are not enlarged in HIGM II patients with C-terminal mutations, who retain SHM (Imai *et al.*, 2005). It is therefore important to carefully assess the gut microbiota in HIGM II patients.

C. Phenotypes of AID C-Terminal Mutations

Most AID C-terminal mutations have one of two phenotypes. One accumulates AID in the nucleus because of a defective NES localized at the C-terminus, and the other loses CSR without reducing SHM. A series of mutations in the NES motif (183–198) all cause a specific CSR defect associated with augmented SHM activity and nuclear AID accumulation (Barreto *et al.*, 2003; Ito *et al.*, 2004; Ta *et al.*, 2003). Interestingly, the insertion of 34 amino acids at residue 182 (a clinical mutation called the P20 mutation) specifically causes CSR loss without accumulating the mutant AID protein in the nucleus (Ito *et al.*, 2004). This appears to indicate that the P20 mutation disrupts the domain that interacts with the CSR-specific cofactor. This domain appears to partially overlap the NES motif, as most NES-motif mutants lose CSR. In all of these C-terminal mutants, the DNA cleavage activity is normal or augmented in both the S and V regions (Doi *et al.*, 2009). These results clearly indicate that CSR requires not only DNA cleavage, but also an additional C-terminal-specific AID activity. Although the nature of this C-terminal-specific activity is not clear, we propose that it is involved in synapsis formation between the cleaved ends (Doi *et al.*, 2009).

In summary, it is evident that AID has two clear functions (Fig. 1), one involving DNA cleavage of the S and V regions localized in the N-terminus, and the other involving CSR-specific activity in the C-terminus, probably related to synapsis formation. The results can be most easily interpreted by the assumption that two separate cofactors interacting at the N-terminus and C-terminus of AID are responsible for DNA cleavage and putative synapsis formation, respectively. Cleavage may become more efficient in C-terminal mutants because the cofactor for DNA cleavage monopolizes the catalytic center, assuming that AID's catalytic activity is required for both activities.

III. DNA CLEAVAGE MECHANISM BY AID

While it seems that AID's involvement in tumorigenesis must be closely linked with DNA cleavage, AID's molecular mechanism for DNA cleavage is still controversial. There are two contrasting hypotheses to explain how AID introduces DNA cleavage in the genome (Neuberger *et al.*, 2003; Petersen-Mahrt *et al.*, 2002; Muramatsu *et al.*, 2007).

A. The DNA Deamination Hypothesis

According to the DNA deamination hypothesis, AID first recognizes C bases on DNA and deaminates them to generate U (Di Noia and Neuberger, 2002). The resultant U/G mismatch is recognized by the base excision repair pathway enzymes. The U's are then removed by uracil-DNA glycosylase or uracil nucleotide glycosylase (UNG). The abasic sites thus created are attacked by apurine/apyrimidine (AP) endonuclease, which cleaves phosphodiester bonds at these sites.

1. AID DEAMINATION ACTIVITY

There are several lines of evidence to support the DNA deamination model. First, AID deaminates C to U on single-stranded DNA *in vitro*. Numerous experiments have revealed the properties of the *in vitro* reaction of DNA deamination by AID, including the nucleotide preference of the cleavage sites (Bransteitter *et al.*, 2003; Chaudhuri *et al.*, 2003; Shen *et al.*, 2005). These experiments showed that AID prefers single-stranded DNA, which may be generated *in vivo* by an R-loop or a transient single-strand bubble during transcription. The single-stranded DNA-binding protein RPA was shown to enhance the *in vitro* reaction (Chaudhuri *et al.*, 2004). However, criticism of this *in vitro* assay noted that the enzyme-to-substrate ratio was very high; the molar ratio of the AID added to the DNA far exceeded that seen in the catalytic activity *in vivo*. In addition, the DNA substrate *in vivo* is not naked DNA, but rather a very tight chromatin complex wrapped around histone octamers. Storb's group (Shen *et al.*, 2009) showed that the *in vitro* DNA deamination reaction is substantially reduced if histones are added to the DNA. In addition, it is notable that the *bona fide* RNA-editing enzyme APOBEC1 shows a similar DNA deamination activity *in vitro* (Petersen-Mahrt and Neuberger, 2003).

Another line of evidence consistent with the DNA deamination model is that AID overexpression can introduce C-to-T mutations in the *E. coli* and yeast genomes (Petersen-Mahrt *et al.*, 2002; Poltoratsky *et al.*, 2004). However, under these conditions there is no clear target specificity, in contrast to the clear-cut preference of the immunoglobulin gene in vertebrates. Further, APOBEC1 overexpression shows similar phenotypes (Harris *et al.*, 2002). Therefore, these phenotypes very likely reflect an artificial situation due to overexpression. Further, Shivarov *et al.* (2008) screened AID mutants *in vitro*, and found a series of mutants in which the DNA deamination activity is deficient or entirely lost. They then introduced these mutants into AID deficient B cells and examined whether the CSR activity was reduced. Interestingly, the N51A mutation almost completely abolished

the *in vitro* DNA deamination activity but retained the CSR activity, indicating that the *in vitro* DNA deamination activity is not directly related to the CSR activity *in vivo*. The *in vitro* DNA deamination activity is also abolished in the homologous APOBEC1 N51A mutant. However, this mutation does not eliminate APOBEC1's RNA-editing activity, again confirming that APOBEC1's *in vitro* DNA deamination activity is not essential for its RNA-editing activity.

2. UNG INVOLVEMENT IN CSR

Another observation supporting the DNA deamination model is that UNG deficiency reduces CSR and gene conversion (Rada *et al.*, 2002; Saribasak *et al.*, 2006). While UNG deficiency reduces CSR by 80–90%, there is always residual activity (Rada *et al.*, 2002). SHM is enhanced in the absence of UNG, which is puzzling (Rada *et al.*, 2002; Saribasak *et al.*, 2006). Although the DNA deamination model attributes the absence of the SHM phenotype in UNG deficiency to compensation with mismatch repair (MMR) enzymes (Rada *et al.*, 1998), a dual deficiency of Ung and Msh2 reduces SHM by only 30%, with a heavy bias toward C-to-T and G-to-A mutations (Rada *et al.*, 2004). In contrast, a dual deficiency in UNG and Msh2 drastically reduces CSR, indicating that these two proteins are active at different steps of CSR (Honjo *et al.*, 2005). Further, this dissociation of CSR and SHM defects suggests that UNG and Msh2 may not be involved in AID-induced DNA cleavage; if they were, a dual UNG and Msh2 deficiency should affect CSR and SHM at similar levels. Gene conversion (GC), unlike SHM, is sensitive to UNG deficiency, although both SHM and GC give rise to point mutations in the V region. GC and CSR are mechanistically more similar to each other than to SHM; while all three processes require DNA cleavage, GC and CSR also require recombination. It is therefore likely that UNG is involved in a step after DNA cleavage, probably having to do with repairing cleaved ends for recombination.

Since UNG deficiency reduces CSR but not SHM, Begum *et al.* (2004) wondered whether this defect is associated with AID's DNA cleavage activity. They showed that even in the absence of UNG, DNA cleavage remains intact in the *S* regions. For this assay, IgM hybridomas were generated from UNG-deficient spleen cells after *in vitro* stimulation. Many IgM hybridomas had levels of *Sμ*–region mutations or deletions comparable to those in wild-type spleen cells, clearly indicating that DNA cleavage takes place in UNG-deficient spleen cells. Similarly, in two assays conducted in CH12F3-2 cells, UNG was completely blocked with its specific protein inhibitor Ugi to determine whether DNA cleavage could be induced by either (a) stimulating γH2AX focus formation, or (b) directly labeling the cleaved ends with biotin-labeled dUTP and terminal deoxynucleotide transferase. In both cases, completely blocking

UNG did not reduce the DNA cleavage activity in the activated CH12F3-2 cell *S* region (Begum *et al.*, 2004). UNG is most likely involved in repairing or pairing DNA after cleavage. In fact, Durandy's group (Kracker *et al.*, 2010) recently showed that UNG-deficient human B cells have much longer microhomology at the switch recombination junction, suggesting that UNG is required to support short heteroduplex regions for recombination, and that only very long microhomology pairing can be stabilized for recombination in the absence of UNG. The effects of UNG deficiency are far more severe in humans than in mice; this probably reflects human CSR's greater dependence on microhomology-mediated endjoining, which uses ligase 3, rather than nonhomologous endjoining, which uses ligase 4 and does not require any homology at the junction.

Begum *et al.* (2007) made a series of UNG catalytic-center mutants, and found that several mutations that abolish the U removal activity still retain the CSR activity in UNG-deficient spleen B cells (Table II). They also found that the WXXF motif required for UNG's interaction with the HIV Vpr protein is critical for UNG CSR. This WxxF motif is essential for recruiting UNG to the HIV virion to support HIV propagation. Thus, UNG appears to serve as a scaffold for forming protein complexes rather than for U removal (Fig. 2).

Accumulating evidence indicates that UNG has a function other than U removal: (a) host UNG is required for HIV viral genome integration, in which UNG interacts with an integrase and DNA preintegration complex, and UNG's catalytic activity has recently been shown to be indispensable for this interaction (Guenzel *et al.*, 2011); (b) although UNG is essential for vaccinia virus genome replication, its catalytic activity is not required (De Silva and Moss, 2003, 2008); and (c) the histone H3 variant CENP-A is required for chromosome segregation during mitosis. CENP-A assembly on DNA depends on UNG, but not on UNG's catalytic activity (Zeitlin *et al.*, 2011).

It was recently reported that AID induces U accumulation in UNG-deficient spleen cells (Maul *et al.*, 2011). However, highly abundant U amounts are accumulated, as though the MMR proteins are not functional. These results should be quantitatively examined to determine whether the amount of U agrees with a known mutation frequency.

3. The AP Endonuclease (APE) Requirement

Another important criterion for DNA deamination is the requirement of APE for CSR. In mammals, there are two types of APE: APE1 and APE2. In APE2-deficient mice, CSR is not reduced and SHM is enhanced (Sabouri *et al.*, 2009). This is consistent with enzymological studies showing APE2 to be an exonuclease rather than endonuclease (Burkovics *et al.*, 2006). Another report indicates that APE2 deficiency reduces CSR slightly under weak stimulation (Guikema *et al.*, 2007). The significance of this defect is not clear, as

Table I CSR and SHM Activities of Mutant AID

AID	Mutation (AA)		Patients		*In vitro*		SHM CSR (%)		
Domain[a]	mAID	hAID	SHM (%)	CSR (%)	SHM (%)	CSR (%)	wtAID		References
N-terminal domain	S3A		ND[b]	ND	207.2	143.6	28×10^{-4}	15.6	Gazumyan *et al.* (2010)
	S3D		ND	ND	ND	142.3			Gazumyan *et al.* (2010)
	M6A		ND	ND	1.1	3.3	18.1×10^{-4}	15	Okazaki *et al.* (2011)
		M6T	(−)[c]	(−)	ND	(−)	ND	ND	Durandy *et al.* (2006)
	N7A		ND	ND	47.5	93.3			Okazaki *et al.* (2011)
	R8A		ND	ND	139.2	106.6			Okazaki *et al.* (2011)
		R8_Y13delinsNfsX19	(−)	(−)	ND	(−)			Durandy *et al.* (2006)
	R9A		ND	ND	92.8	93.3			Okazaki *et al.* (2011)
	K10A		ND	ND	37.6	60			Okazaki *et al.* (2011)
		K10R	ND	ND	60.0±5.2	56.7±17	ND	ND	Ta *et al.* (2003)
	K10R		ND	ND	53.6	80			Okazaki *et al.* (2011)
	Y13H		ND	ND	10.7	63.2	0.84±0.12	38±7	Shinkura *et al.* (2004)
		F15X	0	ND	0.5±0.5	2.7±1.8			Ta *et al.* (2003)
	V18R		ND	ND	10.7	105.3			Shinkura *et al.* (2004)
	V18S/R19V		ND	ND	4.8	52.6			Shinkura *et al.* (2004)
	W20K		ND	ND	16.7	102.6			Shinkura *et al.* (2004)
		L22X	ND	(−)	ND	(−)			Durandy *et al.* (2006)
	G23S		ND	ND	10.7	97.4			Shinkura *et al.* (2004)
		R24W	0.9	ND	0.5±0.5	1.3±0.8			Ta *et al.* (2003)
		Y31X	ND	(−)	ND	(−)			Durandy *et al.* (2006)

(*continues*)

Table I (*continued*)

AID	Mutation (AA)		Patients		*In vitro*		SHM CSR (%)		
Domain[a]	mAID	hAID	SHM (%)	CSR (%)	SHM (%)	CSR (%)	wtAID		References
Active site domain	S38A		ND	ND	30.2	36.5	4.3×10^{-4}	17	McBride *et al.* (2006)
	S38A		ND	ND	43.5	30.6	2.3×10^{-4}	17	McBride *et al.* (2008)
	S38D		ND	ND	ND	37.7			McBride *et al.* (2006)
	D45A		ND	ND	2	22	51×10^{-4}	41	Shivarov *et al.* (2008)
	D45A/F46A		ND	ND	2	7.3			Shivarov *et al.* (2008)
	D45A/R50A		ND	ND	2	7.3			Shivarov *et al.* (2008)
	D45A/N51A		ND	ND	3.9	2.4			Shivarov *et al.* (2008)
	D45A/R50A/ N51A		ND	ND	0	2.4			Shivarov *et al.* (2008)
	F46A		ND	ND	2	43.9			Shivarov *et al.* (2008)
	G47A		ND	ND	2	12.2			Shivarov *et al.* (2008)
	H48A		ND	ND	9.8	100			Shivarov *et al.* (2008)
	L49A		ND	ND	3.9	58.5			Shivarov *et al.* (2008)
	R50A		ND	ND	29.4	129.3			Shivarov *et al.* (2008)
	N51A		ND	ND	2	51.2			Shivarov *et al.* (2008)
	R50A/N51A		ND	ND	0	9.8			Shivarov *et al.* (2008)
	K52A		ND	ND	19.6	119.5			Shivarov *et al.* (2008)
	S53A		ND	ND	117.6	85.4			Shivarov *et al.* (2008)
	G54A		ND	ND	21.6	95.1			Shivarov *et al.* (2008)
	C55A		ND	ND	37.3	109.8			Shivarov *et al.* (2008)
		H56Y	0	ND	0.5±0.5	2.1±1.7			Ta *et al.* (2003)
		H56Y	(−)	(−)	ND	(−)			Durandy *et al.* (2006)
		H56_E58delinsV	(−)	(−)	ND	(−)			Durandy *et al.* (2006)
		W68X	0	ND	0.5±0.5	2.0±0.9			Ta *et al.* (2003)
		W80R	ND	ND	0.5±0.5	3.8±3.2			Ta *et al.* (2003)
		S83P	ND	(−)	ND	(−)			Durandy *et al.* (2006)
		W84X	ND	ND	ND	ND	ND	ND	Minegishi *et al.* (2000)

		S85N	ND	(−)	ND	(−)			Durandy *et al.* (2006)
		C87R	ND	ND	0	2.1±1.1			Ta *et al.* (2003)
		C87S	(−)	(−)	ND	(−)			Durandy *et al.* (2006)
		KSS(H56K,C87S,C90S)	ND	ND	ND	0		16	Doi *et al.* (2009)
		L106P	ND	ND	0	1.4±0.9			Ta *et al.* (2003)
Linker		A111E	(−)	(−)	ND	(−)			Durandy *et al.* (2006)
domain		JP8A (R112C)	ND	ND	0.8±0.75	4.2±3.5			Ta *et al.* (2003)
		R112H	0–0.34	ND	0.5±0.5	1.8±0.9			Ta *et al.* (2003)
		L113P	ND	(−)	ND	(−)			Durandy *et al.* (2006)
		M139T	ND	(−)	ND	(−)			Durandy *et al.* (2006)
		M139V	ND	ND	0.5±0.5	4.6±0.3			Ta *et al.* (2003)
	T140A		ND	ND	87	82.4			McBride *et al.* (2008)
		D143_E163del20	(−)	(−)	ND	(−)			Durandy *et al.* (2006)
		C147X	0.76	ND	0	1.7±0.7			Ta *et al.* (2003)
C-terminal				ND					
domain		F151S	ND	ND	ND	ND		ND	Revy *et al.* (2000)
		S169X	ND	(−)	ND	(−)			Durandy *et al.* (2006)
		L172A	ND	ND	27.7	63.5	13×10^{-4}	26	Doi *et al.* (2009)
		L172A/G197A	ND	ND	ND	11.5		26	Doi *et al.* (2009)
		R174S	(−)	(−)	ND	(−)			Durandy *et al.* (2006)
		L181delinsCfsX26	(+)[d]	(−)	ND	(−)			Durandy *et al.* (2006)
		L181_P182ins31	(+)	(−)	ND	(−)			Durandy *et al.* (2006)
		p20(P182ins)	3.4	ND	71.0±23.4	3.2±1.1			Ta *et al.* (2003)
		JP8B(L183rep)	ND	ND	70.0±8	4.5±2.5			Ta *et al.* (2003)
		JP8Bdel(L183X)	ND	ND	548	ND	7.7×10^{-4}	ND	Ito *et al.* (2004)
		JP8Bdel(L183X)	ND	ND	200	3.6		14	Doi *et al.* (2009)
		L189A	ND	ND	430.8	35.6		26	Doi *et al.* (2009)

(*continues*)

Table I *(continued)*

AID	Mutation (AA)		Patients		*In vitro*		SHM CSR (%)		
Domain[a]	mAID	hAID	SHM (%)	CSR (%)	SHM (%)	CSR (%)	wtAID		References
	L189X		ND	ND	100	8.3	11×10^{-4}	30	Barreto *et al.* (2003)
		R190A	ND	ND	18.5	98.1		26	Doi *et al.* (2009)
		JP41(R190X)	ND	ND	75.0±15	6.6±2			Ta *et al.* (2003)
		R190X	65	(−)	ND	ND	2±1.5	ND	Imai *et al.* (2005)
		D191A	ND	ND	74.6	71.2		26	Doi *et al.* (2009)
		A192G	ND	ND	100	80.8		26	Doi *et al.* (2009)
		F193A	ND	ND	592.3	15.4		26	Doi *et al.* (2009)
		F193X	ND	ND	327.3	ND			Ito *et al.* (2004)
		R194A	ND	ND	39.2	57.7		26	Doi *et al.* (2009)
		T195A	ND	ND	27.7	86.5		26	Doi *et al.* (2009)
		L196A	ND	ND	430.8	21.2		26	Doi *et al.* (2009)
		L196X	ND	ND	436	ND			Ito *et al.* (2004)
		G197A	ND	ND	73.9	51.9		26	Doi *et al.* (2009)
		L198A	ND	ND	376.9	21.2		26	Doi *et al.* (2009)
N/C domain double mutants		S3G/N168S	ND	ND	6.9±2.5	24.1±3			Ta *et al.* (2003)
		ΔN5JP8Bdel	ND	ND	550.7	ND			Ito *et al.* (2004)
Linker/C double mutants		JP8A(R112C)/JP8B (L183rep)	1.3	ND	ND	ND	ND		Ta *et al.* (2003)
		D143_L181delinsAfsX4	(+)	(−)	ND	(−)			Durandy *et al.* (2006)

All activities shown on this table are relative to wtAID values shown on the right column.
del, deletion; fs, frameshift; ins, insertion; X, stop; ΔN5, N-terminal 5AA deletion; Rep, replacement.
[a]As indicated in Fig. 1.
[b]Not done.
[c]Undetectable.
[d]Detectable.

Table II Rescue of CSR by UNG, SMUG1, and Their Mutants in $UNG^{-/-}$ B Cells

Gene/mutation	Feature/property	CSR
Mouse UNG2	Wild type	(+)[a,e]
D145N	Catalytic inactivation	(+)[a,e]
H268L	Catalytic inactivation	(+)[a,e]
N204V	Catalytic inactivation (does not bind U)	(+)[a,e]
F242S[f]	Unknown/unstable/mito-targeted[g]	(+)[a]
D145N+H268L	Catalytic inactivation	(+)[a]
D145N+N204V	Catalytic inactivation	(−)[a]
N204D	CDG and residual UDG	(−)[a,b]
Y147A	TDG and residual UDG	(−)[a,b]
L272A	Unable to flip Uracil	(+)[c]
L272R	Increased binding to DNA	(+)[c]
R276E	Single strand specific activity	(+)[c]
W231A	Single mutation at Vpr binding site (WxxF)	(+)[b]
W231K	Single mutation at Vpr binding site (WxxF)	(−)[b,d]
F234G	Single mutation at Vpr binding site (WxxF)	(+)[b]
F234Q	Single mutation at Vpr binding site (WxxF)	(+)[b]
NΔ28	Lacks interaction motif for PCNA	(+)[d]
NΔ65	Lacks interaction motif for PCNA and NLS	(+)[b]
NΔ77	Lacks interaction motif for PCNA and NLS	(+)[d]
NΔ86	Lacks interaction motif for PCNA and RPA and NLS	(+)[d]
NΔ86.D145N	Catalytic inactivation	(−)[b]
NΔ86.H268L	Catalytic inactivation	(+)[b]
NΔ86.W231A	Single mutation at Vpr binding site (WxxF)	(−)[d,b]
NΔ86.W231K	Single mutation at Vpr binding site (WxxF)	(−)[d,b]
NΔ86.F234Q	Single mutation at Vpr binding site (WxxF)	(−)[d,b]
NΔ86.F234G	Single mutation at Vpr binding site (WxxF)	(−)[d,b]
NΔ86.W231A + F231G	Double mutation at Vpr binding site (WxxF)	(−)[d,b]
E. coli UNA	Wild type	(+)[b]
D64N	Catalytic inactivation	(−)[b]
H187Q	Catalytic inactivation	(+)[b]
Mouse SMUG1	Wild type	(+)[e,b]
N85A	H_2O coordination	(−)[b]
H239L	Stabilization of transition state	(−)[b]
N163D	Substrate binding	(−)[b]
G87Y	Thymine expulsion	(−)[e,b]
G87V	Thymine expulsion	(−)[b]
W144K	WxxF site	(−)[b]

[a]Begum *et al.* (2004).
[b]Begum *et al.* (2009).
[c]Muramatsu *et al.* (2007).
[d]Begum *et al.* (2007).
[e]Di Noia *et al.* (2006).
[f]Imai *et al.* (2003).
[g]Kavli *et al.* (2005).

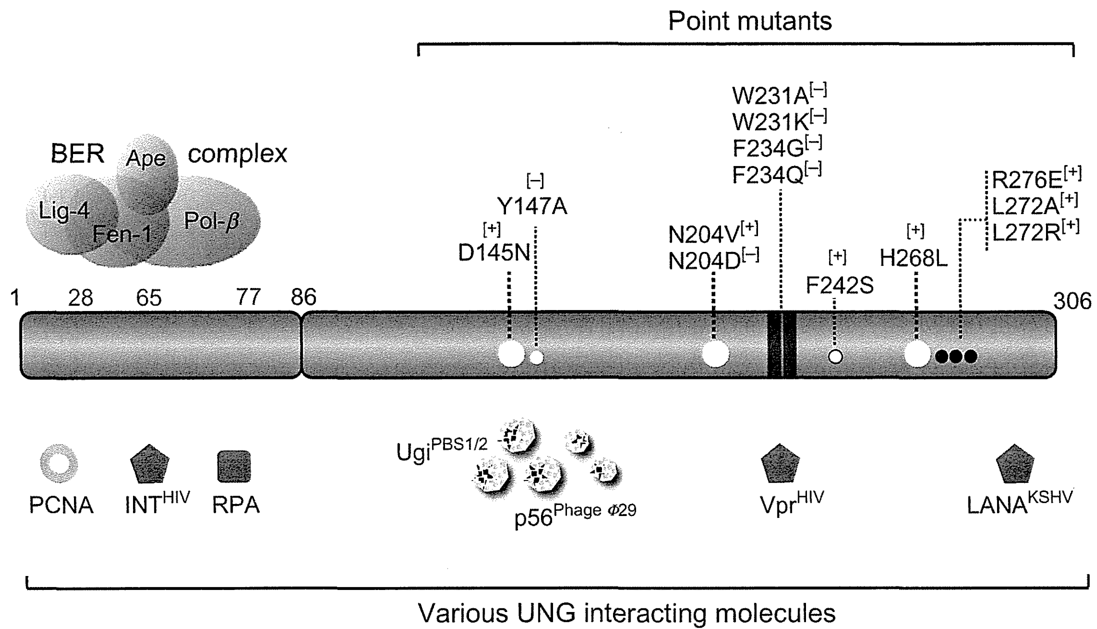

Fig. 2 Structural features of mouse UNG2, showing key mutations and interactive partners that contributed to our understanding of UNG in CSR. Amino acid numbers and the names of individual interacting proteins, along with their origin (upper case) if not cellular, are indicated. Active site residues are shown by wide white circles; two vertical bands denote the WxxF motif position. A mutant's ability [+] or inability [−] to induce or rescue CSR is shown beside its name. *Abbreviations*: UNG, uracil-DNA glycosylase; CDG, cytosine DNA glycosylase; TDG, thymine DNA glycosylase; RPA, replication protein A; PCNA, proliferating cell nuclear antigen; INT, integrase; HIV, human immunodeficiency virus; UGI, uracil-DNA glycosylase inhibitor; Vpr, viral protein R; LANA, latency-associated nuclear antigen; KSHV, Kaposi's sarcoma-associated herpesvirus; CENP-A, centromere protein-A; PBS1/2, phage of *Bacillus subtilis*.

there are no significant differences in serum Ig isotypes in the $APE2^{-/-}$ mice (Ide *et al.*, 2004). Since APE1 is essential for cell survival, *APE1*-knockout mice are lethal at an early stage of embryogenesis (Xanthoudakis *et al.*, 1996). APE1 knockdown in CH12F3-2 cells does not affect CSR (Sabouri *et al.*, 2009). These results clearly indicate that APE 1 and APE2 are not essential for CSR. Taken together, these results indicate that the DNA deamination model has to be reexamined more carefully.

B. The RNA-Editing Model

An alternative hypothesis, called the RNA-editing model, proposes that AID deaminates C bases in RNA, and the edited RNA mediates AID function (Honjo *et al.*, 2005; Muramatsu *et al.*, 2007). Studies on AID mutants suggest that AID has at least two functions: DNA cleavage, and synapsis formation of the cleaved ends. These functions are located at AID's N-terminal and C-terminal regions, respectively. The RNA-editing hypothesis postulates that

AID edits two different types of RNA for these two functions. For DNA cleavage by AID, it is assumed that AID's N-terminal region captures and edits a microRNA precursor associated with the N-terminal cofactor protein, generating a new microRNA that interacts with the 3′ UTR of topoisomerase 1 (Top1) mRNA to suppress its translation (Fig. 3). This reduces the level of Top1, preventing the restoration of excessive DNA supercoiling accumulated during active transcription (Kobayashi *et al.*, 2009). This facilitates the formation of unusual DNA structures, that is, non-B-form in the *S* or *V* region, resulting in irreversible cleavage by Top1 itself. The lack of restoration of transcription-associated negative DNA supercoiling by Top1 induces the DNA helix to loosen in the rear of the transcriptional machinery (Fig. 3). Under these conditions, thermodynamic calculations suggest that both the S and V regions form a non-B structure. Top1 normally cuts, rotates, and religates DNA to correct the aberrant supercoil accumulated by transcription. While Top1 can cut the non-B DNA form, it cannot rotate around the helix, as it is trapped by being covalently bound to the DNA. It has to be removed by resection enzymes such as Ctip and the MRN complex, which remove Spo11 (Top2) in meiotic recombination (Hartsuiker *et al.*, 2009). This model is proposed based on the series of observations described below.

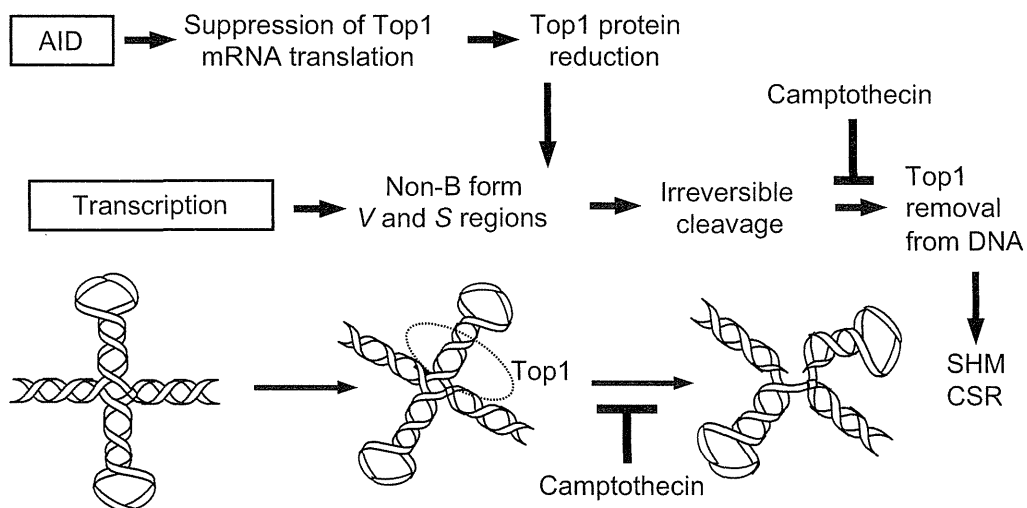

Fig. 3 A hypothetical method for AID-induced DNA cleavage and target transcription. AID suppresses Top1 mRNA translation, thus reducing the level of Top1 protein. Active target DNA transcription causes a negative supercoil accumulation behind the transcription complex. These factors favor the formation of non-B DNA structure in the *V* and *S* regions, which contain inverted repeats or repetitive sequences. Top1 introduces irreversible cleavage in a non-B structure due to steric hindrance of its rotation around the DNA helix. This causes Top1 to bond covalently with DNA, and its subsequent removal from the DNA creates a single-stranded break. Camptothecin intercalates in the Top1 and DNA complex and prevents Top1 from being removed from the DNA, thus inhibiting SHM and CSR.

1. AID Induces DNA Cleavage by Reducing Top1

AID reduces Top1's translation efficiency by half, decreasing Top1 protein levels by half within 24h, in accordance with Top1's half-life of about 3.7h in CH12F3-2A cells (Kobayashi *et al.*, 2009). Artificial Top1 reduction in CH12F3-2 cells by siRNA augments the CSR and DNA cleavage of *S* regions by AID. Similarly, the SHM activity is enhanced in a mutant plasmacytoma cell line (P388/CPT45) that has much less Top1 than the parental cell line (P388) (Kobayashi *et al.*, 2011). Overexpressing Top1 in P388/CPT45 cells strongly reduces SHM, while SHM is greatly augmented in *Top1* heterozygote B cells, which express about half the normal amount of Top1. It has also been reported that reducing Top1 with antisense oligonucleotides causes general genome instability (Miao *et al.*, 2007; Tuduri *et al.*, 2009). Thus, reducing Top1 augments the DNA cleavage in immunoglobulin genes and other loci. Experiments with the Top1-specific inhibitor camptothecin showed that the DNA cleavage activity in CSR and SHM depends on Top1 (Kobayashi *et al.*, 2009, 2011). A screen of endonuclease inhibitors identified camptothecin as the most potent inhibitor of the DNA cleavage in *S*-region CSR and SHM. Camptothecin traps DNA-bound Top1 by inhibiting Top1's release from the DNA protein complex. This release is essential to initiate CSR and SHM.

2. CSR Depends on the Association of AID's C-Terminus with mRNA

AID is proposed to interact with messenger RNA captured by a cofactor that interacts with AID's C-terminus. The edited mRNA is postulated to generate a novel protein responsible for synapsis formation of cleaved ends. This activity is unique to CSR and not required for SHM. Nonaka *et al.* (2009) showed that AID is associated with poly A mRNA, and that this association depends on AID's C-terminal region. The edited mRNA has to be translated, and thus exported from the nucleus to the cytoplasm, in a C-terminal NES motif-dependent manner. In support of this assumption, Doi *et al.* (2003) showed that CSR is sensitive to very brief exposure to protein synthesis inhibitors. When CH12F3-2A cells are treated with cycloheximide or puromycin 1h before AID activation, CSR is completely blocked 3 or 6 h after AID activation, as measured by the formation of $S\mu$–$S\alpha$ recombinant DNA. The requirement of AID nuclear export for CSR has been demonstrated by two types of experiments. First, mutations in the C-terminal NES motif, which cause AID to accumulate in the nucleus, abolish CSR but do not reduce SHM. In addition, a 1-h preincubation with leptomycin B, which specifically inhibits the nuclear export protein CRM1, also blocks CSR (Doi *et al.*, 2003). An association with a CSR-specific cofactor other than the nuclear export cofactor at the C-terminus is supported by the finding that in the P20 mutant,

which has an insertion at residue 182, CSR is completely blocked, but there is no effect on nuclear export of AID. SHM is normal in the P20 mutant, clearly indicating that the DNA cleavage activity and switch recombination depend on different regions of AID (Shinkura *et al.*, 2004; Ta *et al.*, 2003). We proposed that the C-terminal domain of AID is responsible for the recombination step after DNA cleavage (Doi *et al.*, 2009). In agreement with this hypothesis, a heterozygote human AID mutant with a C-terminal truncation (R190X) shows unusually longer junctions at Sμ–Sα recombination (Kracker *et al.*, 2010). Interestingly, many repair-related proteins such as DNA-PKcs, UNG, and MMR were reported to interact with the C-terminal domain of AID (Ranjit *et al.*, 2011; Wu *et al.*, 2005). Another group confirmed the independent role of the C-terminal domain from DNA cleavage and proposed its involvement in stabilization of AID (Ellyard *et al.*, 2011). However, this hypothesis does not agree with the fact that the P20 mutant is stable in spite of its loss of the recombination activity. So far, no experimental evidence against the RNA-editing model has been obtained. However, no direct evidence for RNA editing has been obtained, either. To prove that AID edits RNA, it is important to identify the microRNAs and mRNAs that are edited for DNA cleavage and for synapsis formation, respectively.

C. AID Target Specificity for DNA Cleavage

1. NON-IG AID TARGETS

Although AID was originally believed to specifically target immunoglobulin gene V and S regions, it has become evident that AID also cleaves other genes. AID-transgenic mice show tumors developed in various cells, in which mutations are accumulated in non-Ig genes, including proto-oncogenes (Kotani *et al.*, 2005; Okazaki *et al.*, 2003). However, the number of genes AID actually targets is not clear. There have been several attempts to identify AID's target genes by analyzing AID-induced mutations. Schatz and his colleagues showed that AID could introduce SHM in about 30 genes they examined by PCR (Liu *et al.*, 2008). Since this study only included preselected targets, it is uncertain whether AID preferentially targets these genes over the rest of the genome. Indeed, in the Peyer's patch B cells of 6-month-old mice, the mutation frequencies of all the non-Ig genes are less than half those of the J_H loci (Liu *et al.*, 2008). Recently, several groups have tried to identify AID target genes using the ChIP microarray or ChIP sequence method with various markers. Stavnezer's group, using Nbs1 as a DSB marker, reported that Nbs1 ChIP detected hundreds of genes after AID expression (Staszewski *et al.*, 2011). Since the authors did not confirm the mutations in these sites, it is not clear whether all the Nbs1-binding sites

were cleaved by AID expression. Curiously, this report showed that Nbs1 accumulated in many nontranscribed loci, even though it has been well established that Ig gene transcription is mandatory for CSR and SHM (Betz *et al.*, 1994; Jung *et al.*, 1993; Peters and Storb, 1996; Zhang *et al.*, 1993). This puzzle will be clarified when Nbs1's exact function in DNA metabolism in the genome is elucidated. Another group carried out ChIP analysis across the whole genome to identify AID-associated targets in mice (Yamane *et al.*, 2011). This study listed about 5900 genes as possible AID targets. However, the order of the targets' AID-binding abundance did not correlate with their mutation frequencies. A physical association of AID and chromatin may or may not indicate loci that are functionally targeted by AID. Nussenzweig and his colleagues used Spt5 as a ChIP marker, assuming that since Spt5 interacts with AID, it is a target locus guiding factor for AID to directly deaminate DNA (Pavri *et al.*, 2010). In this study, approximately 9000 genes were crosslinked with Spt5, which is a component of the transcriptional machinery. Not surprisingly, most of the genes associated with Spt5 are also identified by ChIP against RNA polymerase II. Here again, the physical interaction of Spt5 and AID does not necessarily indicate their functional involvement. It is also not certain whether these targets are actually cleaved or not; the 10 genes with the highest Spt5 binding have mutation frequencies as low as those of the β2 microglobulin and β actin genes, which are known to be inert AID targets. We examined the genes functionally affected by Spt5 knockdown, and found that only 196 of them were upregulated, and 16 were downregulated (unpublished). These results suggest that physical Spt5 binding does not confirm its functional involvement in these loci, as has also been shown for RAG1 (Zhang and Swanson, 2008).

Recently, we took a different approach to the same question. We treated the BL2 cell line, which expresses a C-terminally truncated AID mutant fused with the ER domain (JP8del-ER), with tamoxifen for 3h and directly labeled DNA cleavage sites by a DNA linker containing biotin-labeled dU (Kato *et al.*, 2012). DNA fragments carrying this biotin-dU linker were concentrated by streptavidin beads and sequenced by high-throughput DNA sequencing, to compare the distribution of linker tags between tamoxifen-treated and nontreated BL2 JP8del-ER cells. The same tagged libraries were also analyzed by the promoter microarray method. Statistically significant targets identified by whole-genome sequencing were compared with those identified by microarray data. The expression profiles of candidate targets were examined, and the differential enrichment of target fragments in the two libraries was further confirmed by qPCR (Kato *et al.*, 2012). Some of the targets were further analyzed for mutations. MALAT1 and SNHG3, two unexpected AID target sites identified by these studies, accumulate mutations as efficiently as the V region gene in BL2 JP8Bdel cells, and are

known to be chromosomal translocation targets in various tumors (Davis *et al.*, 2003; Levin *et al.*, 2009; Rajaram *et al.*, 2007).

2. MARKERS FOR AID TARGETS

A big question is how AID chooses its Ig and non-Ig targets from among other sites in the genome. It is also not known how AID differentially regulates CSR and SHM. Any model that tries to explain AID-dependent DNA cleavage has to provide the mechanism for the target specificity. To answer this question according to the DNA deamination model, extensive studies were carried out to identify AID-binding cofactor(s) that might account for the target specificity of the AID function. Many proteins have been reported to interact with AID, including RNA polymerase II (Nambu *et al.*, 2003), replication protein A (RPA) (Chaudhuri *et al.*, 2004), protein kinase A (PKA) (Basu *et al.*, 2005; Pasqualucci *et al.*, 2006), DNA-PKcs (Wu *et al.*, 2005), MDM2 (MacDuff *et al.*, 2006), CTNNBL1 (Conticello *et al.*, 2008), Spt5 (Pavri *et al.*, 2010), PTBP2 (Nowak *et al.*, 2011), Spt6 (Jeevan-Raj *et al.*, 2011; Okazaki *et al.*, 2011), and Trim28/KAP1 (Jeevan-Raj *et al.*, 2011; Okazaki *et al.*, 2011). Unfortunately, however, no functional correlation has been shown for any of these proteins to support their role as an AID-specific target. Most of the proteins, such as RNA polymerase II, PKA, Spt5, and PTBP2 interact with many proteins other than AID. PTBP2 is a splicing factor, and Spt5 is one of the transcription elongation factors that associate with RNA polymerase II. RPA, DNA-PKcs, and MDM2 are proteins involved in general DNA repair. CTNNBL1 has since been shown to be dispensable for CSR (Han *et al.*, 2010). Among 10 molecules we found coimmunoprecipitated with AID (Okazaki *et al.*, 2011), Spt6 was the only functionally important one. Spt6 binds to AID's N-terminal region and regulates CSR but not SHM. Since AID's N-terminal region is responsible for SHM and for DNA cleavage in CSR, Spt6's binding to AID may not be directly relevant to the target specificity. In fact, Spt6 was later shown to regulate CSR as a histone chaperon to modify an epigenetic marker of target chromatin, as described below (unpublished data). Interestingly, in an experiment using CD19-cre and Trim28 conditional knockout mice, Trim28/KAP1 regulated CSR without affecting SHM (Jeevan-Raj *et al.*, 2011). However, the AID expression was drastically reduced in a Trim28 conditional knockout using mb1-cre (Okazaki *et al.*, 2011). This discrepancy is most likely due to the insufficient deletion of a floxed gene by CD19-cre compared with mb1-cre (Hobeika *et al.*, 2006).

Generally speaking, the target specificity of known specific recombinations, such as VDJ and meiotic recombination, is determined by the combination of the *cis* (DNA sequence/structure) and *trans* (DNA-binding proteins and the chromatin modification mark of the target locus) elements (Table III). In VDJ recombination, the recombination signal sequence is

Table III Recombination Target Specificity Determinants

Cleaving enzymes	*Cis*	*Trans*
Rag1, 2 (VDJ Rb)	RSS (12/23 bp)	H3K4me3
Spo11 (meiotic Rb)	13 mer	H3K4me3
Top1 (CSR, SHM)	Repetitive sequences and/or non-B form	H3K4me3

widely distributed in the genome, but the chromatin modification, for instance the histone3 lysine4 trimethylation (H3K4me3) recognized by the RAG2 PHD domain, is essential to cleave the target accurately (Liu *et al.*, 2007; Matthews *et al.*, 2007). In meiotic recombination, Spo11 cleaves at loosely conserved DNA target sequences that are also recognized by zinc finger-histone methyltransferase (PRDM9) to generate H3K4me3 at the target chromatin (Baudat *et al.*, 2010; Myers *et al.*, 2010; Parvanov *et al.*, 2010; Wahls and Davidson, 2010). Without PRDM9, meiotic recombination is abortive. We have also shown that H3K4me3 at the target region is essential for CSR and SHM (Stanlie *et al.*, 2010). The FACT complex, which is a histone chaperone composed of SSRP1 and Spt16, modulates the trans-histone modification cascade and is essential for CSR (Stanlie *et al.*, 2010). In the absence of FACT, H3K4me3 modifications are defective at the Sμ and Sα regions, and this defect is associated with an S-region cleavage defect, although transcription of the Sμ and Sα regions is not reduced. Similarly, Spt6 and Spt5 are also required for CSR and assist in generating an H3K4me3 mark at the S region (our unpublished data). The RNA-editing model proposes that the target specificity may be determined by transcription-induced changes in *cis* and *trans* elements, which are represented by non-B-DNA structure formation and chromatin modification, respectively (Kobayashi *et al.*, 2009, 2011; Stanlie *et al.*, 2010).

IV. AID INVOLVEMENT IN GENOME INSTABILITY AND TUMORIGENESIS

A. Evolutionary Consideration of AID-Induced Genome Instability

1. CSR AND MEIOTIC RECOMBINATION HOMOLOGY

As mentioned above, H3K4me3 histone modification is an essential marker for all known specific recombinations—VDJ, meiotic, and CSR. This finding led us to compare the overall molecular mechanism of meiotic

Table IV Similar Molecules and Mechanisms in Meiotic Recombination and CSR

Function	Meiotic Rb	CSR
Recognized DNA	Hotspot 13-bp	Non-B (?)
Transcription dependency	Yes	Yes
Epigenetic mark	H3K4me3	H3K4me3
Cleaving enzyme	Spo11 (Top2)	Top1
Cleaved ends	Spo11-5′P-DNA	Top1-3′P-DNA
Resection of Top-DNA	CtIP and MRN	CtIP and MRN (?)
Stabilization of DNA structure	Msh4/5, Mlh1/Mlh3	Msh2/6, Mlh1/Pms2 (?)
$5' \rightarrow 3'$ exonuclease	Exo1	Exo1
Endjoining	Crossing over	C-NHEJ or A-EJ

recombination with CSR (Table IV). It became clear that the two recombination mechanisms share many aspects and players in addition to the H3K4me3 mark. First, both recombination mechanisms depend on topoisomerase for DNA cleaving—Spo11, a member of the Top2 family, in meiotic recombination, and Top1 in CSR. Second, both depend on the transcription of the target locus. Third, meiotic recombination requires Ctip and the MRN complex to resect covalently bound Spo11 from DNA to initiate recombination. CSR also requires Ctip and the MRN complex, although their precise roles in CSR are not established (Lee-Theilen *et al.*, 2011). Finally, both recombinations depend on MMR proteins. In meiotic recombination, Msh4 and 5 are required for stabilizing the synapsis of strand invasion products during homologous recombination. CSR requires Msh2 and 6, and the DNA deamination model proposes that Msh2 and 6 are involved in the DNA cleavage step by recognizing mismatches generated by AID cytosine deamination. However, Msh2 knockout reduces CSR by shortening the average length of recombination junction microhomology sequences involved, indicating a reduced amount of atypical endjoining relative to nonhomologous endjoining (Schrader *et al.*, 2002). Msh2 deficiency drastically reduces CSR in $S\mu^{-/-}$ mice (Min *et al.*, 2003). These results suggest that Msh2 might be also involved in stabilizing single-stranded pairing at the CSR junction. Without Msh2 stabilization, atypical endjoining might become inefficient, because it depends on single-stranded pairing with many mismatches. Such conservation between meiotic recombination and CSR suggests that CSR evolved by borrowing the basic mechanism for DNA cleavage in meiotic recombination. If so, AID's loose target specificity is probably inevitable, since the target specificity of meiotic recombination is limited but not absolute.

2. TOP1 INVOLVEMENT IN TRANSCRIPTION-RELATED GENOME INSTABILITY

Both CSR and SHM depend completely on target transcription, which is by nature dangerous to genome integrity. Transcription inevitably exposes single-stranded DNA, either by local denaturation or by R-loop formation. In eukaryotes, transcription involves disassembling the tightly packed chromatin structure, exposing naked DNA, and then reassembling the chromatin. Further, transcription creates local distortion of the superhelix, positive in the front and negative in the rear of the transcription machinery. Because of these situations, excessive transcription has been shown to be associated with mutations, recombination, and other genome instability events. Among these, transcription-associated mutagenesis (TAM) has been extensively studied in yeast and has been shown to occur in mammals (Aguilera, 2002).

Several mechanisms have been proposed to explain why excessive transcription is prone to induce mutation, especially when associated with inefficient repair. Yeast genetic studies have shown that replacement mutations depend heavily on error-prone polymerase zeta, which is also involved in SHM (Zan *et al.*, 2001). The nucleotide excision repair pathway, but not the base excision repair pathway, plays an important role in preventing TAM (Morey *et al.*, 2000). Recently, a type of TAM called 2–5 base deletion was shown to depend almost totally on Top1's catalytic activity (Lippert *et al.*, 2011; Takahashi *et al.*, 2011). Top1's covalent association with the enhanced transcription target was demonstrated by chromatin immunoprecipitation. In addition, 2–5 base deletion takes place at hot spots with di- or trinucleotide repeats. More recently, triplet contraction/expansion, which is associated with many human genetic diseases including Huntington's disease, was also shown to depend on Top1 (Hubert *et al.*, 2011). It is well known that tandem repeats of triplets form a non-B structure when transcribed (Bacolla *et al.*, 2006). Here again, DNA cleavage leading to genome instability appears to share several features—transcription, non-B structure, and irreversible cleavage by Top1—with CSR and SHM. Thus, Top1's DNA cleavage mechanism in TAM and triplet contraction may be evolutionarily related to that in CSR and SHM.

B. Evidence for AID's Involvement in Tumorigenesis

1. AID IN MURINE TUMORS

The first evidence of AID's involvement in tumorigenesis was obtained through transgenic mouse studies. Ubiquitous AID expression in mice causes several types of tumors, most frequently in T cells, in association with

mutations in many genes, including *T cell receptors*, *CD4*, *CD5*, *c-myc*, *P53*, and *Pim1* (Kotani *et al.*, 2005; Morisawa *et al.*, 2008; Okazaki *et al.*, 2003). These findings clearly indicate that AID can cause tumors by targeting several different genes, even in T cells, when strongly and continuously expressed. AID deficiency was shown to block *c-myc-Ig* translocation, which is associated with plasmacytomagenesis in mice (Ramiro *et al.*, 2004). Lymphomagenesis studies *in vivo* indicated that AID is required for a typical *c-myc-Ig* translocation-associated B lymphomagenesis (Kotani *et al.*, 2007; Takizawa *et al.*, 2008). AID-deficient Bcl-xL transgenic mice develop delayed atypical plasma cell tumors with the unusual Ig/myc chromosomal rearrangements (Kovalchuk *et al.*, 2007). *C-myc* transgenic animals develop pre-B lymphomas or B lymphomas without SHM. Interestingly, AID deficiency reduces the incidence of B lymphomas, but not pre-B lymphomas (Kotani *et al.*, 2007). AID deficiency prevents GC- and post-GC-derived lymphoma, but not marginal zone lymphoma development, in Iμ Bcl6 transgenic mice. These results indicate that AID is involved in tumorigenesis in mature activated B cells.

2. AID IN GC-DERIVED B-CELL LYMPHOMAS

AID is expressed physiologically in GC B cells (Muramatsu *et al.*, 2000; Yang *et al.*, 2005). Thus, after AID was discovered, initial studies on its expression in human hematological malignancy were performed in GC-derived human B-cell lymphomas, such as diffuse large B-cell lymphoma (DLBCL), follicular B-cell lymphoma (FL), and Burkitt lymphoma (BL). The majority, though not all, of these lymphomas express AID constitutively (Faili *et al.*, 2002; Greeve *et al.*, 2003; Hardianti *et al.*, 2004a,b; Muto *et al.*, 2000; Pasqualucci *et al.*, 2001; Smit *et al.*, 2003). These lymphomas frequently carry chromosomal translocations involving IgH, such as IgH/bcl6, IgH/bcl2, and IgH/c-myc; some of these are thought to stem from illegitimate CSR (Kuppers and Dalla-Favera, 2001). These lymphomas also frequently display hypermutations in oncogenes such as *Bcl6*, *c-myc*, *Pim1*, *RhoH/TTF1*, and *Pax5* that are similar to the SHM in Ig genes (Gordon *et al.*, 2003; Migliazza *et al.*, 1995; Pasqualucci *et al.*, 2001; Shen *et al.*, 1998).

AID is consistently expressed in neoplastic cells in nodular lymphocyte-predominant Hodgkin's lymphoma (LPHL), but infrequently in those of classical Hodgkin's lymphoma (CHL) (Greiner *et al.*, 2005; Mottok *et al.*, 2005). This finding is consistent with the idea that lymphocytic and histiocytic (L&H) cells, the neoplastic cells in LPHL, carry highly mutated Ig genes with ongoing SHM, and are therefore probably derived from GC B cells (Braeuninger *et al.*, 1997). In contrast, Hodgkin and Reed-Sternberg cells, the neoplastic cells in CHL, carry highly mutated Ig genes without ongoing SHM, and are probably derived from pre-apoptotic GC B cells, in which

B-cell-specific gene expression is downregulated (Kanzler *et al.*, 1996; Kuppers *et al.*, 2002; Marafioti *et al.*, 2000). An intriguing possibility is that AID expressed in L&H cells plays a role in transforming LPHL, an indolent subtype of Hodgkin's lymphoma, into aggressive DLBCL by introducing additional transforming mutations (Greiner *et al.*, 2005; Mottok *et al.*, 2005).

3. AID IN NON-GC-DERIVED B-CELL LYMPHOMAS

AID expression has also been extensively studied in human B-cell chronic lymphocytic leukemia (B-CLL), which is a non-GC-derived B-cell lymphoma and the most common leukemia in the western world (Albesiano *et al.*, 2003; Cerutti *et al.*, 2002; Heintel *et al.*, 2004; McCarthy *et al.*, 2003; Oppezzo *et al.*, 2003). B-CLL cells were originally considered to be the neoplastic counterpart of naïve B cells, which do not undergo SHM (Hamblin, 2002). However, this view changed when it was demonstrated that approximately 50% of B-CLL cases express mutated IgV_H genes (Schroeder and Dighiero, 1994). It is currently well accepted that there are at least two distinct B-CLL subsets, one with unmutated IgV_H genes (UM B-CLL) and the other with mutated IgV_H genes (M B-CLL); importantly, M B-CLL cases have a better prognosis than UM B-CLL cases (Damle *et al.*, 1999). Gene expression profiles suggest that both B-CLL subtypes resemble memory B cells but not naïve B cells (Klein *et al.*, 2001; Rosenwald *et al.*, 2001). Surprisingly, AID is constitutively expressed in UM B-CLL but not M B-CLL cells (Albesiano *et al.*, 2003; Cerutti *et al.*, 2002; Heintel *et al.*, 2004; McCarthy *et al.*, 2003; Oppezzo *et al.*, 2003), suggesting that the SHM machinery in UM B-CLL cells is defective or inactivated. In contrast, B-CLL cells that constitutively express AID undergo active CSR without any stimulation (Cerutti *et al.*, 2002; Oppezzo *et al.*, 2003), indicating a dissociation between SHM and CSR in CLL cells. Since AID is differentially expressed in the two subsets of CLL, even though their gene expression profiles are almost identical (Klein *et al.*, 2001; Rosenwald *et al.*, 2001), it is tempting to think that AID is involved in the poor prognosis of UM B-CLL. In addition, several AID splicing variants are expressed in UM B-CLL cells (Marantidou *et al.*, 2010; McCarthy *et al.*, 2003; Oppezzo *et al.*, 2003). It will be interesting to learn whether these splicing variants are involved in UM B-CLL pathogenesis.

Recently, the study of AID expression in human B-cell leukemia or lymphoma has extended beyond GC-derived B-cell lymphomas and CLL to other kinds of human B-lineage leukemias and lymphomas. Several studies showed that AID is constitutively expressed in some cases of MALT lymphoma, which derives from marginal zone B cells in mucosa-associated lymphoid tissue; in immunocytoma, which is derived from plasma cells, and in hairy cell leukemia, which is derived from memory B cells, but not in multiple myeloma, which is derived from plasma cells (Forconi *et al.*, 2004; Greeve *et al.*, 2003;