

Uptake and Organ Distribution of Feed Introduced Plasmid DNA in Growing or Pregnant Rats*

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ABSTRACT

Fragments of DNA present in food and feed are taken up by the gastrointestinal tract (GIT) of mammals. The extent of uptake varies according to organism, study design and DNA source. This study explores the hypothesis that actively growing, as well as pregnant rats, are more likely to take up DNA from the GIT than mature animals due to the high demand for nutrients for tissue and organ development. Plasmid DNA (pDNA) was added to standard feed for growing, and pregnant rats. The young rats received one pDNA (50 µg) containing meal by gavage. Blood, organ and tissue samples were harvested at 2 h to 3 days post feeding (p.f). The pregnant females were fed pellets containing pDNA (100 µg) daily, starting at day 5 after established pregnancy. Females and foeti were killed at days 7 and 14 of gestation, and pups at the time of weaning. Genomic DNA was analyzed by PCR followed by Southern blot and real-time PCR. A 201 bp target sequence was detected in mesenteric lymph nodes, spleen, liver and pancreas from growing rats 2 h p.f. At 6 h, target DNA was detectable in the kidneys, and at three days p.f. in the liver. Target DNA was not detected in samples from pregnant rats, their foeti or pups. In conclusion, low level of feed introduced DNA could be transiently detected in organs of young, growing rats. However, indications of increased DNA uptake levels in the GIT of growing rats were not found.

Keywords: DNA Uptake, DNA Persistence, DNA Degradation, Rodents, Feeding Trial

1. Introduction

Several published studies now suggest that minor portions of feed introduced DNA is taken up by the gastrointestinal tract (GIT) of mammals. For instance, the digestive fate of the recombinant portion of DNA from GM crops has been investigated in mice, rats, fish, cattle, poultry and pigs [1-35]. Food is often processed by different treatments prior to consumption that fragment DNA such as high temperatures and acidic conditions [36-39]. The extent of DNA fragmentation will, however, also be determined by other protective factors as well as the storage conditions [38-39].

The first site of possible degradation in the GIT is the oral cavity. Studies by Duggan *et al.* [10], and Mercer *et al.* [40-42] demonstrated that plasmid DNA survived for a short time in human saliva and was able to transform *Escherichia coli* or *Streptococcus gordonii*; implying that

DNA released from the diet may survive and provide a source of transforming DNA for bacteria present in the oral cavity. Feeding experiments in adult mice performed by Walter Doerfler's group in Cologne, Germany, have shown that most foreign DNA is substantially degraded during passage through the lower GIT [1-3,5]. However, the persistence of fragments up to about 1700 bp in length was demonstrated in the caecum and large intestine. Fragments were subsequently also detected in leucocytes, spleen, liver and kidney. Moreover, these studies indicated that fragments of phage DNA added to the feed had become covalently linked to chromosomal DNA after transplacental transfer [1-3,5]. Other studies have also reported detection of feed introduced DNA in mammals. For instance, fragments of plant DNA have been observed in muscle, liver, spleen, and kidney of chicken after feeding with corn [17-20]. In pigs, sheep and cattle, fragments of ingested DNA from corn have been detected in GIT contents as well as in tissues and

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organs [7-9,12,15,21,31,33]. In contrast, a study in fallow deer fed on GM plants concluded that no recombinant DNA fragments were detected in GIT contents or the visceral tissues [23].

The cellular processes governing the degradation, time-limited survival or cellular uptake of feed-introduced DNA remain poorly understood and characterized despite the many observational studies available. Schubbert *et al.* [2] suggested that extracellular DNA enters the organism via the gut mucosa and then reaches the different organs via the blood stream. Small amounts of feed introduced DNA are therefore expected to circulate in human blood after feed intake and to be cleared from circulation by the liver [43,44].

The high demand for efficient nutrient uptake from the GIT (to supply energy and nutrients, including DNA metabolites) for tissue and organ development in growing individuals suggest the frequency of uptake of feed introduced DNA may differ from those recorded in previous studies of adult individuals. Only few studies have examined the fate of feed introduced DNA in rapidly growing/developing animals. In this study the hypothesis that young actively growing, as well as pregnant rats, are more susceptible to and more likely to take up and integrate DNA from the GIT than mature animals was explored. A plasmid that contained a *gfp* (green fluorescent protein) gene under the control of the 35S cauliflower mosaic virus (CaMV) promoter was added to the standard, semi-synthetic DNA void, rat feed source to determine if: 1) feed introduced DNA was taken up from the GIT and distributed to internal organs, 2) there was a difference in DNA uptake efficiency between young, actively growing and adult, pregnant female rats, 3) feed introduced DNA sequences crossed the placental “barrier” and could be detected in foeti and pups, and if 4) there was a difference in uptake frequency between linear and circular pDNA.

2. Materials and Methods

2.1. Preparation of DNA for the Rat Feeding Experiments

The plasmid p_{hr}GFP-CaMV [45] carries the gene for the green fluorescent protein (GFP) under control of the 35S Cauliflower Mosaic virus (CaMV) promoter. Plasmid DNA was isolated from *E. coli* strain DH5 α and was solubilized in sterilized water according to the Qiagen Plasmid Maxi Kit protocol (Qiagen, Germany). The DNA concentration was quantified spectrophotometrically at OD₂₆₀ with a SmartSpec TM Plus (Bio-Rad, USA). Linear plasmids were obtained by incubation with the *EcoRI* enzyme (New England BioLabs, USA) at 37°C according to the manufacturer’s instructions. The restriction reac-

tion was terminated with thermal inactivation of the enzyme at 65°C for 20 minutes.

2.2. Preparation of Rat Feed

The basic feed for the young, actively growing rats was a breast-milk substitute (NAN, AS Nestle, Norway), which had a composition closely related to the fully balanced semi-synthetic lactalbumin based diet (LA; 150 g protein/kg diet) [46]. The pregnant rats were fed rat chow AIN-93G recommended for growth, pregnancy and lactation and AIN-93M, recommended for maintenance after pregnancy [47] (Scanbur BK AS, Norway). All feedlots used in these experiments were tested for the absence of the target PCR DNA. Food pellets were made by mixing 80 ml sterile, autoclaved water with 200 g rat feed-powder. The solution was poured into a box, lined with aluminum foil, and left at 4°C overnight. The solidified material was then cut into feed pellets (1.5 × 1.5 × 1.5 cm pellets) and left on a tray covered with baking paper overnight to dry at room temperature. This work was performed in a sterile laminar airflow cabinet. Pellets were then frozen at -20°C until they were used.

2.3. DNA Stability in Feed

A total of 50 μ g plasmid DNA pKTG from *E. coli* strain DH5 α [48] was mixed with the feed pellets in 3 parallels and left at room temperature for 0, 1, 12, 36 or 72 hours. The pellets were frozen at -20°C until DNA extraction. Frozen pellet samples (0.5 g) were crushed to a fine powder using a mortar. The DNA was extracted using the FastDNA SPIN kit (Q-Biogene, Heidelberg, Germany). Absorbance at OD₂₆₀ was used to determine the DNA concentrations. To test whether the isolated DNA remained functionally intact over the incubation period, a transformation experiment was performed using the naturally competent *Acinetobacter baylyi* strain BD413 [49]. For the filter-based transformation assay, 1 μ g of DNA isolated from each rat feed pellet was mixed with 80 μ l of bacterial cells and the filter transformation, with appropriate controls, was carried out as described by Ray and Nielsen [50]. The transformation frequency was determined as the number of transformants (expressing the pKTG harbored *nptII* kanamycin resistance gene) divided by the total number of recipient BD413 cells.

2.4. Feeding Experiments

Wistar rats, Mol:WIST Han, M&B Denmark, bred at the rat facility of the Department of Comparative Medicine of the University of Tromsø, Norway, were used for the feeding experiments. The animal experiments and housing procedures were approved by The National Animal Research Authority, Norway. Two series of experiments were conducted:

Experiment series 1: Young actively growing rats (80 ± 3 grams) were divided into three different groups. Each group consisted of 6 rats: 3 females and 3 males. The target meal contained the circular plasmid DNA (pDNA) for group one, the linear pDNA for group two, and no pDNA (negative control group) for group three. For the target meal, which was administered by gavaging, a total of 50 μ g pDNA was mixed with the feed to group one and two. Two rats from each group were killed in a CO₂ chamber 2 h, 6 h and 3 days after receiving the target meal.

Experiment series 2: Six pregnant rats were separated into two groups. Group one did not receive any pDNA (control group). Group two were given pDNA mixed with their food daily from day 5 of established pregnancy. pDNA consisted of 50% circular and 50% linearized pDNA in a total of 100 μ g added (pipetted into the core region of the pellets) to 3 feed pellets that were given in the morning. In the afternoon, and after finishing the 3 feed pellets with DNA, the rats were given the same feed but without pDNA. The pregnant rats did not receive any pDNA the day they were killed. The adult rats and their foeti were killed (one rat from each group) in a CO₂ chamber at days 7 and 14 of gestation, and at weaning. The pups were killed by decapitation at weaning.

2.5. Rat Organs

Organs from both experiments were sampled and dissected as described by Pusztai *et al.* [46] and frozen in liquid nitrogen within 5 minutes *post mortem*. The following organs were sampled: mesenteric lymph nodes, pancreas, spleen, liver, kidney, thymus, heart, ovaries/uterus, brain, lung, gastronomic muscle and blood. The fetuses and placentas were collected from the pregnant rats.

2.6. DNA Isolation

The different rat organs were weighed and homogenized in a digestion buffer (10 mM Tris/HCl pH 8.0, 100 mM NaCl, 25 mM EDTA pH 8.0, 0.5% SDS) using an Ultra Turax T25 Basic homogenizer (Ika-Labortechnik, Germany). DNA was isolated using the QIAamp DNA isolation kit (Qiagen) according to the manufacturer's instructions. For mesenteric lymph nodes and spleen, 10 mg tissue was used for DNA isolation per sample (according to recommendations given in the instructions of the QIAamp DNA isolation kit) as they have a very high cell number for a given mass of tissue. For all other organs, 25 mg tissue was used. The DNA was isolated from baby rat organs, fetuses, rat feed and gastrointestinal contents using the FastPrep (FP120) shaker (Q-Biogene,) and the FastDNA kit (Q-Biogene). The DNA was isolated from blood using the QIAamp DNA blood kit (Qiagen). The

quantity and purity of DNA were measured spectrophotometrically (BioRad SmartSpec TM Plus) at OD_{260/280}.

2.7. PCR of a Rat Housekeeping Gene

Extracted DNA was analyzed for the presence of inhibitors by PCR amplification of a 1277 bp part of the rat Bradykinin B2 receptor gene [51,52]. The reactions were performed in three parallels in a total volume of 50 μ l containing the following: 1.3 μ l of primers (forward: 5-GTC CTT CGT TTT GAG TCT GG -3, reverse: 5- GGT TCT GTG TTG TAG GGA GT -3) at 10 μ M, 25 μ l DYNAzyme™ II PCR Master Mix (Finnzymes Oy, Finland), 17.4 μ l water and 5 μ l DNA template (200 ng). PCR was performed with the following thermal cycling conditions: 94°C for 4 min; 30 cycles of 94°C for 30 sec, 55°C for 30 sec and 74°C for 1 min and 30 sec, final extension at 74°C for 3 min. The PCR products were run on agarose gels for visualization.

2.8. PCR and Southern Hybridization of PhrGFPCaMV Amplicons

DNA isolated from the various tissues was analyzed for the presence of plasmid sequences. The PCR was designed to amplify a 391 bp (primers CaMV08F and CaMV08R-2) and a 922 bp (primers CaMV08F and CaMV08R) fragment, including the 35S CaMV promoter of the plasmid phrGFPCaMV (**Figure 2**). The reactions were performed in a total volume of 50 μ l containing the following: 1.0 μ l of primers (CaMV08F: 5-GAG TCG ACC TCG AGA CCA T-3, CaMV08R-2: 5-GCG GTA CAC GAA CAT CTC CT-3, CaMV08R: 5-AAA TGA GCC TTG GGA CTA TG) at 10 μ M, 25 μ l DYNAzyme™ II PCR Master Mix (Finnzymes Oy, Finland), 18 μ l water and 5 μ l DNA template (200 ng). Negative PCR controls (no template), negative rat controls (no DNA in feed), commercial rat genomic DNA (BD Bioscience) and positive controls (plasmid dilution series) were always included in each set of PCR analysis. PCR for the 391 bp sequence was performed with the following thermal cycling conditions: 94°C for 5 min; 35 cycles of 94°C for 30 sec, 64°C for 30 sec, and 72°C for 40 sec, final extension at 72°C for 7 min. and for the 922 bp sequence: 94°C for 5 min; 35 cycles of 94°C for 30 sec, 65°C for 30 sec, and 72°C for 1 min, final extension at 72°C for 7 min. The PCR products were run on 1% agarose gels and blotted onto Nytran N membranes (Schleicher & Schoell, Germany) with the TurboBlotter system (Schleicher & Schoell) before chemoluminescence detection using sequence specific dig-labeled oligonucleotide probes for the detection of the corresponding region in the CaMV promoter region. Signals were developed using the substrate CDP-Star (Roche, Germany) and visualized using a Lumi-Ima-

ger F1 (Roche).

2.9. Real time PCR Standard Curves, Amplification Efficiencies and Statistics

To assess the quantification range of the real time PCR system, a 10-fold dilution of the plasmid phrGFPCaMV ranging from 23 100 to 2.3 copies was prepared and analyzed by real time PCR as described below. The standard curve for phrGFPCaMV resulting from Ct values of each reaction plotted against the log of estimated copy numbers is presented in **Figure 1(a)**. The curve is linear in the range tested by triplicate reactions. The slope of the standard curve in **Figure 1(a)** is -3.35 and the square regression coefficient after the linear regression (R^2) is 0.99. The R^2 indicated a good correlation between Ct value of the amplification and the amount of template (DNA). The 10-fold diluted phrGFPCaMV samples were also spiked with the amount of DNA used (200 ng).

2.10. Real Time PCR Analysis

The DNA samples were analyzed for the presence of a 201 bp region within the 35S-CaMV promoter of the phrGFPCaMV plasmid using real-time PCR. Primers and probes were designed using the Primer Express program (Primer Express Software V. 2.0, Applied Biosystems, US). The real time PCR was performed on an ABI 7900 HT Fast Real Time PCR system (Applied Biosystems) with the primers 5-TGC CAT CAT TGC GAT AAA GG-3 and 5-GGA AGG GTC TTG CGA AGG AT-3 (Eurogentec, Belgium). The amplified PCR fragments were detected by a TaqMan probe with a 5'-FAM fluorescent dye and 3'-Dark Quencher label (5-ATC GTT GAA GAT GCC TCT GCC GAC A-3) (Eurogentec). The real time PCR included denaturation at 95°C for 10 sec., and 60°C for 1 min for 50 cycles. For amplification, the qPCR Mastermix Plus reagent (Eurogentec) was used. All samples were analyzed in triplicate, including positive (isolated plasmid DNA), negative control samples (no DNA in feed) and no template controls (dH₂O). A total of 200 ng DNA was analyzed in each well. This corresponds to approximately 28,500 cells as 1 mammalian cell in average contains 7 pg DNA [53]. For blood and sera, the DNA concentrations were lower due to overall lower DNA content and difficulties in extracting enough DNA.

3. Results

3.1. DNA Stability in Feed Pellets

Plasmid DNA was as added to rat feed pellets and incubated over time (0 to 72 hours) to determine whether this treatment would result in quantifiable pDNA degradation. The added DNA showed no signs of degradation over the 72 h incubation period at room temperature (**Table 1**). A

bacterial transformation assay was subsequently performed to verify that the feed-incubated pDNA retained its biological functionality if acquired by competent cells. As seen in **Table 2**, no significant reduction in the transformation frequencies could be seen when the bacteria were exposed to DNA extracted from feed pellets after up to 72 hours of incubation.

3.2. Target DNA Isolation, Housekeeping Gene PCR, and Real-Time PCR Standard Curves

The DNA was isolated from mesenteric lymph nodes, pancreas, spleen, liver, kidney, thymus, heart, ovaries, uterus, brain, lung, gastronomic muscle and blood. The % sample weight of each organ is given in **Table 3**. For each analysis 200 ng total DNA was used. Prior to regular PCR and real time PCR, a rat BK2-targeted PCR was run to detect any inhibition of the PCR amplification and no indication of the presence of inhibitors was found. The standard curve used in the real-time PCR was spiked with rat genomic DNA (200 ng) to check whether the PCR efficiency was affected. The Ct values and R^2 for these runs indicated that the amount of total DNA present have no effect on the amplification efficiency for any of the plasmid dilutions (**Figure 1(b)**).

3.3. Detection of Target DNA in Rat Organ and Tissue Samples

To detect the presence of feed introduced pDNA in the organ and tissue samples, a regular PCR amplification and subsequent Southern blotting were performed with primers and probes targeting part of the CaMV promoter. However, pDNA was not detected in organs, blood/sera or foeti of the pregnant rats and their offspring.

The real-time PCR quantification was based on detecting a 201 bp sequence inside the 35S CaMV promoter, and calculating copy numbers according to the standard curve based on serial dilutions of the pDNA (described in Materials and Methods) (**Figure 1(a)**). The mean value of estimated copy numbers was >2.3 if considered positive (see Material and Methods). All negative

Table 1. Quantification of DNA isolated from feed pellets.

Hours ^a	With plasmid DNA ^b	No DNA ^c
0	97.1 (± 16.9)	<0.0001
1	86.4 (± 03.8)	<0.0001
12	79.1 (± 11.3)	<0.0001
36	88.9 (± 29.2)	<0.0001
72	90.6 (± 11.5)	<0.0001

^aHours of DNA incubation in the pellets before sampling. ^bThe numbers are based on ABS₂₆₀ measurements of 3 parallels of DNA isolated from 0.5 g rat feed. Standard deviations are given in brackets. ^cDNA extracted from feed added H₂O (negative control).

Table 2. Natural transformation assay of *Acinetobacter baylyi* by plasmid DNA isolated from rat feed pellets.

Treatment (incubation time in pellet)	Total Bacteria (CFU)	Transformants (CFU)	Transformation Frequency ^a
Plasmid (0 h)	$4.7 (\pm 0.5) \times 10^8$	$3.3 (\pm 5.8) \times 10^2$	7.1×10^{-5}
Plasmid (1 h)	$3.6 (\pm 0.5) \times 10^8$	11.7 (± 7.9)	3.3×10^{-6}
Plasmid (12 h)	$3.3 (\pm 0.7) \times 10^8$	$3.5 (\pm 0.5) \times 10^2$	1.1×10^{-4}
Plasmid (36 h)	$4.0 (\pm 0.7) \times 10^8$	$2.7 (\pm 0.3) \times 10^2$	6.6×10^{-5}
Plasmid (72 h)	$1.8 (\pm 0.3) \times 10^8$	39.6 (± 3.5)	2.2×10^{-5}
pKTG (no inc.) ^b	$4.1 (\pm 0.6) \times 10^8$	$1.9 (\pm 1.0) \times 10^3$	4.5×10^{-4}
H ₂ O (0 h)	$3.4 (\pm 0.8) \times 10^8$	N.D.	$<3.0 \times 10^{-9}$
H ₂ O (72 h)	$3.1 (\pm 0.3) \times 10^8$	N.D.	$<3.2 \times 10^{-9}$
H ₂ O (no inc.) ^c	$1.8 (\pm 0.1) \times 10^8$	N.D.	$<5.6 \times 10^{-9}$

^aTransformation frequency: the number of transformants divided to the total number of recipients, after a 24 h transformation period. CFU: colony forming units. ^bPurified plasmid pKTG (not incubated with the feed pellets) was used as a positive control. ^cH₂O (instead of extracted DNA) was used as a negative control. ^dN.D.; Not detected.

Table 3. Amount of rat tissue used for DNA extraction and PCR analyses.

Organ (<i>n</i> = 12)	Mean organ weight (mg)	Tissue (mg) for DNA extraction	% total organ weight	DNA (μ g) obtained per extraction	% extracted DNA analyzed ^a
Mesenteric lymph nodes	294	10	3.4	19.9	1.0
Pancreas	353	25	7.1	32.2	0.6
Spleen	178	10	5.6	70.5	0.3
Liver	3798	25	0.7	43.1	0.5
Kidney	912	25	2.7	37.0	0.5
Thymus	243	25	10.3	79.5	0.3
Heart	350	25	7.1	10.1	2.0
Ovaries/Uterus	347	25	7.2	26.8	0.9
Testes/Prostates	858	25	2.9	N.A	N.A
Brain	1207	25	2.1	10.1	2.0
Lung	788	25	3.2	28.6	0.7
Gastronemius muscle	730	25	3.4	8.7	2.3

^aPer PCR tube; 200 ng DNA analyzed per reaction and/or real-time PCR. Each PCR/Real-time PCR was run in triplicate per organ sample. N.A = Not analyzed.

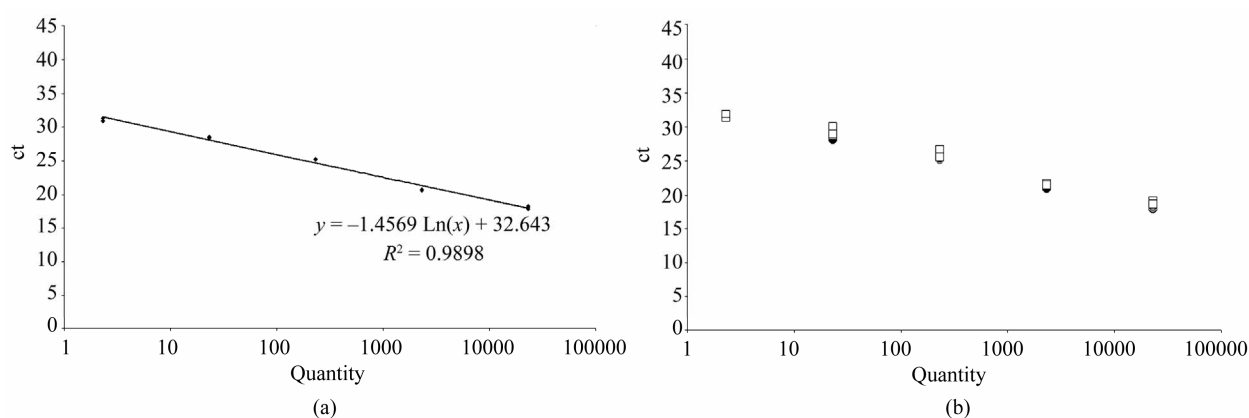


Figure 1. (a) Standard curve for real time PCR. Ten-fold dilutions of the plasmid phrGFPCaMV ranging from 100 fg to 0.01 fg (23 100 to 2.3 copies) were used and each dilution was run in triplicate. The slope and correlation coefficients were calculated for each run. The Ct value is the threshold cycle at which the system starts to detect the increase in signal associated with an exponential growth of the PCR product during the log-linear phase. (b) Standard curve for real time PCR with added rat DNA. The plasmid dilutions, ranging from 23,100 to 2.3 copies, were also in parallel added 200 ng genomic rat DNA. Squared open points; not added genomic DNA, and filled black circles; added genomic DNA.

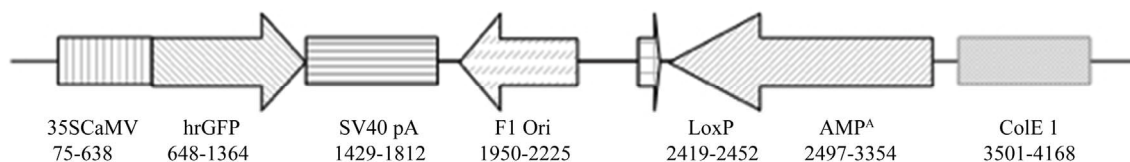


Figure 2. Linear map of the phrGFP-CaMV plasmid (4220 bp)(not to scale).

sample controls (rats fed with no DNA) were negative under these conditions. The results for young, actively growing rats are summarized below. The results are given as estimated plasmid copy numbers using the standard curve. The pDNA targets detected were below 64 copies per analyzed sample. Collectively, through the 3 days sampling period after the pDNA feeding, target pDNA was detected in 4 out of 12 rats (three female and one male). Of these four, three had received linear pDNA and one received circular pDNA. It is noteworthy that pDNA was still detectable in the liver of one rat 3 days after the single pDNA-containing meal. More specifically, pDNA was detected in the mesenteric lymph node (11 copies), the spleen (5 copies), the liver (3 copies) and the pancreas (63 copies) of one female rat fed linear plasmid DNA two hours after feeding, (Rat 4). In another female rat fed linear DNA and sampled after two hours, pDNA was detected in the pancreas only (6 copies) (Rat 3). The pDNA was detected in the kidney (6 copies) of one female rat (Rat 6) after 6 hours. After 3 days, 2 pDNA copies were detected in the liver of one female rat fed linear DNA (Rat 12). No pDNA detections were made in the samples from the 6 control rats (numbers 13 - 18) that received a meal without pDNA. Tissue samples from pregnant rats, foeti, and offspring pups were also investigated by real-time PCR. However, no pDNA from organs, blood/sera and foeti samples could be detected (detection limit >2 copies per sample).

4. Discussion

The data presented here demonstrate uptake and intra-organismal spread of feed-introduced DNA. They also extend the observations made in previous feeding studies [1-35] as only few studies published so far have examined the fate of feed introduced DNA in rapidly growing/developing animals. This study examined the hypothesis that the frequency of uptake of feed introduced DNA from the GIT may differ in rapidly growing individuals from those recorded in previous studies of adult individuals due to the high demand for nutrient uptake during growth. However, although pDNA was more readily detected in the organs of young actively growing rats than the pregnant adults, no indications of a substantially elevated uptake rate in young, growing rats as compared to adults were found. Additional novel observations reported here include that feed introduced DNA

is present in the pancreas. This has not been shown in previous feeding studies. Interestingly, three of the four rats with detectable pDNA in tissues were feed linear DNA, possibly indicating that linear DNA may be more easily taken up from the GIT or persist longer than circular DNA. Two previous studies in mice have also indicated that linearized polyomavirus DNA is more infectious than circular versions [54,55].

The current study also showed several differences to previous reports. Importantly, our study was not able to detect feed introduced DNA in any organs or in offspring foeti/pups of the continuously pDNA exposed pregnant rats as previously demonstrated by Schubert *et al.* [3]. The amount of pDNA used in our study (50 μ g plasmid DNA per rat) corresponds to approximately 10^{13} phrGFP plasmid molecules. Schubert *et al.* [3] detected low-level DNA transfer from gut to liver and spleen after feeding 10^{13} plasmids molecules per mouse. This latter feeding design therefore represents a higher dose relative to body weight, when compared to the current study. However, both studies are within the normal range of DNA exposures as rodents ingest milligram quantities of foreign DNA with their daily food intake [53].

Our study did not detect pDNA in blood, although DNA has previously been reported to persist in blood samples from mammals, including humans and mice [24, 27,56,57]. The reason for this is unclear. However, the different feeding regimes used in the various published studies might have influenced the time-dependent distribution of feed-introduced DNA and caused the differences seen. For instance, gavage feeding avoids initial DNA degradation in the oral cavity, and the amount of DNA that reaches the GIT will be higher compared to the experimental designs using orally ingested pDNA. Duggan *et al.* [10] demonstrated that approximately 70% of pDNA was extensively degraded after incubation for one minute *in vivo* in the ovine oral cavity. The results that differ from other reports may also be due to variations in sampling in relation to a time-dependent DNA uptake, clearance and degradation of DNA in different rodent species.

In summary, although it is known that the penetration/transfer of macromolecules over the GIT barrier and their further distribution/clearance is influenced by age and that mammals are especially susceptible to macromolecules uptake in the neonatal period [58,59], no evidence

was found suggesting actively growing rats are more susceptible to take up feed introduced DNA. Other novel findings include the observation of feed DNA in the pancreas, and possibly increased uptake rates of linear DNA as compared to circular DNA.

The presented and previous studies have all focused on the mechanistic aspects of DNA uptake and distribution. Few studies have examined the biological outcome of rare uptake and integration events of feed-derived DNA in mammalian cells [28,29,60]. However, alimentary administration of DNA vaccines provide evidence that DNA, if taken up from the GIT, can be transcribed, translated, and presented to the immune system as a functional protein [61-64]. Therefore, further studies on the biological effect and physical/functional state of food-ingested DNA in mammals' GIT should be undertaken.

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