Class switch recombination (CSR) is a process by which antibody diversity is generated. This is achieved through the recombination of highly repetitive DNA elements, which are separated by 60–200 kbp. CSR involves the production of multiple DNA double-strand breaks (DSBs) in switch regions, which are then repaired by a mechanism that requires an intact DNA damage response and classical or alternative nonhomologous end joining (A-NHEJ). Among the DNA damage response factors, 53BP1 has the most profound effect on CSR. We explore the role of 53BP1 in intrachromosomal DNA repair using I-SceI to introduce paired DSBs in the IgH locus. We find that the absence of 53BP1 results in an ataxia telangiectasia mutated–dependent increase in DNA end resection and that resected DNA is preferentially repaired by microhomology-mediated A-NHEJ. We propose that 53BP1 favors long-range CSR in part by protecting DNA ends against resection, which prevents A-NHEJ–dependent short-range rejoining of intra–switch region DSBs.

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Soulsas-Spraul et al., 2007; Yan et al., 2007; Boboila et al., 2010a,b). C-NHEJ ligates DSBs with little or no microhomology and appears to be the dominant pathway involved in CSR, based on the physiological predominance of blunt or small microhomology switch joins (Yan et al., 2007; Boboila et al., 2010a,b). In contrast, little is known about the factors that mediate A-NHEJ (Haber, 2008). However, this is a robust pathway that makes extensive use of junctional microhomologies and can reconstitute up to 20–50% of CSR in the absence of C-NHEJ (Yan et al., 2007; Boboila et al., 2010a,b). A-NHEJ appears to be kinetically slower than C-NHEJ and mediates many of the translocations that are rare byproducts of V(D)J recombination and CSR (Zhu et al., 2002; Han and Yu, 2008; Wang et al., 2008, 2009; Xie et al., 2009; Boboila et al., 2010b).

DSBs incurred during CSR activate the DNA damage response, as indicated by the accumulation of foci of Mre11/Rad50/Nbs1 (MRN), H2AX, and 53BP1 on the IgH locus during CSR (Petersen et al., 2001; Reina-San-Martin et al., 2003). DNA damage response factors are also required for efficient CSR. Deficiency in any of these or ataxia telangiectasia mutated (ATM), a key mediator of the DNA damage response, leads to inefficient switching and concomitant accumulation of DNA damage on chromosome 12 (Reina-San-Martin et al., 2004; Franco et al., 2006; Ramiro et al., 2006; Jankovic et al., 2007). Among DNA damage response factors, the most pronounced defect in CSR occurs upon loss of 53BP1, a chromatin binding protein that is also an ATM-dependent protein kinase (DNA-PKcs substrate (DiTullio et al., 2002; Manis et al., 2004; Ward et al., 2004; Callén et al., 2007). Similarly, the absence of 53BP1 leads to a joining defect between distal DSBs during V(D)J recombination and a reduced rate of transchromosomal fusions of unprotected telomeres (Difilippantonio et al., 2008; Dimitrova et al., 2008). In contrast, ISDs and chromosome translocations, both of which appear to be mediated primarily by A-NHEJ, are either increased or not affected by the loss of 53BP1 (Ramiro et al., 2006; Reina-San-Martin et al., 2007). In addition, overexpression of a dominant-negative fragment of 53BP1 enhances homologous recombination (HR), as determined using a reporter substrate (Xie et al., 2007).

Several nonmutually exclusive models have been proposed to account for the effects of 53BP1 on CSR, V(D)J recombination, HR, telomere fusion, and chromosome translocation. 53BP1 may enhance synopsis and long-range interactions between two distal DSBs (Manis et al., 2004; Ward et al., 2004; Reina-San-Martin et al., 2007), possibly by altering local chromatin structure (Difilippantonio et al., 2008) or increasing chromatin mobility (Dimitrova et al., 2008). Alternatively, it may regulate repair by favoring one end-joining pathway or another, and thereby control the relative importance of microhomology in the joining process. However, there is little direct experimental data to support these ideas.

In this paper, we report experiments that investigate the function of 53BP1 using the I-SceI meganuclease to introduce site-specific DSBs in IgH in B lymphocytes undergoing CSR. We show that 53BP1 regulates the choice of end-joining pathways by interfering with resection, thereby favoring C-NHEJ.
Cre-induced CSR in AID-deficient IgH\textsuperscript{I-96k} B cells and control IgH\textsuperscript{I-96k} B cells (Fig. 1). B cells were stimulated with LPS and IL-4, and infected with a Cre-encoding retrovirus or an inactive retrovirus Cre\textsuperscript{*}. Infected cells were identified by GFP expression and CSR was measured by flow cytometry. Cre expression in AID-sufficient IgH\textsuperscript{I-96k} B lymphocytes increased CSR to IgG1 from 3.8 to 14.4% and from 16.2 to 32.2% at 72 and 96 h after stimulation, respectively (Fig. 1 B). However, the rate of CSR in IgH\textsuperscript{I-96k} B cells was similar in the presence or absence of AID 96 h after stimulation (Fig. 1, B and C).

To determine whether the loss of 53BP1 alters the efficiency of Cre-mediated CSR, we compared the frequency of recombination between IgH\textsuperscript{I-96k}AID\textsuperscript{-/-}53BP1\textsuperscript{-/-} and IgH\textsuperscript{I-96k}AID\textsuperscript{-/-} B cells. We found that CSR in Cre-infected IgH\textsuperscript{I-96k}AID\textsuperscript{-/-}53BP1\textsuperscript{-/-} B cells was indistinguishable from 53BP1-proficient cells (Fig. 1, C and D). We conclude that neither AID nor 53BP1 alters the overall structure of the IgH locus sufficiently to change the rate of synapsis between the IgH\textsuperscript{I-96k} loxP sites.

I-SceI endonuclease–induced CSR

To further investigate the role of 53BP1 in CSR, we made use of I-SceI, which produces DSBs independently of synapsis. IgH\textsuperscript{I-96k}AID\textsuperscript{-/-} B cells were stimulated with LPS and IL-4, and infected with retroviruses encoding I-SceI or an inactive form of the enzyme I-SceI\textsuperscript{*} (Fig. S2). Switching to IgG1 by retrovirally infected B cells was initially measured by flow cytometry (Fig. 2 B).

In contrast to Cre, I-SceI mediated CSR was inefficient in IgH\textsuperscript{I-96k}AID\textsuperscript{-/-} B cells, with a mean recombination frequency of 0.54% 3 d after retroviral infection (five independent experiments; Fig. 2 C and Table S1). The observed rate of I-SceI–induced CSR was somewhat lower than the rate obtained in B cells derived by RAG-blastocyst complementation (Zarrin et al., 2007). However, our experiments differ from previous experiments in several respects: AID was deleted and switch regions were present in our experiments, also, we used only a single I-SceI site in each location as opposed to two I-SceI sites, which doubles the number of breaks.

To determine whether loss of 53BP1 affects the joining efficiency of I-SceI breaks, we assayed IgH\textsuperscript{I-96k}AID\textsuperscript{-/-}53BP1\textsuperscript{-/-} B cells. In contrast to Cre, where we observed no effects of 53BP1 deficiency, the I-SceI recombination frequency was significantly reduced in IgH\textsuperscript{I-96k}AID\textsuperscript{-/-}53BP1\textsuperscript{-/-} B cells to 0.31% (five independent experiments; P = 0.0037; Fig. 2 C and Table S1). To obtain an independent measure of recombination frequency, we examined joining between I-SceI sites by sample dilution PCR. In agreement with the

Figure 1. Cre recombinase induces efficient CSR to IgG1 independently of AID and 53BP1. (A) Schematic representation of the IgH\textsuperscript{I-96k} allele (top) and the Cre-induced recombinant that encodes IgG1 (bottom). LoxP sites are indicated as red triangles, and I-SceI sites are indicated as blue circles. (B) Representative flow cytometry experiments showing CSR to IgG1 of IgH\textsuperscript{I-96k} B cells infected with retroviruses encoding Cre or catalytically inactive Cre\textsuperscript{*}. IgG1 expression was analyzed at 72 and 96 h after LPS and IL-4 stimulation. (C) Experiment as in B but for IgH\textsuperscript{I-96k}AID\textsuperscript{-/-} and IgH\textsuperscript{I-96k}AID\textsuperscript{-/-}53BP1\textsuperscript{-/-} B cells analyzed at 96 h after LPS and IL-4 stimulation. (D) Graph shows the results of three independent flow cytometry experiments measuring CSR to IgG1 after Cre infection of IgH\textsuperscript{I-96k}AID\textsuperscript{-/-} and IgH\textsuperscript{I-96k}AID\textsuperscript{-/-}53BP1\textsuperscript{-/-} B cells. The means are shown as horizontal lines. FSC, forward scatter.
flow cytometry analysis, we found that the joining efficiency in stimulated IgH<sup>I-96kAID<sup>-/-</sup></sup> B lymphocytes was significantly reduced from 0.76 to 0.48% in the absence of 53BP1 (P = 0.0002; Fig. 2 D and Table S2). We conclude that loss of 53BP1 decreases the efficiency of recombination between I-SceI sites in IgH<sup>I-96kAID<sup>-/-</sup></sup> B cells; however, the effect is far less penetrant than it is for CSR.

### 53BP1 prevents processing of broken ends

53BP1 has been implicated in regulating the choice between HR and NHEJ DNA repair pathways in the S or G2 phase of the cell cycle. CSR does not appear to involve HR and occurs in the G1 phase of the cell cycle (Petersen et al., 2001). Nevertheless, CSR-induced DSBs can be joined by two different pathways: C-NHEJ or A-NHEJ (Yan et al., 2007; Boboila et al., 2010a,b).

To determine whether 53BP1 affects the choice between C-NHEJ and A-NHEJ, we characterized the joins between I-SceI sites from IgH<sup>I-96kAID<sup>-/-</sup></sup>53BP1<sup>-/-</sup> B cells and IgH<sup>I-96kAID<sup>-/-</sup></sup> controls. Precise joining between I-SceI sites yields a 336-nt PCR product (Fig. 3 A), which was the predominant species in control IgH<sup>I-96kAID<sup>-/-</sup></sup> B cells (Fig. 3 B). End processing results in lower molecular weight species, which can be scored directly by counting the number of products that run ≤300 nt (i.e., more than ~30 nt total end resection). The loss of 53BP1 resulted in an overall increase in the number of PCR products, with lower than expected molecular weight to 51.6% of all products. (P = 0.0001; Fig. 3 C and Table S3).

The precise molecular structure of the joins was determined by sequencing. Although the mean extent of end processing was 34.7 nt in IgH<sup>I-96kAID<sup>-/-</sup></sup> B cells, it was 66.8 nt in the absence of 53BP1 (P = 0.012; Fig. 3 D and Table S4). Consistent with the idea that ends are more frequently processed in the absence of 53BP1, the number of precise joins that reconstituted the I-SceI site also decreased from 30.9 to 13.3% in 53BP1<sup>-/-</sup> B cells (P = 0.0041; Fig. 3 E and

![Figure 2. Loss of 53BP1 decreases the joining efficiency of two distal I-SceI–induced DSBs.](image-url)

**Figure 2.** Loss of 53BP1 decreases the joining efficiency of two distal I-SceI–induced DSBs. (A) Schematic representation of the IgH<sup>I-96k</sup> allele (top) and the I-SceI–induced recombinant that encodes IgG1 (bottom). LoxP sites are indicated as red triangles, and I-SceI sites are indicated as blue circles. (B) Representative flow cytometry experiments showing CSR to IgG1 of IgH<sup>I-96kAID<sup>-/-</sup></sup> and IgH<sup>I-96kAID<sup>-/-</sup>53BP1<sup>-/-</sup></sup> B cells 72 h after the first infection with an I-SceI–encoding retrovirus. (C) Graph shows the results of five independent flow cytometry experiments, with each dot representing an individual experiment. The p-value was calculated using a two-tailed paired Student’s t test. The means are shown as horizontal lines. (D) Bar graph showing I-SceI to I-SceI recombination frequency in the presence and absence of 53BP1, determined by nine independent PCR experiments. Error bars indicate standard deviation. The p-value was calculated using a two-tailed paired Student’s t test. FSC, forward scatter.
Inhibition of ATM kinase decreases resection DNA end resection by combined action of MRN, CtIP, Bloom’s helicase, and Exo1 is essential for HR (Mimitou and Symington, 2009). Optimal resection and formation of the single-stranded DNA (ssDNA) substrate for HR requires ATM, because this kinase facilitates the recruitment and activation of the nucleases that attack DNA ends (Jazayeri et al., 2006; You et al., 2009). Although HR does not appear to be involved in CSR, we investigated whether a related mechanism is involved in processing DNA ends for microhomology-based A-NHEJ.

To determine how the loss of ATM activity might affect the processing of I-SceI breaks, we treated IgH I-96k AID−/− and IgH I-96k AID−/− 53BP1−/− B cells, respectively, which is characteristic of C-NHEJ (Fig. 4 and Fig. S3). In contrast, the majority of joining events that involved extensive resection (≥30 nt) used 3 nt or more of junctional microhomology, with a mean of 3.6 and 4 nt for AID−/− and AID−/− 53BP1−/− B cells, respectively, indicative of A-NHEJ (P < 0.0001 for AID−/−; P = 0.0004 for AID−/− 53BP1−/−; Fig. 4 C and Fig. S3). These data show that loss of 53BP1 leads to increased resection of DNA ends, and that resection is associated with microhomology-mediated end joining irrespective of 53BP1.

Table S5). Interestingly, all of the minimally processed joins showed 0–4 nt of junctional microhomology, with a mean of 1.5 and 2 nt for AID−/− and AID−/− 53BP1−/− B cells, respectively, which is characteristic of C-NHEJ (Fig. 4 and Fig. S3). In contrast, the majority of joining events that involved extensive resection (≥30 nt) used 3 nt or more of junctional microhomology, with a mean of 3.6 and 4 nt for AID−/− and AID−/− 53BP1−/− B cells, respectively, indicative of A-NHEJ (P < 0.0001 for AID−/−; P = 0.0004 for AID−/− 53BP1−/−; Fig. 4 C and Fig. S3). These data show that loss of 53BP1 leads to increased resection of DNA ends, and that resection is associated with microhomology-mediated end joining irrespective of 53BP1.

Figure 3. Loss of 53BP1 leads to increased end resection. (A) Schematic representation of IgH I-96k allele (top) with the PCR primers used to amplify a 336-nt recombination product, indicated as arrows (bottom). LoxP sites are indicated as red triangles, and I-SceI sites are indicated as blue circles. (B) Representative ethidium bromide–stained agarose gels showing PCR products obtained after I-SceI–induced recombination in IgH I-96k AID−/− and IgH I-96k AID−/− 53BP1−/− B cells. (C) Bar graphs showing frequency of I-SceI–induced recombination products running ≤300 nt for IgH I-96k AID−/− and IgH I-96k AID−/− 53BP1−/− B cells, determined by 12 independent PCR experiments. Error bars indicate standard deviation. The p-value was calculated using a two-tailed Student’s t test. (D) Dot plot showing total resection of I-SceI–infected IgH I-96k AID−/− and IgH I-96k AID−/− 53BP1−/− B cells. Each dot represents one sequence. The p-value was calculated using a two-tailed Student’s t test. (E) Bar graph shows the frequency of perfect I-SceI joins in I-SceI–infected IgH I-96k AID−/− and IgH I-96k AID−/− 53BP1−/− B cells in five independent experiments. Error bars indicate standard deviation. The p-value was calculated using a two-tailed Student’s t test.
B cells with a small molecule ATM inhibitor (ATMi). In contrast to loss of 53BP1, ATMi did not significantly affect the joining rate of paired I-SceI breaks on the IgH locus, as measured by flow cytometry (Fig. 5, A and B; and Table S6). However, ATMi-treated IgH^{+/−} AID−/− B cells showed significantly reduced DNA end resection (17.8% ATMi vs. 34.1% control; P = 0.0024; Fig. 5, C and D; and Tables S3 and S7) and a concomitant increase in the number of precise I-SceI joins (47.6% ATMi treated vs. 30.9% control; P = 0.026; Fig. 5 E; and Tables S5 and S8).

To determine whether the increased end resection in the absence of 53BP1 is ATM dependent, we examined end resection in ATMi-treated IgH^{+/−} AID−/− 53BP1−/− B cells. ATMi induced a pronounced decrease in end resection in IgH^{+/−} AID−/− 53BP1−/− B cells (18.1% ATMi treated vs. 51.6% untreated IgH^{+/−} AID−/− 53BP1−/− control; P = 0.0003; Fig. 5, C and D; and Tables S3 and S7) and a concomitant increase in precise I-SceI joins (34.2% ATMi vs. 13.3% untreated control; P = 0.0021; Fig. 5 E; and Tables S5 and S8). Thus, the increase in I-SceI–induced DNA end processing observed in the absence of 53BP1 is dependent on ATM.

Loss of 53BP1 interferes with CSR, but enhances recombination between repeat DNA within switch regions (Manis et al., 2004; Ward et al., 2004; Ramiro et al., 2006; Reina-San-Martín et al., 2007). Because the latter requires DNA end processing, we examined whether inhibition of DNA end processing by ATMi might enhance CSR in 53BP1−/− B cells. In agreement with published studies (Lumsden et al., 2004; Reina-San-Martín et al., 2004; Callén et al., 2007), ATMi reduces CSR to IgG1 in WT B cells (11.6% ATMi vs. 21.5% untreated control; P = 0.0004; Fig. 6, A and B; and Table S9). In striking contrast, ATMi enhanced CSR in 53BP1−/− B cells (3.2% ATMi vs. 1.3% untreated controls; P = 0.0012; Fig. 6, A and B; and Table S9). We conclude that ATMi ameliorates the severe defect in CSR observed in the absence of 53BP1.

DISCUSSION

DSBs are dangerous lesions, which, if left unrepaired, can lead to genomic instability and genome rearrangements. These potentially cytotoxic lesions are repaired either by HR, which is conservative and error free, or by NHEJ, which frequently leads to deletions and insertions. NHEJ is mediated by at least two separate pathways: C-NHEJ, which does not require DNA end resection and produces direct joins with little or no microhomology (Lieber, 2008), and A-NHEJ, which is a robust alternative pathway that makes extensive use of microhomology and therefore requires resection of ends (Haber, 2008). Of the two NHEJ pathways, C-NHEJ is the more conservative because there is no obligatory loss of genetic material.

DSBs can be repaired with a high degree of fidelity during the S and G2 phases of the cell cycle by HR, which uses the undamaged sister chromatid as a template for repair. HR requires 5′ to 3′ resection of DNA ends to produce ssDNA, which recruits replication protein A, leading to deposition of Rad51, a factor that is essential in homology search (Sung and Klein, 2006). End resection is believed to occur during two stages: in the first phase, relatively short stretches of ssDNA are produced by the combined action of MRN and CtIP; in the second phase, longer stretches of ssDNA are produced by the combined action of Bloom’s helicase and Exo1 (Sartori et al., 2007; Gravel et al., 2008; Mimitou and Symington, 2008; Nimonkar et al., 2008; Zhu et al., 2008; Rass et al., 2009; Xie et al., 2009). ATM is implicated as a regulator of the resection process because it phosphorylates all of the enzymes known to be involved in resection (Cortez et al., 2001), and is required for optimal ATR activation and for CtIP recruitment to DNA ends (Cuadrado et al., 2006;
Jazayeri et al., 2006; Myers and Cortez, 2006; Sung and Klein, 2006). Recent work from several laboratories indicates that the critical choice between HR and NHEJ in the S/G2/M phases of the cell cycle is regulated at the level of DNA end resection (Huertas et al., 2008; Huertas and Jackson, 2009; Yun and Hiom, 2009).

In the absence of a template sister chromatid during CSR in G1, DSBs are repaired by error-prone C-NHEJ or Jazayeri et al., 2006; Myers and Cortez, 2006; Sung and Klein, 2006). Recent work from several laboratories indicates that the critical choice between HR and NHEJ in the S/G2/M phases of the cell cycle is regulated at the level of DNA end resection (Huertas et al., 2008; Huertas and Jackson, 2009; Yun and Hiom, 2009).

Figure 5. Increased DNA end resection in the absence of 53BP1 is dependent on ATM. (A) Representative flow cytometry experiment showing CSR to IgG1 by IgH I-96k AID−/− B cells infected with an I-SceI-encoding retrovirus in the presence or absence of ATMi. (B) Graph shows the results of three independent flow cytometry experiments, with each dot representing an individual experiment. The means are shown as horizontal lines. (C) Representative ethidium bromide-stained agarose gels showing the PCR amplification products after I-SceI–induced recombination of IgH I-96k AID−/− and IgH I-96k AID−/− 53BP1−/− B cells in the presence or absence of ATMi. (D) Bar graph showing the frequency of I-SceI–induced recombination products running ≤300 nt for IgH I-96k AID−/− and IgH I-96k AID−/− 53BP1−/− B cells in the presence or absence of ATMi. Error bars indicate standard deviation. p-values were calculated using a two-tailed Student’s t test (three independent experiments). (E) Frequency of direct I-SceI joins, reconstituting an I-SceI site, determined by sequencing of individual molecular products of I-SceI–infected IgH I-96k AID−/− and IgH I-96k AID−/− 53BP1−/− B cells in the presence or absence of ATMi. Error bars indicate standard deviation. p-values were calculated using a two-tailed Student’s t test (three independent sequencing experiments). FSC, forward scatter.
A-NHEJ. However, little is known about the choice between C-NHEJ and A-NHEJ. Under physiological circumstances, the majority of switch joins are blunt or show minimal microhomology, suggesting that C-NHEJ is the dominant pathway (Stavnezer et al., 2008). Nevertheless, A-NHEJ is a robust pathway that can reconstitute up to 50% of normal levels of CSR in the absence of core C-NHEJ factors such as ligase IV, XRCC4, or Ku70/80, or even the combination of Ku70 and ligase IV (Corneo et al., 2007; Yan et al., 2007; Boboila et al., 2010a,b). A-NHEJ can also mediate plasmid recircularization in transfected cells (Kabotyanski et al., 1998), joining of I-SceI breaks (Guirouilh-Barbat et al., 2007), oncogenic translocations (Zhu et al., 2002; Ramiro et al., 2006; Wang et al., 2008, 2009), and, finally, V(D)J recombination when the end protection function of the RAG recombinase is disabled (Lee et al., 2004). However, other than the preponderance of microhomologies found at the junctions (Haber, 2008; Zha et al., 2009) and the suggestion that MRN is required for A-NHEJ (Deng et al., 2009; Dinkelmann et al., 2009; Rass et al., 2009; Xie et al., 2009), this pathway remains poorly defined.

In addition to NHEJ, the DNA damage response is also essential for physiological CSR (Jankovic et al., 2007). Among the factors that mediate this response, 53BP1 has the most profound effect on CSR and specifically affects long-range joining between different switch regions (Manis et al., 2004; Reina-San-Martin et al., 2007). In contrast, 53BP1-deficient B cells show increased short-range intra-switch joining, which involves ligation of highly repetitive DNA (Reina-San-Martin et al., 2007). These observations and the related findings that loss of 53BP1 results in decreased V(D)J recombination between distal gene segments (Difilippantonio et al., 2008) and transchromosomal fusions of deprotected telomeres (Dimitrova et al., 2008) support the hypothesis that 53BP1 plays a role in synopsis during CSR. An alternative, nonexclusive explanation of the dominant effect of 53BP1 on long-range joining, which is strongly supported by our data, is that this protein disfavors short-range joining between repetitive sequences in the switch region by blocking the processing of DNA ends. Specifically, end processing is essential for the production of the ssDNA required for microhomology-based A-NHEJ. Therefore, by interfering with end processing, 53BP1 normally impairs A-NHEJ, which leads to enhanced C-NHEJ. Conversely, loss of 53BP1 favors ISDs by enhancing resection-dependent A-NHEJ and disfavors C-NHEJ-dependent long-range recombination between two nonhomologous switch regions. Consistent with this idea, inhibition of end resection by interfering with ATM activity enhances CSR in 53BP1-deficient B cells.

However, a switch from C-NHEJ to A-NHEJ is not likely to account for the profound defect in CSR in 53BP1 mutant B cells, as there is persistent CSR at up to 50% of normal levels despite increased ISDs in the complete absence of C-NHEJ (Yan et al., 2007; Han and Yu, 2008; Boboila et al., 2010a,b). We therefore propose a model in which 53BP1 has two roles in CSR: it favors synapsis between distal DSBs and also interferes with end resection and A-NHEJ. Alternatively, the rapid formation of a synapse in the presence of 53BP1 could prevent nuclease access and extensive DNA end resection. However, the preponderance of microhomologies found at the junctions (Haber, 2008; Zha et al., 2009) and the suggestion that MRN is required for A-NHEJ (Deng et al., 2009; Dinkelmann et al., 2009; Rass et al., 2009; Xie et al., 2009), this pathway remains poorly defined.

Figure 6. Inhibition of ATM leads to partial rescue of the CSR defect in 53BP1−/− B cells. (A) Representative flow cytometry experiment showing CSR to IgG1 by WT and 53BP1−/− B cells 96 h after LPS, IL-4, and RP105 stimulation in the presence or absence of ATMi. (B) Graph summarizing CSR to IgG1 by WT and 53BP1−/− B cells in four independent experiments in the presence or absence of ATMi. The means are shown as horizontal lines. p-values were calculated using a two-tailed Student’s t test. FSC, forward scatter.
appears to be a key event in the choice between C-NHEJ and A-NHEJ. Furthermore, as is the case for HR, where ATM is required for the efficient recruitment and activation of the factors mediating DNA end resection (Cudradato et al., 2006; Jazayeri et al., 2006; Myers and Cortez, 2006; Sung and Klein, 2006; You et al., 2009), ATM also regulates the resection that precedes A-NHEJ. However, in contrast to S and G2, where end resection stimulates HR and preservation of genetic material, resection in G1 leads to its obligate loss. Our data suggest a model wherein 53BP1 blocks access of nucleases to DNA ends, which favors C-NHEJ. We speculate that the requirement for obligate deletion of genetic information during A-NHEJ may explain why C-NHEJ, which is more conservative, has evolved as the preferential end-joining pathway in the G1 phase of the cell cycle.

MATERIALS AND METHODS

Mice. IgH Δ94 mice were produced by sequential gene targeting of C57BL/6 embryonic stem cells (Fig. S1). AID−/− mice (Muramatsu et al., 2000) were backcrossed to C57BL/6 for at least 10 generations, and 53BP1−/− mice (Ward et al., 2003) were backcrossed to C57BL/6 for at least 5 generations. All experiments were in agreement with protocols approved by the Rockefeller University and the National Institutes of Health Institutional Animal Care and Use Committee.

B cell cultures. B lymphocytes were isolated from mouse spleen using anti-CD43 MicroBeads (Miltenyi Biotec) and cultured at 0.5 × 10⁶ cells/ml in R10 medium (RPMI 1640 supplemented with 1-glutamine, sodium pyruvate, Hepes, 50 µM 2-mercaptoethanol, antibiotic/antimycotic, and 10% fetal calf serum; Hyclone). For B cell stimulation 25 µg/ml LPS (Sigma-Aldrich) and 5 ng/ml of mouse recombinant IL-4 (Sigma-Aldrich) were added to the cultures. After 6 h, retroviral supernatants were replaced with LPS-stimulation, and spinoculated at 1,150 g for 10 min in 5 mM CFSE. After 72 h, retroviral supernatants were filtered and added to B cell cultures at 20 and 44 h after start of stimulation, and spinoculated at 2.5 µM AID or 53BP1 Δ545 for 10 cycles at 92°C for 10 s, 55°C for 30 s, and 68°C for 40 s, with 2 s of additional extension time per cycle. PCR reactions were performed with the Expand Long Template PCR System (Roche). The first round of PCR was performed with the following primers and conditions: 5′-AGACAGGACAGGACAGGACCAAAC-3′ and 5′-AGACAGGACAGGACAGGACCAAAC-3′ for 10 cycles at 92°C for 10 s, 56°C for 30 s, and 68°C for 40 s, followed by 25 cycles at 92°C for 15 s, 56°C for 30 s, and 68°C for 40 s, with 2 s of additional extension time per cycle. The second round of PCR was performed with the following primers and conditions: 5′-CTTCCATTATGGTGGTCTTTCGGA-3′ and 5′-CTTCCATTATGGTGGTCTTTCGGA-3′ for 10 cycles at 92°C for 10 s, 55°C for 30 s, and 68°C for 30 s, followed by 15 cycles at 92°C for 15 s, 55°C for 30 s, and 68°C for 30 s, with 2 s of additional extension time per cycle. PCR products were isolated from gels and sequenced.

Online supplemental material. Fig. S1 shows the gene targeting strategy and phenotype analysis of IgH Δ94+ mice. Fig. S2 shows that catalytically inactive I-SceI* does not induce recombination between I-SceI sites. Fig. S3 is a summary of all junctional analysis data for IgH Δ94/AID−/− and IgH Δ94/AID−/−53BP1−/− B cells. Tables S1 and S2 are summaries of I-SceI−/− induced recombination frequency by FACS and PCR. Tables S3–S5 show the resection data of I-SceI−/−infected IgH Δ94/AID−/− and IgH Δ94/AID−/−53BP1−/− B cells. Tables S6–S8 are summaries of joining frequencies and resection data of I-SceI−/−infected IgH Δ94/AID−/− and IgH Δ94/AID−/−53BP1−/− B cells in the presence of ATMi. Table S9 is a summary of IgG1 expression in WT and 53BP1−/− B cells in the presence or absence of ATMi. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20100244/DC1.

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