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Effects of static magnetic fields on the development and aging of *Caenorhabditis elegans*

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SUMMARY

The current study investigated the possible effects of static magnetic fields (SMFs) on the developmental and aging processes of *Caenorhabditis elegans*. Nematodes were grown in the presence of SMFs of strengths varying from 0 to 200 mT. The rate of development and the lifespan were recorded. Treatment with a 200 mT SMF reduced the development time from the L2 to the L3 stage by 20%, from L3 to L4 by 23%, and from L4 to young adult by 31%. After SMF treatment, the average lifespan was reduced from 31 days to 24 days for wild-type nematodes. The up-regulation of *clk-1*, *lim-7*, *daf-2*, *unc-3* and *age-1* after SMF treatment was verified by quantitative real-time RT-PCR. Apparently, induction of gene expression is selective and dose dependent. The total developmental time was significantly reduced for the *lin-4*, *lin-14*, *lin-41* and *lim-7* mutants, but not for the *let-7*, *clk-1*, *unc-3* and *age-1* mutants. Lifespan analyses revealed that the *let-7*, *unc-3* and *age-1* mutants were not affected by SMF treatment. Here we show that SMFs accelerate nematode development and shorten nematode lifespan through pathways associated with *let-7*, *clk-1*, *unc-3* and *age-1*.

Key words: aging, *C. elegans*, static magnetic fields, life cycle.

INTRODUCTION

The safety of static magnetic fields (SMFs) has been discussed for decades (Drinker and Thomson, 1921). Various studies have examined the effects of SMFs, but their results were often inconclusive (Valentina et al., 2009). With the application of SMFs in clinical practice, the incidence of exposure has greatly increased. A general concern is that the knowledge regarding the health hazards of SMFs lags behind the development of medical technologies, such as magnetic resonance imaging (MRI). Stronger SMFs are required for higher resolution imaging. Weak magnetic fields are used in the treatment of parkinsonism and the motor complications of chronic levodopa therapy (Sandyk et al., 1992). The hazardous effects associated with SMFs on human health have become conspicuous. New studies are needed to fill in the gaps in our knowledge and provide the assurance that novel medical technologies will not cause unwanted health hazards (Leszczynski, 2005).

There have been few studies on the effects of SMFs at the cellular level (Miyakoshi, 2005). SMFs alone do not have a lethal effect on the basic properties of cell growth and survival under normal culture conditions (Nakahara et al., 2002; Sakurai et al., 2009). Most studies have also suggested that SMFs do not affect cell proliferation (Hiraoka et al., 1992) or influence the cell cycle. Morphological analyses indicated that SMFs could induce modifications in cell shape, the cell surface and the cytoskeleton (Yamaguchi et al., 1993; Buemi et al., 2001; Teodori et al., 2002; Pacini et al., 2003; Pate et al., 2003; Pagliara et al., 2005; Tarantino et al., 2005; Gamboa et al., 2007). SMFs may also modulate apoptosis by influencing cytoplasmic calcium ion concentrations (Tarantino et al., 2005). SMFs were able to induce double-stranded DNA breaks in rat brain cells (Lai and Singh, 2004). SMFs are also associated with an increased risk of cancer (McCann, 1998). It is likely that SMFs also affect longevity.

In multicellular organisms, SMFs may cause minor effects at the cellular level that then accumulate and eventually cause distinct symptoms. Early studies did not show any effect of exposure to SMFs on the organogenesis, fetal development or cortical development of mice (Sikov et al., 1979; Konermann and Monig, 1986; Murakami et al., 1992; Okazaki et al., 2001). However, the International Agency for Research on Cancer (IARC) proposed that SMFs might affect embryonic development in amphibians (International Agency for Research on Cancer, 2002). SMFs have been reported to be toxic to rat embryos (Mevissen et al., 1994), and to cause significant time- and dose-dependent increases in the frequency of micronuclei in mice (Suzuki et al., 2001) and in the rates of germination and early growth in *Cicer arietinum* L. (Ananta and Shantha, 2008). Various studies have focused on the effects of SMFs on the nervous system, behavior, genotoxicity and cancer incidence. Only a few studies have examined the effect of SMFs on reproduction and development, and the results are inconclusive (Valentina et al., 2009).

Caenorhabditis elegans, Maupas, 1900, are a valuable resource for genetic and molecular investigations in a multicellular organism. With a short lifespan, the nematode serves as a powerful model system for studying the molecular mechanisms underlying aging and development. The median lifespan of *C. elegans* ranges from 11.8 days (Van Voorhies, 1992) to 20 days (Kenyon et al., 1993) when grown on agar plates at 20°C with *Escherichia coli*. *Caenorhabditis elegans* populations exist primarily as hermaphrodites. The short lifespan of *C. elegans* makes it attractive for whole-organism compound screening (Hertweck et al., 2003).

Furthermore, *C. elegans* may help us to fill the gaps in our knowledge regarding the possible health hazards of SMFs. In *C. elegans*, a short-term treatment with SMFs induced fluctuations in

heat shock protein gene expression (Miyakawa et al., 2001; Kimura et al., 2008). The current study is based on the hypothesis that, given sufficient intensity, SMFs can shorten the life cycle and cause premature aging in nematodes.

MATERIALS AND METHODS

Strains and chemicals

The strains of *C. elegans* used in this research, N2 (wild-type), *lim-7*, *lin-14*, *lin-41*, *clk-1*, *age-1* and *unc-3*, were obtained from the Caenorhabditis Genetics Center (CGC), University of Minnesota, St Paul (MN, USA). *Caenorhabditis elegans* populations exist primarily as hermaphrodites. Nematodes were propagated at 20°C on nematode growth medium (NGM) plates (Brenner, 1974) with the *E. coli* strain OP50 as a food source. All culture media and related chemicals, including Bacto agar, Bacto tryptone and yeast extract, were purchased from Gibco Co. (Gaithersburg, MD, USA). Other chemicals (of analytical grade or higher) were purchased from Sigma (St Louis, MO, USA) or Merck (Hsinchu, Taiwan, ROC). Nd-Fe-B magnets were purchased from the Taiwan Magnetic Corp. Ltd (Taipei, Taiwan, ROC). Magnetic field strength was measured at the National Measurement Laboratory, Taiwan.

Isolation of nematode developmental stages

The developmental stages of nematodes were defined according to the timing of the molting period and the body length, as described previously (Hope, 1999; Cassada and Russell, 1975).

L1: the first larval stage, about 11.5 h after fertilization. General structure is similar to the adult with a body length of 250 μm.

L2: 12 h after hatching. The body length of the nematode is 360–380 μm.

L3: 20 h after hatching. The body length of the nematode is 490–510 μm.

L4: 28 h after hatching. The body length of the nematode is 620–650 μm.

Young adult: 38 h after hatching. The body length of the nematode is 900–940 μm.

Adult: 46 h after hatching. The body length of the nematode is 1110–1150 μm, and the nematode is capable of egg laying.

Quantitative real-time RT-PCR

Total RNA was extracted from 100 worms using TRI-reagent (Talron Biotech, Taipei, Taiwan, ROC) according to the manufacturer's specifications. Worms were picked and washed three times with M9 medium and collected in a 2 ml Eppendorf tube. The pellet was dissolved in 1 ml Tri-reagent and disrupted with a homogenizer on ice; the RNA was isolated using chloroform extraction and isopropanol precipitation. The crude RNA extract was immediately purified with an RNeasy Mini Kit (Qiagen, Taipei, Taiwan, ROC) to remove impurities and unwanted organic substances. Purified RNA was resuspended in DEPC water and quantified by absorbance at OD_{260}. The OD_{260} to OD_{280} absorbance ratio usually exceeded 2.0 at this stage. For cDNA synthesis, 1 μg total RNA was annealed with 1 μg oligo-dT, followed by reverse transcription using SuperScript® III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) in a total volume of 50 μl. Between 0.2 and 0.5 μl of the reverse transcription reactions was used for quantitative real-time PCR using SYBR Green I on an iCycler iQ5 (Bio-Rad Laboratories, Hercules, CA, USA). Cycling conditions were as follows: 1× (5 min at 95°C) and 50× (20 s at 95°C, 20 s at 60°C, and 40 s at 72°C); fluorescence was measured after each 72°C step. Expression levels were obtained as threshold cycles (Ct), which were determined by the iCycler iQ Detection System software. Because there is no *let-7* gene sequence provided by Wormbase and *lin-41* gave no results for quantitative real-time PCR, the *let-7* and *lin-41* data were not available. Relative transcript quantities were calculated using the ΔΔCt method. The ribosomal proteins L18 and L21 were used as reference genes and were amplified from the same cDNA samples. The difference in threshold cycles of the sample mRNA relative to ribosomal protein (L18 or L21) mRNA was defined as ΔCt. The difference between the ΔCt of the untreated control and the ΔCt of the SMF-treated sample was defined as ΔΔCt. The fold change in mRNA expression was expressed as $2^{\Delta\Delta Ct}$. The results are expressed as the mean ± s.d. of six experiments.

Lifespan assay

Synchronized wild-type nematodes were placed on NGM plates with the *E. coli* strain OP50 as a food source. The next day, 60 L1 nematode larvae were placed onto three new NGM plates, with 20 nematodes on each plate. We defined this day as the first day. Two groups were developed under the same conditions. One group was treated with a SMF, and the other was given no SMF treatment. The nematodes were moved every day to a new NGM plate with the *E. coli* strain OP50 as a food source, and the number of live nematodes was recorded to calculate the percentage of living nematodes. The results are expressed as the mean ± s.d. All experiments were performed at 20°C.

RESULTS

A SMF of 200 mT shortens the lifespan of *C. elegans*

The SMF device was composed of two Nd-Fe-B permanent magnets that sandwich a Petri dish. The field intensity at the center of the SMF device was measured. Adjusting the distance between the magnets varied the intensity from 0 to 200 mT (Fig. 1). To investigate the possible effects of SMFs on *C. elegans* lifespan, synchronized nematodes were grown under a 200 mT SMF at 20°C. A lifespan assay was performed, and the results were compared with those of the untreated group (Fig. 2). The median lifespan of *C. elegans* was 16 days for the untreated group and was reduced to 13 days upon treatment with a 200 mT SMF. The normal lifespan for *C. elegans* is 31 days at 20°C. SMF treatment shortened the lifespan to 24 days. Based on these results, there was a 23% reduction in the normal *C. elegans* lifespan upon treatment with a 200 mT SMF.

SMFs stronger than 150 mT accelerate nematode development time

It is possible that SMFs lead to a shorter nematode lifespan because they interfere with the time required for the early stages of nematode development. The nematode life cycle was measured from the egg stage to the adult stage. Synchronized wild-type nematodes (N2) were treated with 0–200 mT SMFs for 3 days. The total development time from egg to adult was measured (Fig. 3A). At 20°C, the complete life cycle of wild-type nematodes takes 57.5 h. The life cycle time was reduced by SMFs in a dose-dependent manner. Treatment with a 200 mT SMF led to a 22% reduction in life cycle time compared with the untreated group. Because there is a distinct difference in appearance at each stage of nematode development, it was possible to measure the duration of each stage from egg to adult. No significant differences were observed for the stages from egg to L2 (Fig. 3B). A significant difference in development time occurred from L2 to L3; there was an 18% reduction after 150 mT treatment and a 23% reduction after 200 mT treatment (Fig. 3C). Based on these results, SMF treatment seems to accelerate nematode development. For later stages, the development time tended to decrease upon SMF treatment. Although SMFs did not induce a

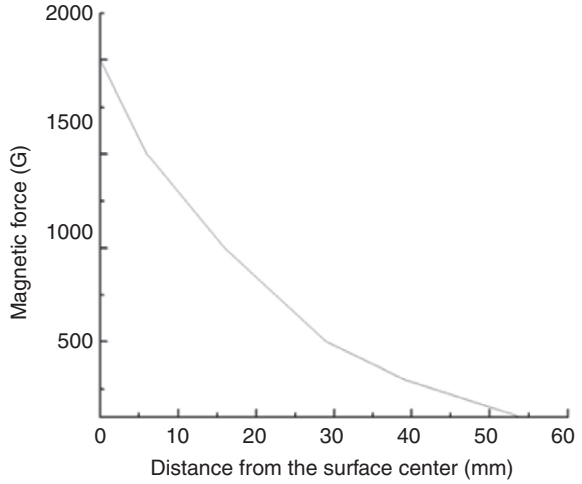

Fig. 1. Magnetic field strength at the center of the static magnetic fields (SMFs) used in the current study. The magnetic force (in gauss, G) is plotted against the distance (in mm) between two permanent magnets sandwiching a Petri dish.

statistically significant difference in development time from the L3 stage to the L4 stage (Fig. 3D), there was a 19% reduction in the development time from the L4 stage to the young adult stage and a 23% reduction from the young adult stage to the adult stage after 200 mT SMF treatment (Fig. 3E,F). SMFs accelerated nematode development and shortened the development time from the L2 stage to the adult stage. These results are also reflected in the shortened lifespan after SMF treatment.

Genes associated with development and aging are differentially expressed after treatment with SMFs

The shortened nematode life cycle indicated that pathways associated with development and aging may have been altered by SMF treatment. To verify the differential expression of genes in these pathways due to SMF treatment, quantitative real-time RT-PCR was performed. For the preliminary screening, seven genes were selected, and their primer pairs were designed based on the sequences provided in GenBank (Table 1). These genes are associated with cell development (*lin-4*, *lin-14*, *lin-41*), growth rate (*clk-1*), growth of somatic gonadal sheath cells from the L4 stage to adulthood (*lim-7*), regulation of development and differentiation of B lymphocytes, adipocytes and nerve cells (*unc-3*), and aging (*age-1*). Quantitative real-time RT-PCR consistently showed up-regulation of *clk-1*, *lim-7*, *unc-3* and *age-1* when the worms were cultured in the presence of a 200 mT SMF (Fig. 4A). There was a 13.9-fold, 2450-fold, 5.7-fold and 324-fold up-regulation of *clk-1*, *lim-7*, *unc-3* and *age-1*, respectively. These results imply that pathways associated with development and aging play a role in the SMF-induced reduction of lifespan and development time.

Our studies indicated that the life cycle time was reduced by SMFs in a dose-dependent manner. It is not clear whether the aging-related genes exhibit dose-dependent expression. We chose *clk-1*, *daf-2*, *age-1* and *lim-7* to investigate dose-dependent gene expression by quantitative real-time RT-PCR. These four genes exhibited similar trends in expression levels when various intensities of SMFs were applied (Fig. 4B). A SMF of 30 mT stimulated a minor increase in gene expression. The expression levels increased rapidly, reached a plateau when a 50 mT SMF was applied, and remained constant under 100, 150 and 200 mT SMFs. The up-regulation of *clk-1*, *daf-2*, *age-1* and *lim-7* was triggered by SMFs in a dose-dependent manner. Under SMF treatments ranging from 50 to 200 mT, the average fold change in expression level was 1470, 161, 377 and 2350 for *clk-1*, *daf-2*, *age-1* and *lim-7*, respectively.

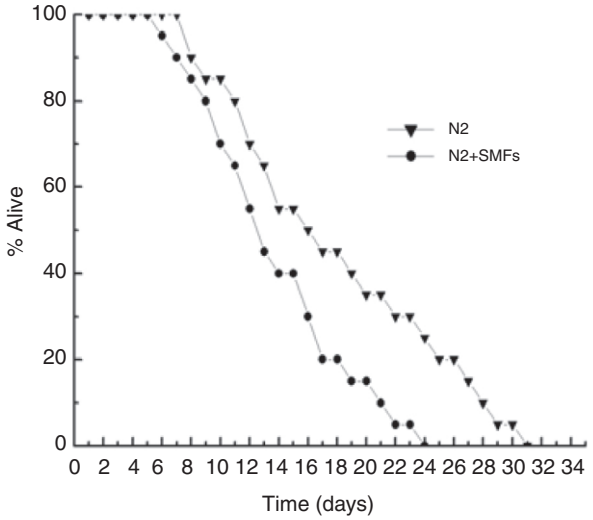

Fig. 2. The effects of SMFs on the lifespan of wild-type nematodes. Sixty wild-type nematodes were transferred onto nematode growth medium (NGM) plates with the *Escherichia coli* strain OP50 as a food source. Lifespan was measured at 20°C. The median lifespan of wild-type nematodes was 16 days for the untreated group and was reduced to 13 days after treatment with a 200 mT SMF. The normal lifespan for wild-type nematodes was 31 days at 20°C. SMF treatment shortened the lifespan to 24 days. N2 is the wild-type nematode strain.

Nematodes mutant for developmental and aging genes are resistant to SMF-induced life cycle reduction

Although the expression levels of genes associated with development and aging were altered upon treatment with SMFs, it has not yet been demonstrated that the inactivation of these genes causes nematodes to be resistant to SMF treatment. Given the expression levels of genes associated with development and aging were altered upon treatment with SMFs, we wanted to find out whether inactivation of these genes causes nematodes to be resistant to SMF treatment. We took advantage of mutant nematodes that carry mutations in genes involved in developmental and aging pathways. Strains mutant for *let-7*, *lin-14*, *lin-41*, *clk-1*, *lim-7*, *unc-3* and *age-1* were scored for total development time with or without 200 mT SMF treatment (Fig. 5). The gene *let-7* is directly involved in the transition from the late larval to adult cell fates. For wild-type nematodes, SMF treatment significantly reduced development time by 22%, from 58 h to 45 h. The life cycles of *lim-7*, *lin-14*, *lin-41* and *lin-4* mutants were significantly reduced by 20%, 23%, 21% and 22%, respectively, upon SMF treatment. However, the life cycles of *let-7*, *unc-3*, *clk-1* and *age-1* mutants were not affected by SMF treatment. Apparently, pathways associated with development and aging (*let-7*, *unc-3*, *clk-1* and *age-1*) are involved in mediating the SMF-induced life cycle reduction in nematodes.

Nematodes mutant for developmental and aging genes are resistant to SMF-induced lifespan reduction

To investigate the possible effects of SMFs on the *C. elegans* lifespan, synchronized wild-type (N2) and mutant nematodes were grown under a 200 mT SMF at 20°C. Lifespan assays were performed on N2, *let-7*, *unc-3* and *age-1* nematodes, with or without SMF treatment. The median lifespan of wild-type nematodes was

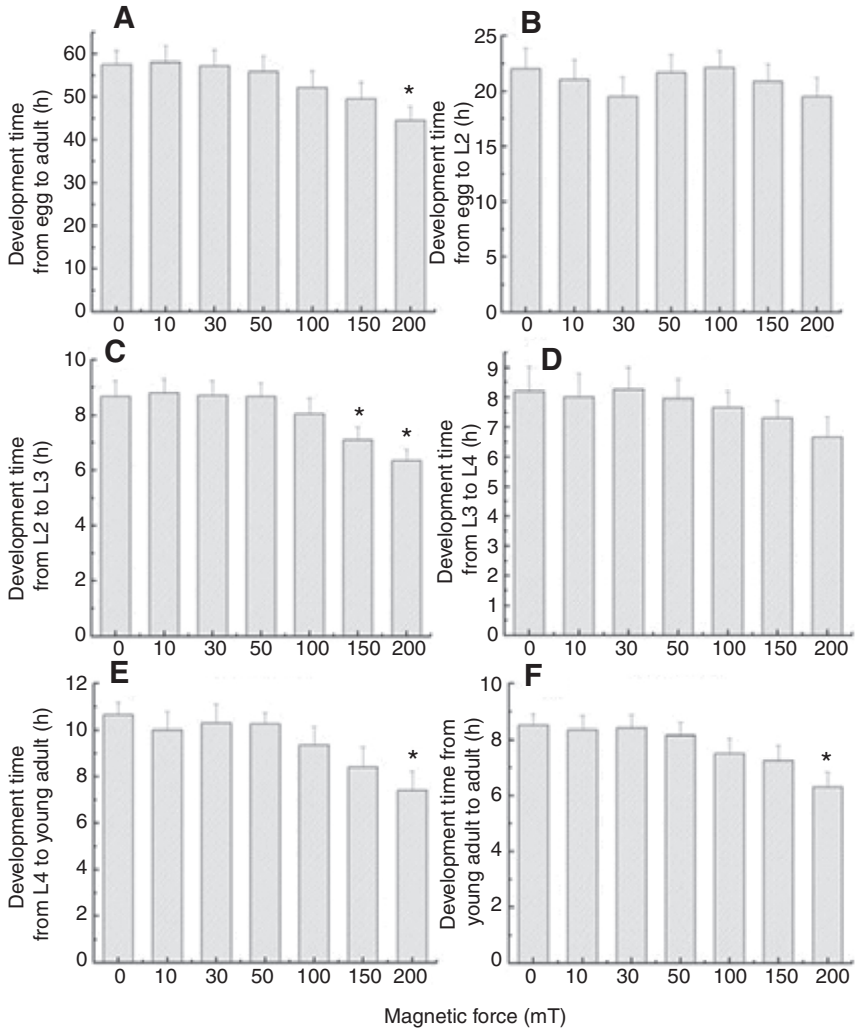

Fig. 3. The effects of SMFs on the life cycle of wild-type nematodes. Synchronized wild-type nematodes were transferred onto NGM plates with the *E. coli* strain OP50 as a food source. Larval worms were grown under SMFs from 0 to 200 mT, and the development time at each stage was measured at 20°C. (A) The total development time of wild-type nematodes was significantly reduced by 23% with a 200 mT SMF. (B) No significant reduction was observed in the development time from egg to the L2 stage under SMF treatment. (C) The development time from L2 to L3 was significantly reduced by 18% and 23% with 150 mT and 200 mT SMFs, respectively. (D) There was no significant reduction in the development time from L3 to L4 after SMF treatment of 0–200 mT. (E) The development time from L4 to young adult was significantly reduced by 19% with 200 mT SMF treatment. (F) The development time from young adult to adult was significantly reduced by 23% with 200 mT SMF treatment. Each group contains at least 60 worms. Data are presented as means and s.d. The comparisons between groups were performed through a one-way analysis of variance. The differences between groups were considered statistically significant at $P<0.05$ (*).

16 days for the untreated group and was reduced to 13 days after treatment with a 200 mT SMF. The normal lifespan for a wild-type nematode is 31 days at 20°C. SMF treatment shortened this lifespan to 24 days (Fig. 6A). The lifespan of the *let-7* mutants showed only a minimal reduction, from 30 to 29 days. The lifespan of the *unc-3* mutants also showed only a minimal reduction, from 34 to 33 days (Fig. 6B). Finally, the lifespan of the *age-1* mutants also showed only a minimal reduction, from 33 to 32 days (Fig. 6C). In summary, the lifespans of *let-7*, *unc-3* and *age-1* mutants were not affected by SMF treatment. These results show that the developmental and aging pathways associated with these genes are correlated with a SMF-induced reduction in lifespan.

Table 1. Primer sequences for genes involved in development and aging

Symbol	Annotation	Primer sequence (5′→3′)
lin-4	Abnormal cell lineage	F: gtgccagcctcacggaaagg
		R: gggaggagtagctgaaggag
lin-14	Abnormal cell lineage	F: aaccagcatcgccgacattac
		R: ggagtggtggagctgtttcaac
lin-41	Abnormal cell lineage	F: tcccgcaagactcctttcgg
		R: gcgtcggagacaggtacatc
lim-7	LIM domain family	F: accaccgatggcagtttgtgc
		R: caggcaacacacgcaaagcag
clk-1	Clock (biological timing) abnormality	F: aggtgcaatggcttgtacaattgc
		R: tccatcgtgttctactccagtatc
unc-3	Uncoordinated	F: gatgtgccgagtgcttctcac
		R: gcatcggtgcgcttagttctc
age-1	Aging alteration	F: agagctccacggcactttcc
		R: ctcagcttggcagccttgac
daf-2	Abnormal dauer formation (insulin/IGF-1-like receptor)	F: tactgtttgaagacactctgcca
		R: aaactgtgctacacgaaaacgat

F denotes the sequence of the forward primer.
R denotes the sequence of the reverse primer.

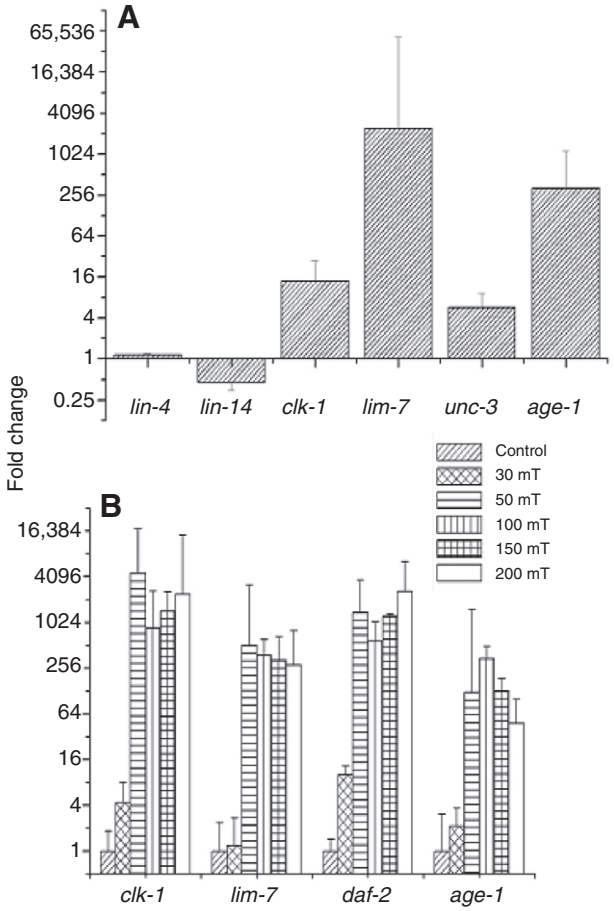

Fig. 4. Quantitative real-time RT-PCR for genes associated with development and aging pathways. (A) Treatment with a 200 mT SMF elevated the expression levels of *clk-1*, *lim-7*, *unc-3* and *age-1*. The expression level of the corresponding gene from an untreated group served as the normalization control. The fold changes were calculated from the difference in cycle numbers of real-time RT-PCR by the ΔΔCt method as described in Materials and methods. (B) Upregulation of *age-1*, *daf-2*, *lim-7* and *clk-1* corresponds to increasing doses of SMFs. Nematodes were cultured under SMFs at strengths of 30, 50, 100, 150 and 200 mT. The expression levels of *age-1*, *daf-2*, *lim-7* and *clk-1* for the treated worms relative to the untreated controls were obtained by quantitative real-time RT-PCR and are expressed as a fold change. The data are averaged from six independent experiments. Data are presented as means and s.d.

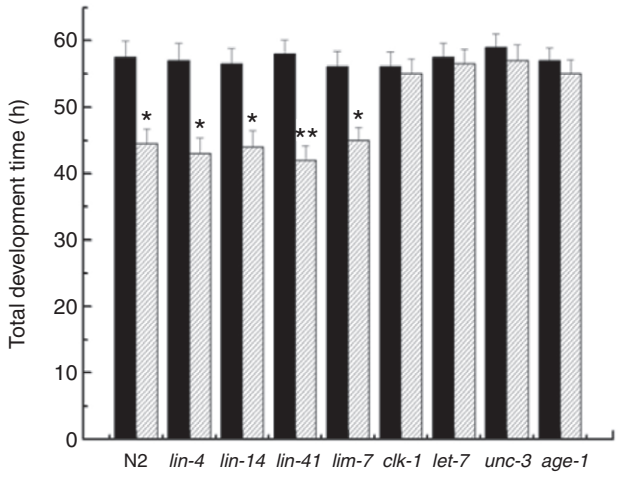

Fig. 5. The effects of SMFs on the life cycle of mutant nematodes. Synchronized wild-type nematodes were transferred onto NGM plates with the *E. coli* strain OP50 as a food source. Larval worms were grown at 20°C under SMFs from 0 to 200 mT, and the development time of each stage was measured. Each group contained at least 60 worms. Data are presented as means and s.d. Comparisons between groups were performed through a one-way analysis of variance. The differences between groups were considered statistically significant at $P<0.05$ (*) or $P<0.01$ (**). The hatched bars depict data for the 200 mT SMF-treated group. The filled bars depict data for the untreated group. For wild-type nematodes, SMF treatment significantly reduced the development time by 22%, from 58 h to 45 h. The life cycles of *let-7*, *unc-3*, *clk-1* and *age-1* mutants were not affected by SMF treatment. The life cycles of *lim-7*, *lin-14*, *lin-41* and *lin-4* mutants were significantly reduced by 20%, 23%, 21% and 22%, respectively, after SMF treatment.

DISCUSSION

SMFs accelerate the development and aging of nematodes

Few studies have examined the effect of SMFs on fertility and the development of embryos and fetuses. Slight changes in spermatogenesis and embryogenesis in mice exposed to 1.5 T for 30 min have been reported (Narra et al., 1996). The maturation of sperm movement in mice as well as postnatal testicular and epididymis development were largely unaffected by either single, short-term exposure or continuous, long-term exposure at 500–700 mT (Tablado et al., 1996; Tablado et al., 1998; Tablado et al., 2000).

Strong magnetic field gradients may affect embryonic development in amphibians (International Agency for Research on Cancer, 2002). The abnormal growth and increased incidence of malformations in embryos exposed to a static field of 1 T have been observed (Neurath, 1968; Ueno et al., 1984). Later studies indicated a lack of developmental effects after exposure to a SMF (Ueno et al., 1994). Exposure to SMFs of up to 17 T induced abnormalities in the first three cleavages of the African clawed toad, *Xenopus laevis*, embryo (Denegre et al., 1998; Ueno et al., 1984; Ueno et al., 1994).

Teratogenic effects did not occur in mammalian embryos when they were exposed to a static field of up to 6.3 T (Sikov et al., 1979; Konermann and Monig, 1986; Okazaki et al., 2001; Murakami et al., 1992). However, when rats were exposed to a 30 mT static field during the entire gestation period, a significant decrease in the number of live fetuses per litter was reported (Mevissen et al., 1994).

Abnormalities in mammalian development as a result of strong MRI exposures of up to 5 T have been reported (Tyndall, 1993; Tyndall, 1990; Tyndall and Sulik, 1990; Carnes and Magin, 1996; Magin et al., 2000). These include neuronal and eye abnormalities. However, despite the differences in the experimental protocols, the effects described in the two studies are not likely to be reproducible.

Although developmental abnormalities in individuals have been observed, the statistical analyses and molecular studies are missing. The current study provides, for the first time, quantitative measurements of gene expression that correlate with developmental and aging processes. The functional analyses using nematode mutants further provide strong evidence for molecular pathways mediating the SMF-induced acceleration of both the development and aging processes.

Global effects of SMFs

We expect the effects of SMFs to be global. Among the genes selected for measurement of transcriptional activity, most were

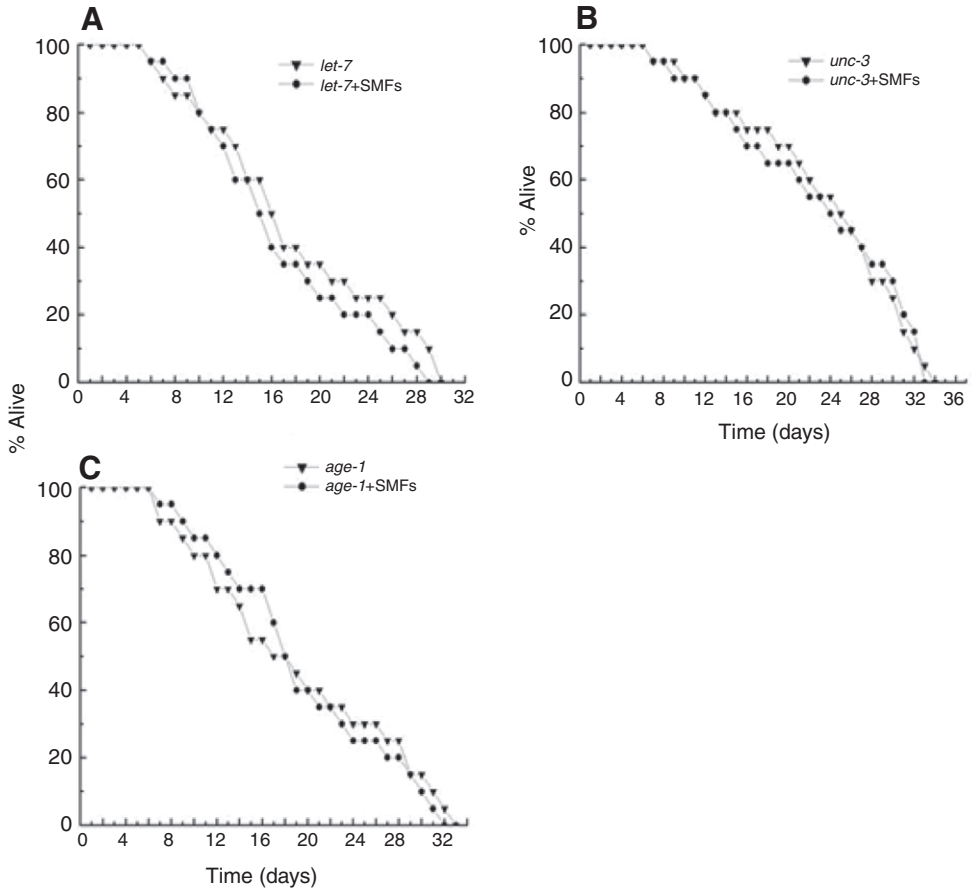

Fig. 6. The effects of SMFs on the lifespan of mutant nematodes. (A) The lifespan of *let-7* mutants in the presence and absence of a 200 mT SMF. (B) The lifespan of *unc-3* mutants in the presence and absence of a 200 mT SMF. (C) The lifespan of *age-1* mutants in the presence and absence of a 200 mT SMF. Each experiment contained 60 nematodes. The lifespan experiment was performed at 20°C.

affected to different extents. However, genes associated with development and aging were particularly up-regulated. The *clk-1*, *lim-7*, *daf-2* and *age-1* genes are associated with development and aging; however, these genes are members of distinct pathways within the development and aging processes. The *clk-1* (clock abnormal protein 1) gene encodes an enzyme (demethoxyubiquinone monooxygenase) that is necessary for ubiquinone biosynthesis in the worm *C. elegans* and other eukaryotes (Liu et al., 2005). The mouse version of the gene is called *mclk1*, and the human, fruit fly and yeast homologs are called *COQ7*. The gene *lim-7* encodes one of seven *C. elegans* LIM-homeodomain (LIM-HD) proteins and is the sole *C. elegans* member of the Islet subclass of LIM-HD transcription factors. Loss of *lim-7* activity results in L1 larval lethality characterized by locomotion and morphological defects. A later role for *lim-7* in gonad development has been suggested. The gene *age-1* encodes a homolog of the p110 subunit of phosphatidylinositol 3-kinase, and *daf-2* encodes an insulin receptor family member. *daf-2* and *age-1* are involved in regulating the entry into an alternative developmental pathway, the dauer pathway. For these genes, the increase in lifespan is primarily due to an increase in adult lifespan. Although we focused on genes and pathways associated with development and aging, it is likely that SMFs may affect pathways of other biological processes. A genomic survey will reveal the global effect of SMFs on the biological system.

Triggering effects of SMFs on transcription

Given the minor effects of SMF on development time and lifespan, it is intriguing to observe the sensitive and enhanced response of gene expression after application of SMFs. SMFs higher than 50 mT seem to trigger the up-regulation of *clk-1*, *lim-7*, *unc-3* and *age-1*, and gene expression instantly reached a plateau. A switch in expression seemed to occur if the applied SMF was higher than a threshold level, which resides somewhere between 30 and 50 mT. The strong change in gene expression at 50 mT exhibited by the nematode was unexpected because a SMF at this intensity did not induce a significant variation in development time. Further translational studies and functional investigations will reveal the molecular correlation between SMFs and development.

Genetic analyses of mutants reveal possible mechanisms underlying the SMF-induced gene expression

Here we took advantage of nematode genetics to quantify expression levels with quantitative real-time RT-PCR. The results are consistent with the mutant analyses in both development time and lifespan (Figs 5 and 6). The only exception occurs with *lim-7*. SMFs up-regulated *lim-7*; however, development in the mutant strain occurred at wild-type rates. It is likely that SMFs affect development through alternative pathways, since we have shown the global effect of SMFs. Thus, although the expression levels of *lim-7* were not increased in the mutant nematode, SMFs shortened the development time through other pathways.

CONCLUSIONS

Here, we have shown that exposing nematodes to SMFs above 150 mT significantly shortens the life cycle of *C. elegans*. The application of SMFs accelerated nematode development from the L2 to adult stages. The greatest effect, a 23% reduction in development time, was seen in the progression from the L2 stage to the L3 stage. Using real-time quantitative RT-PCR, we identified genes (*clk-1*, *lim-7*, *unc-3* and *age-1*) that were differentially

expressed following SMF treatment. A dose–response analysis indicated that a 50 mT SMF was sufficient to trigger the induction of transcription. Genetic analysis using mutant strains indicated that developmental and aging pathways, including the insulin receptor pathway, are involved in the SMF-induced development time and lifespan reductions. SMFs of sufficient intensity induced a reduction in life cycle time and accelerated the aging process in nematodes. The current study provides insight into the molecular consequences and mechanisms underlying SMF treatment.

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