Science in China Series C: Life Sciences

Springer

www.scichina.com
life.scichina.com
www.springer.com/scp
www.springerlink.com

Article

Generation of *fad2* transgenic mice that produce omega-6 fatty acids

CHEN Qing[1], LIU Qing[2], WU ZhiFang[1], WANG ZongYi[3] & GOU KeMian[1†]

[1] State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing 100193, China;
[2] Blackmountain Laboratory, CSIRO Plant Industry, Canberra, Australia;
[3] College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

Fatty acid desaturase-2 (FAD2) introduces a double bond in position Δ12 in oleic acid (18∶1) to form linoleic acid (18∶2 *n*-6) in higher plants and microbes. A new transgenic expression cassette, containing CMV promoter/*fad2* cDNA/SV40 polyA, was constructedto produce transgenic mice. Among 63 healthy offspring, 10 founders (15.9%) integrated the cotton *fad2* transgene into their genomes, as demonstrated by PCR and Southern blotting analysis. All founder mice were fertile and heterozygous *fad2* female and nontransgenic littermates were used for fatty acid analysis using gas chromatography. One *fad*2 transgenic line showed substantial differences in the fatty acid profiles and the level of linoleic acid was increased 19% (*P*<0.05) in transgenic muscles compared to their nontransgenic littermates. Moreover, it exhibited an 87% and a 9% increase (*P*<0.05) in arachidonic acid (20∶4 *n*-6) in muscles and liver, compared to their nontransgenic littermates. The results indicate that the plant *fad2* gene can be functionally expressed in transgenic mice and may playan active role in conversion of oleic acid into linoleic acid.

fatty acid desaturase, FAD2, transgenic mouse, oleic acid, linoleic acid, arachidonic acid

Plant fatty acid desaturase-2 (FAD2) introduces a double bond in position Δ12 in oleic acid (18∶1) to form linoleic acid (18∶2 *n*-6, LA). Because mammals lack the FAD2 enzyme and cannot synthesize *de novo n*-6 fatty acids, they must take it from plant or seafood in their diets. Polyunsaturated fatty acids (PUFAs) including *n*-6 fatty acids are constituents of cellular membrane phospholipids which affect membrane fluidity and the function of many membrane proteins (enzymes and receptors)[1]. The serve as signaling molecules or precursors of other signaling molecules, and influence the physiological function of cardiovascular, brain and nerve systems[2]. *n*-6 fatty acids may be associated with several diseases such as hyperinsulinism, arterosclerosis, and cancer[3]. Therefore *fad2* transgenic mice are used to study the mechanism of the endogenous synthesis of polyunsaturated fatty acids. Which are also significant factorsin clinical fields and livestock breeding. Saeki and colleagues in 2004 showed functional expression of a spinach (*Spinacia oleracea*) desaturase gene in transgenic pigs[4]. It was the first study wherein a plant gene was functionally expressed in transgenic animals[5]. In their study, the level of LA in white adipose tissue was increased by 20% in the *fad2* transgenic pigs, but the level of arachidonic acid (20∶4 *n*-6, AA) did not change[4]. In early 2005, we constructed a cotton (*Gossypium hirsutum*) *fad2* expression vector (pFAD2) driven by alpha-*actin* promoter and produced the transgenic mice. Unfortunately, the transgenic mouse failed to express the exogenous gene[6]. In this study, the *fad2* gene wasconstructed into the expression vector (pCMV-FAD2) driven by a CMV promoter and successfully produced

Received March 11, 2009; accepted June 3, 2009
doi: 10.1007/s11427-009-0143-z
†Corresponding author (email: goukm@cau.edu.cn)
Supported by the National Basic Research and Development Program of China (Grant No. 2004CB117500), the National Natural Science Foundation of China (Grant No. 30571332), and the National Major Special Project on New Varieties Cultivation for Transgenic Organisms (Grant No. 2008ZX08008-003)

Citation: CHEN Q, LIU Q, WU Z F, et al. Generation of *fad2* transgenic mice that produce omega-6 fatty acids. Sci China Ser C-Life Sci, 2009, 52(11): 1048-1054, doi: 10.1007/s11427-009-0143-z

transgenic mice expressing cotton FAD2 desaturase. The transgenic line endogenously synthesize not only LA but also AA in the tested tissues. This transgenic model may have value in the investigation of the mechanism of related diseases.

1 Materials and methods

1.1 Chemicals

All chemicals for manipulating the mouse embryo and PUFA Mix No. 2 (47015-U) as the lipid analytical standard were purchased from Sigma/Aldrich (St. Louis, MO, USA). Restriction enzymes and T*aq* polymerase were purchased from Takara Biomedical(Dalian,China). DNase, Klenow enzyme, T4 ligase, oligo-dT primers and MMLV reverse transcriptase were purchased from Promega Incorporation (Madison, USA). DNA markers, TRIzol Reagent and TIANgel Midi Purification Kit were purchased from Tiangen Company (China).

1.2 Animals

Mice C57BL/6 and DBA/2 at 8 weeks of age were obtained from the Beijing Experimental Animal Center. Hybrid B6D2F1 mice were produced by the natural mating of C57BL/6 female and DBA2 males in our animal facility and used to produce the transgenic mice. Transgenic mice were backcrossed with C57BL/6 mice through at least four generations before PUFA analysis. All animals were raised with a diet deficient in omega-3 fatty acids and maintained in a light-controlled room (14L∶10D, lights on at 06: 00 AM) at a temperature of 22℃. All animal procedures in the present study were approved by the Committee for Experimental Animals of our university.

1.3 Construction of the expression vector

The vector pCMV-FAD2 was constructed by modifying a plasmid pFAD2 (6.3 kb) used in our previous study[6]. Briefly, the pFAD2 was digested by restriction enzymes *Nhe* I and *Hind* III to remove the 0.9 kb alpha-actin promoter, and then polished with a Klenow enzyme, ligating the vector using T4 ligase (Figure 1). The new plasmid was named pCMV-FAD2, which carried the *fad2* cDNA driven by the CMV promoter and was confirmed by enzyme digestion and DNA sequencing. The 5.4-kb transgene was digested using the restriction enzyme *Afl* II, isolated by preparative electrophoresis on an agarose gel, purified using a TIANgel Midi Purification Kit, and dissolved in TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0) at a final concentration of 5 μg/mL for DNA microinjection.

1.4 Generation of transgenic mice

B6D2F1 female mice, 6—8 weeks old, were i.p. injected with 5.0 IU pregnant mare's serum gonadotropin (Hua-Fu Hi-tech Biological Company, Tianjin, China) followed by 5.0 IU human chorionic gonadotropin (hCG; Hua-Fu Hi-tech) 48 h later, and mated with B6D2F1 males of proven fertility. Pronuclear-stage embryos were collected from excised oviducts at 24-h post-hCG, freed from the cumulus by treatment with 300 IU/mL hyaluronidase (Sigma) in a modified CZB with 20 mmol/L

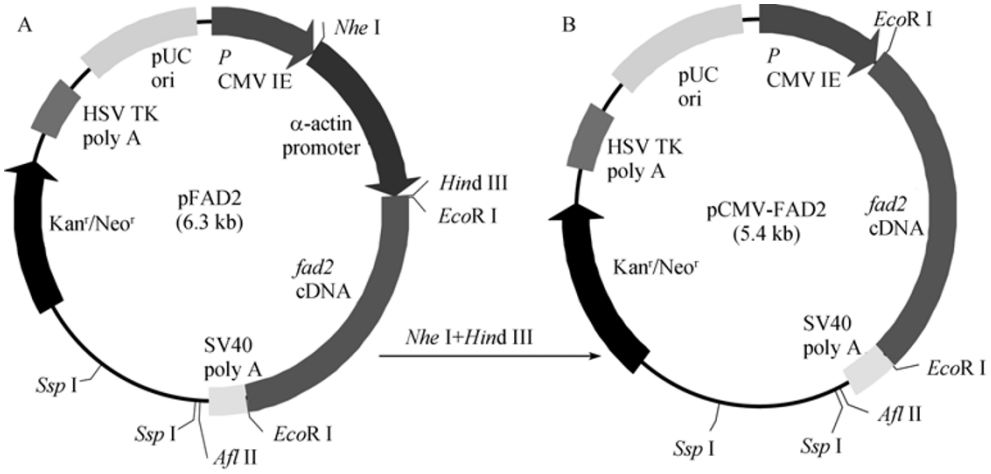

Figure 1 Construction of the expression vector pCMV-FAD2. The vector pFAD2 (Left) driven by an alpha-*actin* promoter is digested with *Nhe* I and *Hin*d III and re-ligate to construct the pCMV-FAD2 vector (Right).

HEPES and 5 mmol/L $NaHCO_3$ for 1—2 min at 37℃, thoroughly rinsed and kept in CZB medium at 37℃ with 5% CO_2 until DNA microinjection. The purified transgene was microinjected into the pronuclei of embryos. All surviving embryos were cultured in CZB medium. One h later, these embryos were then transferred to the oviducts of 0.5-dpc pseudopregnant females, which mated naturally with vasectomized males.19—20 d later, the babies were delivered naturally, with their tails biopsied at 3 weeks of age and tested for the transgene by PCR and Southern blotting as described below.

1.5 Transgene detection by PCR and Southern blot analysis

Approximately, 0.5 cm of mouse tail was digested by using SNET solution containing 0.4 mg/mL proteinase K (Invitrogen) at 55℃ overnight. DNA was extracted using phenol∶chloroform∶isoamyl alcohol (25∶24∶1) and precipitated by isopropyl alcohol. The extracted DNA was dissolved in TE Buffer (pH 8.0), and 0.5—1.0 μg DNA were used for PCR amplification. The PCR primers (Invitrogen, China) used to probe for the *fad2* gene were sense 5′-tttcactttctcccacaacc-3′ and antisense 5′-ccaatccc attcagacga-3′. The PCR reaction was carried out under the following conditions: preincubation at 95℃ for 5 min, followed by amplification for 35 cycles. Each amplification cycle consisted of desaturation at 94℃ for 1 min, annealing at 57℃ for 1 min, and extension at 72℃ for 1 min. Cycling was followed by a final extension step at 72℃ for 10 min, and then cooled down to 4℃. Wild-type and positive (wild-type mixed with *fad2* expression plasmid) DNA samples were included as controls.

Southern blotting was performed according to standard procedures[7]. In brief, genomic DNA (10 μg) was digested with *Eco*R I and *Ssp* I for 12—16 h at 37℃. The digested products were subjected to overnight 0.8% agarose gel electrophoresis. The gel was stained with Ethidium Bromide and photographed using a Versadoc imaging system (Bio-Rad, USA). The DNA was denatured by using an alkaline transfer buffer and transferred from the agarose gel to Hybond-N^+ membrane (Amersham, UK) by downward capillary transfer. The 3.1-kb *fad2* fragments digested by *Eco*R I & *Ssp* I were labeled by random priming (Rediprime II Random Prime Labeling System, Amersham, UK) with [α-^{32}P] dCTP (Furui, China) and used as probes. The membrane was subsequently hybridized with labeled probes according to standard protocols, using storage phosphor autoradiography (GE Healthcare, USA) for 5 d to obtain an autoradiographical image. Wild-type genome alone, or mixed with *fad2* fragments, were used as controls.

1.6 RT-PCR

RT-PCR was carried out to detect the expression of *fad2* in the offspring of transgenic founders, which included both transgenic and wild-type littermates. The total RNA was extracted from muscle tissue using the TRIzol Reagent according to the manufacturer's instructions. RNA samples were treated with DNase prior to RT-PCR. Purified RNA (500 ng) was used for first-strand cDNA synthesis. Reverse transcription was performed using oligo-dT primers at 42℃ for 1 h and then at 90℃ for 5 min. The resulting cDNA was used in PCRs with primers specific for *fad2* (5′-tacatcagcgacacaggcatc-3′ and 5′-gtatttgtgagccagggcatt-3′) as described above. The amplification of *hprt* was used as an internal control using the sense (5′-cctgctggattacattaaagcactg-3′) and antisense (5′-gtcaagggcatatccaacaacaaac-3′) primers. PCR was carried out by heating at 95℃ for 5 min, followed by 35 cycles consisting of 94℃ for 15 s, 55℃ for 30 s and 72℃ for 1 min, and a final extension at 72℃ for 7 min. An RNA sample from the muscle tissue of a wild-type mouse was used as a negative control and wild-type genomic DNA mixed with *fad2* fragment was used as a positive control.

1.7 PUFAs analysis

The male transgenic mice were mated with female C57BL/6 mice and which yielded nontransgenic and transgenic littermates. Female offspring at 6 weeks of age was used for the PUFA analysis using gas chromatography as previously described[8]. Fresh mouse tissue was homogenized by grinding it in liquid nitrogen and an aliquot of tissue homogenate in a glass methylation tube was mixed with 1.5 mL of hexane and 1.5 mL of 14% BF_3/MeOH reagent (Sigma). After being blanketed with nitrogen, the mixture was heated at 100℃ for 1 h, cooled down to room temperature after which methyl esters were extracted in the hexane phase following addition of 1.5 mL of H_2O. The samples were centrifuged at 3000 r/min for 1 min, and then the upper hexane layer was removed. Fatty acid methyl esters were analyzed by gas chromatography using a fully automated 6890 Network GC System (Agilent Technologies, Palo Alto, CA,

USA) equipped with a flame-ionization detector. The chromatography utilized an Agilent J&W fused-silica capillary column (DB-23; 60 m, 0.25-μm film thickness, 0.25-mm i.d.; Agilent). The injector and detector ports were set at 250℃. FAME were eluted using a temperature program set initially at 180℃ and held for 6 min, increased at 6.5℃/min and held at 205℃ for 3 min, increased at 3℃/min, and held at 240℃ for 3 min to complete the run. The carrier gas was helium, set to a 230 kPa constant pressure. Peaks were identified by comparison with fatty acid standards, and the area percentage for all resolved peaks was analyzed using GC ChemStation Software (Agilent Technologies).

1.8 Statistical analysis

All data in this study were expressed as mean±SD. Differences in fatty acid composition were assessed by an unpaired Student's *t*-test. Statistical significance was accepted at the level of $P<0.05$ and Prism 4 for Windows Software (GraphPad) was used for all calculations.

2 Results and discussion

2.1 Generation of transgenic mice

The linear transgenic cassettes were microinjected into the pronuclei of 1-c embryos. Seven recipients transferred with 184 embryos were pregnant and gave birth to 63 (34.2%) offspring. PCR analysis of DNA samples derived from the 63 offspring showed the presence of transgenes in ten mice (Figure 2A). To confirm the integration of the transgene, Southern blotting analysis (Figure 2B) was carried out using a *fad2* DNA probe, which was designed to hybridize a 3100 bp fragment and demonstrated that all PCR positive mice integrated foreign genes with a frequency of 15.9% (10/63). The integration rate was consistent with the previous study in our laboratory (10%—20%), and was also similar to the international integration rate (10%—40%) by microinjection[9]. All 10 founders were fertile and normally developed to adulthood.

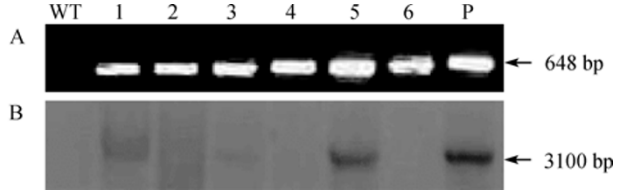

Figure 2 Transgene analysis.

PCR (A) and Southern blotting (B) analysis of total DNA from control (wt), and transgenic mice (1—6) showed that mice 1, 3 and 5 were *fad*2 transgenic, whereas mice 2, 4 and 6 were nontransgenic. Positive samples in lane P were the mixture of the wt genome and the *fad*2 plasmid. PCR products were 648-bp spanning the *fad*2 gene. The 3100-bp fragments of *fad2* functioned as probes during Southern blotting analysis.

2.2 RT-PCR

To assess the expression potential of the *fad*2 transgene *in vivo,* we extracted the total RNA from various tissues and analyzed the mRNA by RT-PCR with specific primers for *fad*2 and housekeeping *hprt* genes. RT-PCR analysis indicated that the transgene was expressed in all ten transgenic lines. As partial results showed in Figure 3, the *fad*2 gene was expressed in transgenic muscle tissues, while no transgenic signal was detected in the nontransgenic littermates.

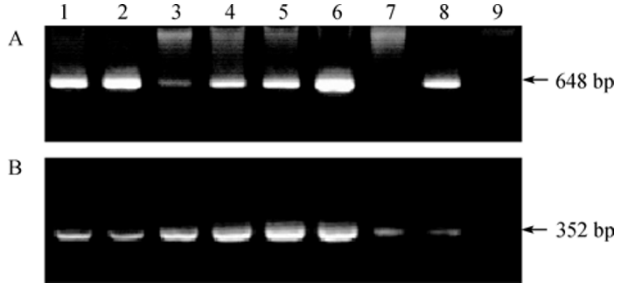

Figure 3 RT-PCR analysis of transgenic mice. The total RNA was extracted from muscle tissues from different transgenic lines and wild-type mice. The *fad2* specific 648-bp fragments A, were amplified by RT-PCR, as well as the internal control, the *hprt* specific 352-bp fragments B. In both panels, lane 1—6, offspring of different transgenic founder; lane 7, wild-type; lane 8, wild-type mixed with *fad2* expression plasmid; and lane 9, wild-type without reverse transcriptase.

2.3 Elevated level of *n*-6 fatty acids in tissues of transgenic mice

To determine whether or not the transgenic mice expressing FAD2 enzymes catalyzed the synthesis of fatty acid contents in their tissues, the heterozygous *fad*2 and nontransgenic littermates were used for PUFA analysis. Among the ten *fad*2 transgenic lines, there was only one line which showed substantial differences in the fatty acid profiles. Figure 4 showed the fatty acid profile of muscle samples from heterozygous transgenic and nontransgenic littermates derived from the transgenic expression line. As shown in Table 1, although the concentrations of oleic acid (18∶1) were not significantly

(P>0.05) changed in transgenic muscle tissues ((8.580± 1.232) vs (10.812±1.244)) and liver tissue ((9.236±0.965) vs (9.638±0.593)) compared with the nontransgenic littermates, the concentrations of linoleic acid in transgenic muscle ((15.653±0.557) vs (13.168± 0.634)) and liver ((15.962±0.552) vs (15.200±0.170)) were significantly (P<0.05) higher than that of their nontransgenic littermates. Consequently, the level of linoleic acid was increased 19% in these transgenic muscle and 5% in liver tissues. Saeki et al.[4] showed that the level of LA in white adipose tissue from pigs expressing spinach FAD2 was increased by 20% in comparison to wild-type controls. Taken together, our results, as well as those of Saeki et al.[4] indicated that the *fad2* is functionally expressed in a complex mammalian system and the expressed *fad*2 in transgenic animals actively converts oleic acid into LA.

Due to the lack of the FAD2 desaturase, mammals were unable to synthesize LA, and therefore relied on a dietary supply. The *n*-6 fatty acids including 18∶3, 20∶3, 20∶4, 24∶5 and 22∶5 are derived from dietary LA synthesized via a series of desaturation and elongation reactions (Figure 5). In *fad2* transgenic mice, it promoted the *de novo* biosynthesis of the other *n*-6 classes. Therefore, we analyzed the downstream *n*-6 fatty acids including γ-linolenic acid (18∶3 *n*-6) and AA. No significant difference ($P\geqslant$0.05) in the level of γ-linolenic acid (18∶3 *n*-6) from muscle tissues was detected between transgenic (0.227±0.026) and wild-type mice (0.290±0.341). However, muscle tissue from transgenic mice contained 87% more AA than that from the wild-type controls ((12.850±1.479)vs.(6.871±0.665), P<0.01). In addition, the level of AA from the transgenic liver tissues (12.607±0.623) was increased by 9% compared to wild-type samples (11.601±0.492; P<0.05). These results suggested that the *fad*2 transgenic mice had efficiently converted oleic acid into LA and resulted in an increase of the AA composition in their bodies. The study of the transgenic pigs expressing spinach *fad2* indicated the increase of the amount of LA with no AA change detected in the white adipose tissue[4]. Our results showed that the increase of substrate LA from muscle tissues in transgenic mice resulted in a significant increase of the synthesis of AA, which is one kind of active *n*-6 PUFA extremely rich in the animal nervous system delivered to their offspring through the placenta and through milk. It is also critical for brain development during the fetal and postnatal development in the same manner as *n*-3 fatty acid DHA (22∶6 *n*-3)[10,11]. AA is a precursor of families of signaling molecules including prostaglandins and leukotrienes (LTB4) in humans and animals (Figure 5).

Previous studies about PUFAs mainly focused on changing the fatty acid constituents to make them more

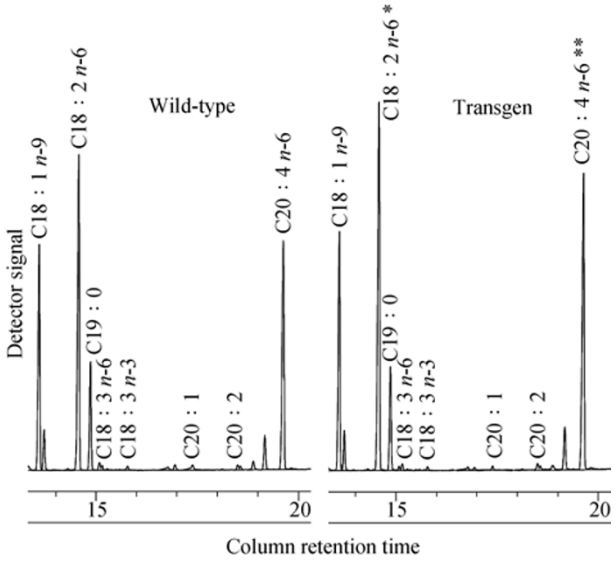

Figure 4 Partial gas chromatogram traces showing fatty acid profiles of total lipid extracted from mouse muscle. Both the wild-type (wt, left) and heterogeneous *fad*2 transgenic (tg, right) littermates were 6-week-old females that were fed the same diet. The lipid profiles showed that levels of *n*-6 fatty acids (18∶2 *n*-6 and 20∶4 *n*-6, asterisks) were markedly higher in tg than in wt muscle whereas the concentrations of oleic acid (18∶1) showed no difference.

Table 1 Profiles of partial polyunsaturated fatty acids in muscle and liver tissues of transgenic (tg) and nontransgenic (wt) littermates

Fatty acids	Muscle		Liver	
	wt (n=8)	tg (n=8)	wt (n=4)	tg (n=4)
18∶1 *n*-9	10.812±1.244	8.580±1.232	9.638±0.593	9.236±0.965
18∶2 *n*-6	13.168±0.634	15.653±0.557*	15.200±0.170	15.962±0.552*
18∶3 *n*-6	0.290±0.341	0.227±0.026	0.210±0.013	0.256±0.036*
20∶4 *n*-6	6.871±0.665	12.850±1.479**	11.601±0.492	12.607±0.623*

Muscle tissue samples are taken from eight transgenic (tg) or nontransgenic (wt) female littermates at 6 weeks of age. Liver tissue samples are taken from four tg or wt female littermates at 6 weeks of age. The values (% of total fatty acids) are expressed as means±SD. *, P<0.05; **, P<0.01.

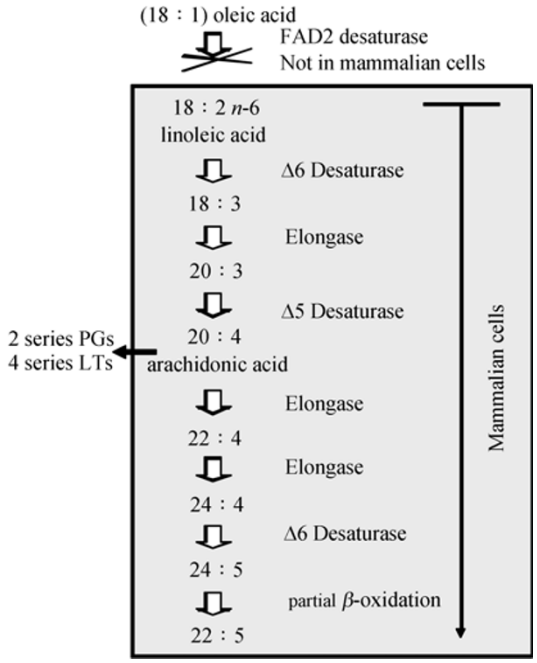

Figure 5 The biosynthetic pathway of *n*-6 fatty acids.

beneficial for health by adjusting the diet. Such studies might yield conflicting results due to the inevitable variance in dietary composition and eating habits of humans or animal[12]. In general, vegetable oils, meats from beefs, pigs, and chicken (sourcing from plants) contain a high content of *n*-6 fatty acids, whereas fish oils contain high content of *n*-3 fatty acids. Changes in fatty acid content by expressing foreign desaturase genes in transgenic animal models might reveal the essential effect of fatty acids on the body[13]. A transgenic animal model expressing the *C. elegans fat-1* gene encoding an *n*-3 fatty acid desaturase that converts *n*-6 to *n*-3 fatty acids has been generated[14]. The *fat-1* mice have now been widely used in studying the effects of fatty acids on various adverse health conditions[15–19]. *fad2* transgenic pigs and *fat-1* transgenic mice may serve as references for the commercial production of transgenic animals[4,20]. PUFAs serve as signaling molecules or precursors of other signaling molecules, such as prostaglandins, leukotrienes, and thromboxanes, and influence a wide range of biological effects. Deficiency of *n*-6 or *n*-3 series' fatty acids may cause quite different symptoms in mammals. Lack of *n*-3 fatty acid results in several nervous system diseases, whereas lack of *n*-6 fatty acid results in several monneuronal abnormalities, including reduced growth, reproductive failure, skin lesions, fatty liver, and polydipsia[1]. Furthermore, *n*-6 fatty acid have been indicated in several diseases such as hyperinsulinism, arteriosclerosis, and cancer, etc[21–24]. Besides the *fad2* transgenic pigs created by Saeki et al.[4], the *fad2* transgenic mice we produced may offer an opportunity for future study of these diseases.

We are grateful for Prof. An Xiaorong and Dr. Wang Yu in our lab for the help in designing and carrying out the experiments.

1 Marszalek J R, Lodish H F. Docosahexaenoic acid, fatty acid-interacting proteins, and neuronal function: breastmilk and fish are good for you. Annu Rev Cell Dev Biol, 2005, 21: 633—657
2 Kang J X. The importance of omega-6/omega-3 fatty acid ratio in cell function. The gene transfer of omega-3 fatty acid desaturase. World Rev Nutr Diet, 2003, 92: 23—36
3 Simopoulos A P. Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr, 1991, 54: 438—463
4 Saeki K, Matsumoto K, Kinoshita M, et al. Functional expression of a Delta12 fatty acid desaturase gene from spinach in transgenic pigs. Proc Natl Acad Sci USA, 2004, 101: 6361—6366
5 Niemann H. Transgenic pigs expressing plant genes. Proc Natl Acad Sci USA, 2004, 101: 7211—7212
6 Wang Y, An X R, Liu Q, et al. Establishment of transgenic mice integrated with gene encoding cotton omega-6 fatty acid desaturase-2. J Agri Biotechnol, 2005, 13: 207—211
7 Sambrook J, Fritsch E F, Maniatis T. Molecular Cloning: A Laboratory Mannual 3th ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press. 2001.
8 Kang J X, Wang J. A simplified method for analysis of polyunsaturated fatty acids. BMC Biochem, 2005, 6: 5
9 Giraldo P, Montoliu L. Size matters: Use of YACs, BACs and PACs in transgenic animals. Transgenic Res, 2001, 10: 83—103
10 Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr, 1992, 120: S129—S138
11 Innis S M. Polyunsaturated fatty acids in human milk: An essential role in infant development. Adv Exp Med Biol, 2004, 554: 27—43
12 Madsen L, Pedersen L M, Liaset B, et al. cAMP-dependent signaling regulates the adipogenic effect of *n*-6 polyunsaturated fatty acids. J Biol Chem, 2008, 283: 7196—7205
13 Morimoto K C, Van Eenennaam A L, DePeters E J, et al. Endogenous production of n-3 and *n*-6 fatty acids in mammalian cells. J Dairy Sci, 2005, 88: 1142—1146
14 Kang J X, Wang J, Wu L, et al. Transgenic mice: Fat-1 mice convert *n*-6 to *n*-3 fatty acids. Nature, 2004, 427: 504
15 Jia Q, Lupton J R, Smith R, et al. Reduced colitis-associated colon cancer in Fat-1 (*n*-3 fatty acid desaturase) transgenic mice. Cancer Res, 2008, 68: 3985—3991

16 Nowak J, Weylandt K H, Habbel P, et al. Colitis-associated colon tumorigenesis is suppressed in transgenic mice rich in endogenous n-3 fatty acids. Carcinogenesis, 2007, 28: 1991—1995
17 Hudert C A, Weylandt K H, Lu Y, et al. Transgenic mice rich in endogenous omega-3 fatty acids are protected from colitis. Proc Natl Acad Sci USA, 2006, 103: 11276—11281
18 Xia S, Lu Y, Wang J, et al. Melanoma growth is reduced in fat-1 transgenic mice: Impact of omega-6/omega-3 essential fatty acids. Proc Natl Acad Sci USA, 2006, 103: 12499—12504
19 Connor K M, SanGiovanni J P, Lofqvist C, et al. Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. Nat Med, 2007, 13: 868—873
20 Lai L, Kang J X, Li R, et al. Generation of cloned transgenic pigs rich in omega-3 fatty acids. Nat Biotechnol, 2006, 24: 435—436
21 Almendingen K, Hostmark A T, Fausa O, et al. Familial adenomatous polyposis patients have high levels of arachidonic acid and docosahexaenoic acid and low levels of linoleic acid and alpha-linolenic acid in serum phospholipids. Int. J Cancer, 2007, 120: 632—637
22 Polesel J, Talamini R, Montella M, et al. Linoleic acid, vitamin D and other nutrient intakes in the risk of non-Hodgkin lymphoma: An Italian case-control study. Ann Oncol, 2006, 17: 713—718
23 Reyes N, Reyes I, Tiwari R, et al. Effect of linoleic acid on proliferation and gene expression in the breast cancer cell line T47D. Cancer Lett, 2004, 209: 25—35
24 Kilian M, Mautsch I, Gregor J I, et al. Influence of conjugated vs. conventional linoleic acid on liver metastasis and hepatic lipidperoxidation in BOP-induced pancreatic cancer in Syrian hamster. Prostaglandins Leukot. Essent Fatty Acids, 2002, 67: 223—228