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Comment on the mechanisms of APOBEC3G-catalyzed processive deamination of C on single-stranded DNA

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To the editor

The article by Nowarski and colleagues¹, “Hypermethylation by intersegmental transfer of APOBEC3G cytidine deaminase” claims first that APOBEC3G deaminates cytidines by a mechanism predominantly involving intersegmental transfer. A protein moving by intersegmental transfer undergoes direct transfers between segments of DNA by a “doubly bound” intermediate state, rather than by completely releasing and reassociating with the DNA as implied by the terms, hopping or jumping^{2–4}. Secondly, although Nowarski and colleagues¹ find that APOBEC3G can act processively, they also suggest that the enzyme is not inherently processive based on the observation that it cannot deaminate consecutive cytidines within a hotspot 5’CCC motif. This letter is intended to comment on, and hopefully clarify, several points regarding these interpretations.

Taking the second point first, we begin by defining processivity. A general definition, which we have applied previously to APOBEC3G and to AID (activation-induced cytidine deaminase)⁵, states that a processive enzyme catalyzes multiple reactions on a single substrate molecule prior to acting on another molecule. For APOBEC3G, deaminations occur preferentially at the third C in 5’CCC hotspot motifs⁶. Our experiments measured APOBEC3G-catalyzed deaminations occurring at two 5’CCC motifs separated by various numbers of nucleotides on a single-stranded (ss)DNA substrate. The data showed that APOBEC3G is able to catalyze processive deaminations at two 5’CCC motifs separated by as few as 1 nt and by as many as 100 nt^{7,8}. From the data we calculate a ‘processivity factor’, by comparing the observed fraction of double deaminations occurring at two CCC motifs on the same ssDNA molecule with the predicted fraction of double deaminations catalyzed independently by two separate APOBEC3G molecules^{7,8}. If the processivity factor is substantially larger than 1, the majority of double deaminations are caused by a single APOBEC3G molecule acting at both CCC motifs. The APOBEC3G processivity factor on single-stranded DNA is about 7 on average^{7,8}, thus the majority of double deaminations are caused by a single APOBEC3G molecule. In these experiments, typically less than 10% of the ssDNA substrates are deaminated, so that the fraction of double deaminations catalyzed by more than a single APOBEC3G per strand is insignificant, according to Poisson statistics. This conclusion doesn’t depend on the APOBEC3G/DNA ratio, nor does it depend on the concentration of ssDNA. It depends only on the fraction of ssDNA deaminated. We have measured similar APOBEC3G processivities at enzyme/DNA ratios of 1/40 to 1/1, over a 30-fold range of DNA concentrations (0.03 to 1 μ M). However, if ssDNA is present at sufficiently high concentrations, it may act as a competitor and thereby transfer APOBEC3G from one substrate to a different substrate, thus reducing the enzyme’s measured processivity, but not its *intrinsic* processivity.

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In contrast, Nowarski and colleagues¹ define processivity on the basis of whether each C in a single CCC motif is deaminated during a single encounter with an APOBEC3G molecule. This definition does not, in our view, serve to establish APOBEC3G's inherent processivity because processive movement does not impose a requirement for deaminating adjacent Cs, but instead the enzyme can catalyze numerous deaminations on different regions of the same ssDNA molecule before acting on a different DNA substrate^{7,9}. We also point out that consecutive deaminations are unlikely to occur at adjacent Cs in a single CCC motif because there is a large differential deamination efficiency within the trinucleotide motif that Nowarski et al.¹, ourselves^{7,8,10} and others^{6,11} have reported. The entire 5'CCC motif should be viewed as a single target analogous to the WRC (W=A/T, R=purine) hot spot deamination motif for the APOBEC family enzyme AID⁹.

Returning to the issue of intersegmental transfer, the authors have proposed that their data do not support the sliding and jumping model suggested by us earlier⁷, because they do not observe consecutive deaminations occurring in adjacent CCC motifs¹. Contrary to the author's interpretation, we have suggested for APOBEC3G⁷ and shown for AID^{9,12}, that these deaminases act stochastically and processively on ssDNA substrates⁵. For example, using a 365 nucleotide *lacZ* reporter sequence, we have observed sizable numbers of closely and distantly spaced deaminations at multiple C targets on individual ssDNA substrates with AID⁹ and APOBEC3G (Pham, *et al.*, unpublished data). AID catalyzes an average of about 20 deaminations in a *lacZ* target, whereas APOBEC3G catalyzes an average of 8 deaminations, while having available about 5-fold fewer target motifs compared to AID. The key point is that these multiple, but not necessarily consecutive, deamination events take place on an individual ssDNA substrate *prior* to reactions occurring on a different substrate; in other words, APOBEC3G and AID are intrinsically processive. The idea of stochastic processive deamination is contained in our models for APOBEC3G (Fig. 7, Ref. 7) and AID (Fig. 5, Ref. 12), in which we suggest that a transition, e.g., jumping, to another location on the DNA will surely be facilitated by the flexible ssDNA structure putting otherwise distal C targets in close proximity to APOBEC3G or AID. Our depiction of jumping⁷, through microscopic dissociation and reassociation⁴, does not exclude intersegmental transfer, which may well be occurring.

Whether APOBEC3G completely dissociates from the DNA as it travels to a distal site (jumping), or one DNA binding domain hangs on while a search is taking place elsewhere (intersegmental transfer), or both types of movement occur, a sliding motion is likely to be involved as well, for the following reason. Since APOBEC3G binds to ssDNA irrespective of nucleotide sequence, even in the absence of C⁵, it is highly unlikely that a jump or an intersegmental transfer would place the enzyme immediately at a CCC motif. Therefore, contrary to what Nowarski and colleagues¹ suggest, the enzyme would have to sample nearby bases, most likely by sliding, after the initial binding event occurs, otherwise the search would not be efficient. APOBEC3G can deaminate closely spaced 5'CCC motifs, e.g., separated by 1 or 5 nt, and this is likely accomplished by sliding^{7,8}. Furthermore, using stopped-flow kinetics we found that processive double deaminations occur within 5 seconds (Fig. 3A–B, Ref 8). Therefore, our data indicate that distally correlated APOBEC3G-catalyzed deaminations are efficient and thus likely involve sliding along with larger movements such as jumping and/or intersegmental transfer. Since our data cannot establish the degree of dissociation in the process of jumping or intersegmental transfer, nor can the data of Nowarski and colleagues¹ in our view, the actual nature of the translocation mechanism remains uncertain.

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