer pairs used in these PCR analyses were indicated in the M13 DNA map in Fig. 3b. The data demonstrate the presence of M13 DNA molecules of a length of about 830 bp in the DNAs from the sources listed (Fig. 3a). The foreign DNA can be readily detected by both PCR and FISH in several organs of M13 DNA-fed animals up to 18h after feeding, and in fetuses and newborn animals whose mothers were fed M13 DNA. The maximal fragment length in fetal DNA, as determined by PCR, is about 830 bp; longer fragments have not been detected by PCR in the experiments shown in Fig. 3a.

Association of food-ingested foreign DNA with both chromatids in chromosomes of fetuses

As described under Materials and methods, cells from fetuses whose mothers had been fed with pEGFP-C1 DNA during gestation, were propagated in culture. Upon treatment with colchicine, chromosomal spreads of these cells were investigated by the FISH technique

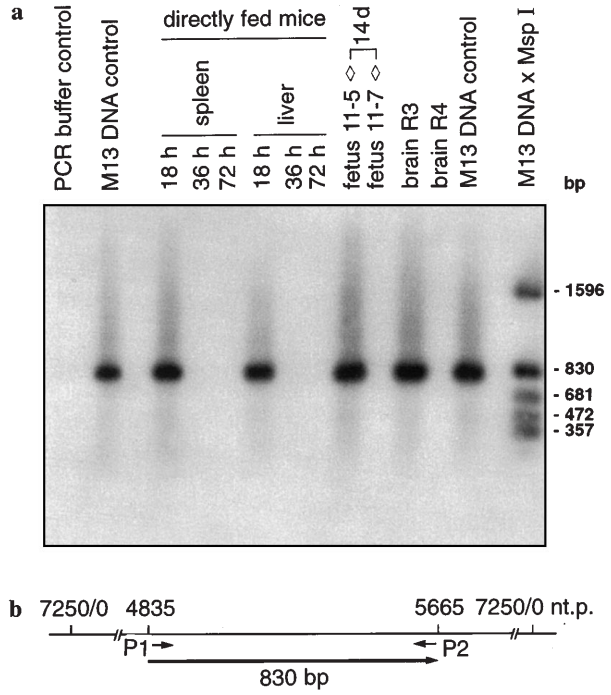

Fig. 3a Detection by PCR of the M13 test DNA in organs of mice that had been fed with M13 DNA or in the offspring (fetuses) of mice fed regularly during pregnancy. DNA from the sources indicated was isolated and analyzed by PCR for the presence of M13 DNA. (**b**) Map of the bacteriophage M13 DNA and the locations of the primer oligodeoxyribonucleotides (P1, P2) that were used for PCR analyses. *Msp*I fragments of M13 DNA were coelectrophoresed as size markers. For experimental details, see the text

(Lichter et al. 1990; Heller et al. 1995) for the linkage of M13 DNA to the chromatids. Chromosomal association of the orally ingested test DNA with both chromatids could be demonstrated in rare instances. The example provided in Fig. 2g was one of three instances in fetuses from different litters of foreign DNA-fed mice. These data further argue in favor of the chromosomal integration of M13 DNA, even in cells of the offspring of female mice that were fed this foreign DNA during pregnancy.

Ingested foreign DNA thus not only traverses the gastrointestinal barrier and can be integrated into DNA very similar to the nuclear DNA, probably pseudogenes, of the recipient animals (Table 2), but can also penetrate the placental circulatory system and reach the offspring of pregnant animals. It is conceivable that the newborn animals could have picked up some of the foreign DNA from the mothers' milk. In all experiments performed so far, the ingested foreign DNA transferred to the next generation of mice has been found in the nuclei of occasional single cells, sometimes in small clusters of cells, but never in all cells of the offspring. This distribution pattern of foreign DNA signals excludes germline transmission and suggests transplacental entry into the fetus. It has been reported elsewhere that foreign DNA intravenously injected into pregnant mice can be recovered in the offspring (Tsukamoto et al. 1995). This finding also supports the notion of transplacental transfer of foreign DNA from the maternal to the fetal circulation. We have now initiated experiments in which we feed animals a daily dose of 50 μg of pEGFP-C1 DNA for several generations. In these experiments the question of germline transfer and expression of orally administered foreign DNA can be more critically addressed than in the experimental design used so far.

The linkage of foreign DNA uptake via the gastrointestinal tract and by transplacental routes to the daily and vital uptake of food prompts reflections on the consequences of this permanent contamination of established organisms by DNA fragments derived from their prey. What role has this process played in evolution, what medical implications have to be weighed? Can DNA ingested by the mother during or before pregnancy elicit mutations in the offspring? The not infrequent association of ingested foreign DNA with cells in the Peyer's patches, peripheral white blood cells and spleen cells (Schubbert et al. 1997) renders it likely that the organism's defense systems can recognize and possibly dispose of foreign genes.

Conclusions

The natural environment is heavily "contaminated" with DNA and its highly recombinogenic cleavage products. The gastrointestinal tracts, e.g. of mammals, are constantly exposed to DNA from very different sources. Bacteriophage M13 DNA or plasmid pEGFP-C1 DNA was chosen as tracer DNA for studies on the persistence of this DNA in the mouse. Although we have used several different methods, we have never detected homologies between M13 or pEGFP-C1 DNA and mouse DNA. We have previously documented entry of orally ingested foreign DNA into several organ systems of the mouse and linkage to mouse-like DNA (Schubbert et al. 1997). The data presented here corroborate and extend these results. M13 DNA recloned from spleen cells has again been found in covalent linkage to DNA with 81% homology to mouse DNA – perhaps a pseudogene – or to *E. coli* DNA (Table 2). The latter observation suggests that *E. coli* DNA, which must be abundant in the intestinal contents and on other surfaces of the animals, can reach the DNA in cells of the host organism.

The ingested foreign DNA can transgress not only the intestinal but also the placental barrier and reach the nuclei of cells of fetuses or newborns from female mice that have been fed the test DNA daily for longer periods during pregnancy. FISH (Fig. 2) and PCR data (Fig. 3) reveal the presence of the test DNA in the nuclei of clusters of cells in the fetus or in newborn mice. Some of this DNA is associated with chromosomes (Fig. 2g), and is probably integrated into host DNA. The length of the persisting M13 DNA fragments extends to maximally 830 bp. The patterns of distribution of foreign DNA

molecules in the offspring organisms are consistent with transplacental transfer. Germline transmission would probably have been difficult to detect under the experimental regimen chosen. The intimate association of the foreign test DNA with both chromatids in cells of the offspring (Fig. 2g) and the long-term persistence of foreign DNA in newborn animals for up to 3 months after birth (Table 3) raise novel questions about the genetic effects that this intrusion might have on the fetus. It will be a challenging task to investigate the possible consequences of foreign DNA insertion into the genomes of the recipient organisms.

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