PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The breast cancer susceptibility gene, *BRCA2*: at the crossroads between DNA replication and recombination?

Ashok R. Venkitaraman

Phil. Trans. R. Soc. Lond. B 2000 **355**, 191-198
doi: 10.1098/rstb.2000.0558

THE ROYAL SOCIETY

The breast cancer susceptibility gene, *BRCA2*: at the crossroads between DNA replication and recombination?

Ashok R. Venkitaraman

CRC Department of Oncology, University of Cambridge, and
The Wellcome Trust Centre for the Study of Molecular Mechanisms in Disease, The Cambridge Institute for Medical Research, Wellcome Trust—MRC Building, Hills Road, Cambridge CB2 2XY, UK

The identification and cloning of the familial breast cancer susceptibility gene, *BRCA2*, has excited much interest in its biological functions. Here, evidence is reviewed that the protein encoded by *BRCA2* has an essential role in DNA repair through its association with mRad51, a mammalian homologue of bacterial and yeast proteins involved in homologous recombination. A model is proposed that the critical requirement for BRCA2 in cell division and the maintenance of chromosome stability stems from its participation in recombinational processes essential for DNA replication.

Keywords: BRCA2; DNA replication; homologous recombination; cancer

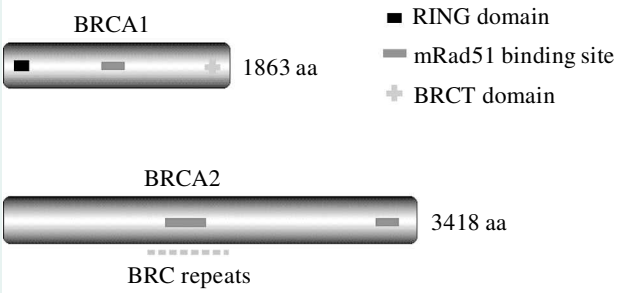
1. BREAST CANCER SUSCEPTIBILITY GENES AND CANCER PREDISPOSITION

nherited mutations in the *BRCA2* gene predispose humans o familial, early-onset breast cancer (Wooster *et al.* 1994, 995; Tavtigian *et al.* 1996). The gene was first identified by positional cloning approach through the analysis of amilies exhibiting an increased susceptibility. Moreover, ounder mutations in the *BRCA2* gene associated with ncreased cancer susceptibility have been identified in everal geographically or ethnically restricted human opulations (for example, Gudmundsson *et al.* 1996; ohannesdottir *et al.* 1996; Neuhausen *et al.* 1996; Thorlacius *et al.* 1996). In contrast, mutations in *BRCA2* do not appear o be a feature of sporadic breast cancer (reviewed in Lahman & Stratton 1998).

BRCA2 is not simply a breast cancer susceptibility gene. The spectrum of cancer predisposition associated with *BRCA2* mutations has not fully been characterized. It is already clear, for example, that *BRCA2* mutation carriers re also susceptible to familial ovarian cancer (Wooster *et al.* 1994; Tavtigian *et al.* 1996). Cancers of the prostate, ancreas and male breast may also be associated with *BRCA2* mutations (Thorlacius *et al.* 1996). The occurrence f thymic lymphomas in currently available mouse nodels for *BRCA2* deficiency (Connor *et al.* 1997; Friedman *et al.* 1998), to be discussed elsewhere in this aper, deserves mention. Although there does not appear o be an excess risk of lymphoma in human *BRCA2* muta- ion carriers, the incidence of *BRCA2* mutations in poradic cases of lymphoma remains to be evaluated.

Despite the wide scientific and public interest engen- ered by the cloning of *BRCA2*, little information has een gleaned merely by analysis of the gene's sequence. It ncodes a large protein of 3418 amino acids in humans, which bears no significant resemblance to molecules of known function. The only remarkable feature is the presence of a cluster of eight repeated sequences, the so-called BRC repeats (Bork *et al.* 1996), located within *BRCA2* exon 11 (figure 1). The sequence of the BRC repeats is highly conserved between several species of mammals, although the intervening sequences are not (Bignell *et al.* 1997). Viewed in the light of the generally limited (*ca.* 60%) homology between murine and human BRCA2 proteins (Sharan & Bradley 1997), this suggests that the BRC repeats may have a conserved and essential role in the function of BRCA2. It may be significant that the *BRCA2* mutations associated with familial ovarian cancer tend to cluster within the BRC repeat region encoded by exon 11 (Gayther *et al.* 1997). It must be noted, however, that these are truncating mutations, making it difficult to explain why truncations positioned more 5′ in the gene, which also result in the loss of BRC repeats, are not associated with a similar phenotype.

Positional cloning has also identified another breast cancer susceptibility gene, *BRCA1*, which in humans encodes a protein of 1863 amino acids (Miki *et al.* 1994). It is important to emphasize that despite the similarity in their acronyms, the molecules themselves are highly distinct in sequence (figure 1). There is much circumstantial evidence, however, to suggest some commonality in their functions. Mutations in either gene confer a marked increase in breast cancer risk in humans. Targeted truncations in the murine homologues of *BRCA1* (Gowen *et al.* 1996; Hakem *et al.* 1996; Liu *et al.* 1996; Ludwig *et al.* 1997) or *BRCA2* (Ludwig *et al.* 1997; Sharan *et al.* 1997; Suzuki *et al.* 1997) result in either early embryonic lethality at day 7–9 or, if positioned more carboxyl-terminally in *BRCA2* (Connor *et al.* 1997; Friedman *et al.* 1998), severe embryonal growth retardation, perinatal lethality and the development of thymic tumours in surviving adults. Both genes encode nuclear proteins that are highly expressed during



igure 1. Shows a schematic representation of the human RCA1 and BRCA2 proteins. Known structural features are arked, but are not drawn to scale. The RING and BRCT omains in BRCA1 are putative protein–protein interaction otifs. The Rad51 binding sites in both proteins, and the ructural motifs found in BRCA2, are discussed in §§ 1 and 3.

he S phase of the cell cycle (Rajan *et al.* 1996; Bertwistle *et l.* 1997; Jin *et al.* 1997). Finally, there is evidence that RCA1 and BRCA2 proteins co-localize to subnuclear ructures in mitotic and meiotic cells, and may even hysically associate at low stoichiometry (Chen *et al.* 998*c*).

For both *BRCA1* and *BRCA2*, inheritance of a single efective allele is sufficient to confer cancer predisposition, but tumours isolated from mutation carriers almost lways exhibit a loss of heterozygosity (Collins *et al.* 1995; udmundsson *et al.* 1995). By contrast, there are no eports of tumour predisposition in mice heterozygous for argeted mutations in *BRCA1* or *BRCA2*. No satisfactory xplanation is yet available for this species-specific difference in the phenotype of *BRCA2* heterozygotes.

2. *BRCA2* AND DNA REPAIR

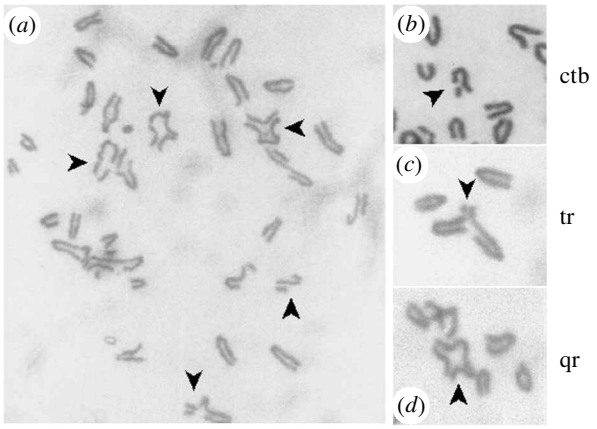
There is little in the nucleotide sequence of *BRCA2* that suggestive of its biological function. The first insights ave therefore emerged from studies of mice harbouring argeted mutations in the murine homologue of the *BRCA2* gene (hereafter termed *Brca2* in accordance with he accepted nomenclature). An essential role in embryonal cellular proliferation and as a consequence, in ntrauterine viability, has been inferred from the marked rowth retardation and early embryonal lethality pparent in animals homozygous for truncations in *Brca2* xon 10 (Ludwig *et al.* 1997; Sharan *et al.* 1997; Suzuki *et l.* 1997). Perhaps more amenable to further study, a role or *Brca2* in the cellular response to DNA damage has een postulated from the observation that it associates ith mammalian (m)Rad51 (Chen *et al.* 1998*b*; Mizuta *et l.* 1997; Sharan *et al.* 1997; Wong *et al.* 1997), a homologue f the bacterial protein RecA, which is known to function n the repair of DNA double-strand breaks (DSBs) by enetic recombination. Consistent with this notion, urine blastocysts homozygous for a *Brca2* exon 10 truncation exhibit X-ray sensitivity (Sharan *et al.* 1997), as do broblasts harbouring a truncation in the 3′ region of xon 11 (Connor *et al.* 1997).

Although these observations hint at a role for Brca2 in NA repair, they admit of several explanations. The ellular response to DNA damage (reviewed in Friedberg *et al.* 1995) involves the activation of cell-cycle checkpoints to prevent the replication of damaged DNA templates, the recruitment of the machinery for DNA repair and sometimes, the induction of apoptosis in the face of overwhelming, irreparable genetic damage. Thus, an increased sensitivity to X-rays is only indicative of a potential dysfunction in any of the cellular responses to DNA damage, and not necessarily of defective DNA repair *per se*.

It is therefore important that checkpoint activation and apoptosis appear to be largely intact in fibroblasts and lymphocytes isolated from mice harbouring a targeted truncation at residue 1492 in *Brca2* exon 11 (termed the *Brca2*^{Tr} allele) (Patel *et al.* 1998). Brca2$^{Tr/Tr}$ cells exhibit arrest in the G1 and G2/M phases of the cell cycle following exposure to X-rays or UV light, and abrogate DNA synthesis after treatment with hydroxyurea. Moreover, even small doses of X-rays can induce the apoptotic death of Brca2$^{Tr/Tr}$ cells just as efficiently as in wild-type cells. Thus, these observations indicate that Brca2 has an essential function in some aspect of the DNA damage response that is distinct from participation in checkpoint enforcement or apoptosis. Since the Brca2^{Tr} allele leaves the exons encoding residues 1–1492 intact, a role for these amino-terminal domains in checkpoints or apoptosis is not, of course, excluded by these findings (Patel *et al.* 1998). It is noteworthy in this context that a *Brca1* exon 11 in-frame deletion gives rise to abnormalities in the G2/M checkpoints (Xu *et al.* 1999), although the significance of this observation to Brca2 function remains to be ascertained.

The spectrum of genotoxin sensitivity exhibited by Brca2$^{Tr/Tr}$ cells is consistent with a defect in DNA repair by homologous recombination (Patel *et al.* 1998). Like Brca2$^{Tr/Tr}$ cells, yeast mutants in the *RAD52* epistasis group of genes (including *RAD51*, *RAD52*, *RAD55*, *RAD57*, *RAD59*, *MRE11* and *Xrs2*) involved in the repair of DSBs by homologous recombination generally exhibit cross-sensitivity to X-rays, UV light and monofunctional alkylating agents. Similar sensitivities are observed in vertebrate cells deficient in *Rad54* (Bezzubova *et al.* 1997; Essers *et al.* 1997), as well as in *Rad51*-related genes (Jones *et al.* 1987; Lim & Hasty 1996; Tsuzuki *et al.* 1996; Liu *et al.* 1998; Sonoda *et al.* 1998).

Strikingly, Brca2$^{Tr/Tr}$ cells spontaneously accumulate numerous chromosomal aberrations (Patel *et al.* 1998) during passage in culture (figure 2). The aberrations include chromatid and chromosome breaks, and the formation of structures termed tri-radial and quadri-radial chromosomes previously noted in the human genetic disease Bloom syndrome (German 1993). These latter abnormalities (figure 2) reflect defective chromatid exchange during homologous recombination in mitotic cells, with tri-radials (Y-shaped chromosome structures with three arms) indicative of incomplete separation following isochromatid exchange and quadri-radials (star-shaped structures with four arms) arising from a flawed exchange between chromosomes. More recently, similar abnormalities have been described in *Brca1* exon-11-deficient murine cells (Xu *et al.* 1999). These observations demonstrate that Brcal deficiency or Brca2 deficiency in mouse models may give rise to a phenotype reminiscent of other human diseases (table 1) in which chromosomal instability is associated with cancer



igure 2. Chromosomal abnormalities that spontaneously ccumulate in cultures of murine fibroblasts homozygous or a targeted truncation in the *Brca2* gene. Note the high requency of the aberrations, and the occurrence of (*a*) chromatid (ctb) and chromosome breaks, (*b*) tri-radial tr), and (*c*) quadri-radial (qr) chromosomes. Reproduced om Patel *et al*. 1998, with the permission of Cell Press.

redisposition. More specifically, they suggest that some eatures of this phenotype may arise from a defect in the bility to correctly execute or regulate DNA repair by omologous recombination.

3.DNA REPAIR BY HOMOLOGOUS RECOMBINATION

The mechanism for homologous recombination is most learly understood in *Escherichia coli*. The bacterial RecA rotein plays a central role in the process (reviewed in Loca & Cox 1997). It recognizes and aligns homologous egions in the two DNA molecules that are to undergo ecombination, and mediates the strand exchange reac- ion, which generates a crossover between them. A umber of additional proteins are necessary to initiate, egulate and complete the reactions catalysed by RecA. The RecBCD protein complex, which includes DNA heli- ase, ATPase, exo- and endonuclease activities, acts at SBs to generate a stretch of single-strand DNA that erves as a substrate for homologous strand pairing by RecA. The single-strand DNA substrate is coated by RecA polymers to form a nucleoprotein filament. Fila- ent assembly is regulated by the RecFOR proteins and he single-strand binding protein SSB. Finally, the RuvA- proteins act to resolve the recombination intermediates ormed by the action of RecA through effects on branch igration, and on Holliday junction resolution by endo- ucleolytic cleavage.

Homologous recombination in *E. coli* has classically een studied as a mechanism for the generation of diver- ty during conjugation, a process for which the RecA athway is essential. There is much evidence, however, hat the pathway also plays an important role in DNA epair, and may in particular be an effective response to NA strand gaps or DSBs (Meselson & Radding 1975; Vest *et al*. 1981; Szostak *et al*. 1983). This is certainly true n yeast, where homologous recombination is the major athway for the repair of DSBs. It has been asserted that, n contrast, mammalian cells repair DSBs primarily by on-homology-dependent mechanisms such as non-homologous end joining (NHEJ) (reviewed in Friedberg *et al*. 1995; Jackson & Jeggo 1995). The evidence to support this assertion is largely indirect, coming from studies in which integration, as opposed to homologous recombination, of transfected DNA predominates in mammalian cells. More recent work, however, challenges the notion that homologous recombination is a minor or unimportant pathway for DSB repair in mammalian cells. DSBs experimentally induced into mammalian chromosomes by the activity of rare-cutting endonucleases enhance homologous recombination by two to three orders of magnitude (Rouet *et al*. 1994). As many as 30–50% of DSBs introduced in this way at direct repeat sequences are resolved by homology-directed repair mechanisms (Liang *et al*. 1998). Indeed, although rodent cells deficient in the major NHEJ pathway dependent on the *xrcc4–7* genes are highly sensitive to ionizing radiation during G1, they are relatively resistant late in the S phase or in G2, when homologous recombination can occur between replicated DNA strands (Stamato *et al*. 1988; Whitmore *et al*. 1989; Cheong *et al*. 1994). A similar phenotype is apparent in Ku70-deficient or RAD54-deficient avian cells, defective in NHEJ or recombination mechanisms, respectively (Takata *et al*. 1998). Collectively, these observations suggest that homologous recombination may be of particular importance for DNA repair during DNA replication in mammalian cells.

Table 1. *Genetic diseases where aberrations in chromosome structure are associated with cancer predisposition*

Bloom syndrome	*Brca1* deficiency (mouse model)
Fanconi anaemia	
Ataxia–telangiectasia	*Brca2* deficiency (mouse model)
Nijmegen breakage syndrome	

It should be emphasized that, quite apart from its potential role in DNA replication and/or repair in mitotic cells, homologous recombination initiated at DSBs is essential for the normal completion of meiosis (Haber 1997; Keeney *et al*. 1997). It is therefore provocative that BRCA2 (as well as BRCA1 and mRad51) co-localize to the synaptonemal complexes formed at meiotic chromosomes (Chen *et al*. 1998*c*). Indeed, the reproductive sterility and germ cell abnormalities observed in *Brca2*-deficient mice (Connor *et al*. 1997; Friedman *et al*. 1998) are consistent with defective meiosis, although this has yet to be firmly established.

In eukaryotes, Rad51 plays a central role in homologous recombination analogous to that of RecA. RAD51 was first identified in yeast (Shinohara *et al*. 1992*b*), as was its meiosis-specific homologue; Dmc1 (Bishop *et al*. 1992). Mammalian homologues of both proteins exist. Interestingly, however, at least five further mRad51-like molecules are also present in vertebrate cells, including the products encoded by the *xrcc2* and *xrcc3* genes isolated by complementation of X-ray-sensitive CHO cell mutants (Cartwright *et al*. 1998; Liu *et al*. 1998), as well as *Rad51B*, *Rad51C* and *Rad51D* (Albala *et al*. 1997; Dosanjh *et al*. 1998; Pittman *et al*. 1998). The reason for the multiplicity of mRad51-like molecules in vertebrates is uncertain. Yet there appears to be little redundancy for those functions of mRad51 that are essential for cell division and viability,

nce its targeted disruption is lethal both to murine and vian cells (Lim & Hasty 1996; Tsuzuki *et al*. 1996; Sonoda *et al*. 1998). In contrast, mutations in yeast *RAD51* ngender defective homologous recombination and eiosis, but do not cause lethality (Shinohara *et al*. 1992*a*).

Despite this important phenotypic difference, the avail-ble biochemical evidence suggests a great similarity etween the function of yeast and mammalian Rad51 olecules in homologous recombination. Both proteins orm helical filaments on DNA in a manner similar to acterial RecA (Ogawa *et al*. 1993; Sung & Robberson 995). In concert with Rad52 (Sung 1997; Benson *et al*. 998; New *et al*. 1998; Shinohara *et al*. 1998), they mediate omologous pairing between DNA strands (Sung 1994; ung & Robberson 1995; Baumann *et al*. 1997) to romote a strand exchange reaction. The single-strand NA binding protein, RPA, considerably enhances this eaction, which is associated with (Sung 1994; Baumann *et al*. 1997; Gupta *et al*. 1997), but may not depend on Sung & Stratton 1996), a DNA-dependent ATPase ctivity intrinsic to yeast and mammalian Rad51.

Differences between RecA and eukaryotic Rad51 are lso considerable. The strand exchange reaction promoted y the eukaryotic enzymes is far weaker than that ediated by RecA (Sung 1994; Baumann *et al*. 1997). Moreover, their ATPase activity is some 100-fold less than hat of RecA (Gupta *et al*. 1997). These biochemical easurements in *in vitro* experimental systems hint at the ecessity for additional eukaryotic proteins to modulate he activity of Rad51 and to regulate—in a manner not equired in *E. coli*—the *in vivo* progress and extent of the ecombination reaction. It is in this context that the hysical association of mRad51 with tumour suppressor roteins such as p53 (Sturzbecher *et al*. 1996), BRCA1 Scully *et al*. 1997) and BRCA2 (Chen *et al*. 1998*b*; Mizuta *et al*. 1997; Sharan *et al*. 1997; Wong *et al*. 1997) ay be of considerable importance to its essential role in ell division and viability.

Given the central role established for mRad51 in homolo-ous recombination, it is striking that several lines of ircumstantial evidence indicate a shared function with rca2. The genes encoding these proteins show a similar xpression pattern in mammalian cells, with low levels xpressed in many tissues and with particularly high levels ound in the thymus and testes. Expression is restricted, as n the case of *BRCA1*, primarily to the S phase of the cell ycle. As mentioned earlier, mRad51 and Brca2 are hysically associated at relatively high stoichiometry. Co-ocalization can be demonstrated to nuclear foci in mitotic ells, and to synaptonemal complexes in meiotic cells. inally, like $Brca2^{Tr/Tr}$ cells, Rad51-deficient vertebrate cells pontaneously accumulate chromosomal abnormalities and re unable to maintain continued division in culture.

4. *BRCA2*: AT THE CROSSROADS BETWEEN DNA REPLICATION AND RECOMBINATION?

What are the common biological functions that may nderlie the essential requirement for Rad51 and Brca2 n cell division and chromosome stability? Paradigms rom work on *E. coli* suggest that they may be related to he necessity for RecA-catalysed strand exchanges to nable the error-free resumption of DNA replication when replication fork progression is stalled (reviewed in West *et al*. 1981; Kuzminov 1995; Kogoma 1996, 1997; Cox 1997). Several lesions encountered by a replication fork could lead to its collapse, including strand gaps, base adducts causing template distortion, or DSBs. In these situations, replication restart in *E. coli* is dependent on the use of recombinational mechanisms to bypass the lesion and enable origin-independent reinitiation of the replication fork from recombination intermediates (for example, Kogoma 1996; Liu *et al*. 1999). Failure to carry out replication restart would result not only in defective DNA synthesis and cell proliferation, but could also provoke the occurrence of discontinuities in replicated chromosomes.

An important element in this scheme is the notion that replication forks are frequently stalled during normal cell division. Conditions that precipitate stalling could conceivably arise in many different ways. These may include base lesions created by DNA modification (reviewed in Lindahl 1993, 1996) through hydrolysis, oxidation and other reactions, UV-induced strand alterations, base mismatches caused by replication errors, or simply by replication fork encounter with DNA-bound proteins. No direct measurement of the extent of replication stalling during normal growth is available. In *E. coli* RecBCD or RecARecD mutants, stalling of replication forks results in the generation of DSBs (Michel *et al*. 1997; Seigneur *et al*. 1998). It is provocative that in these strains, DSBs spontaneously accumulate to high levels during normal growth indicative of the considerable frequency of replication fork stalling which must occur during DNA replication. Moreover, DSB accumulation is even further elevated in this background when DNA replication is impeded by disruption of replicative helicases (Michel *et al*. 1997), emphasizing the role of homologous recombination in the repair of DNA damage induced by defective replication.

To what extent can the paradigms developed in work on bacteria be extended to eukaryotes? There is limited but intriguing evidence that homologous recombination is stimulated by, and necessary for, eukaryotic DNA replication. Recombination intermediates representing unresolved Holliday junctions accumulate spontaneously during the S phase of the cell cycle in synchronously dividing yeast cells in the absence of exogenously induced DNA damage, suggesting that recombination operates to repair replication-associated lesions (Zou & Rothstein 1997). Indeed, mutations affecting components of the replicative machinery increase the level of recombination intermediates detected in this system. This may provide some mechanistic substance to the oft-noted observation that in mammalian cells, mutations in DNA polymerases, ligases or helicases such as the RecQ homologue altered in Bloom syndrome, greatly stimulate genetic exchange between sister chromatids as detected by differential BrdU labelling of newly replicated DNA strands (reviewed in Rothstein & Gangloff 1995).

In the model proposed here, in which recombinational processes dependent on Rad51 and Brca2 are required for normal DNA replication, the proliferative impediment apparent in Rad51-deficient avian cells and in $Brca2^{Tr/Tr}$ mouse fibroblasts is of particular interest. Rad51 deletion results in a gradual loss of replicative capacity (Sonoda *et al*. 1998), with progressive accumulation in the G2/M phases of the cell cycle consistent with arrest at the check-

oints that monitor the completeness of DNA replication. rca2$^{Tr/Tr}$ fibroblasts also display a progressive impedi- ent to their capacity to undergo cell division (Patel *et al*. 998). When freshly isolated from murine embryos, they re roughly similar in their proliferative competence to ild-type or heterozygous control cells. A severe prolif- rative defect becomes apparent, and progressively orsens, the more often the cells are passed in culture, ccompanied by increasing G1 and G2/M phase arrest. hese phenotypes of Rad51-deficient and Brca2$^{Tr/Tr}$ cells re reminiscent of RecF pathway mutants of *E. coli*, which lso exhibit an inability to sustain ongoing rounds of NA replication even in the absence of exogenously nduced DNA damage (Courcelle *et al*. 1997).

Studies of co-localization also lend support to the idea at mRad51 and BRCA2 participate in repair processes ssociated with DNA replication. Nuclear foci containing Rad51, BRCA1 and BRCA2 are formed during late S nd G2 (Chen *et al*. 1998*c*). Following exposure to hydro- yurea, which depletes nucleotide pools required for NA replication, BRCA2-containing foci costain with CNA (Chen *et al*. 1998), suggesting localization at repli- ation foci. These observations are consistent with a odel in which the activity of BRCA2 and Rad51 is ecessary to overcome the replication fork stalling at rand gaps induced by hydroxyurea. It is currently nclear if BRCA2 or Rad51 interact directly with compo- ents of the eukaryotic DNA replication machinery or ith the accessory molecules involved in regulating the pigenetic effects associated with DNA replication. This is prediction of the model proposed here, and is likely to e a worthwhile focus for further analysis.

The model proposed here for the function of BRCA2–Rad51 in eukaryotic DNA replication has important mplications for the role of BRCA2 mutations in cancer redisposition. Defects in components of the replication achinery have clearly been associated with a mutator henotype in eukaryotes (Chen *et al*. 1998*a*), suggesting at BRCA2-deficient cells may also sustain elevated utation rates even without exogenously induced DNA amage. The replication defect and chromosomal nstability observed in Brca2-deficient cells at first glance eem at odds with the unrestrained proliferation ssociated with cancer. It can therefore be predicted that econdary mutations—incurred at a high frequency ecause of the mutator phenotype—must be selected uring the process of transformation to yield cancer cells, hich have in some way ameliorated or compensated for ny underlying replication and/or repair defect (Lee *et al*. 999). If this model is correct, the identification and nalysis of genes that consistently undergo secondary utation in BRCA2-deficient tumour cells are likely to rovide valuable biological insights into the mechanism of eplication-associated recombination in mammalian cells.

Over-reliance on analogies to the bacterial RecA athway in interpreting or predicting the manner in which omologous recombination and replication may interact n mammalian cells is, of course, to be avoided. For a start, here are many significant differences between the activ- ies of the enzymes involved. As discussed previously, Rad51 is far less proficient at promoting strand xchange, exhibits poor ATPase activity and appears to ork with the opposite strand polarity, when compared to RecA. Eukaryotic DNA replication undoubtedly occurs in a more complicated molecular and cellular milieu than in prokaryotes, necessitating the action of additional molecules for which there may be no counterparts in the simpler system. For similar reasons, analogies to yeast may also be of limited value. Homologues to p53, BRCA1 or BRCA2—all known to interact with mRad51—do not appear to exist in yeast. Mammalian cells contain a multiplicity of Rad51-like proteins. Yeast *RAD52*, but not *RAD51*, *RAD55* or *RAD57*, appears to be essential for the formation of recombination intermediates associated with replication (Zou & Rothstein 1997). Many of these discrepancies will ultimately be resolved by biochemical studies to elucidate the activities of the mammalian enzymes involved in homologous recombination and to define their inter-molecular interactions, an effort now underway in several laboratories. Despite these limitations to a model for mRad51/Brca2 activity based on bacterial parallels, its formulation does provide a useful framework for further work in which informative predictions can be made for experimental analysis.

While this review is focused on the functions of BRCA2 in DNA repair in relation to its interaction with mRad51, it bears reiteration that this exceptionally large nuclear protein is very likely to have multiple functions within a cell that may or may not be relevant in this context. For example, functions in transcription activation (Chapman & Verma 1996; Fuks *et al*, 1998) and transcription-coupled DNA repair (Gowen *et al*. 1998) have been ascribed to both BRCA1 and BRCA2. The relationship of these putative roles to the phenotype of Brca2-deficient murine cells is uncertain, and their potential contribution to chromosome instability and cancer predisposition remains to be fully explored.

Finally, it remains unclear to what extent the study of BRCA2 will enlarge our understanding of breast cancer pathogenesis in general. Somatic BRCA2 mutations do not occur in non-familial breast cancers, which account for over 90% of incidence, undermining (but not entirely excluding) the conjecture that a common pathway involving the molecule will be dysfunctional in sporadic as well as familial tumours. Moreover, BRCA2 is widely expressed and appears to have important functions in cellular processes such as DNA repair and transcription apparently fundamental to all tissues. Why should BRCA2 mutations therefore result in predisposition to breast and ovarian cancer in particular? These are important gaps in our current understanding (Venkitaraman 1999), which cannot easily be resolved without invoking additional—and perhaps tissue-specific—functions for BRCA2 which remain to be identified.

I am indebted to current members of my laboratory for many helpful and stimulating discussions. Our research is supported by the Cancer Research Campaign, the Medical Research Council and the Leukaemia Research Fund. The generosity of the late Dr F. A. Zöellner in endowing the Ursula Zöellner Professorship of Cancer Research is gratefully acknowledged.

REFERENCES

Albala, J. S., Thelen, M. P., Prange, C., Fan, W., Christensen, M., Thompson, L. H. & Lennon, G. G. 1997 Identification of a novel human RAD51 homolog, RAD51B. *Genomics* **46**, 476–479.

aumann, P., Benson, F. E. & West, S. C. 1997 Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. *Cell* **87**, 757–766.

enson, F. E., Baumann, P. & West, S. C. 1998 Synergistic actions of Rad51 and Rad52 in recombination and DNA repair [see comments]. *Nature* **391**, 401–404.

ertwistle, D., Swift, S., Marston, N. J., Jackson, L. E., Crossland, S., Crompton, M. R., Marshall, C. J. & Ashworth, A. 1997 Nuclear location and cell cycle regulation of the BRCA2 protein. *Cancer Res.* **57**, 5485–5488.

ezzubova, O., Silbergleit, A., Yamaguchi-Iwai, Y., Takeda, S. & Buerstedde, J. M. 1997 Reduced X-ray resistance and homologous recombination frequencies in a RAD54-/- mutant of the chicken DT40 cell line. *Cell* **89**, 185–193.

ignell, G., Micklem, G., Stratton, M. R., Ashworth, A. & Wooster, R. 1997 The BRC repeats are conserved in mammalian BRCA2 proteins. *Hum. Mol. Genet.* **6**, 53–58.

ishop, D. K., Park, D., Xu, L. & Kleckner, N. 1992 DMC1: a meiosis-specific yeast homolog of *E. coli* recA required for recombination, synaptonemal complex formation, and cell cycle progression. *Cell* **69**, 439–456.

ork, P., Blomberg, N. & Nilges, M. 1996 Internal repeats in the BRCA2 protein sequence [letter]. *Nature Genet.* **13**, 22–23.

artwright, R., Tambini, C. E., Simpson, P. J. & Thacker, J. 1998 The XRCC2 DNA repair gene from human and mouse encodes a novel member of the recA/RAD51 family. *Nucl. Acids Res.* **26**, 3084–3089.

hapman, M. S. & Verma, I. M. 1996 Transcriptional activation by BRCA1 [letter; comment]. *Nature* **382**, 678–679.

hen, C., Umezu, K. & Kolodner, R. D. 1998*a* Chromosomal rearrangements occur in *S. cerevisiae* rfa1 mutator mutants due to mutagenic lesions processed by double-strand-break repair. *Mol. Cell* **2**, 9–22.

hen, P. L., Chen, C. F., Chen, Y., Xiao, J., Sharp, Z. D. & Lee, W. H. 1998*b* The BRC repeats in BRCA2 are critical for RAD51 binding and resistance to methyl methanesulfonate treatment. *Proc. Natl Acad. Sci. USA* **95**, 5287–5292.

hen, J. (and 10 others) 1998*c* Stable interaction between the products of the BRCA1 and BRCA2 tumour suppressor genes in mitotic and meiotic cells. *Mol. Cell* **2**, 317–328.

heong, N., Wang, X., Wang, Y. & Iliakis, G. 1994 Loss of S-phase dependent radioresistance in irs-1 cells exposed to cross-linking agents. *Mutat. Res.* **314**, 77–85.

ollins, N., McManus, R., Wooster, R., Mangion, J., Seal, S., Lakhani, S., Ormiston, W. & Daly, P. A. 1995 Consistent loss of the wild-type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12–13. *Oncogene* **10**, 1673–1675.

onnor, F., Bertwistle, D., Mee, P. J., Ross, G. M., Swift, S., Grigorieva, E., Tybulewicz, V. L. & Ashworth, A. 1997 Tumorigenesis and a DNA repair defect in mice with a truncating Brca2 mutation. *Nature Genet.* **17**, 423–430.

ourcelle, J., Carswell-Crumpton, C. & Hanawalt, P. C. 1997 recF and recR are required for the resumption of replication at DNA replication forks in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **94**, 3714–3719.

ox, M. M. 1997 Recombinational crossroads: eukaryotic enzymes and the limits of bacterial precedents. *Proc. Natl Acad. Sci. USA* **94**, 11 764–11 766.

osanjh, M. K., Collins, D. W., Fan, W., Lennon, G. G., Albala, J. S., Shen, Z. & Schild, D. 1998 Isolation and characterization of RAD51C, a new human member of the RAD51 family of related genes. *Nucl. Acids Res.* **26**, 1179–1184.

ssers, J., Hendriks, R. W., Swagemakers, S. M., Troelstra, C., De Wit, J., Bootsma, D., Hoeijmakers, J. H. & Kanaar, R. 1997 Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination. *Cell* **89**, 195–204.

Friedberg, E. C., Walker, G. C. & Siede, W. 1995 *DNA repair and mutagenesis*. Washington, DC: ASM Press.

Friedman, L. S. (and 11 others) 1998 Thymic lymphomas in mice with a truncating mutation in Brca2. *Cancer Res.* **58**, 1338–1343.

Fuks, F., Milner, J. & Kouzarides, T. 1998 BRCA2 associates with acetyltransferase activity when bound to P/CAF. *Oncogene* **17**, 2531–2534.

Gayther, S. A., Mangion, J., Russell, P., Seal, S., Barfoot, R., Ponder, B. A., Stratton, M. R. & Easton, D. 1997 Variation of risks of breast and ovarian cancer associated with different germline mutations of the BRCA2 gene. *Nature Genet.* **15**, 103–105.

German, J. 1993 Bloom syndrome: a Mendelian prototype of somatic mutational disease. *Medicine* **72**, 393–406.

Gowen, L. C., Johnson, B. L., Latour, A. M., Sulik, K. K. & Koller, B. H. 1996 Brca1 deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. *Nature Genet.* **12**, 191–194.

Gowen, L. C., Avrutskaya, A. V., Latour, A. M., Koller, B. H. & Leadon, S. A. 1998 BRCA1 required for transcription-coupled repair of oxidative DNA damage. *Science* **281**, 1009–1012.

Gudmundsson, J., Johannesdottir, G., Bergthorsson, J., Arason, A., Ingvarsson, S., Egilsson, V. & Barkardottir, R. 1995 Different tumour types from BRCA2 carriers show wild-type chromosome deletions on 13q12q13. *Cancer Res.* **55**, 4830–4832.

Gudmundsson, J., Johannesdottir, G., Arason, A., Bergthorsson, J. T., Ingvarsson, S., Egilsson, V. & Barkardottir, R. B. 1996 Frequent occurrence of BRCA2 linkage in Icelandic breast cancer families and segregation of a common BRCA2 haplotype. *Am. J. Hum. Genet.* **58**, 749–756.

Gupta, R. C., Bazemore, L. R., Golub, E. I. & Radding, C. M. 1997 Activities of human recombination protein Rad51. *Proc. Natl Acad. Sci. USA* **94**, 463–468.

Haber, J. E. 1997 A super new twist on the initiation of meiotic recombination. *Cell* **89**, 163–166.

Hakem, R. (and 14 others) 1996 The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. *Cell* **85**, 1009–1023.

Jackson, S. P. & Jeggo, P. A. 1995 DNA double-strand break repair and V(D)J recombination: involvement of DNA-PK. *Trends Biochem. Sci.* **20**, 412–415.

Jin, Y., Xu, X. L., Yang, M. C., Wei, F., Ayi, T. C., Bowcock, A. M. & Baer, R. 1997 Cell cycle-dependent colocalization of BARD1 and BRCA1 proteins in discrete nuclear domains. *Proc. Natl Acad. Sci. USA* **94**, 12 075–12 080.

Johannesdottir, G. (and 11 others) 1996 High prevalence of the 999del5 mutation in icelandic breast and ovarian cancer patients. *Cancer Res.* **56**, 3663–3665.

Jones, N. J., Cox, R. & Thacker, J. 1987 Isolation and cross-sensitivity of X-ray-sensitive mutants of V79–4 hamster cells. *Mutat. Res.* **183**, 279–286.

Keeney, S., Giroux, C. N. & Kleckner, N. 1997 Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* **88**, 375–384.

Kogoma, T. 1996 Recombination by replication. *Cell* **85**, 625–627.

Kogoma, T. 1997 Stable DNA replication: an interplay between DNA replication, homologous recombination and transcription. *Microbiol. Mol. Biol. Rev.* **61**, 212–238.

Kuzminov, A. 1995 Collapse and repair of replication forks in *Escherichia coli*. *Mol. Microbiol.* **16**, 373–384.

Lee, H., Trainer, A. H., Thistlethwaite, F. C., Evans, M. J., Friedman, L. S., Ponder, B. A. J. & Venkitaraman, A. R. 1999 Mitotic checkpoint inactivation fosters the transformation of cells lacking the breast cancer susceptibility gene BRCA2. *Mol. Cell.* **4**, 1–10.

iang, F., Han, M., Romanienko, P. J. & Jasin, M. 1998 Homology-directed repair is a major double-strand break repair pathway in mammalian cells. *Proc. Natl Acad. Sci. USA* **95**, 5172–5177.

im, D. S. & Hasty, P. 1996 A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. *Mol. Cell. Biol.* **16**, 7133–7143.

indahl, T. 1993 Instability and decay of the primary structure of DNA [see comments]. *Nature* **362**, 709–715.

indahl, T. 1996 The Croonian Lecture, 1996. Endogenous damage to DNA. *Phil. Trans. R. Soc. Lond.* B **351**, 1529–1538.

iu, C. Y., Flesken-Nikitin, A., Li, S., Zeng, Y. & Lee, W. H. 1996 Inactivation of the mouse Brca1 gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. *Genes Dev.* **10**, 1835–1843.

iu, J., Xu, L., Sandler, S. J. & Marians, K. J. 1999 Replication fork assembly at recombination intermediates is required for bacterial growth. *Proc. Natl Acad. Sci. USA* **96**, 3552–3555.

iu, N. (and 16 others) 1998 XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol. Cell* **1**, 783–793.

udwig, T., Chapman, D. L., Papaioannou, V. E. & Efstratiadis, A. 1997 Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. *Genes Dev.* **11**, 1226–1241.

Meselson, M. S. & Radding, C. M. 1975 A general model for genetic recombination. *Proc. Natl Acad. Sci. USA* **72**, 358–361.

Michel, B., Ehrlich, S. D. & Uzest, M. 1997 DNA double-strand breaks caused by replication arrest. *EMBO J.* **16**, 430–438.

Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P., Harshmann, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L. M. & Ding, W. 1994 A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* **266**, 66–71.

Mizuta, R., LaSalle, J. M., Cheng, H., Shinohara, A., Ogawa, H., Copeland, N., Jenkins, N. A., Lalande, M. & Alt, F. W. 1997 RAB22 and RAB163/mouse BRCA2: proteins that interact specifically with the RAD51 protein. *Proc. Natl Acad. Sci. USA* **94**, 6927–6932.

Neuhausen, S. (and 13 others) 1996 Recurrent BRCA2 6174delT mutations in Ashkenazi Jewish women affected by breast cancer. *Nature Genet.* **13**, 126–128.

New, J. H., Sugiyama, T., Zaitseva, E. & Kowalczykowski, S. C. 1998 Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A [see comments]. *Nature* **391**, 407–410.

Ogawa, T., Yu, X., Shinohara, A. & Egelman, E. H. 1993 Similarity of the yeast RAD51 filament to the bacterial RecA filament. *Science* **259**, 1896–1899.

Patel, K. J., Yu, V. P. C. C., Lee, H., Corcoran, A., Thistlethwaite, F. C., Evans, M. J., Colledge, W. H., Friedman, L. S., Ponder, B. A. & Venkitaraman, A. R. 1998 Involvement of Brca2 in DNA repair. *Mol. Cell* **1**, 347–357.

Pittman, D. L., Weinberg, L. R. & Schimenti, J. C. 1998 Identification, characterization, and genetic mapping of Rad51d, a new mouse and human RAD51/RecA-related gene. *Genomics* **49**, 103–111.

Rahman, N. & Stratton, M. R. 1998 The genetics of breast cancer susceptibility. *A. Rev. Genet.* **32**, 95–121.

Rajan, J. V., Wang, M., Marquis, S. T. & Chodosh, L. A. 1996 Brca2 is co-ordinately regulated with Brca1 during proliferation and differentiation in mammary epithelial cells. *Proc. Natl Acad. Sci. USA* **93**, 7131–7136.

Roca, A. I. & Cox, M. M. 1997 RecA protein: structure, function, and role in recombinational DNA repair. *Prog. Nucl. Acids Res. Mol. Biol.* **56**, 129–223.

Rothstein, R. & Gangloff, S. 1995 Hyper-recombination and Bloom's syndrome: microbes again provide clues about cancer. *Genome Res.* **5**, 421–426.

Rouet, P., Smih, F. & Jasin, M. 1994 Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. *Mol. Cell. Biol.* **14**, 8096–8106.

Scully, R., Chen, J., Plug, A., Xiao, Y., Weaver, D., Feunteun, J., Ashley, T. & Livingston, D. M. 1997 Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell* **88**, 265–275.

Seigneur, M., Bidnenko, V., Ehrlich, S. D. & Michel, B. 1998 RuvAB acts at arrested replication forks. *Cell* **95**, 419–430.

Sharan, S. K. & Bradley, A. 1997 Murine Brca2: sequence, map position, and expression pattern. *Genomics* **40**, 234–241.

Sharan, S. K., Morimatsu, M., Albrecht, U., Lim, D., Regel, E., Dinh, C., Sands, A., Eichele, G., Hasty, P. & Bradley, A. 1997 Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature* **386**, 804–810.

Shinohara, A., Ogawa, H. & Ogawa, T. 1992*a* Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. *Cell* **69**, 457–470.

Shinohara, A., Ogawa, H. & Ogawa, T. 1992*b* Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. *Cell* **69**, 457–470. [Published erratum appears in *Cell* **71**, following 180 (1992)].

Shinohara, A., Shinohara, M., Ohta, T., Matsuda, S. & Ogawa, T. 1998 Rad52 forms ring structures and co-operates with RPA in single-strand DNA annealing. *Genes Cells* **3**, 145–156.

Sonoda, E., Sasaki, M. S., Buerstedde, J. M., Bezzubova, O., Shinohara, A., Ogawa, H., Takata, M., Yamaguchi-Iwai, Y. & Takeda, S. 1998 Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. *EMBO J.* **17**, 598–608.

Stamato, T. D., Dipatri, A. & Giaccia, A. 1988 Cell-cycle-dependent repair of potentially lethal damage in the XR-1 gamma-ray-sensitive Chinese hamster ovary cell. *Radiat. Res.* **115**, 325–333.

Sturzbecher, H. W., Donzelmann, B., Henning, W., Knippschild, U. & Buchhop, S. 1996 p53 is linked directly to homologous recombination processes via RAD51/RecA protein interaction. *EMBO J.* **15**, 1992–2002.

Sung, P. 1994 Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. *Science* **265**, 1241–1243.

Sung, P. 1997 Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase. *J. Biol. Chem.* **272**, 28 194–28 197.

Sung, P. & Robberson, D. L. 1995 DNA strand exchange mediated by a RAD51-ssDNA nucleoprotein filament with polarity opposite to that of RecA. *Cell* **82**, 453–461.

Sung, P. & Stratton, S. A. 1996 Yeast Rad51 recombinase mediates polar DNA strand exchange in the absence of ATP hydrolysis. *J. Biol. Chem.* **271**, 27 983–27 986.

Suzuki, A. (and 15 others) 1997 Brca2 is required for embryonic cellular proliferation in the mouse. *Genes Dev.* **11**, 1242–1252.

Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J. & Stahl, F. W. 1983 The double-strand-break repair model for recombination. *Cell* **33**, 25–35.

Takata, M., Sasaki, M. S., Sonoda, E., Morrison, C., Hashimoto, M., Utsumi, H., Yamaguchi-Iwai, Y., Shinohara, A. & Takeda, S. 1998 Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *EMBO J.* **17**, 5497–5508.

Tavtigian, S. V. (and 27 others) 1996 The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds [see comments]. *Nature Genet.* **12**, 333–337.

Thorlacius, S., Olafsdottir, G., Tryggvadottir, L., Neuhausen, S., Jonasson, J. G., Tavtigian, S. V., Tulinius, H., Ogmundsdottir, H. M. & Eyfjord, J. E. 1996 A single BRCA2 mutation in male and female breast cancer families from Iceland with varied cancer phenotypes [see comments]. *Nature Genet.* **13**, 117–119.

Tsuzuki, T., Fujii, Y., Sakumi, K., Tominaga, Y., Nakao, K., Sekiguchi, M., Matsushiro, A., Yoshimura, Y. & Morita, T. 1996 Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc. Natl Acad. Sci. USA* **93**, 6236–6240.

Venkitaraman, A. R. 1999 What makes breast cancer cells tick? *Cell* **99**, 270–272.

West, S. C., Cassuto, E. & Howard-Flanders, P. 1981 Mechanism of *E. coli* RecA protein directed strand exchanges in post-replication repair of DNA. *Nature* **294**, 659–662.

Whitmore, G. F., Varghese, A. J. & Gulyas, S. 1989 Cell cycle responses of two X-ray sensitive mutants defective in DNA repair. *Int. J. Radiat. Biol.* **56**, 657–665.

Wong, A. K. C., Pero, R., Ormonde, P. A., Tavtigian, S. V. & Bartel, P. L. 1997 RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene brca2. *J. Biol. Chem.* **272**, 31941–31944.

Wooster, R. (and 40 others) 1994 Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12–13. *Science* **265**, 2088–2090.

Wooster, R., Bignell, J., Lancaster, S., Swift, S., Seal, S., Mangion, N., Collins, S., Gregory, C., Gumbs, G. & Micklem, R. 1995 Identification of the breast-cancer susceptibility gene BRCA2. *Nature* **378**, 789–792.

Xu, X., Weaver, Z., Linke, S. P., Li, C., Gotay, J., Wang, X. W., Harris, C. C., Ried, T. & Deng, C. X. 1999 Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. *Mol. Cell* **3**, 389–395.

Zou, H. & Rothstein, R. 1997 Holliday junctions accumulate in replication mutants via a RecA homolog-independent mechanism. *Cell* **90**, 87–96.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES