Biochemistry, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK
e-mail: sorahill@hgmp.mrc.ac.uk
†University Department of Medicine, City Hospital, Dudley Road, Birmingham B18 7QH, UK
‡MRC Human Nutrition Research, Fulbourn Road, Cambridge CB1 9NL, UK
Departments of §Medical Genetics and ||Paediatric Endocrinology, Alberta Children's Hospital, 18200 Richmond Road Southwest, Alberta, Calgary T2T 5C7, Canada

1. Zhang, Y. *et al. Nature* **372,** 425–432 (1994).
2. Montague, C. T. *et al. Nature* **387,** 903–908 (1997).
3. Farooqi, I. S. *et al. N. Engl. J. Med.* **341,** 879–884 (1999).
4. Havel, P. J. *Proc. Nutr. Soc.* **59,** 359–371 (2000).
5. Deurenberg, P., Weststrate, J. A. & Seidell, J. C. *Br. J. Nutr.* **65,** 105–114 (1991).
6. Chung, W. K. *et al. Am. J. Physiol.* **274,** 985–990 (1998).
7. Flier, J. S. *J. Clin. Endocrinol. Metab.* **83,** 1407–1413 (1998).
8. Heymsfield, S. *et al. J. Am. Med. Assoc.* **282,** 1568–1575 (1999).
9. Ravussin, E. *et al. Nature Med.* **3,** 238–240 (1997).

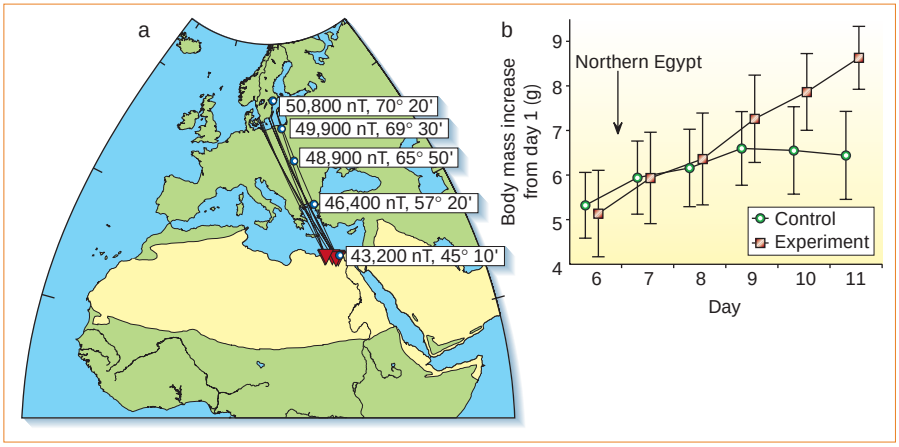

Figure 1 Effect of magnetic-field simulation on migratory refuelling in thrush nightingales (*Luscinia luscinia*). **a,** Map showing the points of autumn recovery of migrating birds originally ringed in Sweden ($n = 9$) and the stopover sites for which magnetic fields were simulated (total intensity (values in nanotesla) and inclination). **b,** Average body-mass increase (± s.e.) during the experiment (registered automatically using a Precisa Balance 310C). The field experienced by experimental birds was changed to that of four localities during the course of the experiment, calculated according to IGRF2000 (ref. 12). Experimental birds remained from day 7 in the magnetic field of northern Egypt until the end of the experiment at day 11, when all birds were released back into the wild. The magnetic system consisted of two independent series of four quadratic coils each, arranged orthogonally[13]. We verified the homogeneity of the magnetic field and set the vertical and horizontal components for the stopover sites using a Zeiss Jena theodolite with a fluxgate magnetometer (Bartington Instruments) and a proton magnetometer (GEM Systems). Further details are available from the authors.

Bird migration

Magnetic cues trigger extensive refuelling

Long stretches of sea and desert often interrupt the migration routes of small songbirds, whose fat reserves must be restored before these can be crossed as they provide no opportunity for refuelling. To investigate whether magnetic cues might enable inexperienced migratory birds to recognize a region where they need to replenish their body fat, we caught and held thrush nightingales (*Luscinia luscinia*) in Sweden just before their first migration and exposed them to a magnetic field simulating that at a migratory stopover in northern Egypt, before the Sahara Desert. We found that this magnetic field stimulated the birds to extend their fat-deposition period, indicating that magnetic cues may help small migratory birds to confront large ecological barriers.

Long-distance migration is common in birds, with some species migrating for more than 6 months a year[1]. Many rely on circannual rhythms, which are fine-tuned by photoperiod, for the timing of activities such as breeding, migration and moulting[2]. Most songbirds migrate alone in a series of nocturnal flights, using both celestial cues and information from the Earth's magnetic field to select and maintain migratory direction[3]. However, evidence of true magnetic navigation in birds remains circumstantial[4]. The loggerhead sea turtle (*Caretta caretta*) is sensitive to both the inclination angle and the intensity of the Earth's magnetic field[5] (sea-turtle hatchlings respond to different magnetic fields by swimming in directions that keep them within a specific region of the north Atlantic Ocean).

The main fuel used by migrating birds is fat, which is laid down at stopover sites en route[1]. As carrying large fat stores incurs increases in flight cost and predation risk[6,7], most bird species accumulate only small fat deposits (20–30% of lean body mass) and refuel at several successive stopover sites[6]. But to cross large ecological barriers such as the Gulf of Mexico or the Sahara Desert, fuel loading needs to be much greater — some birds have been found to double their mass[8] before crossing the Sahara, which involves flight distances of at least 1,500 km.

The extent and timing of refuelling has been assumed to be governed by the circannual rhythm[9]. But as there is a variation in the timing of migration onset in most populations (for example, because of variation in time of breeding), as well as in weather and feeding conditions during migration, a bird cannot gauge its latitudinal position from purely seasonal parameters. Other external cues may be necessary to indicate where large fuel loads need to be accumulated.

Autumn recoveries of thrush nightingales ringed in Sweden at the start of their migration showed that they congregate in northern Egypt[10] (Fig. 1a), presumably in preparation for crossing the Sahara Desert. To investigate whether the rate of fat deposition in thrush nightingales during this migratory stopover could be influenced by the magnetic field in northern Egypt, we randomly assigned first-year birds, caught at Tovetorp Zoological Research Station, Sweden, in August 2000, to either a magnetic treatment, in which the magnetic field was gradually changed to that of northern Egypt, or a control treatment, in which birds experienced the ambient magnetic field.

Birds subjected to the changing magnetic field had a larger increase in body mass than control birds as a result of extending their fuelling period once the magnetic field became the same as that in Egypt (Fig. 1b). Experimental birds increased in mass by 3.5 g ± 0.8 s.e. ($n = 8$) from days 6 to 11, whereas control birds increased in mass by 1.1 g ± 0.6 s.e. ($n = 8$) ($F_{4,48} = 4.40$, $P = 0.004$). No differences were observed between replications ($F_{1,12} = 0.93$, $P = 0.35$).

We have discovered a surprising external cue that helps to optimize this bird's chances of successful migration, and which works in concordance with orientation behaviour and endogenous rhythm[11] to provide precise information about geographical position when such information is crucial. We cannot yet say whether the fat-deposition response described here is an evolved response to the magnetic field in a specific area, or whether the birds are reacting to the latitudinal change in magnetic field.

Thord Fransson*†, Sven Jakobsson*, Patrik Johansson‡, Cecilia Kullberg*, Johan Lind*, Adrian Vallin*

**Department of Zoology, Stockholm University, SE-106 91 Stockholm, Sweden*
†Swedish Museum of Natural History, Bird Ringing Centre, SE-104 05 Stockholm, Sweden
e-mail: thord.fransson@nrm.se
‡Geological Survey of Sweden, SE-751 28 Uppsala, Sweden

1. Alerstam, T. *Bird Migration* (Cambridge Univ. Press, Cambridge, 1990).
2. Gwinner, E. *Ibis* **138,** 47–63 (1996).
3. Able, K. P. *Trends Ecol. Evol.* **8,** 367–371 (1993).
4. Berthold, P. *Control of Bird Migration* (Chapman & Hall, London, 1996).
5. Lohmann, K. J. & Lohmann, C. M. F. *Nature* **380,** 59–61 (1996).
6. Alerstam, T. & Lindström. Å. in *Bird Migration: The Physiology and Ecophysiology* (ed. Gwinner, E.) 331–351 (Springer, Berlin, 1990).
7. Kullberg, C., Fransson, T. & Jakobsson, S. *Proc. R. Soc. Lond. B* **263,** 619–624 (1996).
8. Fry, C. H., Ash, J. S. & Ferguson-Lees, I. J. *Ibis* **112,** 58–82 (1970).
9. Berthold, P. *Trends Ecol. Evol.* **6,** 254–257 (1991).

10. Swedish Museum of Natural History, Bird Ringing Centre. *Annual Reports of Swedish Bird Ringing 1960–98* (ISSN 0282-390X).
11. Thorup, K. & Rabøl, J. *J. Avian Biol.* **32,** 111–119 (2001).
12. International Association of Geomagnetism and Aeronomy. *Geophys. J. Int.* **141,** 259–262 (2000).
13. Lohmann, K. & Lohmann, C. M. F. *J. Exp. Biol.* **194,** 23–32 (1994).

COMMUNICATIONS ARISING

Tumour suppressors

Effect of DNA damage on a BRCA1 complex

The tumour-suppressor protein BRCA1 mediates its biological functions by interacting with cellular factors[1,2] such as the CtIP polypeptide[3,4], a substrate for the ATM (for 'ataxia telangiectasia mutated') protein kinase[5]. Li *et al.*[6] report that the BRCA1–CtIP interaction is disrupted by ionizing radiation and by other genotoxic stresses that induce phosphorylation of CtIP by ATM kinase, and that this dissociation of the BRCA1–CtIP complex in turn modulates the transcription of DNA-damage-response genes[6]. We have shown that the BRCA1-binding domain of CtIP (amino-acid residues 133–369) is distal to the sites that are phosphorylated by ATM kinase (residues S664 and S745)[7]. We now show that the BRCA1–CtIP complex is stable in irradiated cells, and that the phosphorylated isoforms of CtIP that are induced by ionizing radiation still interact *in vivo* with BRCA1. We conclude that disruption of the BRCA1–CtIP complex cannot account for induction of DNA-damage-response genes in the way proposed by Li *et al.*[6].

To investigate the effect of genotoxic stress on CtIP, we treated human T24 carcinoma cells with ionizing radiation or ultraviolet light, and immunoblotted the cell lysates with a CtIP-specific monoclonal antibody[7]. As expected, ionizing radiation induced the formation of the phosphorylated CtIP isoforms[6] (Fig. 1a, top, lane 3). These species migrate more slowly than CtIP polypeptides from untreated cells (lane 1) and are converted to a faster-migrating form after incubation with λ-phosphatase (lane 4). As expected, BRCA1 was also hyperphosphorylated in cells exposed to ionizing radiation (Fig. 1a, bottom)[8,9].

To determine the effect of ionizing radiation on the BRCA1–CtIP complex, we immunoprecipitated T24-cell lysates with a BRCA1-specific polyclonal antiserum, and monitored each precipitate for CtIP by immunoblotting with a CtIP-specific monoclonal antibody. As expected, CtIP was detected in BRCA1 immunoprecipitates from lysates of untreated and ultraviolet-irradiated cells (Fig. 1b, top , lanes 5, 9), which is consistent with our previous findings[7] but not with those of Li *et al.*[10].

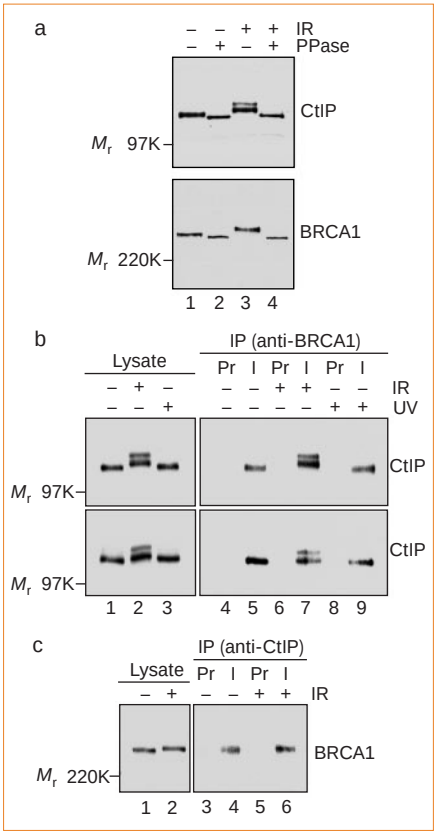

Figure 1 Association of BRCA1 and CtIP in irradiated cells. **a,** Induction of CtIP phosphorylation. Lysates from T24 carcinoma cells (lanes 1, 2, untreated; lanes 3, 4, treated with 40 Gy ionizing radiation (IR) were immunoblotted with CtIP-specific (top) or BRCA1-specific (bottom) monoclonal antibodies[7]; the indicated lysates were pretreated with λ-phosphatase (PPase). **b,** Co-immunoprecipitation of CtIP with BRCA1. T24 cells (top) and GM000637H fibroblasts (bottom) were irradiated with 40 Gy IR or 10 J m^{-2} ultraviolet (UV) light, and lysates were immunoblotted for CtIP (lanes 1–3). Alternatively, lysates were immunoprecipitated with BRCA1-specific antiserum[7] (I) or pre-immune serum (Pr) and then immunoblotted for CtIP (lanes 4–9). **c,** Co-immunoprecipitation of BRCA1 with CtIP. Lysates of HBL100 epithelial cells were either immunoblotted for BRCA1 (lanes 1, 2) or immunoprecipitated with CtIP-specific 210 antiserum[7] and then immunoblotted for BRCA1 (lanes 3–6).

Moreover, we recovered all CtIP species, including the hyperphosphorylated forms, in BRCA1 immunoprecipitates prepared from cells exposed to ionizing radiation (Fig. 1b, lane 7). The same results were obtained in all cell lines tested, including the two lines examined by Li *et al.*[6]: T24 carcinoma cells (Fig. 1b, top) and SV40-transformed GM000637H fibroblasts (Fig. 1b, bottom).

The stability of the CtIP–BRCA1 complex was also evident in reciprocal co-immunoprecipitation experiments, in which comparable amounts of BRCA1 polypeptides were present in CtIP immunoprecipitates from both untreated cells and cells exposed to ionizing radiation (Fig. 1c, lanes 4, 6). Our results indicate that, contrary to the findings of Li *et al.*, the CtIP–BRCA1 complex is stable to genotoxic stress such as ultraviolet or ionizing radiation.

Foon Wu-Baer, Richard Baer
Institute of Cancer Genetics and Department of Pathology, Columbia University College of Physicians and Surgeons, 1150 St Nicholas Avenue, New York, New York 10032, USA
e-mail: rb670@columbia.edu

1. Scully, R. & Livingston, D. M. *Nature* **408,** 429–432 (2000).
2. Deng, C.-X. & Brodie, S. G. *BioEssays* **22,** 728–737 (2000).
3. Yu, X., Wu, L. C., Bowcock, A. M., Aronheim, A. & Baer, R. *J. Biol. Chem.* **273,** 25388–25392 (1998).
4. Wong, A. K. *et al. Oncogene* **17,** 2279–2285 (1998).
5. Kim, S. T., Lim, D. S., Canman, C. E. & Kastan, M. B. *J. Biol. Chem.* **274,** 37538–37543 (1999).
6. Li, S. *et al. Nature* **406,** 210–215 (2000).
7. Yu, X. & Baer, R. *J. Biol. Chem.* **275,** 18541–18549 (2000).
8. Scully, R. *et al. Cell* **90,** 425–435 (1997).
9. Thomas, J. E., Smith, M., Tonkinson, J. L., Rubinfeld, B. & Polakis, P. *Cell Growth Differ.* **8,** 801–809 (1997).
10. Li, S. *et al. J. Biol. Chem.* **274,** 11334–11338 (1999).

Li et al. *reply* — Wu-Baer and Baer confirm our original observation that CtIP is phosphorylated in an ATM-dependent manner in response to γ-radiation. We have shown that phosphorylation by ATM kinase of CtIP at serine residues 664 and 745 is required to liberate DNA-damage-response genes such as *GADD45* from repression. This is consistent with our more recent finding that overexpression in mammalian cells of a phosphorylated CtIP mutant with a double alanine substitution at serines 664 and 745 disrupts the radiation-induced cell-cycle checkpoint between G_2 and M phases. The functional consequence of radiation-induced, ATM-dependent phosphorylation of CtIP is therefore clear.

With respect to the mechanism that underlies this process, we proposed that phosphorylation of CtIP leads to its dissociation from BRCA1, freeing BRCA1 to participate in the activation of DNA-damage-response genes. We do, however, appreciate the potential for experimental artefact in using only soluble co-immunoprecipitation to detect protein–protein interactions, particularly when different antibodies and cell lines are used. (We used human colon cancer cell line HCT 116 and human fibroblasts GM09607A and GM00637G, whereas Wu-Baer and Baer used human bladder carcinoma cell line T24 and fibroblast GM000637H.)

Wu-Baer and Baer report reciprocal co-immunoprecipitation of BRCA1 and CtIP using two different cell lines instead of the same cells. Their observations are inconsistent with ours where the interaction status of BRCA1 and CtIP after γ-irradiation is concerned. This discrepancy should eventually be resolved by systematic investigation using an alternative and complementary method.

Shang Li, Nicholas S.Y. Ting, Lei Zheng, Phang-Lang Chen, Wen-Hwa Lee
Department of Molecular Medicine, University of Texas Health Science Center at San Antonio, Institute of Biotechnology, San Antonio, Texas 78245-3207, USA
e-mail: leew@uthscsa.edu