ORIGINAL

Isolation and characterization of a novel gene *sfig* in rat skeletal muscle up-regulated by spaceflight (STS-90)

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Abstract : We obtained the skeletal muscle of rats exposed to weightless conditions during a 16-day-spaceflight (STS-90). By using a differential display technique, we identified 6 up-regulated and 3 down-regulated genes in the gastrocnemius muscle of the spaceflight rats, as compared to the ground control. The up-regulated genes included those coding Casitas B-lineage lymphoma-b, insulin growth factor binding protein-1, titin and mitochondrial gene 16 S rRNA and two novel genes (function unknown). The down-regulated genes included those encoding RNA polymerase II elongation factor-like protein, NADH dehydrogenase and one novel gene (function unknown). In the present study, we isolated and characterized one of two novel muscle genes that were remarkably up-regulated by spaceflight. The deduced amino acid sequence of the spaceflight-induced gene (*sfig*) comprises 86 amino acid residues and is well conserved from Drosophila to Homo sapiens. A putative leucine-zipper structure located at the N-terminal region of *sfig* suggests that this gene may encode a transcription factor. The up-regulated expression of this gene, confirmed by Northern blot analysis, was observed not only in the muscles of spaceflight rats but also in the muscles of tail-suspended rats, especially in the early stage of tail-suspension when gastrocnemius muscle atrophy initiated. The gene was predominantly expressed in the kidney, liver, small intestine and heart. When rat myoblastic L6 cells were grown to 100% confluence in the cell culture system, the expression of *sfig* was detected regardless of the cell differentiation state. These results suggest that spaceflight has many genetic effects on rat skeletal muscle. J. Med. Invest. 50 : 39-47, 2003

Keywords **:** *spaceflight/differential display approach/skeletal muscle gene/rats*

Abbreviations : ANOVA, analysis of variance ; Cbl, Casitas B-lineage lymphoma ; CK, creatine kinase ; E3, ubiquitin ligase ; FCS, fetal calf serum ; MEF2C, myocyte enhance factor 2C ; NASA, National Aeronautics and Space Administration ; NASDA, National Space Development Agency of Japan ; PCR, polymerase chain reaction, RT, reverse transcription ; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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INTRODUCTION

Skeletal muscles, especially antigravity slow-twitch muscles, are vulnerable to rapid and marked atrophy under microgravity or its simulated conditions (1, 2). We previously reported that spaceflight (STS-90) as well as tail-suspension stimulated the ubiquitination of various proteins, including myosin heavy chain (MHC), and the accumulation of MHC degradation fragments in atrophied rat gastrocnemius muscle (3). In this case, the spaceflight significantly increased mRNA levels of cathepsin L, proteasome components (RC2 and RC9), polyubiquitin and a ubiquitin-conjugating enzyme in gastrocnemius muscle. Based on these findings, we suggest that skeletal muscle may adapt to microgravity conditions by changing its gene expression. However, there are few data concerning the gene expression in skeletal muscle under microgravity conditions. There is only one report that the gene expression in the paraspinal muscles of rats exposed to spaceflight (STS-58) was analyzed by a differential display approach (4). They demonstrated that the expressions of 42 genes changed, including heat shock protein 70, myosin light chain and myocyte enhance factor 2C (MEF2C), and suggested that MEF2C is a key transcriptional factor in skeletal muscle atrophy and regeneration under microgravity.

In this study, we also examined the changes in muscle gene expression after a space shuttle flight (STS-90), using a differential display approach. We identified 9 genes which may play distinct pathological roles in microgravity-induced muscle atrophy, in gastrocnemius muscle atrophied by spaceflight. Among these genes, we focused on a novel spaceflight-induced gene, *sfig*, to elucidate the distinct adaptation of skeletal muscle to microgravity conditions. The deduced amino acid sequence of the *sfig* gene is very similar to that of the Drosophila CG6115 gene, which may function as a transcription factor possessing a leucine zipper structure (5). Tail-suspension up-regulated its expression in gastrocnemius muscle at the early stage, when muscle atrophy caused by suspension initiated (3). Our results suggest that genetic studies on the effects of microgravity are helpful to understand the mechanism of microgravity-induced muscle atrophy.

MATERIALS AND METHODS

Spaceflight and tail-suspended rats.

As described in detail in a previous report (3, 6), the rats were launched into space on April 17, 1998, on the space shuttle Columbia, when they were 8-days old. The shuttle rats were housed with their dams in research animal holding facility (RAHF) cages in the orbiter. The shuttle landed at Kennedy Space Center on May 3, 1998. Approximately 2 hr elapsed under weight-bearing conditions before the animals were killed, and isolation of the gastrocnemius muscles from all animals was completed within 75 min. The asynchronous ground control rats were housed with their dams in cage conditions that simulated the shuttle's environment, including the shuttle's ambient temperature, the facilities and the timing of events of the flight animals.

Six-week-old male Wistar rats were subjected to tail suspension-induced hypokinesia, a model simulating microgravity conditions, for the indicated period by using the apparatus described previously (3, 7). Their tails were suspended to keep their hind legs off the ground. Rats were housed in a room maintained at 23°C on a 12-hr light/dark cycle and were allowed free access to a 20% casein diet and water.

All of the treatments described here were performed according to the Guide for the Care and Use of Laboratory Animals (1985) and were approved by the Animal Care Committee of the National Aeronautics and Space Administration (NASA) or the National Space Development Agency of Japan (NASDA) counterpart.

Cell culture.

Rat myoblastic L6 cells were purchased from Dainippon Pharmaceutical Co. (Osaka, Japan). L6 cells were maintained in tissue culture flasks at 37°C with 5% CO_2/95% air in DMEM, supplemented with 10% fetal calf serum (FCS), 100 units/ml penicillin and 0.1 mg/ml streptomycin. When the cells were grown to 100% of confluence, the medium was changed to DMEM containing 2% horse serum and the same antibiotics to stimulate differentiation.

Differential display analysis.

For the differential display analysis, we used a fluorescence differential display kit (Takara, Kusatsu, Japan) with modifications (8). DNA-free total RNA was isolated with an acid guanidinium thiocyanate-

phenol-chloroform mixture (Nippon Gene, Tokyo, Japan). Total RNA (300 ng) was used for the reverse transcription (RT) reaction (final volume, 20 μl) with 7.5 units of AMV RTase XL, 20 mM dNTP and 2.5 mm downstream primer, which was oligo-dT primer (5'-T_{11}VV-3', V represented A, C or G) for 10 min at 30°C. Following initial denaturation, second-strand synthesis and DNA amplification with 2.5 μM fluorescence-labeled downstream primer, 0.5 μM non-labeled upstream primer, 10 units of Taq DNA polymerase (Takara) and 2 μM dNTP (final volume, 20 μl) were achieved in a thermal cycler through 40 cycles of the following incubations : 30 sec at 90°C, 2 min at 40°C and 30 sec at 72°C. Samples were run on 12% native acrylamide gels, and the gels were analyzed with an image analyzer (FMBIOII, Takara). Differentially expressed bands were cut and each fragment was eluted by boiling in 100 μl of distilled water and re-amplified by polymerase chain reaction (PCR) using the same set of primers. Re-amplified DNA fragments were directly subjected to DNA sequence analysis with a DNA sequencer, model 373A (Perkin Elmer, Foster City, CA, USA).

Cloning and sequencing.

A Uni-ZAP cDNA library from the skeletal muscle of a 12-wk-old Wistar rat was purchased from Stratagen (La Jolla, CA, USA). Re-amplified DNA fragments were used as a probe for plaque hybridization screening, which was performed according to the method of Ishidoh *et al*. (9) with modifications. In short, recombinant plaques were transferred onto nylon membranes (Amersham, Little Chalfont, UK) and fixed with ultraviolet light. After prehybridization in a quick hybridization buffer (Amersham), hybridization was performed overnight at 60°C in the same buffer mixed with an isotope-labeled probe. The membranes were washed and exposed to Kodak X-ray films at -80°C for the appropriate time. Positive plaques hybridizing to probes were subjected to second and third screenings in the same manner.

Phage DNAs containing cDNAs hybridizing the probe were isolated from plate lysates and subcloned into pBluescript SK(-) vectors. The plasmids were introduced into *E. coli* JM109. Plasmid DNAs were prepared by the alkaline-SDS method. The nucleotide sequence of the DNA inserts in the multicloning site of pBluescript vectors was determined by a bi-directional dideoxy sequencing method using T7 DNA polymerase.

Northern blot analysis.

Total RNA (20 μg/lane) was separated in a 1% agarose gel, blotted and ultraviolet crosslinked to a nylon membrane (Amersham). After prehybridization, hybridization of the membrane was performed in a quick buffer with an isotope-labeled cDNA probe, as described above. The membranes were washed and exposed to Kodak X-ray films at -80°C for the appropriate time, and then the films were developed. Autoradiograph signals were quantified by densitometric analysis. Each mRNA level was standardized to that of 18S rRNA.

In vitro translation.

The transcription and translation of cDNA for the *sfig* gene *in vitro* was performed in a rabbit reticulocyte coupled transcription/translation system (Promega) (10). Briefly, 1μg of linearized template (pBluescript SK containing a cDNA for *sfig* gene) was suspended in 50 μl of reaction mixture (25 μl of rabbit reticulocyte lysate, 1 μl of T3 RNA polymerase, 20 μM amino acid mixture, 40 unit of RNase inhibitor and biotinylated lysine-tRNA complex) and then incubated at 30°C for 90 min. This was mixed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer and heated at 95°C for 5 min. The reaction products were subjected to SDS-15%-PAGE and blotted on a nylon membrane. *In vitro* translated protein was detected with streptoavidin-labeled horse radish peroxidase (Promega) in an enhanced chemiluminescence system.

Other biological analyses.

Creatine kinase (CK) activity was measured by the method of Szasz *et al*. (11). Protein concentration was measured according to the method of Lowry *et al*. (12) with bovine serum albumin as a standard.

Statistical analysis.

Data are expressed as mean±SD and were statistically evaluated by analysis of variance (ANOVA) with SPSS software (release 6.1 ; SPSS Japan Inc., Tokyo, Japan). One-way ANOVA was used to determine the significant effects of tail-suspension on the measured variable. Individual differences between groups were assessed using Duncan's multiple range test. The differences were considered significant at $P<0.05$.

RESULTS

Genetic effects of microgravity.

The present study examined the alteration of the muscle gene expression under microgravity using the differential display approach, i.e. a method to identify the actual differences between two well-defined biological situations. We performed differential display analysis for the gastrocnemius muscles of 16-day-spaceflight and asynchronous ground control rats, and obtained 9 PCR fragments whose lengths were 200-500 bp as expected. We then sequenced all of these PCR fragments and compared their DNA homology with the GenBank and the EMBL databases using BLAST. As shown in Table 1, the expression of Casitas B-lineage lymphoma-b (Cbl-b), insulin growth factor binding protein-1, titin and mitochondrial gene 16S rRNA was significantly up-regulated by spaceflight. In contrast, the mRNA levels of RNA polymerase II elongation factor-like protein and NADH dehydrogenase decreased. The physiological functions of three genes similar to KIAA0368, AF412300 or AA275180, are still unknown.

Isolation and sequence of a novel gene, sfig.

In this study, we isolated and fully sequenced the cDNA of *sfig*, one of three novel genes described above (See #6 in Table 1). The nucleotide sequence of the cDNA for this gene comprised 1162 bp containing 94 bp in the 5'-noncoding region, 261 bp in the coding region and 807 bp in the 3'-noncoding region (Fig. 1A). The ATG codon numbered as 1 must be the translation initiation site, since the nucleotide sequence around it (TGAAAATGG) corresponds to Kozak's rule (13) and an in-frame termination codon (-9 to -7) is found in the 5'-upstream sequence. The deduced amino acid sequence of the open reading frame codes for 86 amino acid residues. Calculating from the amino acid sequence, the molecular mass of rat *Sfig* protein is 10405.07. A putative leucine-zipper structure was located at the *N*-terminal portion (Fig. 1A and C). No potential *N*-glycosylation site was found. *In vitro* translation assay revealed that a protein with a molecular mass of about 11 kDa was produced from plasmid containing the cDNA for *sfig* gene, but not from a mock vector (Fig. 1B), suggesting that *sfig* was not a pseudo gene.

The full cDNA sequence of this gene is similar to those of mouse testis full-length cDNA (AK015530), mouse RIKEN cDNA 4930469P12 gene (XM_132933), human gene similar to RIKEN cDNA 4930469P12 (XM_084843), Macaca fascicularis brain cDNA clone QnpA-16830 (AB049871) and Drosophila melanogaster CG6115 gene (AAF53585), besides mouse clone 58 growth hormone-inducible soluble protein mRNA (AF412300). Fig. 1C shows that the amino acid sequence of the *sfig* gene, especially a leucine-repeated sequence (See asterisks) is highly conserved from Drosophila to Homo sapience.

Expression of sfig gene up-regulated by spaceflight and tail-suspension.

To confirm the high expression of the novel gene in the gastrocnemius muscle of spaceflight or tail-suspended rats, Northern blot analysis was performed. The 16-day-spaceflight remarkably increased the mRNA level of *sfig* (Fig. 2A). Tail-suspension also up-regulated the expression of *sfig* transcripts in the early stage and reached the peak value on Day 5, when a significant loss of gastrocnemius muscle started (3), as shown in Fig. 2B. The expression of the transcripts returned to the basal

Table 1. Differentially expressed genes between spaceflight and ground control rats.

No.	Effect of spaceflight	Corresponding rat genes or description
1	up	Cbl-b
2	up	insulin growth factor binding protein-1
3	up	titin
4	up	mitochondrial gene 16S rRNA
5	up	similar to human KIAA0368
6	up	*sfig*, similar to mouse clone 58 growth hormone-inducible soluble protein mRNA (AF412300)
7	down	RNA polymerase II elongation factor-like protein
8	down	similar to mouse AA275180
9	down	NADH dehydrogenase

These genes were searched in the GenBank and EMBL databases using BLAST.

(A)

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-94    G CAC GAG GTC TGA GCA ACC TGT CTT CGG AAC CCC AGA CCT GCT GGT -49
-48  GGT GAA GTA TGT CAT GTA TAA AAG TGG ATT AAT TCC ATA TAA GTG AAA -1
                                                  ***

 1  ATG GCC AAT TCT TTA CGA GGA GAA GTA CTG ACT CTT TAT AAA AAT CTG 48
    Met Ala Asn Ser Leu Arg Gly Glu Val Leu Thr Leu Tyr Lys Asn Leu

49  CTG TAT CTT GGA CGG GAC TAT CCA AAA GGA GCA GAC TAT TTT AAA AGG 96
    Leu Tyr Leu Gly Arg Asp Tyr Pro Lys Gly Ala Asp Tyr Phe Lys Arg

97  CGT TTG AAG AAT GTT TTC CTT AAA AAC AAG GAT GTG AAG GAC CCA GAG 144
    Arg Leu Lys Asn Val Phe Leu Lys Asn Lys Asp Val Lys Asp Pro Glu

145 AAG ATC AAA GAA CTC ATT GCA CGA GGA GAA TTT GTA ATG AAG GAG CTA 192
    Lys Ile Lys Glu Leu Ile Ala Arg Gly Glu Phe Val Met Lys Glu Leu

193 GAG GCC TTA TAT TTC CTT AGG AAA TAC AGA GCT ATG AAG CAA CGT TAC 240
    Glu Ala Leu Tyr Phe Leu Arg Lys Tyr Arg Ala Met Lys Gln Arg Tyr

241 TAT TCA GAT ACC AAA AAC TGA CCA ATC ACT GCC ACC GGC CTA GCT GAC 288
    Tyr Ser Asp Thr Lys Asn ***

289 AAT CAG TGC CAG CTG TTT GCC TGT ACC AAT TAT TAT ACA ATA ATT CAG 336
337 TTT AAA AGG GCA AGA TAC ATG TTT TTT TTT TAA AGT TTC CCT ATT GTA 384
385 CTA ATA TAG GTT TCA ACC CTA TTG ATA CTG AGA GCT TTG CCC ATA ATC 432
433 CTT TAT TAT TGA AAT AGT AAC ATT AGC ACC TTT CAG GAG AAT ACA ATT 480
481 TTG AAA GAA AAT ACA TCT AAT TTT TTA ACA TAT TAT AGC CAA GTA TTC 528
529 TAT TTC TTC ATT TAC TGA TGA GAT TGT CAT TAG CAA ATG GTG TCT GAT 576
577 AGG CCT GCC TGC CCT TTA GTT TGT GGA GTG TCT TTG TTT TTG GTT TTG 624
625 TTA CTC CAT CTA GTA TAC TGA AGT GCA TTT TCA AGG CTC TCT ACA GAC 672
673 ACC TCA AAA TGA TTT GAA TGC AGT TAC CAA AAT GAG ACA TGT GAA GGT 720
721 GGC TTC GAT CTT GAG CTG CCA AGC AGA AAA CTA GCA TTA TGT AGT TAT 768
769 TAT TCC CAT TAC CCT TTG TGC TGC TGG CCT GCC TTA GGT TCC TAA TCT 816
817 GCT TCA ACT GGC CTC CAT GTA GAG TGG TAA TGC TCC TCT ATC TGT GCC 864
865 ATT AAA GCT GGT GCA TTC TGT GCA GTG GTT AAG AGC ACT GAC TGC TCT 912
913 TCC AGA GGT TCC AAG TTC AAT TCT CAG CAA CCA CAT GGT GAC TCA CAA 960
961 CCG TCT GTA ATG GGA TCT GAT GTC CTC TTC TTC TGG TGT GTC TGA AAC 1008
1009 AGA CAG TTA CAG TGT ACT CAT ATA AAT AAA ATA CAA ATA AAC AAA AAA 1056
1057 AAA AAA AAA AAA                                              1068
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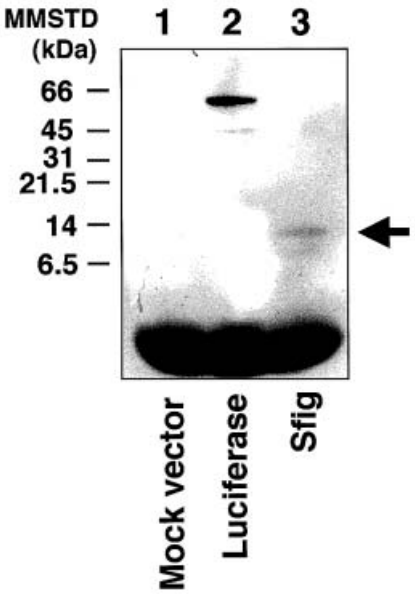
(B)



(C)

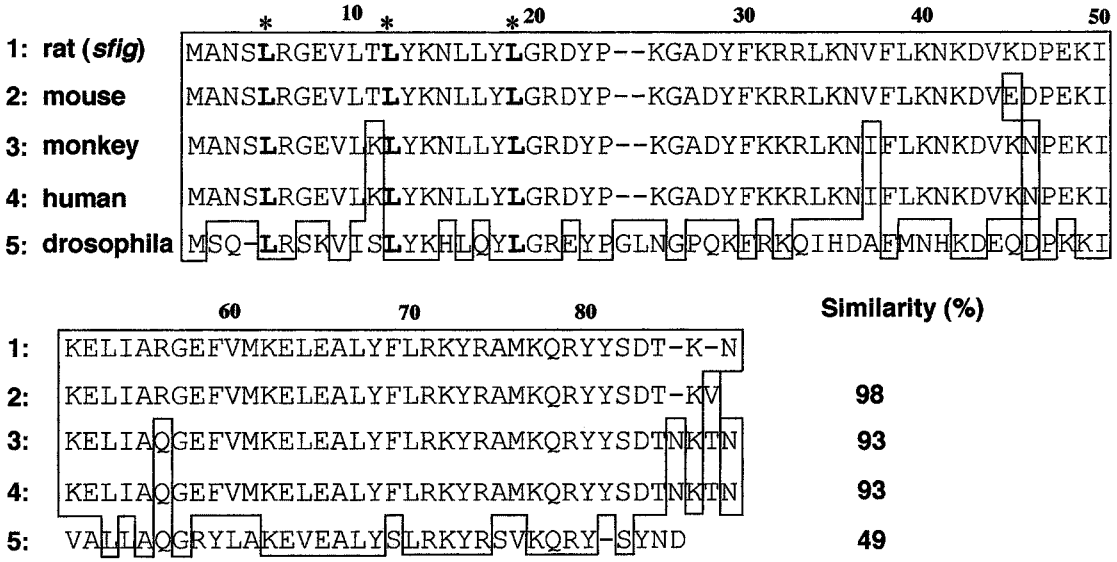


Fig. 1. Nucleotide sequence and deduced amino acid sequence of *sfig* gene. (A) Nucleotide numbering starts at the initiation codon. The *triple asterisks* indicate the termination codon and the in-frame termination codon in the 5'-noncoding region. The underlining indicates the putative leucine zipper structure. The double underlining indicates a poly(A)$^+$ additional signal. (B) Transcription and translation of cDNA for *sfig* gene *in vitro* was performed in a rabbit reticulocyte coupled transcription/translation system. Linearized mock vector (pBluescript SK, lane 1), cDNA for luciferase (lane 2) or linearized template (pBluescript SK containing a cDNA for *sfig* gene, lane 3) was incubated with biotinylated lysine-tRNA complex in the reaction mixture at 30°C for 90 min. The sample was subjected to SDS-15%-PAGE and blotted on a nylon membrane. Translated protein was detected with streptoavidin-labeled horse radish peroxidase. MMSTD, molecular mass standards. (C) Homology of the deduced amino acid of *sfig* gene. The deduced amino acid sequences of mouse AF412300, AK015530 or XM_132933 gene (lane 2), human XM_084843 gene (lane 3), monkey AB049871 gene (lane 4) and Drosophila AAF53585 gene are aligned to give maximal identity to that of rat *sfig* gene (lane 1). Mouse AF412300, AK015530 and XM_132933 gene encode the same peptides. Identical amino acids between the *sfig* gene and other genes are surrounded. *Asterisks* indicate the leucine-repeated sequence in a putative leucine-zipper structure.

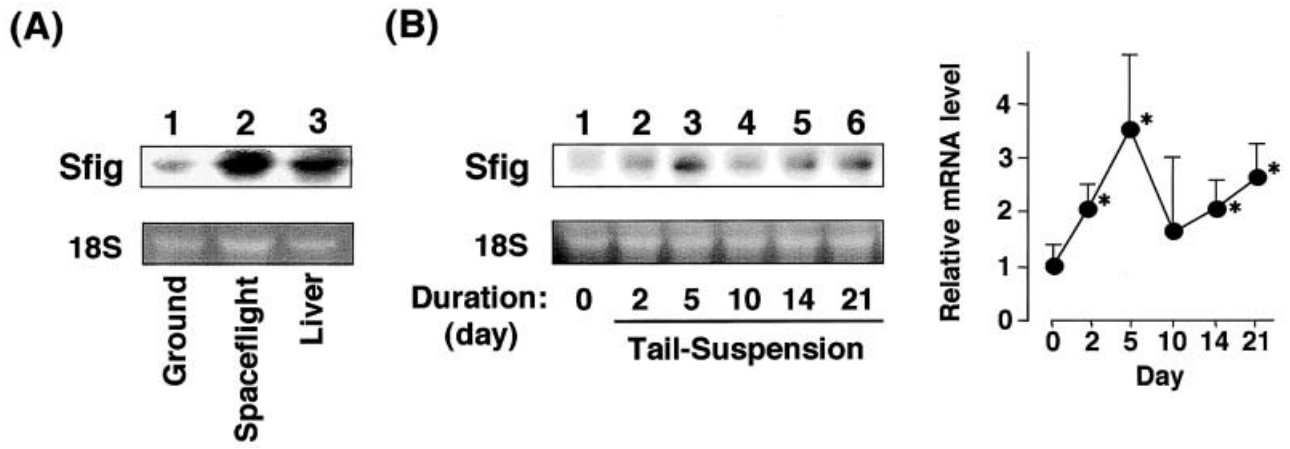


Fig. 2. Up-regulated expression of *sfig* gene in gastrocnemius muscle of spaceflight (A) or tail-suspended rats (B). Total RNA (20 μg/lane) extracted from the indicated muscles was subjected to Northern blot analyses for the *sfig* gene. Since a small amount of RNA from spaceflight rats was available, Northern blot analysis of the space sample was performed once. Similar results for tail-suspension were obtained in three separate experiments. Autoradiograph signals for tail-suspended rats were quantified by densitometric analysis, and each mRNA level was standardized to that of 18S rRNA. Values are mean±SD (n=3). *Significantly different, compared with the control rats before tail-suspension, $P < 0.05$.

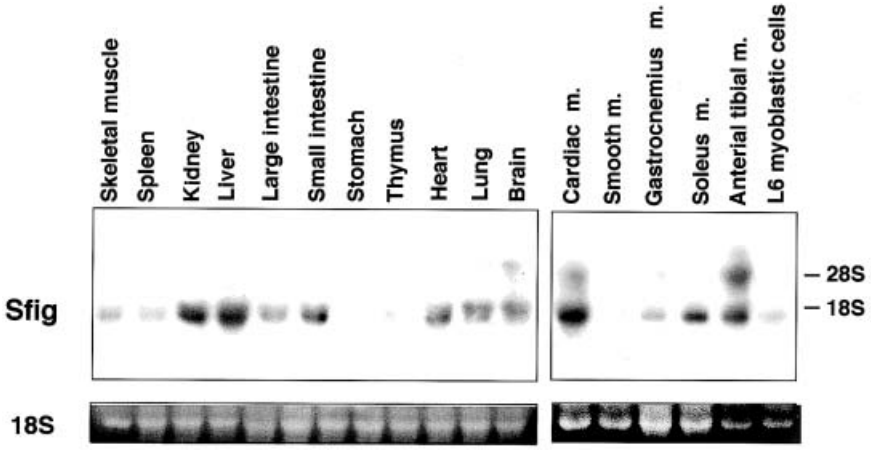


Fig. 3. Expression of *sfig* mRNA in various tissues. Total RNA was extracted from the indicated tissues of Wistar rats or rat L6 myoblastic cell line. Smooth muscle RNA was prepared from the small intestine. In the case of L6 cells, RNA was prepared from the cells in the proliferating stage. Total RNA (20 μg/lane) was subjected to Northern blot analyses for *sfig* gene. The positions of 28 and 18S ribosomal RNA are indicated.

level once after reaching the peak value and then increased gradually.

Tissue distribution.

The levels of *sfig* mRNA were high in the liver, kidney and small intestine, moderate in the large intestine, heart, lung and brain, and low in the skeletal muscle and spleen (Fig. 3). Only small amounts of the *sfig* transcript existed in the stomach and thymus. Among muscles and a myoblastic cell line, the *sfig* transcript was highly expressed in the cardiac and anterior tibial muscles, followed by the soleus and gastrocnemius muscles (Fig. 3). Low levels of the *sfig* transcript were expressed in smooth muscle from the small intestine and proliferating L6 myoblastic cells.

Expression of sfig gene during differentiation of myoblastic cells.

Besides microgravity conditions, the expression of this gene was stimulated during the differentiation of L6 cells. When L6 cells grown to 100% of confluence were treated with DMEM containing 2% horse serum, CK activity, a muscle differentiation marker, in the cells significantly increased on Day 4 (Fig. 4A). The level of *sfig* mRNA remarkably increased on Day 2 prior to the CK activation and then sustained a gradual increase (Fig. 4B).

DISCUSSION

In the present study, we found that 9 muscle genes changed during spaceflight by using a differential display technique. All the genes may have pathological significance for spaceflight-induced

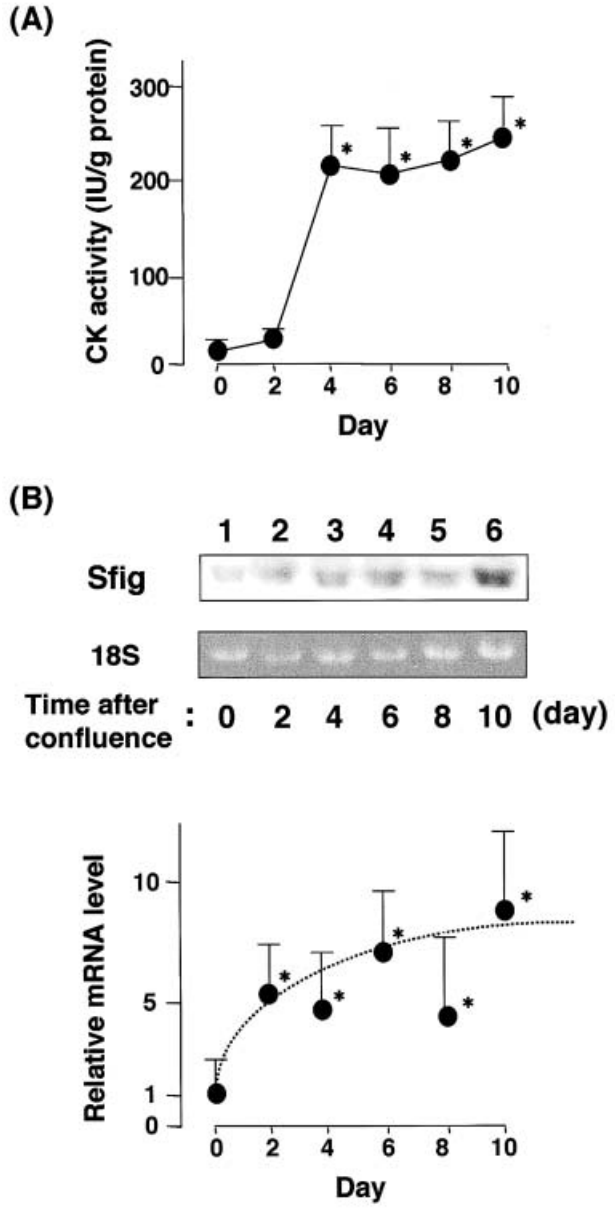


Fig. 4. *Sfig* transcripts in rat myoblastic L6 cells during differentiation. When L6 cells were grown to 100% of confluence (Day 0), the medium was changed to DMEM containing 2% horse serum to stimulate differentiation. (A) CK activity in L6 cells on the indicated days was measured. Values are mean± SD (n=3). *Significantly different, compared with the cells before differentiation (Day 0), $P<0.05$. (B) Total RNA (20 μg/lane), extracted from L6 cells on the indicated days, was subjected to Northern blot analysis, as described in MATERIALS AND METHODS. Autoradiograph signals were quantified by densitometric analysis. Each mRNA level was standardized to that of 18S rRNA. Values are mean±SD (n=3). *Significantly different, compared with the cells before differentiation (Day 0), $P<0.05$.

muscle atrophy. For example, the imbalanced expression of mitochondrial genes, i.e. the up-regulated expression of mitochondrial gene 16S rRNA and the down-regulated expression of NADH dehydrogenase, indicates mitochondria dysfunction under microgravity conditions. We previously reported that tail-suspension caused oxidative stress in hindlimb skeletal muscles (14). These results support our hypothesis that superoxide anions and/or iron ions leaking from mitochondria may contribute to this oxidative stress. Recently, c-Cbl and Cbl-b, adaptor proteins, have been reported to act as ubiquitin-protein ligases (E3s) for several growth factor receptors, including the epidermal growth factor receptor, and to down-regulate the signaling pathway of growth factors (15, 16). We confirmed that spaceflight significantly stimulated Cbl-b expression at the mRNA and protein levels, and Cbl-b played an important role in microgravity-induced muscle atrophy by down-regulating insulin-like growth factor signaling (unpublished observation, manuscript submitted). In general, microgravity conditions decrease the transcription of general genes (17). The down-regulated expression of RNA polymerase II elongation factor-like protein indicates that spaceflight may cause decreased translation as well as transcription. Thus, genetic studies on the effects of spaceflight are helpful to elucidate the mechanisms of microgravity-induced muscle atrophy. We are further examining the pathological roles of other genes identified in the present study.

The genes identified as sensitive to the STS-90 spaceflight are not identical to those sensitive to the STS-58 spaceflight. In the present study, we used younger rats (8-days old at launch) than those (8-wks old at launch) in the STS-58 mission. In addition, we used a fast-twitch muscle (gastrocnemius muscle) for a differential display analysis, rather than a slow-twitch muscle (paraspinal muscle). The duration of spaceflight in the STS-90 mission was 16 days, similar to that (14 days) in the STS-58 mission. Therefore, the age of rats at launch and the type of skeletal muscle may be important factors which affect muscle gene expressions in space.

In this study, we also identified and sequenced a novel skeletal muscle *sfig* gene. Spaceflight and tail-suspension induced the remarkable up-regulated expression of *sfig* mRNA in rat gastrocnemius muscles, whereas they contained small amounts of this transcript under normal conditions (1 *g*). In addition, tail-suspension up-regulated its expression in the early stage (Day 5) when muscle atrophy caused by suspension initiated (3). Changes in *sfig* mRNA expression by denervation, another model simulating microgravity, also showed similar patterns to that by tail-suspension (data not shown). Our results suggest that this gene may be useful as a marker to detect early muscle atrophy caused by microgravity

and its simulated conditions.

At present, the pathophysiological roles of this novel gene for microgravity-induced muscle atrophy are unknown. However, the deduced amino acid sequence of the *sfig* gene was conserved from Drosophila to Homo sapiens. In particular, the Drosophila melanogaster CG6115 gene was reported to code a novel transcription factor, since there is a putative leucine zipper structure at the N-terminal domain (5). During the differentiation of myoblastic cells, the expression of this transcript was significantly up-regulated prior to CK activation, indicating that this *sfig* gene may function as a transcription factor associated with the differentiation of myoblastic cells. In rat skeletal muscles under normal conditions (1 *g*), this mRNA was dominantly expressed in the cardiac and anterior tibial muscles, which are resistant to atrophy caused by microgravity (3, 18). These findings lead us to consider that the expression of this gene may be secondarily up-regulated against muscle atrophy caused by spaceflight. Further examinations are necessary to clarify this hypothesis.

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