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The cytidine deaminases AID and APOBEC-1 exhibit distinct functional properties in a novel yeast selectable system

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Abstract

Activation-induced cytidine deaminase (AID) is indispensable for immunoglobulin maturation by somatic hypermutations and class switch recombination and is supposed to deaminate cytidines in DNA, while its homolog APOBEC-1 edits apolipoprotein (apo) B mRNA by cytidine deamination. We studied the editing activity of APOBEC-1 and AID in yeast using the selectable marker Gal4 linked to its specific inhibitor protein Gal80 via an apo B cassette (Gal4-C) or via the variable region of a mouse immunoglobulin heavy chain gene (Gal4-VH). Expression of APOBEC-1 induced C to U editing in up to 15% of the Gal4-C transcripts, while AID was inactive in this reaction even in the presence of the APOBEC-1 complementation factor. After expression of APOBEC-1 as well as AID approximately 10^{-3} of yeast cells survived low stringency selection and expressed β-galactosidase. Neither AID nor APOBEC-1 mutated the VH sequence of Gal4-VH, and consequently the yeast colonies did not escape high stringent selection. AID, however, induced frequent plasmid recombinations that were only rarely observed with APOBEC-1. In conclusion, AID cannot substitute APOBEC-1 to edit the apo B mRNA, and the expression of AID in yeast is not sufficient for the generation of point mutations in a highly transcribed Gal4-VH sequence. Cofactors for AID induced somatic hypermutations of immunoglobulin variable regions, that are present in B cells and a variety of non-B cells, appear to be missing in yeast. In contrast to APOBEC-1, AID alone does not exhibit an intrinsic specificity for its target sequences.

Keywords: Cytidine deamination; Immunoglobulin; Somatic hypermutations; DNA recombination; Editing

1. Introduction

Activation-induced cytidine deaminase (AID) is absolutely required for antigen-dependent immunoglobulin diversification by somatic hypermutations (SHM) and class switch recombination (CSR) in activated B lymphocytes (Muramatsu et al., 2000, 1999; Revy et al., 2000). APOBEC-1, the catalytic subunit of the apolipoprotein (apo) B mRNA editing enzyme-complex, deaminates C_{6666} of the apoB mRNA together with the mRNA binding protein ACF/ASP (APOBEC-1 complementation factor or APOBEC-1 stimulating protein) (Lellek et al., 2000; Mehta et al., 2000; Teng et al., 1993). AID and APOBEC-1 share a high degree of homology and most probably have arisen by gene-duplication (Espinosa et al., 1994; Muramatsu et al., 1999; Muto et al., 2000). Initially, in analogy to APOBEC-1 also AID was assumed to be an RNA editing enzyme with an unknown RNA target (Muramatsu et al., 2000, 1999). However, AID can deaminate dCs in single stranded DNA in vitro during transcription, preferably at WRCH/DGYW mutational spots (Bransteitter et al., 2004; Chaudhuri et al., 2004, 2003; Dickerson et al., 2003; Pham et al., 2003; Rogozin

Abbreviations: AID, activation-induced cytidine deaminase; APOBEC-1, apolipoprotein B mRNA editing enzyme catalytic polypeptide 1; CSR, class switch recombination; SHM, somatic hypermutations

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and Diaz, 2004; Shen and Storb, 2004; Yu et al., 2004). In *Escherichia coli*, AID induces mutations in transcribed DNA, and a deficiency of uracil-DNA glycosylase (UNG) which removes uracil from the DNA enhances the mutation rate (Petersen-Mahrt et al., 2002; Ramiro et al., 2003). UNG inhibition in DT40 B cells alters the pattern of SHM from transversion dominance to transition dominance, and UNG deficiency in mice in vivo shifts mutations at dC:dG sites to predominantly transitions (Di Noia and Neuberger, 2004; Rada et al., 2002). The DNA deamination model of AID action predicts that AID attacks dC:dG pairs to generate dU:dG lesions that can be replicated over (phase 1A mutation), subjected to uracil excision with generation of an abasic site (phase 1B mutation), or recognized as a mismatch by MSH2/MSH6 (phase 2 mutation) (Petersen-Mahrt et al., 2002). However, this model for AID action is not undisputed entirely. Not only AID, but also the *bona fide* mRNA editing enzyme APOBEC-1 deaminates dCs in single stranded DNA in vitro (Petersen-Mahrt and Neuberger, 2003). In *E. coli*, APOBEC-1 is an even stronger mutator than AID, and transgenic overexpression of both proteins induces cancer, indicating that both AID and APOBEC-1 generate mutations in vivo (Harris et al., 2002; Okazaki et al., 2003; Yamanaka et al., 1995). De novo protein synthesis is required for CSR as well as for DNA cleavage in SHM, in line with an RNA editing mechanism of AID action (Begum et al., 2004; Nagaoka et al., 2005). Moreover, despite its proven DNA mutator activity APOBEC-1 cannot substitute AID to induce SHM or CSR in B cells (Eto et al., 2003; Fugmann et al., 2004).

Here we compare the mode of action of AID and APOBEC-1 in yeast. We used the yeast transcription factor Gal4 as a selectable marker that was fused in frame to its specific inhibitor protein Gal80 either with an apo B sequence containing the apo B editing site (Gal4-C) in between (Lellek et al., 2002), or with a mouse immunoglobulin heavy chain variable region (VH) plus the apo B sequence (Gal4-VH) in between. APOBEC-1 induces mRNA editing specifically at the apo B editing site, while AID has no activity in this reaction even in the presence of ACF/ASP. Neither high level expression of AID nor APOBEC-1 suffice for efficient mutagenesis in a highly transcribed VH DNA sequence. However, AID expression leads to frequent plasmid recombinations that are only rarely observed with APOBEC-1. Thus, AID and APOBEC-1 have distinct functional attributes in this novel yeast selection system.

2. Materials and methods

2.1. Plasmids

2.1.1. pB-Gal4C-AID

The full length cDNA of mouse AID was amplified by RT-PCR from mouse spleen B cells using oligonucleotides mAID10-*Not*I (GCG GCCGCAATGGACAGCCT TCTGATGAAGCAA, nt 93-116 plus *Not*I site) and mAID11 (GCGGATCCTCAAAATC CCAACATAC-GAAATGCA, as, nt 689–665), cloned into the unique *Not*I site of pB-Gal4-ApoB$_C$-Gal80 (Lellek et al., 2002) to generate pBGal4C-AID. *pB-Gal4C-APOBEC-1:* The full length cDNA of rat APOBEC-1 was amplified by PCR from pSVL21-APOBEC-1 (Greeve et al., 1996) with oligonucleotides APOBEC-1-*Not*I (GCGGCCGCAAT-GAGTTCCGAGACAGGC CCTGTA, nt 31–54 plus *Not*I site) and REPV (TCCCAGAAGTCATTTCAACCCTGT, as, nt 729–706) (Greeve et al., 1996), and cloned into the *Not*I site of pB-Gal4-ApoB$_C$-Gal80 to generate pB-Gal4C-APOBEC-1. *pB-Gal4-VH-AID and pBGal4-VH-APOBEC-1:* A mouse immunoglobulin heavy chain variable region derived from a functionally rearranged heavy chain gene of the MPC-11 plasmocytoma (Lang et al., 1982) was amplified by PCR with oligonucleotides VH-5′ (CTTGGTTCCATGGTC-CACTCCCAGGTC, nt 336–352 spanning a *Nco*I site) and VH-3′(TTGTATATCCATGGGTGAGGAGACTG, as, nt spanning a *Nco*I site) and inserted in frame between Gal4 and ApoB in pB-Gal4-C using the unique *Nco*I site to generate pBGal4-VH. The full length cDNAs of AID and APOBEC-1 were inserted into the unique *Not*I site of pBGal4-VH to generate pBGal4-VH-AID and pBGal4-VH-APOBEC-1, respectively. To express AID and APOBEC-1 without the SV40 NLS and HA epitope, their full length cDNAs were amplified with oligonucleotides mAID-*Nde*I (GCCATA TGGACAGCCTTCTGATGAAGCAA, nt 93–116 plus *Nde*I site) and mAID11 or with APOBEC1-*Nde*I(GCCATATGAGTTCCGAGACAGGCCCTGTA, nt 31–54 plus *Nde*I site) and REPV, respectively, cloned into pGEM-Teasy, excised with *Nde*I/*Not*I and inserted into *Nde*I/*Not*I digested pB-Gal4-VH to generate pB-Gal4-VH-AIDØHA and pBGal4-VH-APOBEC-1ØHA, respectively. The *Nde*I/*Not*I restriction enzyme digestion of pB-Gal4-VH releases the SV40 NLS and HA epitope downstream of the *MET25* promoter. All constructs were entirely sequenced to confirm their identity. The generation of pACT-ASP was described previously (Lellek et al., 2002).

2.2. Transformation and growth conditions of yeast CG1945 cells

The pBridge yeast expression plasmids were transformed into the yeast strain CG1945 (Clontech®) by standard methods (Lellek et al., 2002). The genotype of CG1945 is MATa, ura3-52, his3-200, lys2-801, ase2-101, trp1-901, leu2-3, 112, Gal4-542, Gal80-538, cyh-2, Lys2::Gal1$_{UAS}$-Gal1$_{TATA}$-His3, URA3::Gal4$_{17mers(X3)}$-CyC1$_{TATA}$-lacz. After transformation the yeast cells were grown on synthetic drop-out media as described (Lellek et al., 2002). Six to twelve days after growth on −T −M media the yeast colonies were replated onto −T or −T −H media. After growth for further 6 days the colonies were counted and assayed for β-galactosidase activity (Lellek et al., 2002). Cotransforma-