ChAM, a novel motif that mediates PALB2 intrinsic chromatin binding and facilitates DNA repair

Jean-Yves Bleuyard¹, Rémi Buisson², Jean-Yves Masson² & Fumiko Esashi¹⁺

¹Sir William Dunn School of Pathology, University of Oxford, Oxford, UK, and ²Genome Stability Laboratory, Laval University Cancer Research Center, Hôtel-Dieu de Québec, Quebec City, Quebec, Canada

The partner and localizer of breast cancer 2 susceptibility protein (PALB2) is crucial for the repair of DNA damage by homologous recombination. Here, we report that chromatin-association motif (ChAM), an evolutionarily conserved motif in PALB2, is necessary and sufficient to mediate its chromatin association in both unperturbed and damaged cells. ChAM is distinct from the previously described PALB2 DNA-binding regions. Deletion of ChAM decreases PALB2 and Rad51 accumulation at DNA damage sites and confers cellular hypersensitivity to the genotoxic drug mitomycin C. These results suggest that PALB2 chromatin association via ChAM facilitates PALB2 function in the cellular resistance to DNA damage.

Keywords: PALB2; chromatin association; homologous recombination; DNA repair; cancer

EMBO reports (2012) 13, 135–141. doi:10.1038/embor.2011.243

INTRODUCTION

DNA double-strand breaks (DSBs) are the most deleterious lesions a cell can encounter (Jackson & Bartek, 2009; Hiom, 2010), and can arise either from exposure to genotoxic agents (for example, ionizing radiation, anticancer drugs) or as the consequence of normal cellular processes (for example, DNA replication, aerobic metabolism). The partner and localizer of breast cancer 2 susceptibility protein, PALB2, has recently emerged as a crucial factor in homologous recombination (HR), which repairs DSBs using the replicated sister chromatid as a template and thus contributes to the maintenance of genome stability (Xia *et al*, 2006; Tischkowitz & Xia, 2010). Inherited heterozygous mutations in the PALB2 gene are associated with familial breast or pancreatic cancer predisposition (Erkko *et al*, 2007; Rahman *et al*, 2007; Slater *et al*, 2010; Tischkowitz & Xia, 2010), while

homozygous mutations cause the subtype N of the genetic disorder Fanconi anaemia (Reid *et al*, 2007; Xia *et al*, 2007).

PALB2 is the centrepiece of a DNA repair complex, designated the BRCA complex, including the breast cancer 1 (BRCA1) and 2 (BRCA2) susceptibility proteins, the Rad51 recombinase, which catalyses strand invasion and transfer phases of HR, and the chromodomain protein MRG15 (Xia *et al*, 2006; Sy *et al*, 2009a,b; Zhang *et al*, 2009a,b; Buisson *et al*, 2010; Dray *et al*, 2010; Hayakawa *et al*, 2010). Multiple protein interactions including PALB2 oligomerization, BRCA1 binding and MRG15 binding are proposed to help PALB2 recruitment to DSB sites, which in turn promotes the accumulation of BRCA2 and Rad51 and hence stimulates homologous recombinational repair (HRR) (Xia *et al*, 2006; Zhang *et al*, 2009a,b; Sy *et al*, 2009b,c; Hayakawa *et al*, 2010). Furthermore, recent biochemical studies showed that purified PALB2 directly binds DNA via two separate regions (here called P2T1 and P2T3; Fig 1A) and can stimulate Rad51-mediated strand invasion (Buisson *et al*, 2010; Dray *et al*, 2010).

Here, we identify a novel evolutionarily conserved PALB2 motif, named chromatin-association motif (ChAM), comprising 52 amino acids in the N-terminal part of the DNA-binding region P2T3. A PALB2 mutant harbouring a ChAM deletion (PALB2^{ΔChAM}) interacts with other known binding partners, but exhibits reduced association with chromatin in unperturbed and damaged cells. ChAM deletion is sufficient to decrease PALB2 and Rad51 accumulation at DSB sites and to render human cells hypersensitive to the DNA-damaging drug mitomycin C (MMC). Most importantly, although ChAM deletion does not affect PALB2 DNA-binding properties, ChAM on its own associates with nucleosomes. Together, these observations support a model in which ChAM-mediated chromatin association of PALB2 facilitates the recruitment of repair-proficient BRCA complexes at DSB sites and thus the promotion of Rad51-mediated HRR.

RESULTS

Identification of a novel PALB2 conserved region

Human PALB2 (hPALB2) contains two well-documented structural domains: an N-terminal coiled-coil domain, which mediates PALB2 oligomerization and BRCA1 interaction, and C-terminal WD40 repeats, which support binding to BRCA2 and Rad51 (Fig 1A; supplementary Fig S1 online) (Xia *et al*, 2006; Zhang *et al*,

¹Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK

²Genome Stability Laboratory, Laval University Cancer Research Center, Hôtel-Dieu de Québec, Quebec City, Quebec G1R 2J6, Canada

⁺Corresponding author. Tel: +44 1865 275289; Fax: +44 1865 275515;

E-mail: fumiko.esashi@path.ox.ac.uk

Fig 1 | Identification of an evolutionarily conserved region of PALB2. (A) Graph representing the percentage of sequence identity across human, mouse, chicken, opossum, zebrafish and frog PALB2 orthologues. Each data point represents the mean percentage sequence identity over 20 consecutive residues. For illustration purposes, only residues aligned with human PALB2 were implemented. Coloured boxes represent protein- and DNA-interaction regions of human PALB2. (B) Sequence alignment of ChAM. Black boxes indicate residues with >60% identity. (C) Composition of complexes containing EYFP-PALB2 (WT or Δ ChAM) purified from EUFA1341 whole-cell extracts. Mock purifications from parental cells (–) and those expressing EYFP (vector) are shown as controls. Lamin A and H2A.X are markers for extraction of nuclear and chromatin-associated proteins, respectively. BRCA, breast cancer; ChAM, chromatin-association motif; Dr, zebrafish; EYFP, enhanced yellow fluorescent protein; Gg, chicken; Hs, human; IP, immunoprecipitation; Mm, mouse; Md, opossum; PALB2, partner and localizer of breast cancer 2 susceptibility protein; WT, wild type; Xt, frog.

2009a,b; Sy *et al*, 2009b,c; Buisson *et al*, 2010). hPALB2 interactions with MRG15 and DNA are mediated through less characterized regions wherein no known structural domains were identified (Sy *et al*, 2009a; Buisson *et al*, 2010; Dray *et al*, 2010). To gain additional insight into PALB2 protein organization and function, we retrieved from public databases the protein sequences of six PALB2 orthologues, belonging to different vertebrate classes and infraclasses, and performed a basic multiple sequence alignment (supplementary Fig S1 online). Not

surprisingly, our analyses show that the previously described N-terminal coiled-coil domain, C-terminal WD40 repeats and MRG15-binding region are highly conserved among vertebrates (Fig 1A). Most notably, the alignment revealed that hPALB2 residues 395–446 delimit a previously unreported evolutionarily conserved region (Fig 1A,B; supplementary Fig S1 online), which is located within the N-terminal part of PALB2 DNA-binding region P2T3 (Buisson *et al*, 2010). On the basis of our data showing the role of this conserved motif in PALB2 chromatin association (see below), we designated it as ChAM.

ChAM is required for PALB2 chromatin association

To assess properties of PALB2 ChAM *in vivo*, stable cell lines expressing tagged versions of either wild-type PALB2 (PALB2^{WT}) or a ChAM deletion (PALB2 ^{Δ ChAM}) were generated. As shown in Fig 1C, PALB2 ^{Δ ChAM} co-purified with BRCA2, BRCA1, MRG15 and Rad51 with an efficiency comparable to that of PALB2^{WT}, demonstrating that PALB2 ^{Δ ChAM} is as able to form the BRCA complex *in vivo* as is PALB2^{WT}. We next examined the subcellular localization of PALB2 ^{Δ ChAM}, which was expressed in PALB2-defective EUFA1341 or HEK293 cells. Similarly to PALB2^{WT}, PALB2 ^{Δ ChAM} resided predominantly in the nuclear compartment of the cell (Fig 2A, lanes 19–36; supplementary Fig S2A online). Further fractionation revealed that PALB2^{WT} accumulated in the chromatin-enriched fraction (Fig 2A, lanes 22, 31; supplementary Fig S2A online) whereas, in striking contrast, PALB2 ^{Δ ChAM} was present mostly in the nuclear soluble fraction of the cell (Fig 2A, lanes 25, 34; supplementary Fig S2A online). In both cell lines, the accumulation of BRCA2 and Rad51 in the chromatin-enriched fraction proved to be proportional to the amount of PALB2 present (Fig 2A, lanes 31, 34; supplementary Fig S2A online). To evaluate further a potential role of ChAM in the DNA damage response (DDR), cells were treated with ionizing radiation (IR) or MMC, and then subjected to cellular fractionation as above. Whereas we were able to detect characteristic markers of the DDR such as histone H2A.X phosphorylation (γ H2A.X) and increased level of chromatin-associated BRCA1 in all cell lines, the global level of PALB2 remained unchanged or moderately decreased after DNA-damaging treatment (Fig 2A, lanes 31–36; supplementary Fig S2B online). Concurrently, the levels of BRCA2 and Rad51 remained mostly unchanged. Together, these observations demonstrate that ChAM is important for PALB2 constitutive chromatin association in both unperturbed and damaged cells.

Analogously to ChAM, MRG15 has previously been shown to contribute to the chromatin association of PALB2 (Hayakawa *et al*, 2010). To evaluate their relative contributions to the chromatin loading of PALB2, we assessed the effect of MRG15 overexpression or knockdown on the chromatin association of PALB2 ^{Δ ChAM}. When MRG15 was overexpressed, both PALB2^{WT} and PALB2 ^{Δ ChAM} exhibited a moderate increase in their chromatin association (Fig 2B, lanes 21–24). Conversely, the level of PALB2^{WT} associated with chromatin decreased modestly after MRG15 knockdown (Fig 2C, lanes 21, 22), whereas no further reduction of chromatin-associated PALB2 ^{Δ ChAM} was detectable (Fig 2C, lanes 23, 24). These findings suggest that MRG15 is, at least in part, responsible for the residual chromatin-associated PALB2 ^{Δ ChAM} and, most importantly, support the idea that MRG15 and ChAM both contribute to the chromatin association of PALB2.

Fig 2 |ChAM deletion impairs PALB2 chromatin association. EUFA1341 cells expressing EYFP-PALB2 (WT or Δ ChAM) were subjected to DNA-damaging treatment (A) or transfected with 6 $\times$ His-MRG15 expression vector (B) and subjected to cellular fractionation. (C) HT1080 cells expressing Flag-EGFP-PALB2 (WT or Δ ChAM) were treated with control or MRG15-targeting siRNAs and subjected to cellular fractionation. Tagged PALB2 and other components of the BRCA complex were detected by western blotting. Lamin A and histones H2A.X or H3 are markers for extraction of nuclear and chromatin-associated proteins, respectively. γ H2A.X is a marker of the DDR. Asterisks indicate nonspecific bands. γ H2A.X, Ser 139 phosphorylated histone H2A.X; BRCA, breast cancer; ChAM, chromatin-association motif; DDR, DNA damage response; EYFP, enhanced yellow fluorescent protein; PALB2, partner and localizer of breast cancer 2 susceptibility protein; siRNA, small interfering RNA; vec, empty vector; WT, wild type.

Fig 3 | ChAM is sufficient to trigger chromatin association. (A) EMSA performed with ssDNA and recombinant ChAM. (B) Competition EMSAs performed with D-loop, dsDNA, ssDNA and P2T3 (lanes 1–6) or P2T3^{ΔChAM} (lanes 7–12). (C) Competition EMSAs performed as above with PALB2 (lanes 1–6) or PALB2^{ΔChAM} (lanes 7–12). (D) Fluorescence microscopy images of GFP (pDEST53 vector) and GFP–ChAM (pDEST53–ChAM vector) expressed in HEK293T cells. (E) Cellular fractionation of HEK293T cells expressing either GFP or GFP–ChAM. (F) Co-purification of GFP–ChAM with core histones after mild Benzonase DNA digestion. Asterisks indicate nonspecific bands. (G) Analysis of DNA fragment sizes after Benzonase DNA digestion. Total DNA from samples analysed in (F) was recovered with a QIAquick column after RNaseA and Proteinase K treatments. The symbols 1n–4n indicate the nucleosome positions. pDEST53 is a mammalian vector for N-terminal GFP-fusion protein expression. ChAM, chromatin-association motif; cyto., cytoplasmic; chrom., chromatin enriched; DAPI, 4',6-diamidino-2-phenylindole; dsDNA, double-stranded DNA; EMSA, electromobility shift assay; GFP, green fluorescent protein; IP, immunoprecipitation; nucl., nuclear soluble; ssDNA, single-stranded DNA; WCE, whole cell extract.

ChAM supports nucleosome association

To better understand the molecular mechanism underlying PALB2 chromatin association, we first tested whether purified recombinant ChAM (supplementary Fig S3B online) could bind DNA directly. Unlike PALB2 P2T3, which efficiently binds single-stranded DNA (ssDNA), purified ChAM displayed no detectable ssDNA-binding activity on its own (Fig 3A; supplementary Fig S3C online). We next purified WT and ^{ΔChAM} versions of P2T3 or

full-length PALB2 (supplementary Fig S3B online), and evaluated their DNA-binding properties in a competition DNA electromobility shift assay. In agreement with previous reports, P2T3 and PALB2 preferentially bound HR intermediate structures, that is, D-loop and ssDNA, over double-stranded DNA (dsDNA), and we found that P2T3^{ΔChAM} and PALB2^{ΔChAM} showed similar DNA binding preferences for these substrates (Fig 3B,C). We also examined P2T3^{ΔChAM} and PALB2^{ΔChAM} DNA binding

to additional PALB2 substrates (splayed arms and Holliday junctions) and similarly found no differences in the DNA-binding specificities of WT and Δ ChAM proteins (supplementary Fig S3D,E online). Taken together, these data demonstrate that ChAM mediates PALB2 chromatin association independently from the DNA-binding activity associated with P2T3.

Given that ChAM exhibited no direct DNA-binding activity and that PALB2^{WT} and PALB2 ^{Δ ChAM} had similar DNA-binding properties, we explored alternative mechanisms by which ChAM might mediate PALB2 chromatin association. To this end, we generated a green fluorescent protein (GFP)–ChAM fusion and first examined its subcellular localization. Fluorescence microscopy revealed that, in contrast to the diffuse cellular localization of GFP alone, ChAM is sufficient to target the GFP fusion to the nuclear compartment (Fig 3D). Second, when cells expressing GFP–ChAM were fractionated, unlike GFP alone, strong accumulation of GFP–ChAM in the chromatin-enriched fraction was detected (Fig 3E), demonstrating that ChAM is a functional motif and is sufficient to drive chromatin association. Finally, we performed GFP-affinity purification from whole-cell extracts and assessed whether chromatin core components were co-purified. When the affinity purification was performed after extensive DNA digestion with Benzonase (Fig 3G, lanes 1–2), no co-purification of histone H3 or H2B was detectable (Fig 3F, lane 4). Strikingly, however, when the affinity purification was performed after partial Benzonase treatment whereby mononucleosomes retaining ~160bp DNA were mainly produced (Fig 3G, lanes 3–4; supplementary Fig S3F online), we detected a robust co-purification of histones H3 and H2B (Fig 3F, lane 8). Taken together, our data suggest that the chromatin association property of ChAM is mediated through its binding to nucleosome core particles consisting of DNA and a core histone octamer.

ChAM facilitates PALB2 function in DNA damage response

We next addressed whether ChAM-mediated chromatin association of PALB2 is important for its characteristic accumulation in damage-induced nuclear foci after IR or MMC treatment (supplementary Fig S4A online). Immunofluorescence microscopy showed that, in unperturbed cells, both PALB2^{WT} and PALB2 ^{Δ ChAM} displayed a mostly diffuse nuclear localization (supplementary Fig S4A online); approximately one-sixth of the cells exhibited spontaneous focal accumulation (Fig 4A), which is probably associated with DNA replication. By contrast, after induction of DNA damage with IR or MMC, a clear increase in the number of cells containing either PALB2^{WT} or PALB2 ^{Δ ChAM} foci was detected (Fig 4A,E). Importantly, detailed quantitative analysis showed that the percentage of cells containing PALB2 ^{Δ ChAM} foci was significantly lower than that with PALB2^{WT} foci. Similar results were obtained when we assessed its colocalization with γ H2A.X, a well-established marker for DSBs; upon IR and MMC treatment, cells expressing either PALB2 variant exhibited comparable numbers of γ H2A.X foci-positive cells, suggesting that similar numbers of DSBs were generated in both cell lines (Fig 4B), although coincidence of γ H2A.X and PALB2 was significantly lower in cells expressing PALB2 ^{Δ ChAM} than in those expressing PALB2^{WT} (Fig 4C). To further assess the importance of ChAM for PALB2 functions in the DDR, we tested the ability of PALB2^{WT} and PALB2 ^{Δ ChAM} to support MMC-induced Rad51 focus formation

(supplementary Fig S4B online). Treatment with small interfering RNA targeting the 3'-untranslated region of PALB2 mRNA resulted in a significant reduction of the percentage of Rad51 foci-positive cells (Fig 4D). In contrast, exogenous expression of either PALB2^{WT} or PALB2 ^{Δ ChAM} rescued Rad51 focus formation; however, the percentage of Rad51 foci-positive cells was significantly lower in PALB2 ^{Δ ChAM} cells and correlated with the percentage of MMC-induced PALB2 foci-positive cells (Fig 4C,E). Furthermore, we investigated the interplay between ChAM and BRCA1 during the MMC-induced focal accumulation of PALB2. As shown in supplementary Fig S4C online, BRCA1 knockdown severely impairs PALB2 focal accumulation after MMC treatment, and cells expressing PALB2^{WT} or PALB2 ^{Δ ChAM} displayed similar percentages of PALB2 foci-positive cells after BRCA1 knockdown (Fig 4E). These results indicate that BRCA1 has a primary role in efficient damage-induced PALB2 focus formation, whereas ChAM functions as a modifier of PALB2 accumulation at DSB sites.

Impaired PALB2 function confers cellular hypersensitivity to DNA-damaging drugs such as the DNA cross-linking agent MMC or the PARP inhibitor olaparib (Reid *et al*, 2007; Xia *et al*, 2007; Sy *et al*, 2009a,b; Zhang *et al*, 2009a). To further investigate the physiological importance of PALB2 chromatin association in the DDR, we tested whether PALB2 ^{Δ ChAM} could correct the MMC or olaparib hypersensitivity phenotype of PALB2-defective cells. As expected, PALB2^{WT} restored cell survival after either MMC or olaparib treatment (Fig 4F,G; supplementary Fig S4D,E online). In contrast, cells expressing PALB2 ^{Δ ChAM} remained hypersensitive to MMC, and were indistinguishable from those expressing the empty vector (Fig 4F; supplementary Fig S4D online). Intriguingly, we found that PALB2 ^{Δ ChAM} expression partially, but not fully, restored cellular resistance to olaparib (Fig 4G; supplementary Fig S3E online). Taken together, our data show that ChAM-mediated chromatin association of PALB2 contributes to the cellular resistance to MMC, where as it appears less important for the repair of olaparib-induced DNA lesions.

DISCUSSION

In this study, we have identified and characterized ChAM, an evolutionarily conserved motif of PALB2. We show that deletion of the 52-residue ChAM was sufficient to decrease the constitutive chromatin association of PALB2 and, most remarkably, that ChAM is a new functional domain mediating nucleosome association of PALB2. The importance of ChAM for normal PALB2 function is highlighted in cells expressing PALB2 ^{Δ ChAM}, which display a moderate but significant reduction in PALB2 and Rad51 recruitment to DSB sites, and severe MMC sensitivity. These data support the model presented in Fig 5. In unperturbed cells, ChAM, together with MRG15, maintains a fraction of BRCA pre-complex including MRG15, PALB2, BRCA2 and Rad51 on chromatin. Upon DSB formation and DDR activation, this BRCA pre-complex assists efficient reassembly of the BRCA complex and/or further accumulation of BRCA complexes at DSB sites. Although damaged-induced accumulation primarily requires BRCA1, the ChAM-dependent nucleosome binding may help the quality control of HRR, which is catalysed by PALB2, BRCA2 and Rad51.

Deletion of ChAM severely impaired but did not completely abrogate the chromatin association of PALB2, as assessed by cellular fractionation. One of the factors that appear to account for ChAM-independent chromatin association of PALB2 is MRG15,

Fig 4 | ChAM is required for normal PALB2 function *in vivo*. HT1080 cells were challenged with IR or MMC and scored for the percentage of cells with ≥ 10 Flag-EGFP-PALB2 (WT or Δ ChAM) foci (A), γ H2A.X foci (B) and colocalizing γ H2A.X and Flag-EGFP-PALB2 foci (C). HT1080 cells were treated with PALB2 3'-UTR siRNA, challenged with MMC and scored for the percentage of cells with ≥ 10 Rad51 foci (D). HT1080 cells were treated with BRCA1 siRNA, challenged with MMC and scored for the percentage of cells with ≥ 10 Flag-EGFP-PALB2 (WT or Δ ChAM) foci (E). Survival curves after MMC (F) or olaparib (G) treatment of EUFA1341 and derivative cell lines. All data are means ($\pm$ s.d.) of ≥ 3 independent experiments. More than 200 cells were scored for each individual sample. P-values are from the two-tailed Student's *t*-test, and asterisks indicate P-values < 0.05 (*), < 0.01 (**) or < 0.001 (***). γ H2A.X, histone H2A.X phosphorylation; BRCA, breast cancer; ChAM, chromatin-association motif; IR, ionizing radiation; MMC, mitomycin C; PALB2, partner and localizer of breast cancer 2 susceptibility protein; siControl, negative control siRNA; siRNA, small interfering RNA; UTR, untranslated region; Untr., untreated; WT, wild type.

overexpression of which partially restored chromatin association of PALB2^{ΔChAM}. Meanwhile, other factors may also influence PALB2 association with chromatin *in vivo*; first, although BRCA1 is particularly important for PALB2 accumulation at damage sites, which is mediated through the binding of BRCA1 and its accessory proteins to modified chromatin at DSBs (Bekker-Jensen & Mailand, 2010), BRCA1 also associates with undamaged chromatin (Fig 2A), and thus may help the constitutive recruitment of PALB2 to chromatin. Second, PALB2 contains two separate DNA-binding regions (Fig 1A), and although these regions preferentially bind HR-intermediate DNA structures, these DNA-binding properties may also support the residual chromatin association of PALB2^{ΔChAM}. Importantly, PALB2 chromatin association appears to affect the regulation of HRR proteins BRCA2 and Rad51; PALB2 defects or overexpression result in opposed effects on BRCA2 and Rad51 chromatin association (Fig 2; supplementary Fig S2 online), demonstrating a tight correlation between the levels of PALB2, BRCA2 and Rad51 loaded onto chromatin. Further, our analyses of cells expressing PALB2^{WT} or PALB2^{ΔChAM} establish a correlation between constitutive PALB2 chromatin association and the

ability to form Rad51 DNA damage-induced foci. We propose that the regulation of PALB2 chromatin association offers an additional quality control mechanism during HR-mediated DSB repair, and that deregulation of PALB2 chromatin association may contribute to HRR defects, including hyper-recombination phenotypes.

The newly characterized ChAM properties, which mediate chromatin association, provide exciting new perspectives on its role during DNA repair. Even though direct DNA or core histone binding was not detectable, we found that ChAM robustly binds to nucleosomes (Fig 3). Although we cannot exclude the possibility that ChAM binding to nucleosomes is mediated by other nucleosome-binding proteins such as chromatin remodelling complexes (Glatt *et al*, 2011), it is tempting to speculate that PALB2 provides an optimum environment to the chromatin and/or the BRCA complex around DSB sites during HRR. This hypothesis may explain the differences we observed in cell survival, whereby expression of PALB2^{ΔChAM} in PALB2-defective cells failed to rescue MMC hypersensitivity, but partly restored resistance to olaparib treatment. It is noteworthy that although both MMC and olaparib treatments produce DSBs after replication fork collapse, these are

Fig 5 | Model for the role of ChAM-mediated PALB2 chromatin association during HR. See main text for detailed description. BRCA, breast cancer; ChAM, chromatin-association motif; HR, homologous recombination; PALB2, partner and localizer of breast cancer 2 susceptibility protein.

generated through different mechanisms; olaparib treatment generates DSBs when replication forks encounter unrepaired single-strand breaks, whereas MMC-induced DSBs are generated as intermediate products resulting from the repair of interstrand cross-links. Hence, it is possible that an intricate repair process involving additional interstrand cross-links repair factors upon MMC treatment is more dependent on ChAM to retain PALB2 at DSB sites and to assist positioning of the BRCA complex during HRR.

In summary, our study shows for the first time that PALB2 contains a dedicated chromatin association motif, distinct from previously reported DNA-binding regions. Given the evolutionary conservation of ChAM, which is important for survival after DNA damage, this report sheds new light on the functions of PALB2. Further work, along with studies of its binding partners BRCA1, MRG15 and BRCA2, will be required to gain a full appreciation of the role of PALB2 chromatin association properties in DNA damage repair processes.

METHODS

Cells were grown under standard conditions. DNA-binding assay was carried out as described by Buisson *et al* (2010). Cellular fractionation, immunoprecipitation, indirect immunofluorescence and cell survival analyses were performed using standard techniques.

Detailed methods are available in the supplementary information online.

Supplementary information is available at EMBO reports online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

EUFA1341/FA-N fibroblasts were a gift from Dr Hans Joenje (Vrije Universiteit Medical Center, Amsterdam, The Netherlands). Rabbit anti-MRG15 antibody was a gift from Professor Olivia Pereira-Smith (University of Texas Health Science Center, San Antonio, TX, USA). This work was supported by financial support from the Cancer Research UK and Breast Cancer Campaign (F.E.) and the CIHR (J.-Y.M.). R.B. is a FQRNT scholar and J.-Y.M. is a Fonds de la Recherche en Santé du Québec Senior investigator.

Author contributions: J.-Y.B., R.B., J.-Y.M. and F.E. designed the experiments and prepared the manuscript. J.-Y.B. performed *in vivo* analyses and, immunofluorescence and immunoprecipitation experiments. R.B. purified recombinant proteins and performed *in vitro* DNA-binding analyses.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

- Bekker-Jensen S, Møllgaard N (2010) Assembly and function of DNA double-strand break repair foci in mammalian cells. *DNA Repair (Amst)* **9**: 1219–1228
- Buisson R, Dion-Cote AM, Coulombe Y, Launay H, Cai H, Stasiak AZ, Stasiak A, Xia B, Masson JY (2010) Cooperation of breast cancer proteins PALB2 and piccolo BRCA2 in stimulating homologous recombination. *Nat Struct Mol Biol* **17**: 1247–1254
- Dray E *et al* (2010) Enhancement of RAD51 recombinase activity by the tumor suppressor PALB2. *Nat Struct Mol Biol* **17**: 1255–1259
- Erkko H *et al* (2007) A recurrent mutation in PALB2 in Finnish cancer families. *Nature* **446**: 316–319
- Glatt S, Alfieri C, Muller CW (2011) Recognizing and remodeling the nucleosome. *Curr Opin Struct Biol* **21**: 335–341
- Hayakawa T, Zhang F, Hayakawa N, Ohtani Y, Shinmyozu K, Nakayama J, Andreassen PR (2010) MRG15 binds directly to PALB2 and stimulates homology-directed repair of chromosomal breaks. *J Cell Sci* **123**: 1124–1130
- Hiom K (2010) Coping with DNA double strand breaks. *DNA Repair (Amst)* **9**: 1256–1263
- Jackson SP, Bartek J (2009) The DNA-damage response in human biology and disease. *Nature* **461**: 1071–1078
- Rahman N *et al* (2007) PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat Genet* **39**: 165–167
- Reid S *et al* (2007) Biallelic mutations in PALB2 cause Fanconi anemia subtype FA-N and predispose to childhood cancer. *Nat Genet* **39**: 162–164
- Slater EP, Langer P, Niemczyk E, Strauch K, Butler J, Habbe N, Neoptolemos JP, Greenhalf W, Bartsch DK (2010) PALB2 mutations in European familial pancreatic cancer families. *Clin Genet* **78**: 490–494
- Sy SM, Huen MS, Chen J (2009a) MRG15 is a novel PALB2-interacting factor involved in homologous recombination. *J Biol Chem* **284**: 21127–21131
- Sy SM, Huen MS, Chen J (2009b) PALB2 is an integral component of the BRCA complex required for homologous recombination repair. *Proc Natl Acad Sci USA* **106**: 7155–7160
- Sy SM, Huen MS, Zhu Y, Chen J (2009c) PALB2 regulates recombinational repair through chromatin association and oligomerization. *J Biol Chem* **284**: 18302–18310
- Tischkowitz M, Xia B (2010) PALB2/FANCN: recombining cancer and Fanconi anemia. *Cancer Res* **70**: 7353–7359
- Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ, Livingston DM (2006) Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. *Mol Cell* **22**: 719–729
- Xia B *et al* (2007) Fanconi anemia is associated with a defect in the BRCA2 partner PALB2. *Nat Genet* **39**: 159–161
- Zhang F, Fan Q, Ren K, Andreassen PR (2009a) PALB2 functionally connects the breast cancer susceptibility proteins BRCA1 and BRCA2. *Mol Cancer Res* **7**: 1110–1118
- Zhang F, Ma J, Wu J, Ye L, Cai H, Xia B, Yu X (2009b) PALB2 links BRCA1 and BRCA2 in the DNA-damage response. *Curr Biol* **19**: 524–529