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HP1 regulates the localization of FANCJ at sites of DNA double-strand breaks

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Key words

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The breast and ovarian cancer predisposition protein BRCA1 forms three mutually exclusive complexes with Fanconi anemia group J protein (FANCJ, also called BACH1 or BRIP1), CtIP, and Abraxas/RAP80 through its BRCA1 C terminus (BRCT) domains, while its RING domain binds to BRCA1-associated RING domain 1 (BARD1). We recently found that the interaction between heterochromatin protein 1 (HP1) and BARD1 is required for the accumulation of BRCA1 and CtIP at sites of DNA double-strand breaks. Here, we investigated the importance of HP1 and BARD1–HP1 interaction in the localization of FANCJ together with the other BRCA1–BRCT binding proteins to clarify the separate role of the HP1-mediated pathway from the RNF8/RNF168-induced ubiquitin-mediated pathway for BRCA1 function. FANCJ interacts with HP1γ in a BARD1-dependent manner, and this interaction was enhanced by ionizing radiation or irinotecan hydrochloride treatment. Simultaneous depletion of all three HP1 isoforms with shRNAs disrupts the accumulation of FANCJ and CtIP, but not RAP80, at double-strand break sites. Replacement of endogenous BARD1 with a mutant BARD1 that is incapable of binding to HP1 also disrupts the accumulation of FANCJ and CtIP, but not RAP80. In contrast, RNF168 depletion disrupts the accumulation of only RAP80, but not FANCJ or CtIP. Consequently, the accumulation of conjugated ubiquitin was only inhibited by RNF168 depletion, whereas the accumulation of RAD51 and sister chromatid exchange were only inhibited by HP1 depletion or disruption of the BARD1–HP1 interaction. Taken together, the results suggest that the BRCA1–FANCJ and BRCA1–CtIP complexes are not downstream of the RNF8/RNF168/ubiquitin pathway, but are instead regulated by the HP1 pathway that precedes homologous recombination DNA repair.

Recent comprehensive studies of the molecular landscape of cancers have shown that failure of HR repair activity is a hallmark of advanced subtypes of malignant tumors, such as breast, ovarian, and pancreatic cancers, with substantial genomic instability.[1,2] The failure of HR for DSBs has potential implications for therapeutic choices, such as using platinum or the poly-(ADP-ribose) polymerase inhibitor to treat these cancers.[2–4] The breast and ovarian tumor suppressor BRCA1 is a central component of the HR pathway. BRCA1 is essential for several steps in HR, including DNA-end resection to create ssDNA and recruitment of RAD51 through PALB2/BRCA2 to undergo strand invasion of the ssDNA to the sister chromatids.[5–7] The major conformational features of BRCA1 are its N-terminal RING finger domain and its C-terminal tandem BRCT domains. BRCA1 forms three mutually exclusive complexes with FANCJ (also called BACH1 or BRIP1),[8] CtIP (also called RBBP8),[9] and Abraxas (also called FAM175A, ABRA1, or CCDC98)[10–12] through its BRCT domains, whereas the RING domain binds to BARD1, another RING- and BRCT-containing protein, to form a stable E3 ubiquitin ligase complex.[13,14] All three BRCA1–BRCT binding proteins comprise a consensus motif, SXXF, which is phosphorylated at the serine to mediate the interactions.[15,16]

FANCJ is an ATPase-DNA helicase mutated in hereditary breast and ovarian cancers, and its biallelic mutations cause Fanconi anemia, an autosomal recessive bone marrow disorder.[17–19] FANCJ plays important roles in the Fanconi anemia pathway in interstrand cross-link repair, HR, resolution of G-quadruplex DNA structures in replication, and cell cycle checkpoint activation.[17,20,21] Another BRCA1–BRCT binding protein, CtIP, is essential for the initiation of DNA-end resection in HR.[22,23] In collaboration with the MRE11-RAD50-NBS1 (MRN) complex, CtIP removes Ku proteins from DNA ends to generate ssDNA overhangs.[24,25] CtIP and MRN are sufficient for short-range DNA resection, although BRCA1 is required for extensive DNA resection, which is essential for HR. In this process, BRCA1 antagonizes 53BP1- and Ku-dependent DNA-end protection, which leads to NHEJ of DSBs; HR failure and phenotypes caused by BRCA1 deficiency can be rescued by the concomitant loss of 53BP1.[26–29]

The final BRCA1–BRCT binding protein, Abraxas, is an adaptor for RAP80, a ubiquitin-interacting motif-containing protein that is essential for BRCA1 accumulation at DSB sites through RNF8/RNF168-mediated polyubiquitin chains.[10–12,30–35] The role of the BRCA1/Abraxas/RAP80 complex is enigmatic, as it antagonizes rather than promotes HR.[36,37] A recent study has shown its role in cell cycle checkpoint signaling, which is distinct from its repair function.[38]

Although the mechanism for the recruitment of the BRCA1/Abraxas/RAP80 complex through the ATM-dependent RNF8/RNF168-mediated pathway has been studied extensively, that of the BRCA1/FANCJ or BRCA1/CtIP complex has been rather unclear. HP1 and proteins involved in heterochromatin formation have recently emerged as critical in HR[39–41] and comprise an alternative mechanism for BRCA1 accumulation at DSB sites.[42–44] We recently found an essential interaction between BARD and HP1 through direct binding between the BARD1 BRCT domain and the HP1 chromoshadow domain for the accumulation of BRCA1, CtIP, and RAD51 at DSB sites.[45] In this study, we attempted to clarify the role of HP1 and the BARD1–HP1 interaction in FANCJ accumulation in parallel with their role in CtIP and RAP80 accumulation. The role of RNF168 in the accumulation of the three BRCA1–BRCT binding proteins was investigated for comparison. We also analyzed the accumulation of conjugated ubiquitin and RAD51 at DSB sites, and sister chromatid exchange as possible downstream events.

Materials and Methods

Cell lines and culture conditions. HeLa cells and their derivatives were cultured in DMEM supplemented with 10% FBS and 1% antibiotic–antimycotic agent (Life Technologies, Grand Island, NY, USA). Cells expressing specific shRNA(s) and cDNA in a Dox-inducible manner were established as described previously.[45] Briefly, cells expressing BARD1-specific shRNA in a Dox-inducible manner (HeLa-shBARD1 cells) were established with CS-RfA-ETBsd-shBARD1 and blasticidin selection. Cells expressing triple Dox-inducible shRNAs to HP1α, HP1β, and HP1γ were established with CS-RfA-ETPuro-shHP1α, CS-RfA-ETHygro-shHP1β and CS-RfA-ETBsd-shHP1γ, respectively, and triple antibiotic selection with puromycin, hygromycin, and blasticidin. Cells with Dox-inducible replacement of endogenous BARD1 with exogenous BARD1 were established with CSIV-TRE-RfA-UbC-KT-BARD1 (wild-type or PEELI mutant) and CS-RfA-ETBsd-shBARD1, respectively, and were selected with blasticidin and puromycin. The backbone vectors CS-RfA-ETs and CSIV-TRE-RfA-UbC-KT were generous gifts from Dr. Hiroyuki Miyoshi and Dr. Atsushi Miyawaki (RIKEN BioResource Center, Tsukuba, Japan). All of the cells were further maintained in their respective antibiotics. The cells were treated with 1 μg/mL Dox for 48 h unless otherwise indicated for the induction and were then subjected to individual experiments. For IR, the cells were exposed to X-irradiation (10 Gy) and cultured for 1 h before analysis. The chemical agent irinotecan hydrochloride (CPT-11) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Antibodies. The antibodies used against FANCJ (Bethyl Laboratories, Montgomery, TX, USA), BARD1 (BL518; Bethyl Laboratories), BRCA1 (C20; Santa Cruz Biotechnology, Santa Cruz, CA, USA), KAP-pS824 (Bethyl Laboratories), RAP80, (Bethyl Laboratories), RNF168 (ABE367; Millipore, Darmstadt, Germany), and RAD51 (BioAcademia, Osaka, Japan) were rabbit polyclonal antibodies. The antibodies against CtIP (Active Motif, Carlsbad, CA, USA), conjugated ubiquitin (FK2; Nippon Bio-Test Laboratories, Tokyo, Japan), HP1α (Millipore), HP1β (1MOD-1A9; Millipore), HP1γ (2MOD-1G6; Millipore), H3K9me2 (CMA307; Millipore), γH2AX (JBW301; Millipore), and α- and β-tubulin (DMIA+BMIB; Neomarkers, Fremont, CA, USA) were mouse mAbs.

Cell extracts, immunoprecipitation, and Western blot analysis. To analyze the steady-state levels of the proteins in whole cell extracts, cells were lysed with RIPA buffer, clarified, adjusted for protein concentration, and subjected to Western blotting as described previously.[46] For immunoprecipitation of the chromatin extract, 10^7 cells were incubated with 1 mL buffer containing 50 mM Tris–HCl (pH 7.5), 0.5% Nonidet P-40, 150 mM NaCl, 50 mM NaF, 1 mM DTT, 1 mM $NaVO_3$, 1 mM PMSF, 2 μg/mL aprotinin, 2 μg/mL leupeptin, 10 μg/mL trypsin inhibitor, and 150 μg/mL benzamidine. The buffer was supplemented with 125 U/mL benzonase nuclease (Novagen, San Diego, CA, USA) and 200 μg/mL ribonuclease (Sigma-Aldrich) and was incubated with the cells at 4°C for 120 min; the reaction was stopped with 5 mM EDTA. The extract was centrifuged to isolate the chromatin-bound proteins in the soluble fraction, filtered through a 0.45-μm pore size filter, and used for immunoprecipitations as described previously.[46]

RNA interference. The siRNA oligonucleotides targeting RNF168 (5′-GAAAUUCUCUCGUCAACGU-3′) and the non-targeting control siRNA (D-001810-01) were purchased from Dharmacon (Lafayette, CO, USA). RNA duplexes (10 nM final concentration) were transfected into the cells with Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) and were analyzed 48 h after transfection.

Laser microirradiation. To induce DSBs, cells were first sensitized with 10 μM BrdU 12 h prior to irradiation. Laser microirradiation was carried out for indicated lengths of time before analysis using a PALM UV-A pulsed nitrogen laser (100 Hz, λ = 355 nm; P.A.L.M. Microlaser, Bernried, Germany) mounted on an AxioObserver Z1 microscope (Carl Zeiss, Gottingen, Germany) on a custom-designed granite plate.

Immunofluorescence microscopy. Indirect immunofluorescence labeling of cells and fluorescence detection were carried out as described previously[47] with the following modifications. For FANCJ, CtIP, and RAP80 staining, cells were fixed and permeabilized with PBS containing 0.7% Triton X100, 3% paraformaldehyde, and 2% sucrose for 30 min on ice, washed with PBS, and then incubated for 5 min with PBS containing 0.2% Triton X100. For RAD51 staining, cells were pre-extracted with a buffer containing 20 mM HEPES (pH 7.5), 20 mM NaCl, 5 mM $MgCl_2$, and 0.5% IGEPAL (A-630; Sigma-Aldrich) supplemented with proteinase inhibitors for 30 min on ice, washed with PBS, and fixed with 2% formalin in PBS for 20 min at room temperature. After incubation with primary and fluorescence-labeled secondary antibodies, the slides were mounted with ProLong Gold Antifade Mountant with DAPI (Invitrogen) and examined with a confocal laser scanning microscope (LSM 510; Carl Zeiss). For the quantification of the intensity of the proteins accumulated through laser microirradiation-induced DSBs, the straight-line tool from the ZEN 2011 software (line width = 0.12 μm) (Carl Zeiss) was used to draw a line over all of the γH2AX stripes, and the intensities of the corresponding stripes of the repair proteins (FANCJ, CtIP, RAP80, conjugated ubiquitin, and RAD51) were measured. At least 20 stripes were measured in each sample. The relative intensity was calculated as the intensity

of the proteins at the DSB divided by the intensity of the same length of the line of the proteins over an undamaged portion in the same nucleus. The statistical analyses were undertaken with the unpaired Student's *t*-test using GraphPad Prism software.

Sister chromatid exchange assays. Cells were induced or not with Dox for 48 h, and were grown for additional 48 h in the presence of 20 μM BrdU. Cells were then incubated with 0.2 μg/mL colcemid for the last hour before harvest. Cells were collected with trypsin, incubated for 20 min in 75 mM KCl and gently fixed for 30 min in methanol : acetic acid (3:1). Cells were dropped onto ethanol-treated glass slides, air dried, and aged for 3 days. Nuclei were sensitized with 10 μg/mL Hoechst 33258 in 0.5× SSC for 30 min at room temperature. The slides were then bleached with a 352-nm black light for 2 h, heat-treated at 70°C for 60 min, stained with 3% Giemsa for 15 min, and mounted with glass coverslips in Malinol (Muto pure chemicals, Tokyo, Japan). To induce SCEs, 10 nM CPT-11 was added to the culture for 24 h prior to harvest. Slides were analyzed with an Olympus BX53F microscope (Olympus, Tokyo, Japan) equipped with a 100× objective.

Results

FANCJ interacts with HP1γ in response to DNA damage through BARD1. To clarify the functional interaction between HP1 and FANCJ, we first used immunoprecipitation to determine whether they physically interacted with one another *in vivo*. We previously showed that BARD1 primarily interacts with HP1γ among three HP1 family members in physiological conditions, although BARD1 is able to interact with HP1α and β when HP1γ expression is inhibited by shRNA.[45] Therefore, we examined the FANCJ–HP1γ interaction. HeLa cells were harvested after IR or mock treatment and were lysed with benzonase nuclease to solubilize the chromatin proteins. The FANCJ immunocomplex was precipitated and immunoblotted with HP1γ antibody. HP1γ was clearly detected after IR, indicating the IR-induced interaction between FANCJ and HP1γ (Fig. 1a). We previously showed that, among histone modifications, H3K9me2, the histone modification recognized by HP1, was specifically detected by BARD1 immunoprecipitation.[45] Therefore, we tested if H3K9me2 also co-precipitated with FANCJ. As expected, it co-precipitated with FANCJ in an IR-dependent manner (Fig. 1a). Treatment with the topoisomerase I inhibitor irinotecan (CPT-11) also dramatically enhanced the interaction between FANCJ and HP1γ (Fig. 1b). The interactions between FANCJ and BRCA1 and BARD1 were increased after CPT-11 treatment (Fig. 1b). In contrast, the interaction between FANCJ and BRCA1 decreased in response to IR, whereas that between FANCJ and BARD1 was unchanged (Fig. 1a). The mechanism underlying the discrepancy is currently unknown. The decrease of FANCJ–BRCA1 interaction with simultaneous increase of FANCJ–HP1γ interaction may reflect selective decrease of a BRCA1–FANCJ fraction that does not interact with HP1γ. Alternatively, it may suggest direct binding of FANCJ to BARD1 or HP1γ. To explore whether the observed interaction between FANCJ and HP1γ is mediated by BARD1, we next tested the effect of BARD1 depletion on the interaction. HeLa cells stably integrating Dox-inducible BARD1-specific shRNA (HeLa-shBARD1) were exposed to IR, and the chromatin fraction was immunoprecipitated with FANCJ antibody (Fig. 1c). Doxycycline induction effectively inhibited BARD1 and

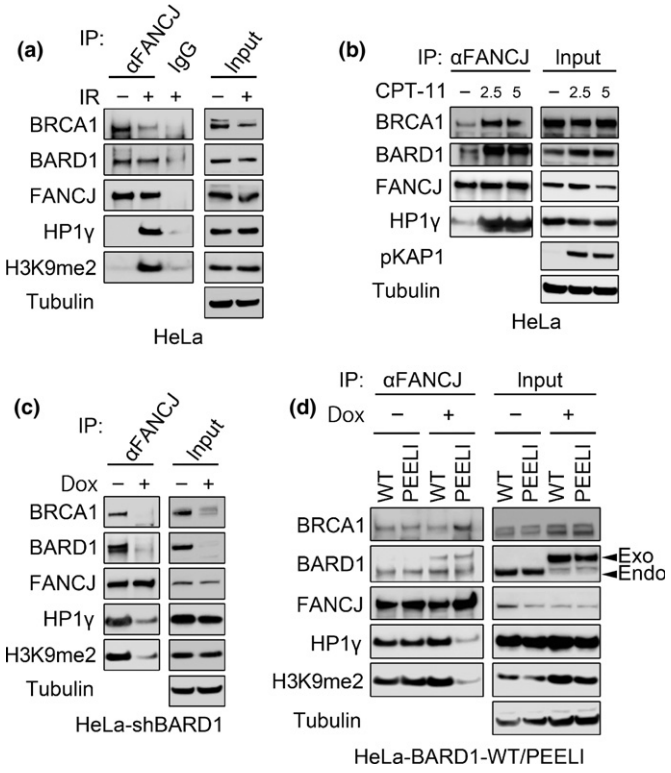

Fig. 1. FANCJ interacts with HP1γ in response to DNA damage. (a) Solubilized chromatin fractions prepared from HeLa cells harvested 1 h after 10 Gy IR (+) or mock treatment (–) were immunoprecipitated (IP) with anti-FANCJ antibody or control IgG, and subjected to immunoblotting with the indicated antibodies. Inputs (1.5%) were also loaded. (b) HeLa cells were treated with indicated concentrations (μM) of CPT-11 or vehicle (–) for 2 h, and subjected to immunoprecipitation and immunoblotting as in (a). (c) HeLa-shBARD1 cells were induced (+) or not (–) with Dox for 48 h, exposed to 10 Gy IR, and subjected to immunoprecipitation and immunoblotting as in (a). (d) HeLa-BARD1-WT and -PEELI cells were induced (+) or not (–) with Dox for 96 h, exposed to 10 Gy IR, and subjected to immunoprecipitation and immunoblotting as in (a). Endo, endogenous BARD1; Exo, exogenous BARD1-EGFP.

BRCA1 expression, whose protein stability is strongly dependent on dimer formation with BARD1.[14,48] The interaction between FANCJ and HP1γ/H3K9me2 was significantly inhibited by Dox induction, indicating that the interaction is mediated by BRCA1/BARD1. Although there still remains the possibility that FANCJ directly interacts with BARD1 in addition to BRCA1, we could not address this possibility due to the interdependence of BRCA1 and BARD1 for their protein stabilities. Next, we further examined whether the FANCJ–HP1γ interaction depends on BARD1–HP1γ interaction. For this purpose, we established stable HeLa cell lines integrating Dox-inducible BARD1-specific shRNA together with the Dox-inducible wild-type or BARD1 mutant L570E/V571E (PEELI) (hereafter named HeLa-BARD1-WT and HeLa-BARD1-PEELI, respectively). We previously found that BARD1 interacts with HP1 through PLVLI residues, which resembles the conserved HP1-recognizing motif PxVxL, and mutations of this motif, including PEELI, disrupt the interaction and result in the failure of DSB accumulation of BRCA1/BARD1 and CtIP. HeLa-BARD1-WT and HeLa-BARD1-PEELI cells were induced with Dox for the substitution and were harvested 1 h after IR. Endogenous BARD1 was effectively replaced with exogenous BARD1 in a Dox-inducible manner with

approximately the same steady-state levels between the wild-type and mutant proteins (Fig. 1d, input). The replacement of either the wild-type or mutant BARD1 did not inhibit BRCA1 steady-state levels, suggesting that the role of BARD1 for BRCA1 stabilization was maintained in both cell lines. Immunoprecipitation with FANCJ antibody revealed that HP1γ and H3K9me2 interact with FANCJ in HeLa-BARD1-WT cells, although this interaction was dramatically inhibited in HeLa-BARD1-PEELI cells by Dox treatment (Fig. 1d). These results indicate that FANCJ interacts with HP1γ through BARD1–HP1γ interaction.

HP1s are required for DSB accumulation of FANCJ and CtIP, but not RAP80. The specific interaction between FANCJ and HP1γ in response to DNA damage prompted us to examine whether HP1 inhibition would affect FANCJ accumulation at DSB sites. We previously showed that, although BARD1 primarily interacts with HP1γ among HP1 family members following DNA damage, HP1γ inhibition is not sufficient to suppress BRCA1 and BARD1 accumulation at DSB sites due to compensation by family members.[45] Therefore, we established stable HeLa cells integrating three Dox-inducible constructs, each expressing shRNAs specific to HP1α, β, or γ (HeLa-tnHP1). The HP1 target members were effectively inhibited by Dox induction (Fig. 2a). Cells were then laser-microirradiated and subjected to immunofluorescent analyses with antibodies specific either to FANCJ, CtIP, or RAP80, together with γH2AX as a marker for DSBs. Time course analyses indicated that FANCJ and CtIP were barely detected at 15 or 30 min after laser-microirradiation and became evident at 1 h after laser-microirradiation (Fig. S1). We therefore analyzed their accumulation at 1 h after laser-microirradiation. Accumulation of FANCJ was readily detected at the DSB sites, but its accumulation was reduced by Dox treatment (Fig. 2b); the average FANCJ intensity was significantly weaker in Dox-treated cells compared to untreated cells ($P = 0.0030$; Fig. 2b, right panel). Accumulation of CtIP at the DSBs was also significantly reduced by Dox treatment ($P < 0.0001$; Fig. 2c). In contrast, RAP80 accumulation at the DSB sites was detected from 15 min after laser-microirradiation, but was not affected by Dox treatment at either 15 min or 1 h after laser-microirradiation (Figs 2d,S1). These results suggest that HP1 is required for the stable accumulation of FANCJ and CtIP, but not RAP80, at DSB sites.

Interaction between BARD1 and HP1 is required for DSB accumulation of FANCJ and CtIP, but not RAP80. The impaired FANCJ accumulation at DSB sites by HP1 inhibition (Fig. 2b) and disruption of FANCJ–HP1γ interaction by inhibition of BARD1–HP1γ interaction (Fig. 1d) prompted us to examine whether inhibition of BARD1–HP1γ interaction by the PEELI mutation would also affect the accumulation of FANCJ at DSB sites. HeLa-BARD1-WT and HeLa-BARD1-PEELI cells were induced with Dox and were either immunoblotted or laser-microirradiated. Endogenous BARD1 was effectively replaced with exogenous BARD1 with approximately the same steady-state levels between the wild-type and mutant proteins (Fig. 3a). The replacement did not inhibit BRCA1 steady-state levels. The laser-microirradiated cells were then subjected to immunofluorescent analyses with antibodies specific either to FANCJ, CtIP, or RAP80 together with γH2AX. Accumulation of FANCJ was detected at the DSB sites in HeLa-BARD1-WT cells; however, the accumulation was significantly reduced in the HeLa-BARD1-PEELI cells ($P < 0.0001$; Fig. 3b). Accumulation of CtIP was also significantly reduced in HeLa-BARD1-PEELI cells ($P < 0.0001$; Fig. 3c). In contrast, RAP80 accumulation was not affected by the BARD1 mutation (Figs 3d,S1). These results suggest that the interaction

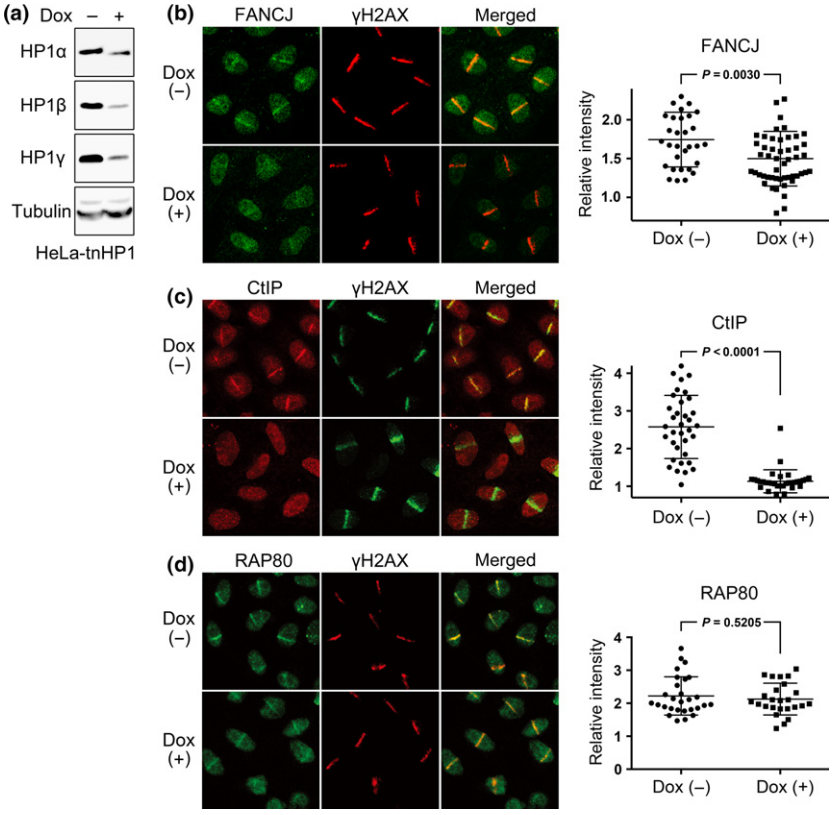

Fig. 2. HP1 inhibition disturbs the accumulation of FANCJ and CtIP, but not RAP80, at DSB sites. (a) HeLa cells conditionally expressing shRNA for all three HP1 family members were induced (+) or not (–) with Dox for 48 h and then subjected to immunoblotting with the indicated antibodies. (b–d) Cells from (a) were laser-microirradiated and immunostained for FANCJ (b), CtIP (c), or RAP80 (d) with γH2AX after 1 h. Right panels, the relative intensities of the indicated proteins are shown in dot plots. Bars and error bars indicate mean and SD, respectively.

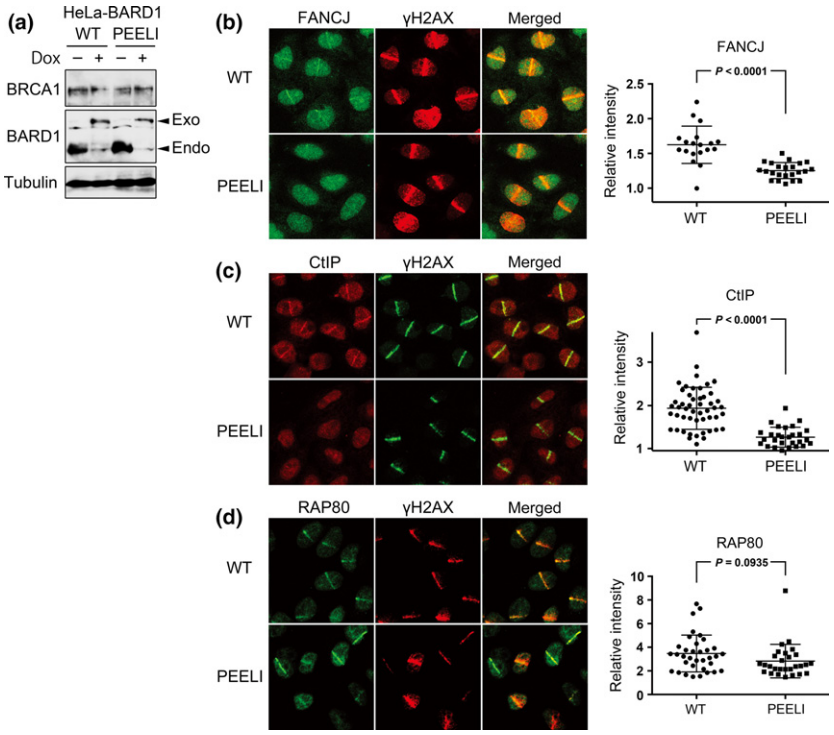

Fig. 3. Inhibition of the interaction between BARD1 and HP1 disturbs the accumulation of FANCJ and CtIP, but not RAP80, at DSB sites. (a) HeLa cells conditionally expressing shRNA for BARD1 together with the wild-type (WT) or PEELI mutant for shRNA-insensitive BARD1-EGFP were induced (+) or not (−) with Dox for 48 h and subjected to immunoblotting with the indicated antibodies. (b–d) Cells from (a) were laser-microirradiated and immunostained for FANCJ (b), CtIP (c), or RAP80 (d) with γH2AX after 1 h. Right panels, the relative intensities of the indicated proteins are shown in dot plots. Bars and error bars indicate mean and SD, respectively. Endo, endogenous BARD1; Exo, exogenous BARD1-EGFP.

between BARD1 and HP1 is required for the stable accumulation of FANCJ and CtIP, but not RAP80, at DSB sites.

RNF168 is required for DSB accumulation of RAP80, but not FANCJ or CtIP. It is well known that ubiquitin ligases RNF8 and RNF168 are required for the formation of polyubiquitin products at DSB sites that recruit the BRCA1–Abraxas complex through ubiquitin-interacting motif-containing protein RAP80.[10–12,30–35] Inhibition of RNF8 or RNF168 significantly reduces BRCA1 accumulation at DSB sites.[30–34] However, we previously showed that the interaction between BARD1 with HP1γ was increased, rather than decreased, by RNF168 depletion,[45] indicating a distinct role for the HP1-mediated pathway in response to DSBs. To clarify this point, we examined the effect of RNF168 depletion on the accumulation of BRCA1–BRCT interacting proteins at DSB sites. HeLa cells were transfected with either control or RNF168-specific siRNAs and then subjected to immunoblotting. RNF168 protein steady-state levels were effectively inhibited by the siRNA treatment (Fig. 4a). The cells were then laser-microirradiated and subjected to immunofluorescent analyses with antibodies specific either to FANCJ, CtIP, or RAP80. Importantly, the DSB accumulation of FANCJ was not affected by RNF168 depletion (Fig. 4b), whereas the DSB accumulation of CtIP was slightly increased (Fig. 4c); only RAP80 accumulation was significantly reduced ($P = 0.0061$; Fig. 4d). The results suggest separate roles for the HP1-mediated pathway from the RNF8/RNF168-induced ubiquitin-mediated pathway; the former is responsible for BRCA1–FANCJ and BRCA1–CtIP accumulation, whereas the latter is responsible for BRCA1–Abraxas–RAP80 accumulation.

RNF168, but not HP1, is required for DSB accumulation of conjugated ubiquitin. To clarify the functional consequences of HP1 and RNF168 on the DSB-responsive ubiquitin pathway, we investigated the effects of HP1 depletion, disruption of BARD1–HP1 binding, and RNF168 depletion on the accumulation of conjugated ubiquitin at the DSB sites. Ubiquitin accumulation was unaffected by either HP1 depletion or disruption of BARD1–HP1 binding (Fig. 5a,b). In contrast, the accumulation of conjugated ubiquitin at the DSB sites was significantly reduced by RNF168 depletion ($P < 0.0001$; Fig. 5c), consistent with previous reports. Because RNF168 depletion did not affect the DSB accumulation of FANCJ or CtIP (Fig. 4), whereas the accumulation of conjugated ubiquitin was inhibited, the results indicate that conjugated ubiquitin is not required for the DSB accumulation of FANDJ and CtIP. The results also indicate that accumulation of conjugated ubiquitin is not sufficient for FANCJ and CtIP accumulation.

HP1 and BARD1–HP1 binding, but not RNF168, are required for DSB accumulation of RAD51. To further define the distinct roles of HP1 and RNF168 on homologous recombination, we investigated the effects of HP1 depletion, disruption of BARD1–HP1 binding, or RNF168 depletion on the accumulation of RAD51, an effector of homologous recombination, at the DSB sites. Time course analyses showed that RAD51 accumulation was undetectable or faint until 60 min after laser-microirradiation and became evident at 3 h after laser-microirradiation (Fig. S1). We therefore analyzed the accumulation at 3 h after laser-microirradiation. Importantly, RAD51 accumulation was significantly reduced by either HP1 depletion or disruption of BARD1–HP1 binding ($P < 0.0001$; Fig. 6a,b). In contrast, it was unaffected by RNF168 depletion (Fig. 6c). Together, these results suggest that HP1-dependent DSB accumulation of FANCJ and CtIP is critical for HR, whereas RNF168-dependent DSB accumulation of conjugated ubiquitin and RAP80 is dispensable, at least for DNA end resection in HR.

HP1 and BARD1–HP1 binding are required for SCE. To further evaluate the role of HP1 and BARD1–HP1 binding on HR, we examined the effects of their inhibition on SCEs (Fig. 7). Sister chromatid exchanges only form after HR repair as a consequence of Holliday junction, but not with other repair pathways in response to DSBs. The rates of SCE were

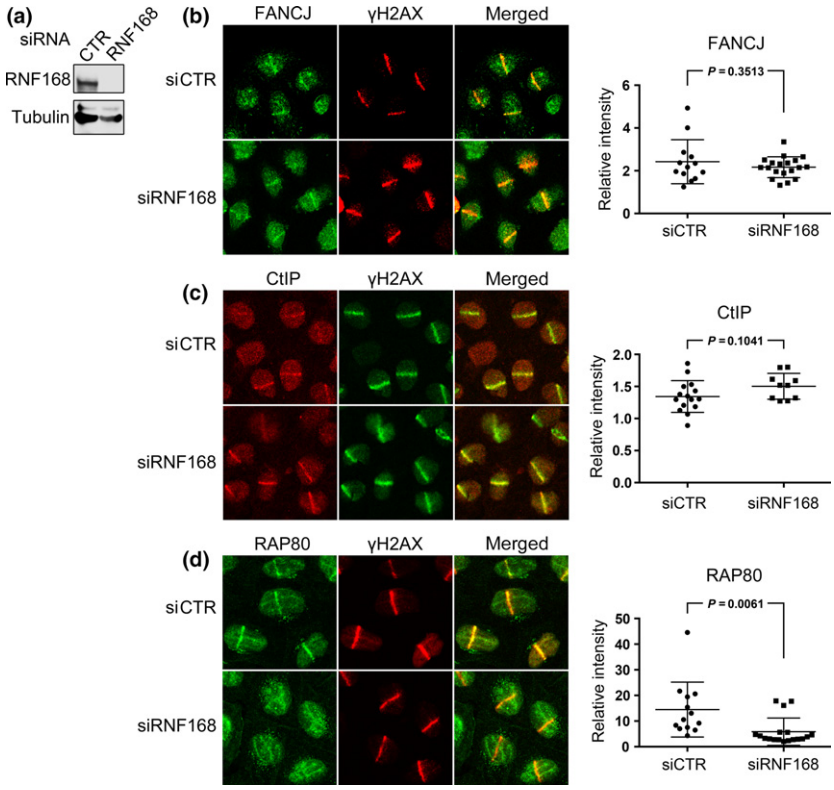

Fig. 4. Depletion of RNF168 disturbs accumulation of RAP80, but not FANCJ or CtIP, at DSB sites. (a) HeLa cells transfected with RNF168-specific or control siRNA (CTR) were subjected to immunoblotting with the indicated antibodies. (b–d) Cells from (a) were laser-microirradiated and immunostained for FANCJ (b), CtIP (c), or RAP80 (d) with γH2AX after 1 h. Right panels, the relative intensities of the indicated proteins are shown in dot plots. Bars and error bars indicate mean and SD, respectively.

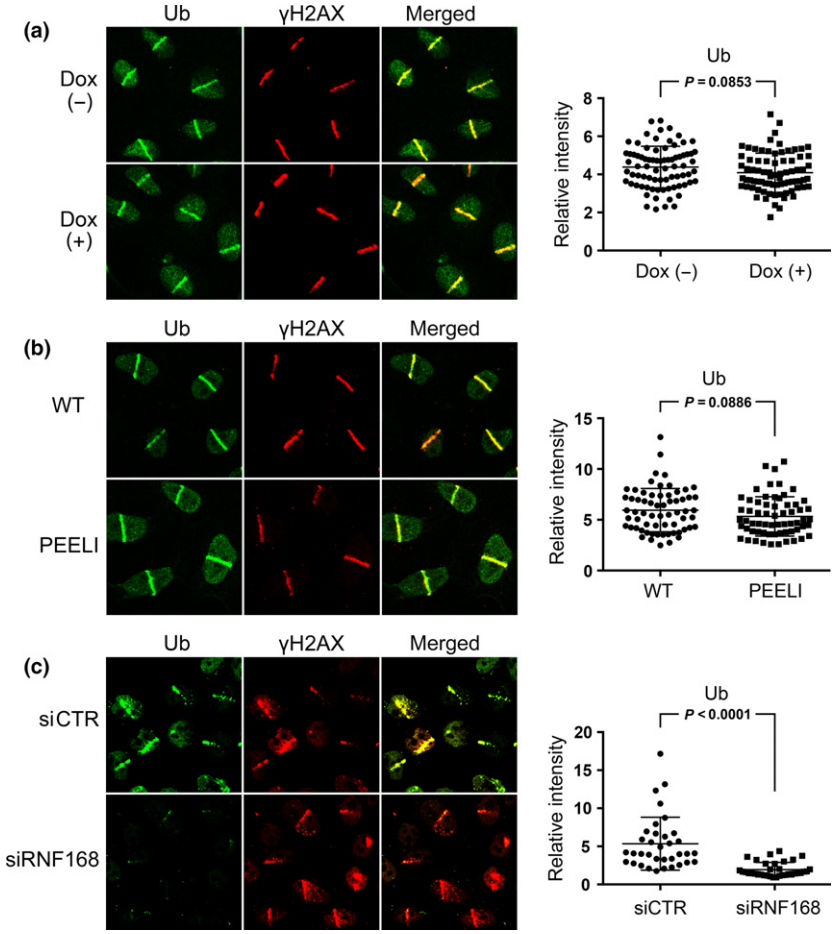

Fig. 5. RNF168, but not HP1 or BARD1–HP1 binding, is required for conjugated ubiquitin accumulation at DSB sites. HeLa cells conditionally expressing shRNA for all three HP1 family members induced (+) or not (–) with Dox (a), HeLa cells conditionally substituted for endogenous BARD1 with wild-type (WT) or BARD1 PEELI mutant (b) or HeLa cells transfected with RNF168-specific or control siRNA (siCTR) (c) were laser-microirradiated and immunostained for conjugated ubiquitin (Ub) with γH2AX after 1 h. Right panels, the relative intensities of the indicated protein are shown in dot plots. Bars and error bars indicate mean and SD.

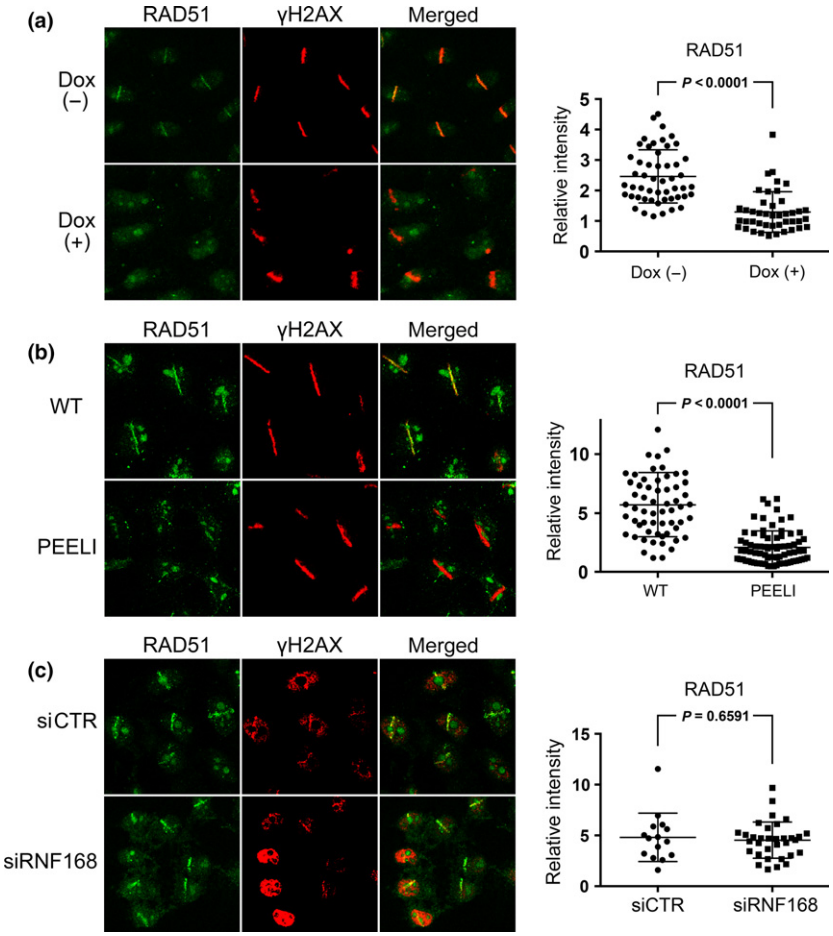

Fig. 6. HP1 and BARD1–HP1 binding, but not RNF168, are required for RAD51 accumulation at DSB sites. HeLa cells conditionally expressing shRNA for all three HP1 family members induced (+) or not (−) with Dox (a), HeLa cells conditionally substituted for endogenous BARD1 with wild-type (WT) or BARD1 PEELI mutant (b), or HeLa cells transfected with RNF168-specific or control siRNA (siCTR) (c) were laser-microirradiated and immunostained for RAD51 with γH2AX after 3 h. Right panels, the relative intensities of the indicated protein are shown in dot plots. Bars and error bars indicate mean and SD, respectively.

comparably low without DNA damage in control and Dox-induced cells, although Dox-induced HeLa-tnHP1 cells showed increased SCE rates (Dox (−), 5.9 ± 0.5 *vs* Dox (+), 11.8 ± 1.0; $P < 0.0001$), possibly due to chromatin decondensation with loss of HP1s that leads DNA vulnerable to spontaneous damage, and increases opportunity of HR that overcomes the negative effect of HP1 loss on HR. Treatment with CPT-11 led to dramatically higher frequencies of SCEs in HeLa-tnHP1 cells without Dox induction (72.2 ± 2.5), but the SCE frequency was significantly attenuated by Dox induction (37.7 ± 3.0; $P < 0.0001$). Similarly, the rates of CPT-11-induced SCE in HeLa-BARD1-PEELI cells were significantly lower (44.1 ± 2.7) than that observed in HeLa-BARD1-WT cells (74.5 ± 4.5; $P < 0.0001$). These results are in contrast to the case of RAP80 depletion that increases rates of damage-induced SCEs,[36,37] and support that HP1 and BARD1–HP1 binding are required for HR.

Discussion

In this study, we reported distinct mechanisms for the localization of the three BRCA1–BRCT binding proteins, FANCJ, CtIP, and RAP80, at DSB sites. Both FANCJ and CtIP localize at DSB sites through BARD1–HP1 interaction, whereas RAP80 localization at the DSB sites depends on the RNF8/RNF168 pathway. There is no cross-dependence; FANCJ and CtIP accumulation is independent of RNF168, and RAP80 accumulation is independent of HP1 (Fig. 8).

In the model, the accumulation of BRCA1 and 53BP1, key players for HR and NHEJ, respectively, is dependent on a common pathway mediated by RNF8/RNF168-induced conjugated ubiquitin. How the common pathway can promote the recruitment of two antagonistic factors to DSB sites has previously been unclear. However, recent evidence indicates that the BRCA1/Abraxas/RAP80 complex does not positively regulate HR. The BRCA1/Abraxas/RAP80 complex suppresses HR by inhibiting excess DNA resection, which is a known tuning function for HR.[36,37,49] Depletion of RAP80 does not inhibit RAD51 accumulation at DSB sites,[36,37] which is consistent with our result that RNF168 depletion does not suppress RAD51 accumulation. Because depletion of BRCA1 itself causes HR failure, these results suggest that distinct fractions of BRCA1 recruited by different mechanisms exist at DSB sites. Supporting this scenario, RNF8 depletion only suppressed the majority of, but not all, fractions of BRCA1 at DSB sites.[50] Inhibition of MDC1, a DNA repair factor that bridges γH2AX and RNF8 at DSB sites, only partially suppressed BRCA1 accumulation at DSB sites.[51] Finally, using the endonuclease I-PpoI-induced DSBs and a ChIP assay, Goldstein and Kastan recently showed that RAP80 is only required to target BRCA1 to chromatin regions flanking the DSBs that are up to 10 kb from the break sites, whereas BRCA1 binding to the DNA break points requires an alternative mechanism involving NBS1.[38] BRCA1 in the Abraxas/RAP80 complex at the flanking regions is phosphorylated at serines 1387 and 1423 by ATM and plays a critical role in S

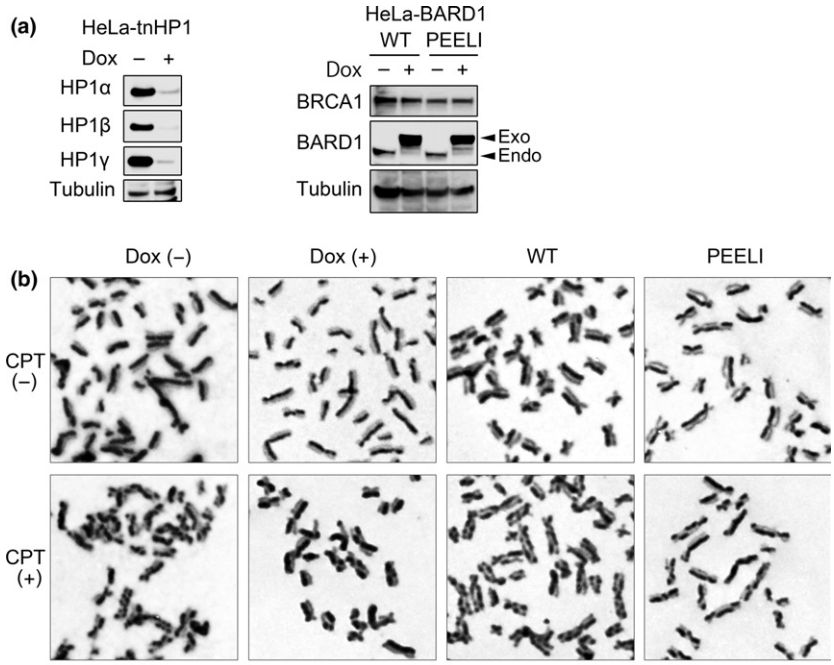

Fig. 7. Sister chromatid exchange (SCE) is suppressed in cells with HP1 deletion or BARD1 PEELI mutation. (a) HeLa cells conditionally depleted (Dox (+)) or not (Dox (−)) of HP1s, or HeLa cells conditionally substituted for endogenous BARD1 with wild-type (WT) or PEELI mutant were subjected to immunoblotting with the indicated antibodies. (b) Cells from (a) were treated with CPT-11 as indicated. Representative metaphase spreads with SCEs are shown. (c) Dot graph depicting the number of SCEs in cells. Each point denotes the total number of SCEs in an individual metaphase spread. Twenty metaphase spreads were analyzed in each sample. Bars and error bars indicate mean and SD, respectively.

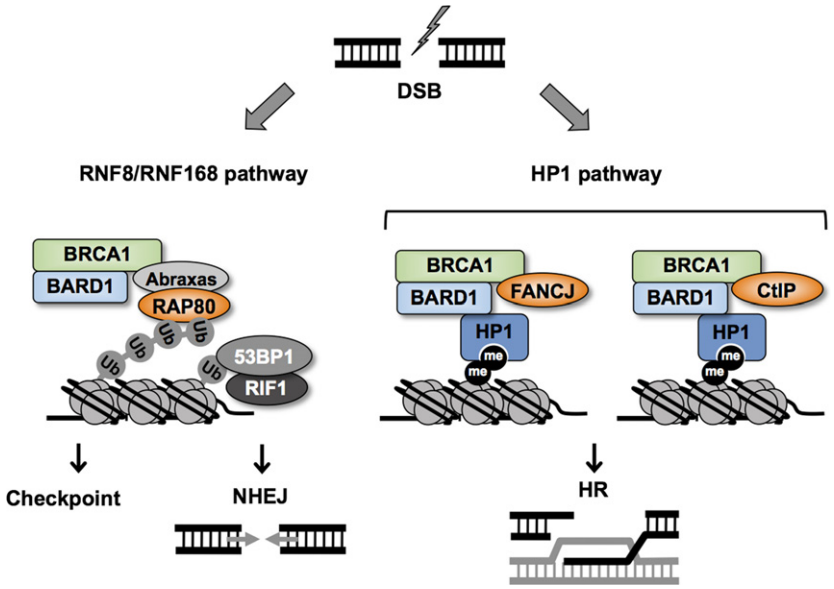

Fig. 8. Proposed model for the accumulation of BRCA1 complexes at DSB sites. BRCA1 constitutes three distinct protein complexes with Abraxas/RAP80, FANCJ, and CtIP through its tandem BRCT domains. In response to DSBs, the RAP80 complex accumulates at chromatin regions flanking DSBs through RNF8/RNF168-induced polyubiquitin chains. This accumulation fine tunes the length of ssDNA by suppressing excess DNA end resection and plays critical roles in S and G_2 phase checkpoints. The 53BP1/RIF1 complex, which is recruited by the same RNF8/RNF168 pathway, mediates NHEJ. In contrast, FANCJ and CtIP are accumulated at the DSB through an alternative pathway involving HP1 interactions with Lys9-methylated histone H3. The BRCA1 complexes in this pathway mediate HR. me, methylation; Ub, ubiquitin.

and G_2 cell cycle checkpoint activation, whereas BRCA1 at the break points was required for DNA religation.[38]

The mechanism required for BRCA1 binding to DNA break points to mediate HR is not fully understood. The I-PpoI system showed that NBS1 is essential for BRCA1 binding to DNA break points and for religation of DNA in both G_1-arrested cells and cycling cells.[38] While this observation indicates essential roles for NBS1 and BRCA1 at break points in NHEJ in G_1-arrested cells, they could also be required for HR in S and G_2 phase cells, especially when considering that

the MRN complex is known to be essential for initiating DSB processing in HR with its exo- and endonuclease activities through the Mre11 subunit.[52,53] CtIP interacts with MRN through NBS1 to undergo effective DNA end resection for HR that is assured by BRCA1 and overcomes the inhibitory effect of 53BP1-RIF1 on end resection.[24,26–29,54] Our results showing that HP1 and BARD1–HP1 interaction is required for the accumulation of BRCA1,[45] CtIP, and RAD51, but not for that of conjugated ubiquitin or RAP80, at DSB sites suggest that a mechanism involving HP1 is required for BRCA1 binding to DNA break points to mediate HR. The CtIP–NBS1 interaction may further strengthen this binding.

Because BRCA1 accumulated at DSB flanking regions theoretically comprises larger parts of a BRCA1 fraction than at DNA break points and because RNF8, RNF168, or RAP80 depletion inhibits the majority of BRCA1 accumulation,[10–12,30–35,50] one may expect that inhibition of the HP1 pathway disrupts only a small portion of BRCA1 accumulation. However, it is interesting that, although there was no cross-dependence between RNF168-mediated RAP80 accumulation and HP1-mediated FANCJ and CtIP accumulation, HP1 depletion or inhibition of the BARD1–HP1 interaction severely disrupted BRCA1 accumulation.[45] This result is consistent with findings that NBS1 depletion abolished BRCA1 accumulation at both DSB break points and flanking regions, whereas RAP80 depletion only inhibits BRCA1 binding at flanking regions.[38] One interpretation could be that BRCA1 complex formation with HP1 and NBS1 at the break points is a prerequisite for BRCA1 accumulation at DSB flanking regions through RAP80.

Although the role of CtIP in strand resection in HR of DSB repair has been relatively well characterized, how FANCJ contributes to DSB repair is rather complicated. With its ATPase/helicase activity, FANCJ contributes to multiple steps in HR.[55] It interacts with Bloom's syndrome helicase and MRE11 nuclease in the MRN complex and plays a critical role in DNA resection by unwinding the DNA duplex in the early phase of HR.[17,56–59] The FANCJ MRE11-associated mechanism is likely specific to DSBs, but not interstrand cross-links, because MRE11 is required for FANCJ accumulation at laser-induced DSBs, but not at psoralen-induced interstrand cross-links.[59] The FANCJ–MRE11 and CtIP–NBS1 interactions[22,23,54,60] suggest that FANCJ and CtIP function together at DNA break points. Indeed, FANCJ-deficient FA-J cells failed to recruit CtIP to DSBs.[59] Our results suggest that HP1 is essential for FANCJ and CtIP functions, which precede HR through BRCA1/BARD1.

In addition to its role in unwinding the DNA duplex, FANCJ is also able to remove RAD51 from ssDNA-RAD51 nucleoprotein filaments and to inhibit the RAD51 strand exchange reaction *in vitro*.[61] However, because FANCJ depletion reduces RAD51 accumulation at laser-induced DSB sites and compromises HR,[59] the destabilization of RAD51 nucleoprotein filament is not likely to simply inhibit HR in response to DSBs, but may contribute to the HR process after strand invasion or to prevent promiscuous recombination. Alternatively, this function of FANCJ could be specific to interstrand cross-link repair or replication stress because RAD51 accumulation is not reduced in FANCJ-depleted cells treated with mitomycin C[62] or hydroxyurea.[17] We showed that HP1 depletion or inhibition of BARD1–HP1 interaction suppresses DSB accumulation of RAD51, FANCJ, and CtIP, accompanied by inhibition of CPT-11-induced SCEs. These results suggest that HP1 is critical for the function of FANCJ and CtIP to positively regulate HR processing through RAD51 recruitment in response to DSBs.

In conclusion, we showed the essential role of HP1 in regulating HR through BRCA1/BARD1-mediated accumulation of FANCJ and CtIP at DSB sites. This mechanism may affect tumorigenesis and chemosensitivity and is thus of high clinical significance.

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Disclosure Statement

The authors have no conflict of interest.

Abbreviations

ATM	ataxia telangiectasia mutated
BARD1	BRCA1-associated RING domain 1
BRCA1	breast cancer 1
BRCT	BRCA1 C terminus
Dox	doxycycline
DSB	DNA double-strand break
FANCJ	Fanconi anemia group J
H3K9me2	Lys-9 dimethylated histone H3
HP1	heterochromatin protein 1
HR	homologous recombination
IR	ionizing irradiation
MRN	MRE11–RAD50–NBS1
NBS1	nibrin
NHEJ	non-homologous end joining
RNF	RING finger protein
SCE	sister chromatid exchange
ssDNA	single-strand DNA

References

1 Koboldt DC, Fulton RS, McLellan MD *et al.* Comprehensive molecular portraits of human breast tumours. *Nature* 2012; **490**: 61–70.
2 Waddell N, Pajic M, Patch A-M *et al.* Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature* 2015; **518**: 495–501.
3 Tutt A, Robson M, Garber JE *et al.* Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. *Lancet* 2010; **376**: 235–44.
4 Patch A-M, Christie EL, Etemadmoghadam D, Garsed DW, George J, Fereday S, *et al.* Whole-genome characterization of chemoresistant ovarian cancer. *Nature* 2015; **521**: 489–94.
5 Panier S, Durocher D. Push back to respond better: regulatory inhibition of the DNA double-strand break response. *Nat Rev Mol Cell Biol* 2013; **14**: 661–72.
6 Venkitaraman AR. Cancer suppression by the chromosome custodians, BRCA1 and BRCA2. *Science* 2014; **343**: 1470–5.
7 Jiang Q, Greenberg RA. Deciphering the BRCA1 tumor suppressor network. *J Biol Chem* 2015; **290**: 17724–32.
8 Cantor SB, Bell DW, Ganesan S *et al.* BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell* 2001; **105**: 149–60.
9 Yu X, Wu LC, Bowcock AM, Aronheim A, Baer R. The C-terminal (BRCT) domains of BRCA1 interact in vivo with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. *J Biol Chem* 1998; **273**: 25388–92.
10 Wang B, Matsuoka S, Ballif BA *et al.* Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. *Science* 2007; **316**: 1194–8.

11 Sobhian B, Shao G, Lilli DR *et al.* RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. *Science* 2007; **316**: 1198–202.
12 Kim H, Chen J, Yu X. Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. *Science* 2007; **316**: 1202–5.
13 Wu LC, Wang ZW, Tsan JT *et al.* Identification of a RING protein that can interact in vivo with the BRCA1 gene product. *Nat Genet* 1996; **14**: 430–40.
14 Hashizume R, Fukuda M, Maeda I *et al.* The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. *J Biol Chem* 2001; **276**: 14537–40.
15 Manke IA, Lowery DM, Nguyen A, Yaffe MB. BRCT repeats as phosphopeptide-binding modules involved in protein targeting. *Science* 2003; **302**: 636–9.
16 Yu X, Chini CCS, He M, Mer G, Chen J. The BRCT domain is a phosphoprotein binding domain. *Science* 2003; **302**: 639–42.
17 Litman R, Peng M, Jin Z *et al.* BACH1 is critical for homologous recombination and appears to be the Fanconi anemia gene product FANCJ. *Cancer Cell* 2005; **8**: 255–65.
18 Levran O, Attwooll C, Henry RT *et al.* The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. *Nat Genet* 2005; **37**: 931–3.
19 Levitus M, Waisfisz Q, Godthelp BC *et al.* The DNA helicase BRIP1 is defective in Fanconi anemia complementation group. *J. Nat Genet* 2005; **37**: 934–5.
20 Brosh RM, Cantor SB. Molecular and cellular functions of the FANCJ DNA helicase defective in cancer and in Fanconi anemia. *Front Genet* 2014; **5**: 372.
21 Greenberg RA, Sobhian B, Pathania S, Cantor SB, Nakatani Y, Livingston DM. Multifactorial contributions to an acute DNA damage response by BRCA1/BARD1-containing complexes. *Genes Dev* 2006; **20**: 34–46.
22 Sartori AA, Lukas C, Coates J *et al.* Human ctip promotes DNA end resection. *Nature* 2007; **450**: 509–14.
23 You Z, Shi LZ, Zhu Q *et al.* CtIP links DNA double-strand break sensing to resection. *Mol Cell* 2009; **36**: 954–69.
24 Limbo O, Chahwan C, Yamada Y, de Bruin RAM, Wittenberg C, Russell P. Ctp1 is a cell-cycle-regulated protein that functions with Mre11 complex to control double-strand break repair by homologous recombination. *Mol Cell* 2007; **28**: 134–46.
25 Chapman JR, Taylor MRG, Boulton SJ. Playing the end game: DNA double-strand break repair pathway choice. *Mol Cell* 2012; **47**: 497–510.
26 Tommiska J, Bartek J, Drapkin R *et al.* 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. *Nat Struct Mol Biol* 2010; **17**: 688–95.
27 Bunting SF, Callén E, Wong N *et al.* 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* 2010; **141**: 243–54.
28 Escribano-Díaz C, Orthwein A, Fradet-Turcotte A *et al.* A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol Cell* 2013; **49**: 872–83.
29 Cruz-García A, López-Saavedra A, Huertas P. BRCA1 accelerates CtIP-mediated DNA-end resection. *Cell Rep* 2014; **9**: 451–9.
30 Kolas NK, Chapman JR, Nakada S *et al.* Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science* 2007; **318**: 1637–40.
31 Mailand N, Bekker-Jensen S, Faustrup H *et al.* RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* 2007; **131**: 887–900.
32 Huen MSY, Grant R, Manke I *et al.* RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. *Cell* 2007; **131**: 901–14.
33 Doil C, Mailand N, Bekker-Jensen S *et al.* RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. *Cell* 2009; **136**: 435–46.
34 Stewart GS, Panier S, Townsend K *et al.* The RIDDLE syndrome protein mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. *Cell* 2009; **136**: 420–34.
35 Mattiroli F, Vissers JHA, van Dijk WJ *et al.* RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. *Cell* 2012; **150**: 1182–95.
36 Hu Y, Scully R, Sobhian B, Xie A, Shestakova E, Livingston DM. RAP80-directed tuning of BRCA1 homologous recombination function at ionizing radiation-induced nuclear foci. *Genes Dev* 2011; **25**: 685–700.
37 Coleman KA, Greenberg RA. The BRCA1-RAP80 complex regulates DNA repair mechanism utilization by restricting end resection. *J Biol Chem* 2011; **286**: 13669–80.
38 Goldstein M, Kastan MB. Repair versus checkpoint functions of BRCA1 are differentially regulated by site of chromatin binding. *Cancer Res* 2015; **75**: 2699–707.
39 Ayoub N, Jeyasekharan AD, Bernal JA, Venkitaraman AR. HP1-β mobilization promotes chromatin changes that initiate the DNA damage response. *Nature* 2008; **453**: 682–6.
40 Price BD, D'Andrea AD. Chromatin remodeling at DNA double-strand breaks. *Cell* 2013; **152**: 1344–54.
41 Luijsterburg MS, Dinant C, Lans H *et al.* Heterochromatin protein 1 is recruited to various types of DNA damage. *J Cell Biol* 2009; **185**: 577–86.
42 Lee Y-H, Kuo C-Y, Stark JM, Shih H-M, Ann DK. HP1 promotes tumor suppressor BRCA1 functions during the DNA damage response. *Nucleic Acids Res* 2013; **41**: 5784–98.
43 Khurana S, Kruhlak MJ, Kim J *et al.* A macrohistone variant links dynamic chromatin compaction to BRCA1-dependent genome maintenance. *Cell Rep* 2014; **21**: 1049–62.
44 Baldeyron C, Soria G, Roche D, Cook AJL, Almouzni G. HP1 recruitment to DNA damage by p150caf-1 promotes homologous recombination repair. *J Cell Biol* 2011; **193**: 81–95.
45 Wu W, Nishikawa H, Fukuda T *et al.* Interaction of BARD1 and HP1 is required for BRCA1 retention at sites of DNA damage. *Cancer Res* 2015; **75**: 1311–21.
46 Nishikawa H, Ooka S, Sato K *et al.* Mass spectrometric and mutational analyses reveal Lys-6-linked polyubiquitin chains catalyzed by BRCA1-BARD1 ubiquitin ligase. *J Biol Chem* 2004; **279**: 3916–24.
47 Sato K, Hayami R, Wu W *et al.* Nucleophosmin/B23 is a candidate substrate for the BRCA1-BARD1 ubiquitin ligase. *J Biol Chem* 2004; **279**: 30919–22.
48 Joukov V, Chen J, Fox EA, Green JB, Livingston DM. Functional communication between endogenous BRCA1 and its partner, BARD1, during *Xenopus laevis* development. *Proc Natl Acad Sci U S A* 2001; **98**: 12078–83.
49 Hu Y, Petit SA, Ficarro SB *et al.* PARP1-driven poly-ADP-ribosylation regulates BRCA1 function in homologous recombination-mediated DNA repair. *Cancer Discov* 2014; **4**: 1430–47.
50 Nakada S, Yonamine RM, Matsuo K. RNF8 regulates assembly of RAD51 at DNA double-strand breaks in the absence of BRCA1 and 53BP1. *Cancer Res* 2012; **72**: 4974–83.
51 Bekker-Jensen S, Lukas C, Kitagawa R *et al.* Spatial organization of the mammalian genome surveillance machinery in response to DNA strand breaks. *J Cell Biol* 2006; **173**: 195–206.
52 Williams RS, Williams JS, Tainer JA. Mre11-Rad50-Nbs1 is a keystone complex connecting DNA repair machinery, double-strand break signaling, and the chromatin template. *Biochem Cell Biol* 2007; **85**: 509–20.
53 Nimonkar AV, Genschel J, Kinoshita E *et al.* BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. *Genes Dev* 2011; **25**: 350–62.
54 Chen L, Nievera CJ, Lee AY-L, Wu X. Cell cycle-dependent complex formation of BRCA1.CtIP.MRN is important for DNA double-strand break repair. *J Biol Chem* 2008; **283**: 7713–20.
55 Brosh RM. DNA helicases involved in DNA repair and their roles in cancer. *Nat Rev Cancer* 2013; **13**: 542–58.
56 Cantor S, Drapkin R, Zhang F *et al.* The BRCA1-associated protein BACH1 is a DNA helicase targeted by clinically relevant inactivating mutations. *Proc Natl Acad Sci U S A* 2004; **101**: 2357–62.
57 Gupta R, Sharma S, Sommers JA, Jin Z, Cantor SB, Brosh RM. Analysis of the DNA substrate specificity of the human BACH1 helicase associated with breast cancer. *J Biol Chem* 2005; **280**: 25450–60.
58 Suhasini AN, Rawtani NA, Wu Y *et al.* Interaction between the helicases genetically linked to Fanconi anemia group J and Bloom's syndrome. *EMBO J* 2011; **30**: 692–705.
59 Suhasini AN, Sommers JA, Muniandy PA *et al.* Fanconi anemia group J helicase and MRE11 nuclease interact to facilitate the DNA damage response. *Mol Cell Biol* 2013; **33**: 2212–27.
60 Yuan J, Chen J. N terminus of CtIP is critical for homologous recombination-mediated double-strand break repair. *J Biol Chem* 2009; **284**: 31746–52.
61 Sommers JA, Rawtani N, Gupta R *et al.* FANCJ uses its motor ATPase to destabilize protein-DNA complexes, unwind triplexes, and inhibit RAD51 strand exchange. *J Biol Chem* 2009; **284**: 7505–17.
62 Peng M, Xie J, Ucher A, Stavnezer J, Cantor SB. Crosstalk between BRCA-Fanconi anemia and mismatch repair pathways prevents MSH2-dependent aberrant DNA damage responses. *EMBO J* 2014; **33**: 1698–712.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Time course of the accumulation of repair proteins at DNA double-strand break sites.