THE ROYAL SOCIETY biology letters

Biogenic magnetite in the nematode *Caenorhabditis elegans*

Charles G. Cranfield[1], Adam Dawe[2], Vassil Karloukovski[3], Rafal E. Dunin-Borkowski[4], David de Pomerai[2] and Jon Dobson[1*]

[1]*Institute for Science and Technology in Medicine, Keele University, Stoke-on-Trent ST4 7QB, UK*
[2]*Molecular Toxicology Division, School of Biology, University of Nottingham, Nottingham NG7 2RD, UK*
[3]*Centre for Environmental Magnetism and Palaeomagnetism, Geography Department, Lancaster University, Bailrigg, Lancaster LA1 4YW, UK*
[4]*Department of Materials Science and Metallurgy, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, UK*
[*]*Author for correspondence (j.p.dobson@bemp.keele.ac.uk).*

Recd 20.01.04; *Accptd* 21.04.04; *Published online* 28.06.04

The nematode *Caenorhabditis elegans* is widely used as a model system in biological research. Recently, examination of the production of heat-shock proteins in this organism in response to mobile phone-type electromagnetic field exposure produced the most robust demonstration to date of a non-thermal, deleterious biological effect. Though these results appear to be a sound demonstration of non-thermal bioeffects, to our knowledge, no mechanism has been proposed to explain them. We show, apparently for the first time, that biogenic magnetite, a ferrimagnetic iron oxide, is present in *C. elegans*. Its presence may have confounding effects on experiments involving electromagnetic fields as well as implications for the use of this nematode as a model system for iron biomineralization in multicellular organisms.

Keywords: magnetite; *Caenorhabditis elegans*; biomineral; electromagnetic fields

1. INTRODUCTION

Magnetite, a ferrimagnetic iron oxide (Fe_3O_4), is produced biogenically in organisms ranging from bacteria to humans (Kirschvink *et al.* 1985, 1992). In many of these organisms its origin and function are unknown, whereas in others it has been demonstrated to play a role in navigation via geomagnetic field sensing (Frankel 1984). Magnetotactic bacteria, which represent the best-understood example of the latter group, produce chains of magnetite particles that act as a 'compass needle' orienting the organism as it swims. In mammals, magnetite has thus far been shown to have a direct neurological connection only in one species—the rainbow trout, *Oncorhynchus mykiss* (Walker *et al.* 1997), though many studies have demonstrated magnetic navigation in other animals, such as pigeons (e.g. Wiltschko & Wiltschko 2002).

In humans, the accumulation of biogenic magnetite in the brain has been linked to Alzheimer's disease (AD) (Hautot *et al.* 2003), and it has been proposed as a potential mechanism for mobile phone bioeffects (Dobson & St Pierre 1996; Kirschvink 1996). In this study, we have examined possible magnetite biomineralization in *Caenorhabditis elegans*, an important model system for biological research on both mobile phone bioeffects and AD, the results of which could be strongly influenced by the presence of magnetite.

Caenorhabditis elegans is a soil nematode of *ca.* 1 mm in length, which is readily grown in culture on a diet of bacteria, usually *Escherichia coli*. Although it has only 959 somatic cells, it possesses a range of differentiated tissues and shares many biological functions with humans. The genome of *C. elegans* has been sequenced and its cell lineage has been delineated (Sulston *et al.* 1983; *C. elegans* Sequencing Consortium 1998).

Recently, it has been reported that *C. elegans* produces stress-response (heat-shock) proteins as a result of exposure to mobile phone-type radiofrequency (RF) electromagnetic fields (EMF) (de Pomerai *et al.* 2000). Though the work is often cited as a clear example of RF bioeffects, the mechanism behind such potential effects is not well understood (de Pomerai *et al.* 2003).

In 1996, mechanisms based on magnetite transduction were proposed to explain potential mobile phone bioeffects (Dobson & St Pierre 1996; Kirschvink 1996). These mechanisms are now being tested experimentally, with interesting results (Cranfield *et al.* 2003*a*,*b*). The coupling of these particles to external fields results in the local absorption of energy within the organism that is many times larger than the thermal background energy at body temperature. Though possible explanations for the effect of relatively low-power RF emissions on cellular function remain uncertain, the potential for magnetite transduction in *C. elegans* in these studies has not been examined.

In light of these recent results, it is important to establish whether biogenic magnetite is present in model organisms such as *C. elegans*. To address this question, we used superconducting quantum interference device (SQUID) magnetometry and transmission electron microscopy (TEM) to examine this nematode. Our results have major implications for the use of *C. elegans* in studies of mobile phone/electromagnetic field effects and in AD research (Link *et al.* 2003).

2. MATERIAL AND METHODS

Synchronous populations of *C. elegans* were cultivated from eggs grown in 150 ml of S-medium (aerated with 0.22 μm of filter-sterilized air), supplemented with 2 g of *E. coli* (strain P90C) and a drop of Anti-Foam A surfactant (Sigma-Aldrich), for 4 days at 20 °C. Before measurement, samples of *C. elegans* were washed six times with ultrapure water to remove any growth medium and *E. coli*. These samples were stored in acid-washed glassware and freeze-dried. Samples of *E. coli*-free S-medium and of *E. coli*-containing S-medium were freeze-dried and compressed into pellets for controls.

Samples were prepared in class I flow hoods and stored in sealed plastic autoclave bags while not in use. Measurement of isothermal remanent magnetization (IRM) was conducted on a Quantum Designs MPMS SQUID magnetometer.

A JEOL JEM-3000F field emission gun TEM with an ultra-high resolution pole-piece, a Gatan (electron energy loss) imaging filter and an Oxford Instruments energy dispersive X-ray (EDX) spectrometer were used for imaging unstained samples of *C. elegans*. The compositions of electron-dense regions were analysed using EDX spectrometry and three-window, background-subtracted, energy-selected imaging (using edges in the energy loss spectrum that included iron $L_{2,3}$ and oxygen K). The lattice spacings of individual crystallites were measured from diffractograms (Fourier transforms).

DOI 10.1098/rsbl.2004.0209

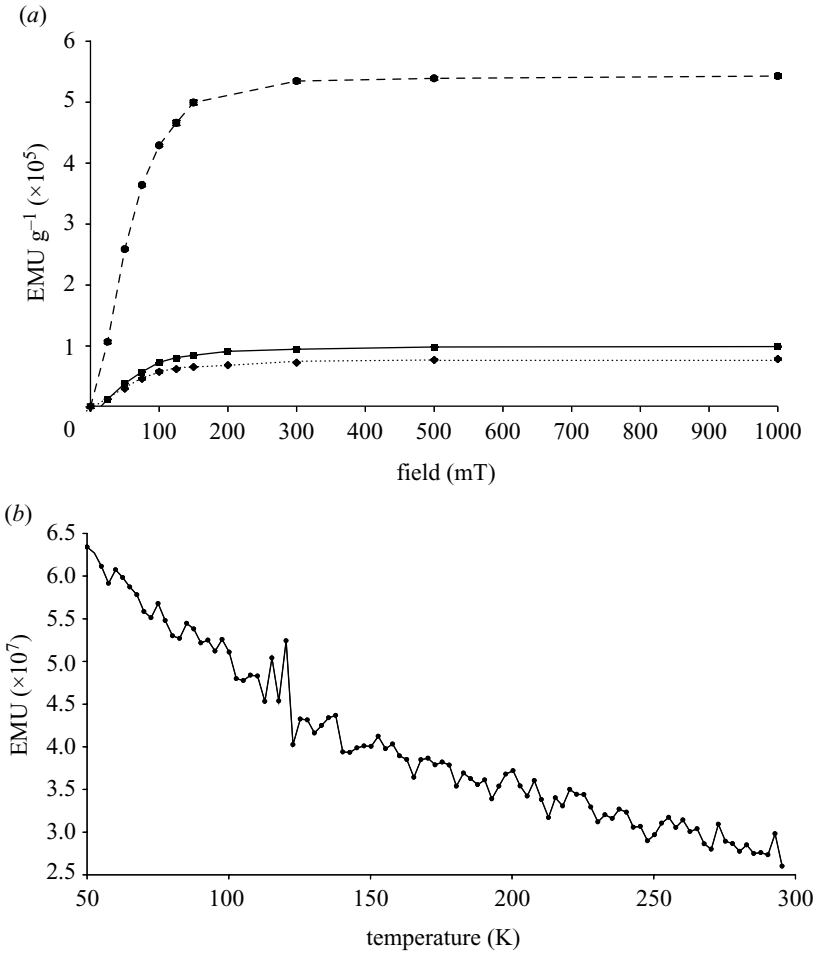

Figure 1. (*a*) IRM curves at 293 K of *Caenorhabditis elegans* (circles, dashed line) compared with S-media (squares, solid line) and S-media with *Escherichia coli* (diamonds, dotted line). Magnetizations are in electromagnetic units per gram (EMU). (*b*) TDR after exposure to a 1 T field at 50 K. A discontinuity and drop in magnetization intensity can be seen at *ca*. 120 K.

3. RESULTS

IRM measurements of *C. elegans*, growth medium and medium plus *E. coli* revealed a remanent magnetization component in all samples, with the highest mass-corrected value observed in *C. elegans* (figure 1). All of the samples reached saturation at fields of between 200 and 250 mT. This saturation field is consistent with the presence of low coercivity iron biominerals such as magnetite (and/or maghemite, γFe_2O_3: an oxidation product of magnetite) or greigite (Fe_3S_4, which is also found in some species of bacteria).

SQUID measurements of the thermal decay of remanence (TDR) showed a discontinuity and a drop in remanent magnetization along with a change in slope at *ca*. 120 K. This is likely to be associated with the Verwey transition—a drop in conductance below *ca*. 125 K associated with a structural transformation from a cubic inverse spinel to a monoclinic crystal structure. The presence of this transition in TDR experiments is diagnostic of the presence of magnetite (Waltz 2002).

TEM imaging of *C. elegans* was carried out only on regions near the edge of the nematode owing to the thickness of the specimen. Electron-dense particles were observed and both EDX and energy-selected imaging demonstrated that these particles, which were surrounded by a Ca-rich crystalline matrix, contained significant amounts of iron and oxygen. No other elements were identified within the particles. The majority of the particles were 2–3 nm in size, and their lattice spacings were consistent with ferrihydrite (the mineral core of the ubiquitous iron storage protein, ferritin). However, several larger iron- and oxygen-rich particles (some tens of nanometres in size) were also observed (figure 2*a*). The dominant spacings in these particles are 0.24–0.25 nm with an angle of 63°. The parameters are consistent with the 222 and 311 interplanar spacings for magnetite oriented along the [−112] zone axis. Owing to the thickness of the samples, the quality of the lattice fringes is poor and there are indications that the crystal contains defects. The size of the crystal (*ca*. 21 nm) and the lattice spacings, however, are not consistent with ferrihydrite, which is generally found within the core of the ferritin protein and that restricts its maximum size to 7–8 nm.

Though only particles along the edges at the anterior region of *C. elegans* could be studied with TEM (figure 2*b*), this does not preclude the presence of magnetite in other regions of the organism. Indeed, the IRM curves imply the presence of a large fraction of blocked magnetite particles, which were not observed in the anterior regions. For magnetite, this would generally indicate particles of larger than 30–50 nm (depending on the shape). Notably, the expression of heat-shock reporter genes in RF-exposed worms was shown to be widespread in both gut tissue and in embryos prior to laying (de Pomerai *et al*. 2000).

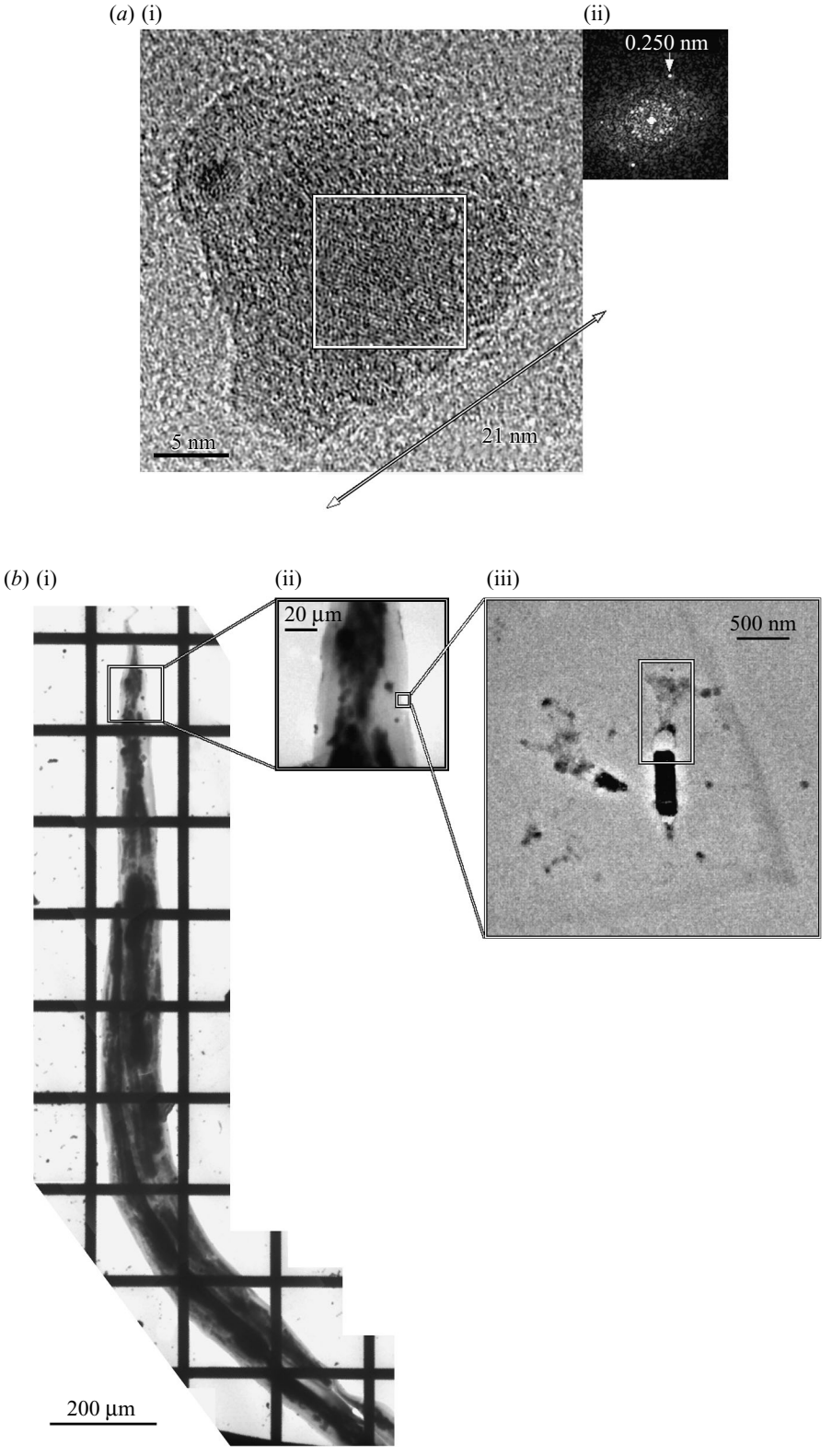

Figure 2. (*a*) (i) High-resolution TEM image of an iron-containing crystallite, whose composition was characterized using EDX and energy-selected imaging, in a sample of *Caenorhabditis elegans*. The double-headed arrow at the bottom right indicates the dimension of the particle (21 nm). (ii) A diffractogram (Fourier transform) of the square region in (i) provides a dominant lattice spacing in the crystallite of 0.25 nm, which is consistent with the 311 lattice spacing in magnetite. (*b*) Bright-field TEM images of a *Caenorhabditis elegans* sample highlighting the location of magnetite discovered within the nematode.

4. DISCUSSION

The magnetometry and TEM results presented provide compelling evidence that wild-type *C. elegans* do in fact contain magnetite. Comparison of the mass-corrected remanent magnetizations indicates that it is unlikely that the magnetite arises from a concentration of magnetite already present in the media and *E. coli*. However, this possibility cannot be ruled out and the proposed biogenic origin of magnetite in *C. elegans* should be considered as preliminary at this stage. Magnetite contamination and

concentration in cells and organisms has been discussed previously in the context of mobile phone experiments (Kobayashi *et al.* 1995) and it is clear that the consequences in this case would be the same with regard to the results of de Pomerai *et al.* (2000).

Lattice defects, such as those observed here, have also been reported in previous studies of bacterial magnetite and bacterial greigite (Devouard *et al.* 1998; Posfai *et al.* 1998*a*,*b*; Taylor *et al.* 2001). It is generally thought that, in these cases, the minerals have formed in the solid state from a non-ferrimagnetic precursor. This may be an indication that biogenic magnetite is forming from a ferritin (ferrihydrite) precursor, as has been proposed in the case of biogenic magnetite associated with human neurodegenerative disease (Dobson 2001, 2004).

The presence of magnetite in these samples may provide a physically plausible explanation for the heat-shock protein responses reported by de Pomerai *et al.* (2000). Other recent studies have shown a stress response in *C. elegans* due to exposure to extremely low frequency (ELF – 50/60 Hz) magnetic fields, which may also have been owing to interactions with biogenic magnetite (Junkersdorf *et al.* 2000; Miyakawa *et al.* 2001). Both of these sets of results are consistent with ferromagnetic transduction via biogenic magnetite and may need to be re-evaluated in light of the discovery of biogenic magnetite in *C. elegans*.

Transgenic *C. elegans* has recently been proposed as a model system for the study of AD (Link *et al.* 2003). As iron compounds are known to play an important role in AD—and indeed in most neurodegenerative disorders—our results have a significant bearing on the use of transgenic *C. elegans* in such a model. This is particularly pertinent as it has been demonstrated that levels of biogenic magnetite are higher than normal in AD subjects and that biogenic magnetite may play a role in β-amyloid (plaque) aggregation (Dobson *et al.* 2001; Hautot *et al.* 2003). The elucidation of the role of these iron compounds in the disease becomes more important if magnetite-producing transgenic organisms are to be used as model systems.

The findings reported here may open a new experimental paradigm for research into the formation of magnetite in multicellular organisms. *Caenorhabditis elegans* would be an ideal multicellular model for the investigation of the biogenic formation of ferrimagnetic compounds and for testing the 'Grand Unified Theory of Biomineralization' proposed by Kirschvink & Hagadorn (2000). The relevance of this is highlighted by the fact that the genome of *C. elegans* has been sequenced and may provide fundamental clues as to the genetic processes involved in magnetite biomineralization. The implications for mobile phone RF exposure through possible ferromagnetic transduction may also be investigated by using the magnetite present in *C. elegans* as an experimental model.

Acknowledgements

The authors thank the EPSRC Nanomagnetics Network and Quentin Pankhurst for financial support. J.D. acknowledges the support of a Wolfson Foundation/Royal Society Merit Award. R.D.B. acknowledges The Royal Society for financial support and Crispin Hetherington and Matthew Weyland for help with TEM. The authors also acknowledge the assistance of Karen Walker and Dimitri Hautot.

C. elegans Sequencing Consortium 1998 Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* **282**, 2012–2018.

Cranfield, C. G., Wieser, H. G., Al Madan, J. & Dobson, J. 2003*a* Evaluation of ferromagnetic transduction mechanisms for mobile phone bioeffects. *IEEE Trans NanoBiosci.* **2**, 40–43.

Cranfield, C. G., Wieser, H. G. & Dobson, J. 2003*b* Exposure of magnetic bacteria to simulated mobile phone type RF radiation has no impact on mortality. *IEEE Trans NanoBiosci.* **2**, 146–149.

de Pomerai, D. I. (and 10 others) 2000 Non-thermal heat-shock response to microwaves. *Nature* **405**, 417–418.

de Pomerai, D. I., Smith, B., Dawe, A., North, K., Smith, T., Archer, D. B., Duce, I. R., Jones, D. & Candido, E. P. M. 2003 Microwave radiation can alter protein conformation without bulk heating. *FEBS Lett.* **543**, 93–97.

Devouard, B., Posfai, M., Hua, X., Bazylinski, D. A., Frankel, R. B. & Buseck, P. R. 1998 Magnetite from magnetotactic bacteria: size distributions and twinning. *Am. Mineral.* **83**, 1387–1398.

Dobson, J. 2001 Nanoscale biogenic iron oxides and neurodegenerative disease. *FEBS Lett.* **496**, 1–5.

Dobson, J. 2004 Magnetic iron compounds in neurological disorders. *Ann. N. Y. Acad. Sci.* **1012**, 183–192.

Dobson, J. & St Pierre, T. G. 1996 Application of the ferromagnetic transduction model to D.C. and pulsed magnetic fields: effects on epileptogenic tissue and implications for cellular phone safety. *Biochem. Biophys. Res. Commun.* **227**, 718–723.

Dobson, J., Gross, B., Exley, C., Mickhailova, A., Batich, C. & Pardoe, H. 2001 Biomedical applications of biogenic and biocompatible magnetic nanoparticles. In *Modern problems of cellular and molecular biophysics* (ed. S. Ayrapetyan & A. North), pp. 121–130. Yerevan, Armenia: Noyan Tapan/UNESCO.

Frankel, R. B. 1984 Magnetic guidance of organisms. *A. Rev. Biophys. Bioengng* **13**, 85–103.

Hautot, D., Pankhurst, Q. A., Khan, N. & Dobson, J. 2003 Preliminary evaluation of nanoscale biogenic magnetite and Alzheimer's disease brain tissue. *Proc. R. Soc. Lond.* B **270**, S62–S64. (DOI 10.1098/rsbl.2003.0022.)

Junkersdorf, B., Bauer, H. & Guteit, H. O. 2000 Electromagnetic fields enhance the stress response at elevated temperatures in the nematode *Caenorhabditis elegans*. *Bioelectromagnetics* **21**, 100–106.

Kirschvink, J. L. 1996 Microwave absorption by magnetite: a possible mechanism for coupling non-thermal levels of radiation to biological systems. *Bioelectromagnetics* **17**, 187–194.

Kirschvink, J. L. & Hagadorn, J. W. 2000 A grand unified theory of biomineralization. In *The biomineralisation of nano- and microstructures* (ed. E. Bäuerlein), pp. 139–149. Weinheim, Germany: Wiley-VCH Verlag GmbH.

Kirshvink, J. L., Jones, D. S. & MacFadden, B. J. (eds) 1985 *Magnetite biomineralisation and magnetoreception by living organisms: a new biomagnetism*. New York: Plenum.

Kirschvink, J. L., Kobayashi-Kirschvink, A. & Woodford, B. J. 1992 Magnetite biomineralization in the human brain. *Proc. Natl Acad. Sci. USA* **89**, 7683–7687.

Kobayashi, A. K., Kirschvink, J. L. & Nesson, M. H. 1995 Ferromagnetism and EMFs. *Nature* **374**, 123.

Link, C. D., Taft, A., Kapulkin, V., Duke, K., Kim, S., Fei, Q., Wood, D. E. & Sahagan, B. G. 2003 Gene expression analysis in a transgenic *Caenorhabditis elegans* Alzheimer's disease model. *Neurobiol. Aging* **24**, 397–413.

Miyakawa, T., Yamada, S., Harada, S., Ishimori, T., Yamamoto, H. & Hosono, R. 2001 Exposure of *Caenorhabditis elegans* to extremely low frequency high magnetic fields induces stress responses. *Bioelectromagnetics* **22**, 333–339.

Posfai, M., Buseck, P. R., Bazylinski, D. A. & Frankel, R. B. 1998*a* Reaction sequence of iron sulfide minerals in bacteria and their use as biomarkers. *Science* **280**, 880–883.

Posfai, M., Buseck, P. R., Bazylinski, D. A. & Frankel, R. B. 1998*b* Iron sulfides from magnetotactic bacteria: structure, composition, and phase transitions. *Am. Mineral* **83**, 1469–1481.

Sulston, J. E., Schierenberg, E., White, J. G. & Thomson, J. N. 1983 The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Devl Biol.* **100**, 64–119.

Taylor, A. P., Barry, J. C. & Webb, R. I. 2001 Structural and morphological anomalies in magnetosomes: possible biogenic origin for magnetite in ALH84001. *J. Microsc.* **201**, 84–106.

Walker, M. M., Diebel, C. E., Haugh, C. V., Pankhurst, P. M., Montgomery, J. C. & Green, C. R. 1997 Structure and function of the vertebrate magnetic sense. *Nature* **390**, 371–376.

Waltz, F. 2002 The Verwey transition—a topical review. *J. Phys. Condens. Matt.* **14**, 285–340.

Wiltschko, W. & Wiltschko, R. 2002 Magnetic compass orientation in birds and its physiological basis. *Naturwissenschaften* **89**, 445–452.