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メタデータ	言語: eng 出版者: 公開日: 2017-12-07 キーワード (Ja): キーワード (En): 作成者: メールアドレス: 所属:
URL	https://doi.org/10.24517/00049208

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Profile of development to genes under extremely low frequency magnetic fields with *Caenorhabditis elegans*

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Abstract. Responses of the small heat shock protein gene, *hsp-16*, were examined in transgenic *Caenorhabditis elegans* exposed to extremely low frequency magnetic fields (ELFMs). Expression of the *hsp-16-lacZ* gene was enhanced when transgenic animals were exposed to magnetic fields up to 0.5 T at 60 Hz. The *hsp-16* promoter was more efficiently expressed at the embryonic than at the post-embryonic stage irrespective of exposure. Promoter activity was more sensitive to the stimulus in the intestine at the post-embryonic stage. Evidence is presented that the induction occurs at the transcriptional step of *hsp-16*.

1. Introduction

Although the biological effects of extremely low frequency magnetic fields (ELFMs) have been well studied, assessments remain contradictory [1,2]. Previously we studied the effects of ELF high magnetic fields (up to 1.2 T) in *C. elegans* on reproductive system, embryonic and post-embryonic development, and behavior [3]. We found that ELFMs affect slightly but significantly the life-cycle of *C. elegans*. That is, the brood size is small, feeding ability decreases, growth rate is slow and result in small body size.

We are interested in why ELFMs have multiple effects on the nematode life-cycle. One possibility is that the nematode recognizes ELFMs as stress. Generally organisms respond to a hard environment by producing stress proteins, called heat shock proteins (HSPs) [4–6]. These proteins have been found in a wide variety of organisms tested, including bacteria, yeast, plants, nematodes, fruit flies and mammalian cells. *C. elegans* is a suitable organism for the study of the relation between ELFMs and biological stress response in that five genes encoding small HSPs have been well characterized in this species by

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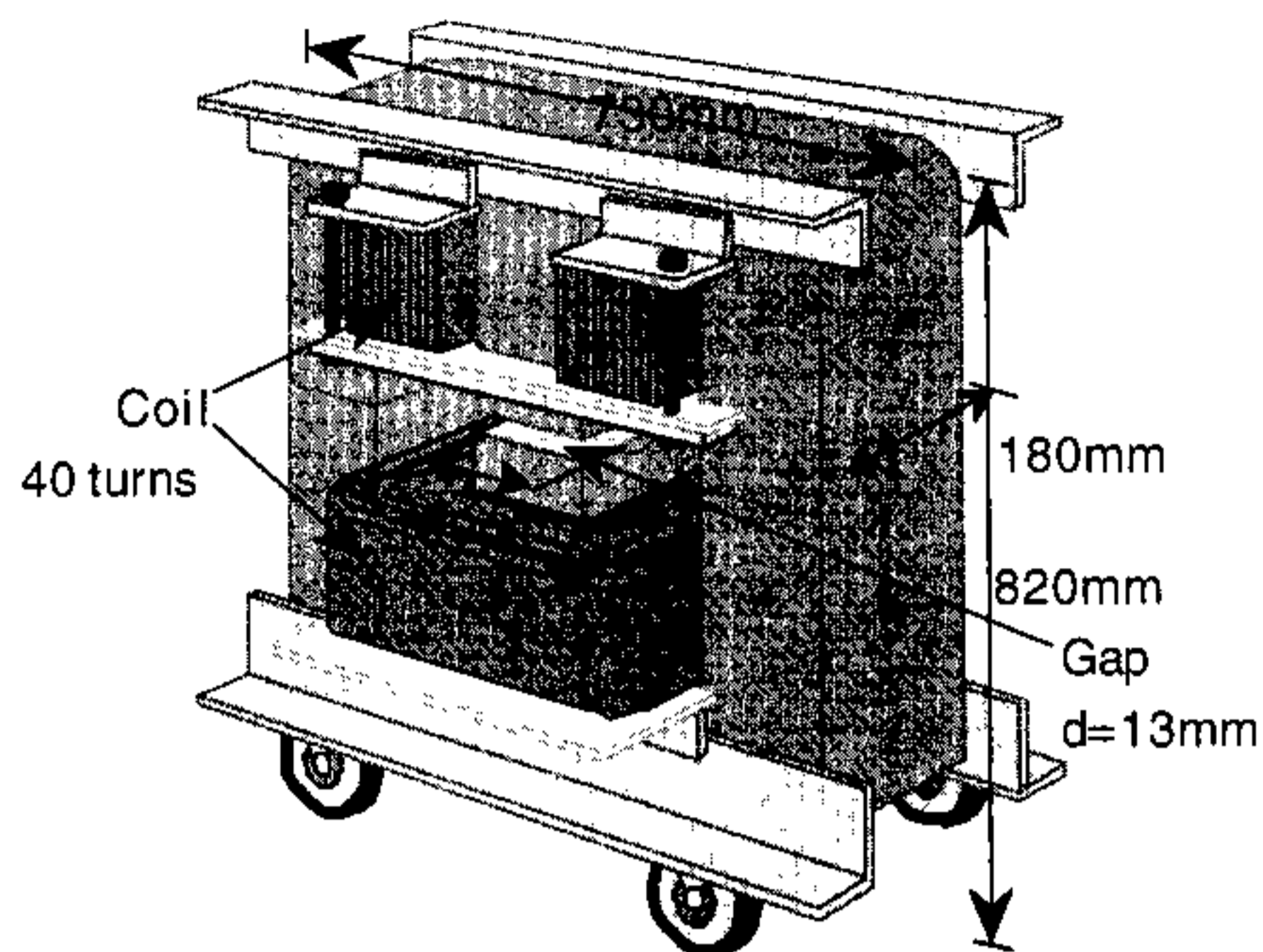


Fig. 1. Magnetic field generator.

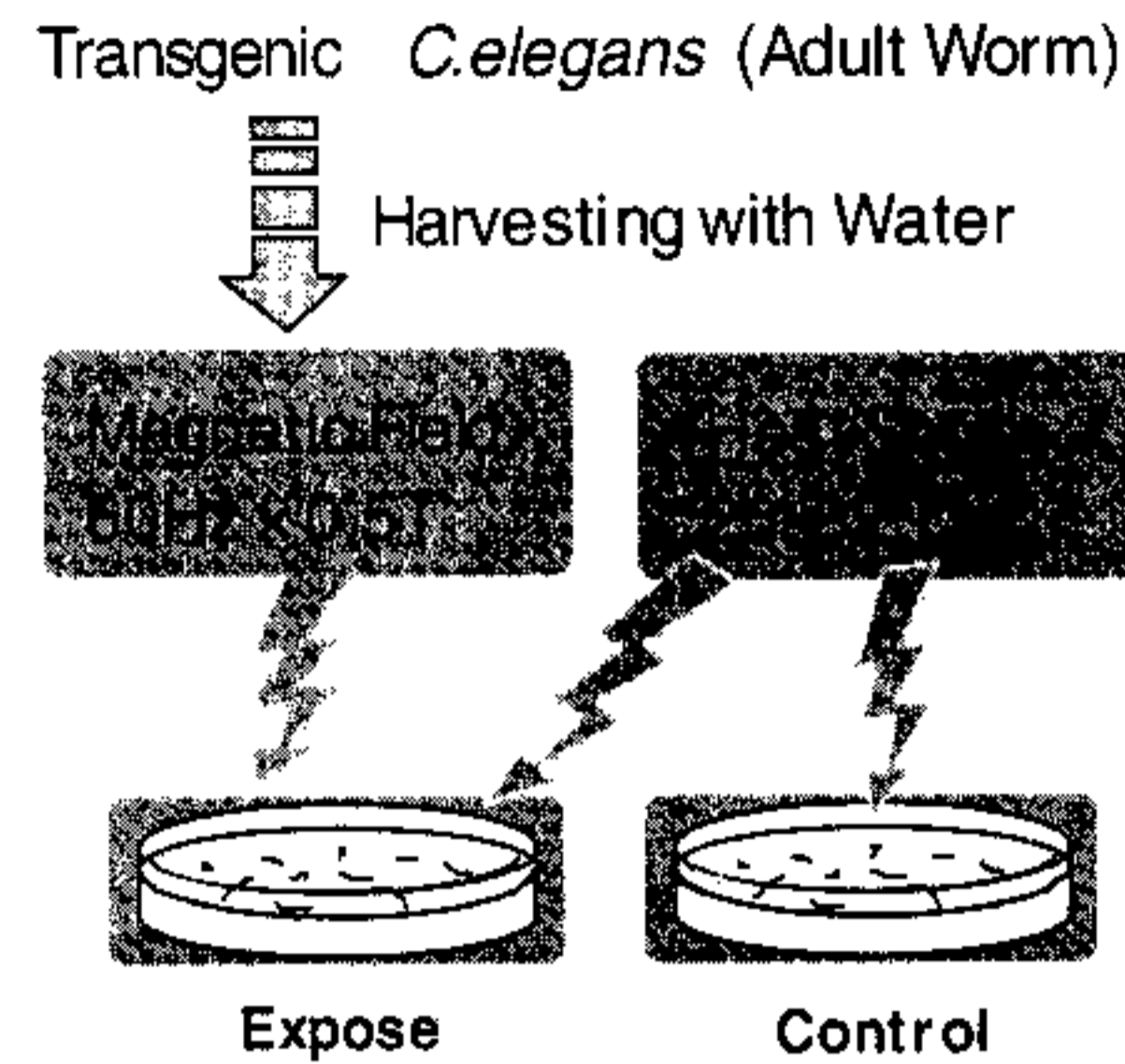


Fig. 2. Outline of ELFMF exposure assay.

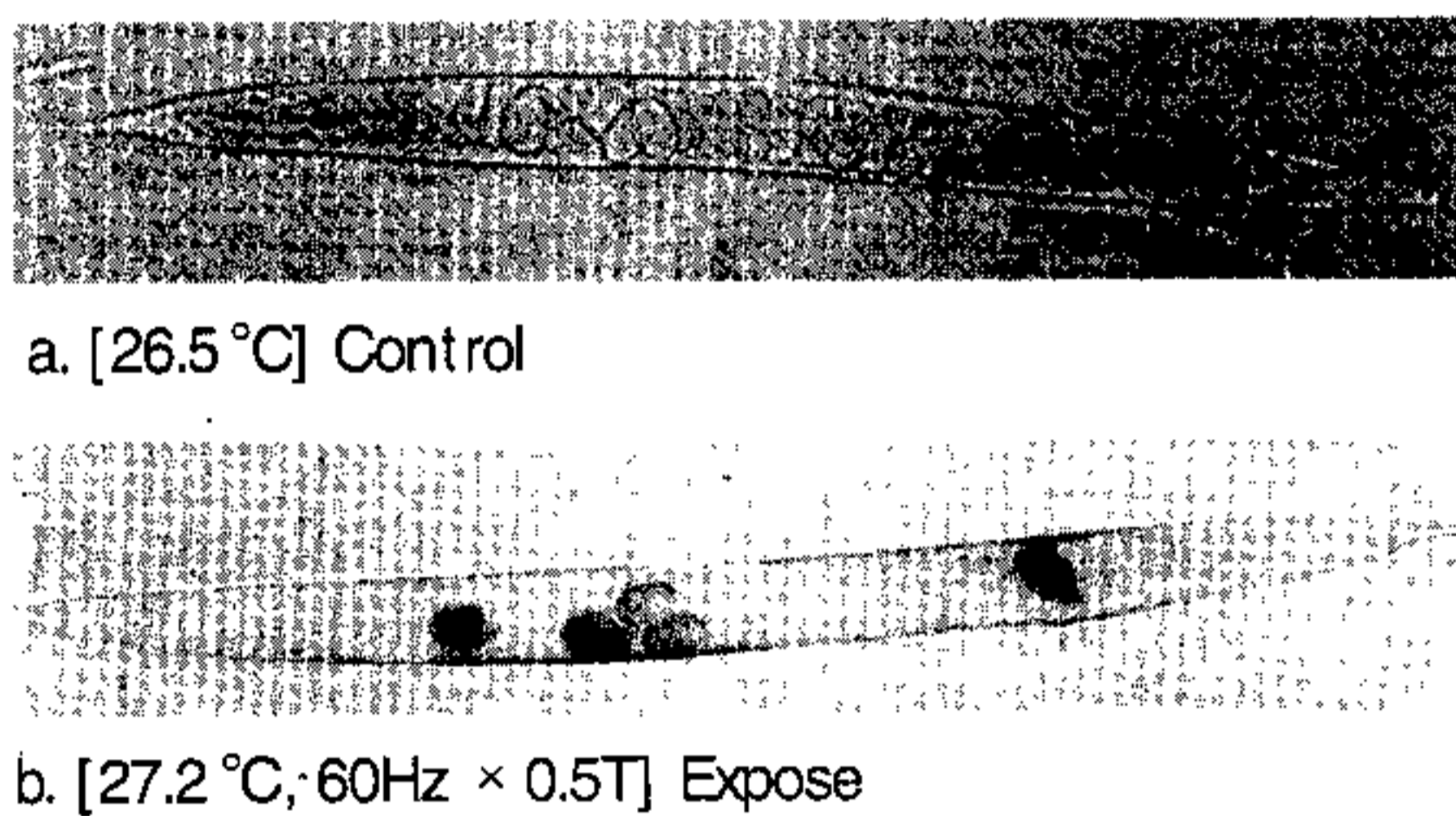


Fig. 3. Expression patterns of HSP16::lacZ.

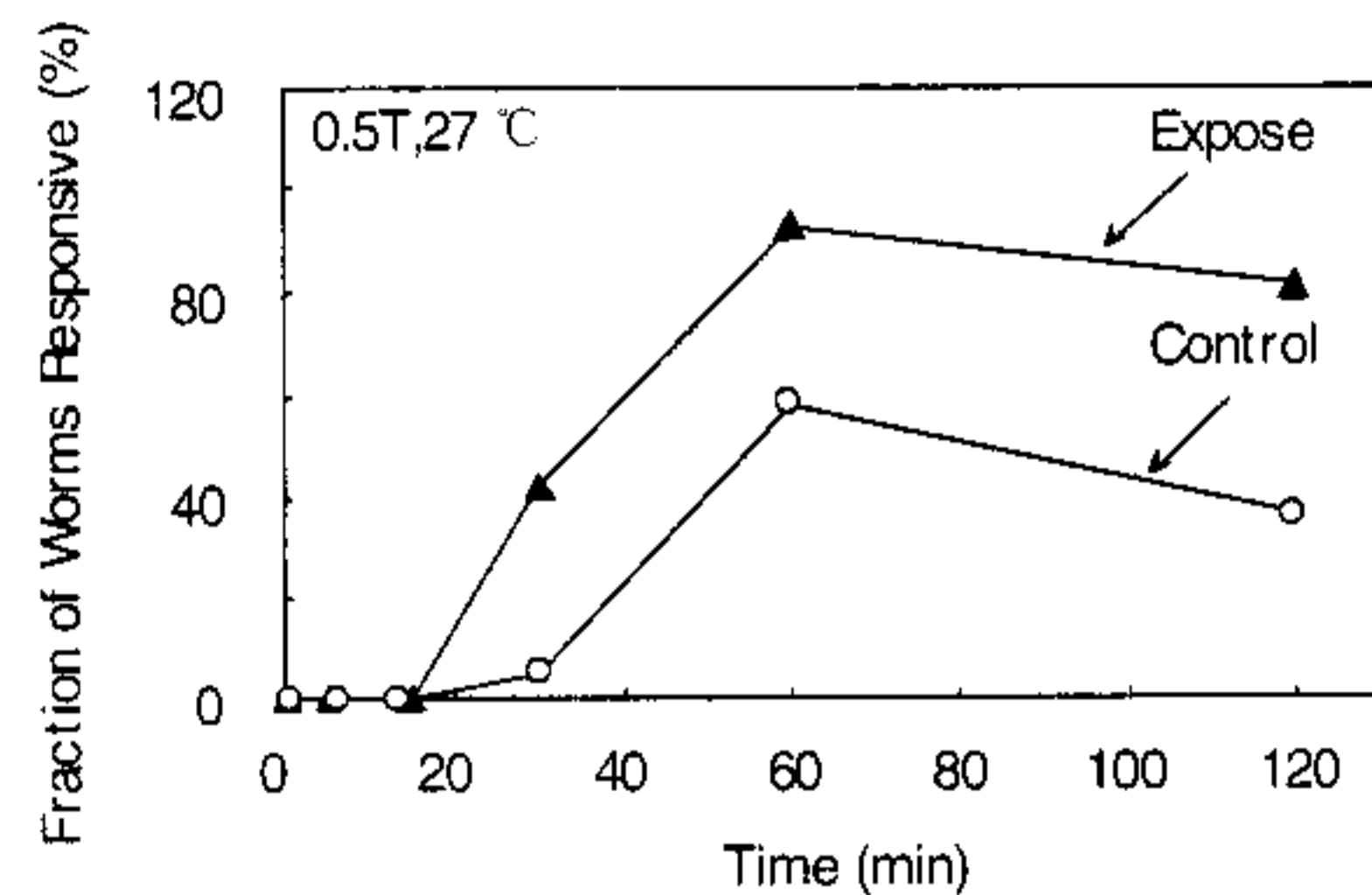


Fig. 4. LacZ expression under ELFMF.

Candido et al. [7] and further several transgenic *C. elegans* strains containing *hsp-lacZ* fusions have already been constructed [8,9].

Pioneering works were done by Goodman et al. [1], as well as others [10] on the response of genes encoding stress responsible heat shock proteins. Here, we studied the expression of *hsp-16* and found the gene was induced by ELFMFs. We conclude that *C. elegans* recognizes ELFMFs as stress.

2. High magnetic field generator

The high magnetic fields generator is composed of two E-type cores with their poles placed face to face (Fig. 1). The experimental space consists of a 13 mm gap in the center. By supplying a 60 Hz, 190 A current, an AC magnetic fields with a peak flux density of 0.5 T can be produced. This machine was cooled by a high performance water circulation system, and a quartz thermometer was used to monitor temperature under the experiment. The fluctuation of temperature in experimental space is less than 0.10°C.

3. Expression of the *hsp-16-lacZ* Transgene Under ELFMFs

We are interested in investigating why ELFMFs multiply affects on *C. elegans* development. Generally organisms respond to a hard environment by producing stress proteins. Therefore, *C. elegans* also may

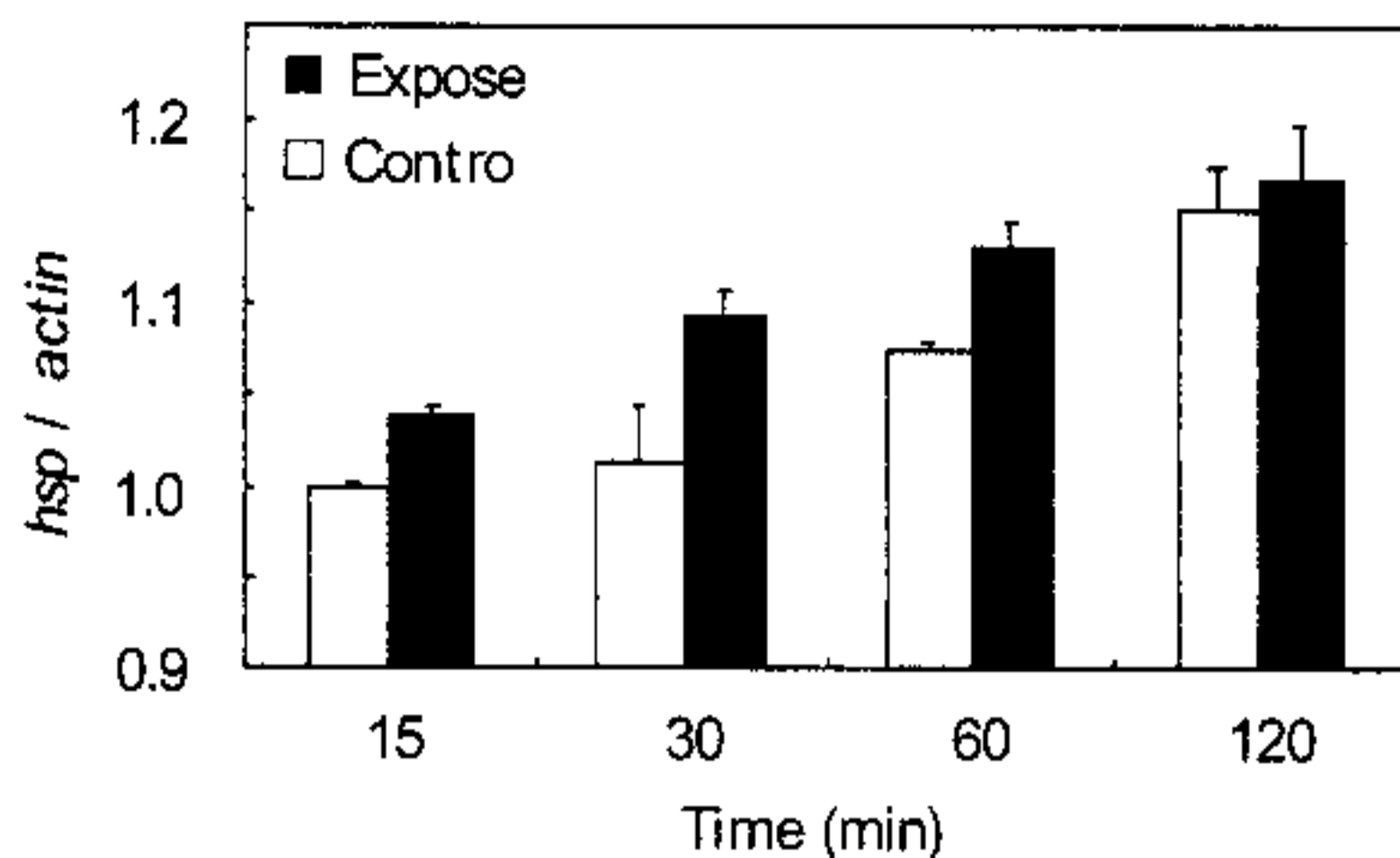


Fig. 5. Transcriptional activation of lacZ expression under ELFMF.

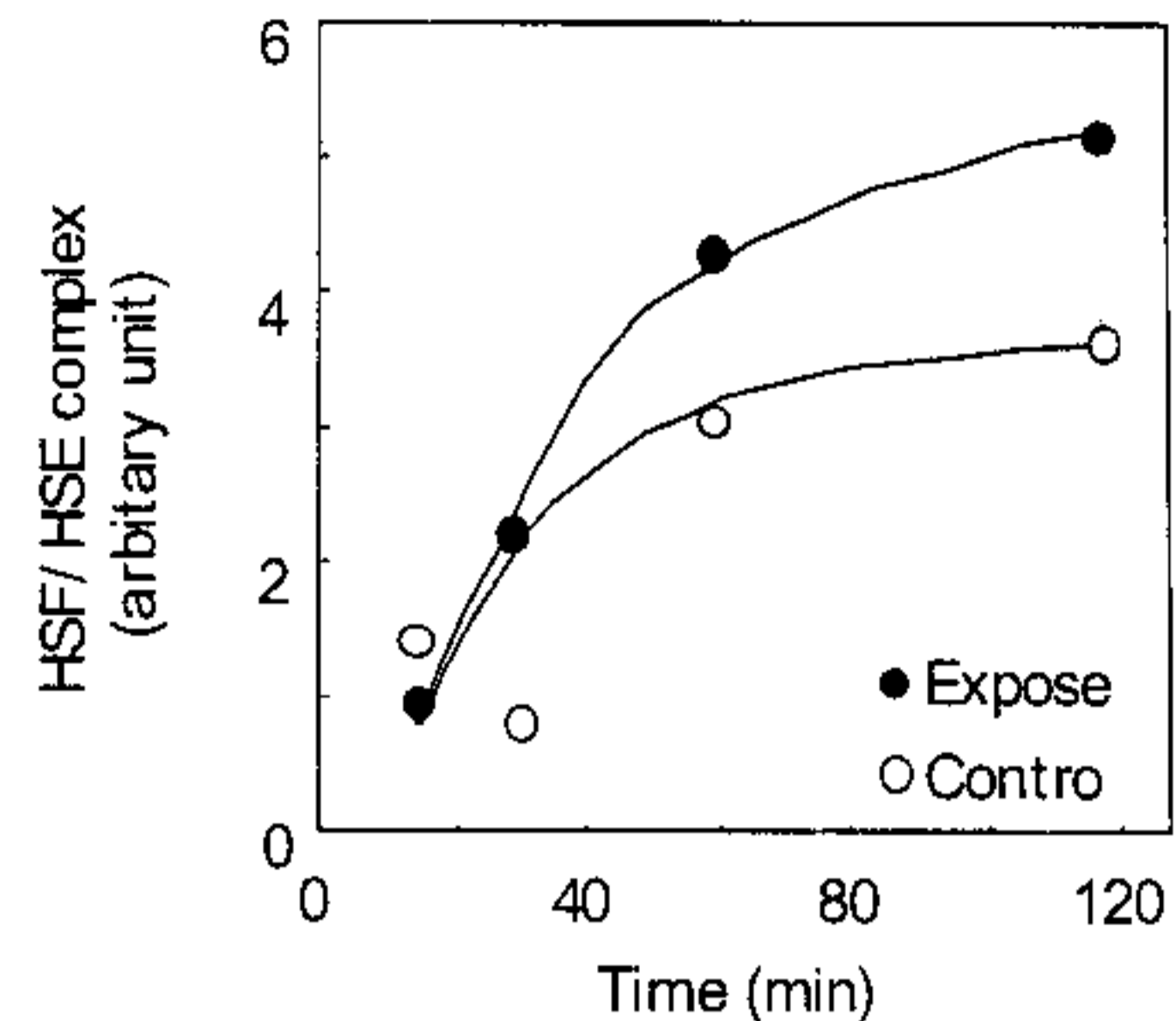


Fig. 6. ELFMF dependent HSF binding to HSE of the *hsp-16* gene.

perceive ELFMFs as stress. We then tested the possibility with the transgenic *C. elegans* containing *hsp-lacZ* fusions provided by Candido (British-Columbia U., Canada) (Fig. 2). Figure 3 shows expression patterns of *HSP16::lacZ* in *C. elegans*. As shown in Fig. 3(a), *C. elegans* without ELFMFs is not stained. In Fig. 3(b), *C. elegans* stained in embryos with ELFMFs is typical expression pattern.

At least 20 min was necessary for the *hsp-16* to be produced. *C. elegans* exposed to ELFMFs showed about a 10-fold increase in expression of the *hsp-16* gene after 30 min as compared with the sham-exposed controls (Fig. 4).

4. Quantification of the *hsp-16* mRNA produced under ELFMFs

Although the *hsp-16* expression significantly increased under ELFMFs, the timing of the induction was approximately the same. Therefore, the induction by ELFMFs is at the transcriptional level, probably either due to the promoter activity, transcriptional elevation or stabilization of the transcript. To distinguish these, we measured mRNA contents by quantitative RT-PCR method. A significant increase in mRNA contents (an average increase of 10% ($n = 3$) after 30 min was observed in Fig. 5, indicating ELFMFs stimulated transcription.

5. Electrophoretic mobility shift assay

Then the effects of ELFMFs on activation of heat shock transcription factor (HSF) and its DNA binding activity were studied with electro mobility shift assay (EMSA). Nuclear extracts were prepared from *C. elegans* exposed to ELFMFs and its binding to HSE was compared with extracts from non-exposed *C. elegans*. As shown in Fig. 6, the level of the complex increased with exposed time and reached the maximum level after 120 min. The level of the complex was at least 1.5 fold higher than the level of the unexposed complex.

6. Conclusion

These results suggest that the organism recognizes the magnetic field as stress and result in multiple response. How does *C. elegans* sense magnetic fields as stress? To determine whether the stress response

induced by the fields stimulation mechanism was mediated by a sequence of events similar to that reported for other physiological stresses, we examined the activation of heat shock transcription factor and its DNA binding activity. We showed transcriptional levels of *hsp-16* were elevated in response to magnetic fields stimulation.

The animals respond to magnetic field stimulation by activating heat shock factor and increasing heat shock element-binding factor via the induction of elevated transcription levels for *hsp-16* gene. Therefore, magnetic fields induction of *hsp-16* gene expression appears to be mediated through binding of the HSF protein. Although the mechanism of the interaction of magnetic fields with *C. elegans* remains unclear, the conditions of the stress response due to magnetic fields stimulation appears to a stimulus that is normally not part of its environment and which may be a general interaction mechanism.

Acknowledgement

We are grateful to E.P.M. Candido for providing us the PC6 and PC72 strains. This work was supported in part by a Grant-in-Aid from the Japan Society for the Promotion of Science (Research for the Future 97L00401) to R.H., and by a Grant-in-Aid for scientific researches from the Ministry of Education, Science, Culture and Sports, Japan (10837004) to H.Y. and (12780581) to S.H.

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