1. INTRODUCTION

Amine groups are vulnerable to modification such as alklyation, carboxylation and oxidation. They can be readily protonated and so provide excellent leaving groups. Of the five bases in nucleic acids, three have primary amino groups all of which are vulnerable to deamination. While this may form part of the metabolism of purines and pyrimidines as well as DNA and RNA precursors, once incorporated into polynucleotides, problems arise because these amino groups are involved in base pairing. Substitution of an amino for a keto group alters Watson–Crick base pairing. Thus, cytidine is changed to uridine, which base-pairs as thymidine, while adenosine is modified to yield inosine, which base-pairs mainly as guanosine. Guanosine deamination is not a major issue in nucleic acid biochemistry and will not be discussed further. In short, deamination can be mutagenic.

2. HOST ENZYMES WITH NUCLEIC ACIDS AS SUBSTRATES

Despite this, numerous cellular enzymes can deaminate RNAs with extraordinary specificity, such as the wobble position in the tRNA anticodon whereby an adenosine residue in the first base of the tRNA anticodon is edited to inosine by a series of genes collectively known as ADATs, adenosine deaminases acting on tRNA (Maas et al. 1999). As inosine is known to be able to base-pair with T, C and less readily A, it is able to decode codons with U or C in the third position. ADAR-1, -2 and -3 (ADAR = adenosine deaminase acting on RNA) can bind to double-stranded RNA (dsRNA) regions within cellular mRNAs and, by a base-flipping mechanism, specifically edit an adenosine residue (Valente & Nishikura 2005). Cytidine deamination too can be remarkably specific. For example, the human APOBEC1 enzyme specifically edits residue 6666 within the 14 121 base human apolipoprotein B (apoB) mRNA within intestinal tissue (Chen et al. 1987; Powell et al. 1987; Innerarity et al. 1996). In so doing, it generates a novel form of the protein that impacts triglyceride metabolism. In this particular case, specificity is conferred by another protein, APOBEC1 complementation factor. Specific editing is understandable, the evolutionary advantage is clear.

For the virologist, high mutation rates are part and parcel of RNA viruses where the genomic mutation rate can be as high as one or two per genome per round of replication (Drake 1993). While the mutation rate can be increased by a factor of 2 or so by the use of drugs (Holland et al. 1990), beyond that limit, the virus is mutated to death. This limit is referred to as the error threshold beyond which the informational integrity of the viral genome is irredeemably compromised (Biebricher & Eigen 2006). Among the 300 or more genes that are upregulated by type I interferons, is ADAR-1L (Samuel 2001). It is a variant of the constitutively expressed ADAR-1 gene. ADAR-1L binds to dsRNA and can edit as many as 90 per cent of adenosines within the same viral RNA. The prototypic example has always been the measles virus in cases of subacute sclerosing panencephalitis (Schmid et al. 1992). Subsequently, ‘blitzkrieg’ adenosine editing of cellular mRNA was also reported, particularly within dsRNA structures or mRNAs containing inverted Alu elements embedded within the 3′ untranslated region of mRNAs, or within introns of pre-mRNAs (Athanasiadis et al. 2004; Blow et al. 2004; Kim et al. 2004; Eisenberg et al. 2005; Levanon et al. 2005). The degree of editing ranges from a few bases to more than 40 per Alu.

Not surprisingly, such phenomena are referred to as hypermutants and the process hypermutation. The
destruction of viral RNA via hypermutation, particularly by an interferon-induced gene, can be readily understood as part of an anti-viral response. The raison d’être of hyperediting mRNA is less clear and, although it could well be coupled to mRNA turnover, there is no clear picture for the moment (Scadden 2005). These observations were generally made by massive mRNA sequencing and computer comparisons with the human genome (Athanasiadis et al. 2004; Kim et al. 2004; Levanon et al. 2004).

Hyperediting of single-stranded DNA (ssDNA) has become de rigueur since the identification of activation-induced deaminase (AID) by Honjo’s group back in 2000 (Muramatsu et al. 1999). There is no need to belabour the point in this volume. This remained a signal example although the homology to APOBEC1, with its specificity for ssRNA, was intriguing. Earlier work on transgenic mice bearing the human, mouse or rabbit APOBEC1 gene under the control of a liver-specific promoter clearly showed the development of hepatocellular carcinomas (HCC; Yamanaka et al. 1995) suggesting, but not proving, that the APOBEC1 enzyme might edit DNA (Harris et al. 2002; Petersen-Mahrt & Neuberger 2003). Interestingly, the same mice showed evidence of hyperediting of apoB mRNA, that is up to 10 edited C bases in the region surrounding residue 6666. Hence, the mechanism by which APOBEC1 expression resulted in these HCCs remained unclear. Direct evidence that APOBEC1 could edit DNA came from a sequence analysis of rifampicin-resistant clones generated by expressing APOBEC1 in Escherichia coli (Harris et al. 2002).

A second human APOBEC gene (APOBEC2) was identified in 1999, yet to date its physiological role remains unknown despite a beautiful crystal structure and knockout mice (Mikl et al. 2005; Prochnow et al. 2007). Navaratnam’s group first identified the human APOBEC3 cluster on chromosome 22 in a remarkably complete paper without ascribing any function to the most studied member APOBEC3G (Jarmuz et al. 2002). They identified all but APOBEC3H (OhAinle et al. 2006). Given the remarkable sequence identity to APOBEC1, it was safe to conclude that APOBEC3s were cytidine deaminases but direct proof only came later (Harris et al. 2003; Lecossier et al. 2003; Manget et al. 2003; Mariani et al. 2003; Zhang et al. 2003; Liddament et al. 2004; Wiegand et al. 2004; Zheng et al. 2004). This helps explain the relative rarity of these sequences in HIV databases. This beautiful virological story is extensively developed in Michael Malim’s accompanying paper. The HIV-1/A3F/A3G example parallels perfectly that of ADAR-1L editing of viral RNA. With the word parallel in mind, it is now clear that the A3G gene is strongly upregulated by type I and type II interferons, both in vitro and in vivo (Bonvin et al. 2006; Peng et al. 2006; Tanaka et al. 2006). Viral hypermutation indicates that nature, as opposed to Nature, has wised up to the fact that blitzkrieg mutation is yet another efficient way of fighting viruses.

3. SELECTIVE AMPLIFICATION OF CYTIDINE- AND ADENOSINE-EDITED HYPERMUTANTS

We realized that PCR could be simply adapted to select for G→A hypermutants. In standard PCR, the denaturation temperature (Td) is invariably 94–95°C and held constant so that DNA is uniformly denatured. Yet it is widely known that AT-rich DNA melts at lower temperatures compared with GC-rich DNA. This is due to the lack of a third hydrogen bond (approx. one-third) and decreased stacking energy (approx. two-thirds). We reasoned that by modulating the Td, conditions could be found allowing selective amplification of AT-rich APOBEC3 edited DNA to the detriment of unedited DNA. This turned out to be the case (figure 1a). Indeed, edited genomes at proportions as low as 10−4 could be selectively recovered (Suspène et al. 2005b). We have extensively used this method, referred to as 3DPCR, in our analyses of retroviral hypermutation (Mahieux et al. 2005; Suspène et al. 2005a,b; Bonvin et al. 2006; Delebecque et al. 2006; Suspène et al. 2008).

Inevitably, this made us wonder how the opposite could be achieved, that is the selective amplification of GC-rich DNA. Given the emerging importance of ADAR editing of a wide variety of RNAs, it would be useful to have a PCR-based method to allow selective amplification of GC-rich alleles. However, in view of the 3:2 hydrogen bonding rule for GC and AT base pairs, differential denaturation of target DNA would appear to be out of the question (Wain-Hobson 2006). Yet the beginnings to a solution lie in ADAR editing itself. Inosine base-pairs with cytidine through two hydrogen bonds rather than the three typical of a GC base pair (figure 2a).
Modified bases are often encountered in DNA bacteriophage genomes, usually as a means to avoid host restriction enzymes (Gommers-Ampt & Borst 1995). Invariably, modifications involve cytidine or thymidine, e.g. 5-hydroxymethyl cytidine in phage T4 DNA. There is, however, just one example of a modified purine, 2,6-diaminopurine, or ‘D’. It is found in the cyanophage S-2L DNA genome where it totally substitutes for adenosine (Kirnos et al. 1977) and has the singular feature of base-pairing with thymidine (T) via three hydrogen bonds (figure 2b). As deoxynosine and deoxydiaminopurine triphosphates (dITP and dDTP) are commercially available, the outlines of a PCR-based method allowing selective amplification of GC-rich alleles becomes clear—a combination of differential denaturation PCR using the modified bases dITP and dDTP. Does it work? Indeed it does and the method is referred to as 3DIPCR, the inverse of 3DPCR (figure 1b). Now we have a pair of methods allowing amplification of AT- or GC-rich DNA. These methods were applied to the problem of IgV hypermutation by AID (Suspène et al. 2008).

Figure 1. Selective amplification of AT- and GC-rich DNA. (a) PCR amplification of molecular clones of HIV-1 differing by between 0 and 18 G→A transitions at the non-restrictive and restrictive temperatures of 95 and 83°C, respectively. At 83°C, only clones containing G→A transitions could be amplified. A sample of a HIVΔvif virus from a molecular clone transfected on to 293T cells and subsequently used to infect PBMCs (peripheral blood mononuclear cells) was also amplified at 83°C. Cloning and sequencing showed the genomes to be G→A hypermutants resulting from APOBEC3G editing of viral cDNA. (b) 3DIPCR amplification of measles virus RNA produced in an interferon type I (i) insensitive (Vero) and (ii) sensitive (MRC5) cell line. (iv) For the MRC5 culture, DNA products could be recovered using a Td as low as 65°C, compared with (iii) the Vero control (67.4°C). Cloning and sequencing showed that the lower temperature products represent measles virus genomes edited by ADAR-1L.

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Complementary DNA corresponding to rearranged Vk1 regions from human CD14+ splenocytes was selectively amplified by 3DPCR and 3DIPCR. Although the latter yielded nothing in particular, 3DPCR recovered a series of exclusively AT-rich V region sequences (figure 3). Both strands were edited while the majority of mutations (68%) mapped to CDR1 and CDR2 (figure 3b). The local context for editing was WGCW, highly indicative of AID editing (figure 3c). These are among the few examples where there is a trace of AID editing alone in IgV genes. What is particularly interesting is that they were derived from cDNA meaning the AID 'only' edited loci that were transcribed before DNA repair. As mentioned earlier, 3DPCR is acutely sensitive, down to $10^{-4}$, so these sequences could well reflect the exception.

Although APOBEC3-associated hypermutation has been described for many retroviruses in a myriad of co-transfection experiments in vitro, for the moment, G→A hypermutants have only been identified in vivo.
for HIV, other lentiviruses and hepatitis B virus (Meyerhans et al. 1989; Günther et al. 1997; Vartanian et al. 2002; Beale et al. 2004; Liddament et al. 2004; Kieffer et al. 2005; Noguchi et al. 2005; Suspène et al. 2005a,b, 2006; Kijak et al. 2008). A single hyperedited HTLV-1 sequence in vivo represents a rather singular case, it being derived from an experimentally infected New World squirrel monkey, which is not its natural host (Vartanian et al. 1997). As species-specific effects have been described in vitro concerning the restriction of lentiviruses, it is probably wise to put this example on hold. Although the primate spumaviruses appear to be quite sensitive to restriction by some APOBEC3 members in vitro, not a single hypermutant has been described in vivo or in virus cultures to date (Delebecque et al. 2006). A recent report of hyperedited human papillomavirus 1 and 16 DNA (HPV1 and HPV16) in vivo will be addressed below (Vartanian et al. 2008).

The database for APOBEC3-edited retroviral DNA is dominated by the primate lentiviruses, with HIV\textsuperscript{\textit{Δvif}} at the top. In the absence of Vif, the proportion of edited genomes is approximately 100 per cent. Certainly, a SIV\textsuperscript{Δvif} virus fails to grow in vivo (Desrosiers et al. 1998). This is an interesting example. SIVs are found only among African monkeys and primates. The sooty mangabey virus (SIVsm) grows to high titres in its natural host without pathology. However, when transposed to the Asian Rhesus macaque, the virus was quickly adapted and resulted in overt pathology. This suggests that despite the many millions of years separating the two monkey lineages, the inter-host differences within the APOBEC3F and 3G genes were small fare for a rapidly evolving lentivirus such as SIV. For hepatitis B virus in vivo, the frequencies were very low, approximately \(10^{-4}\), while similar frequencies pertain to human papillomavirus (HPV). For the moment and from a virological standpoint, APOBEC3 cytidine deaminases are only efficient against the lentivirus subgroup of retroviruses.

4. APOBEC EDITING OF ENDOGENOUS RETROVIRUSES

Much has been written about the possible role of APOBEC3 genes in controlling endogenous retroviral transposition. It is an attractive hypothesis with much in vitro data in its favour (Esnault et al. 2005; Schumacher et al. 2005, 2008; Bogerd et al. 2006; Muckenfuss et al. 2006; Jern et al. 2007). The problem is the relative dearth of examples in vivo. Molecular biology is so powerful these days that hypothesis making can be a relatively easy affair. Hypothesis breaking, making sure that the observations are physiologically relevant, takes time and is frequently fraught with difficulties. The most straightforward rider is the fact that the avian lineage does not encode an APOBEC3 orthologue despite an abundant of endogenous retroviruses (ERVs) and a particularly famous exogenous retrovirus, Rous sarcoma virus. Reptile and fish genomes encode ERVs (Andersen et al. 1979; Huder et al. 2002; Shen & Steiner 2004); indeed some of the piscine ERVs are expressed in vivo (Paul et al. 2006). APOBEC3 cytidine deaminases appear by the rodent lineage, where there is a single gene, moving up to six in equines (Bogerd et al. in press).

By contrast, Macaca mulatta, Pan troglodytes and Homo sapiens encode an expanded seven-gene APOBEC3 loci (A3A, A3B, A3C, A3DE, A3F, A3G and A3H). As Papio, Ceropithecus and some other monkey lineages encode clear homologues to A3G and A3H, it is a reasonable hypothesis for the moment that most monkey and Great Ape species encode seven APOBEC3 genes. An interesting ‘problem’ is afforded by baboon endogenous retrovirus (BaEV, \textit{Papio} sp.), which has not only been identified in the peripheral blood of baboons but is also able to grow on many cell lines (Benveniste & Todaro 1974), suggesting that the virus is not restricted by baboon A3 deaminases. The explanation for this is the xenotropic nature of the surface envelope protein—the virus in unable to infect baboon cells yet is able to infect many non-baboon or foreign cells.

In an experimental setting, endogenous mouse intracisternal A type particles (IAPs) and MusD elements are vulnerable to hyperdeamination by a variety of APOBEC3 genes. However, the experimental system was human because the IAPs do not transpose well in murine cells. Showing a GC→AT mutation bias with an excess of \(G\rightarrow A\) over \(C\rightarrow T\) within IAPs compared with a consensus sequence is not prima\textit{}\textit{fact} evidence because similar biases can be found for pseudogenes compared with their cognate genes having corrected for CpG sites (Gojobori et al. 1982), mutated p53 alleles and disease alleles (Krawczak et al. 1995; Vartanian et al. 1997). As genes are generally GC rich, while transitions are the most frequent of mutations, a GC→AT bias is arguably the null hypothesis for any gene degradation process. Non-coding DNA is generally AT rich, which although proving nothing \textit{per se}, is consistent with the notion that in mammals loss of information tends to end up as AT-rich DNA, if not deleted before.

What is perhaps a little surprising about extant analyses of ERVs is the relative dearth of hypermutants (Esnault et al. 2005; Jern et al. 2007; Jonsson et al. 2007; Armitage et al. 2008; Lee et al. 2008). The majority of ERVs known and studied to date are ‘Moloney like’ or ‘mouse mammary tumour virus (MMTV) like’. Research on Moloney murine leukaemia virus (MoMLV) and MMTV go back to the pre-HIV days when retrovirology was effectively oncoretrovirology. When the first mammalian ERVs were described, it was noted that they had clear homology to either of these two murine retroviruses presumably reflecting past invasion of germinal cells. The effect of murine APOBEC3 on MMTV is slight with no deamination reported so far (Doehle et al. 2005; Abudu et al. 2006; Jern et al. 2007; Okeoma et al. 2007; Rulli et al. 2008). The APOBEC/MLV literature is highly variable, with a clear lack of consensus. Recent papers have suggested that one of the intrinsic resistance factors for Friend-MLV, \textit{Rfe}3, is murine APOBEC3 (Santiago et al. 2008; Takeda et al. 2008). However, as there are multiple restricting factors for this retrovirus, their effects can only be dissected out in highly inbred mice. Whether or not mAPOBEC3 is...
a dominant restriction factor in feral mice would seem to be an open question for the moment. In Balb/c Friend-sensitive mice, hyperdeamination of Fr-MLV is a rare event (Petit et al. 2009).

One glorious study showed that the rabbit genome harbours ERV remnants akin to a lentivirus, delightfully called RELIKs for rabbit endogenous lentivirus type K (Katzourakis et al. 2007). From a consideration of mutation rates, the authors concluded that RELIK had invaded the rabbit genome more than 7 Myr ago. From the present perspective, it must be noted inter alia that RELIK harboured a decayed rif gene meaning that the ‘solution’ to cytoplasmic APOBEC3 deaminases was solved a long time ago. It is not possible to know in which species this occurred for it could be argued that RELIK emerged in another species and crossed over to the rabbit.

5. APOBEC3 AND DNA VIRUSES
Not surprisingly, the current literature is dominated by work on retroviruses for that is where ‘Hurricane APOBEC3’ started. Double-stranded DNA comes alive through transcription and replication via ssDNA. As some APOBEC3 proteins can be found in the nucleus (Boger et al. 2006; Kinomoto et al. 2007), unlike A3F and A3G that are strongly cytoplasmic albeit with the occasional exceptions (Mangeat et al. 2003; Wiegand et al. 2004; Gallois-Montbrun et al. 2007, 2008), the question arises as to whether single-stranded cellular DNA can be edited by APOBEC3 deaminases. AID clearly can be specifically targeted to the nucleus (Bogerd et al. 2007). Albeit not within the APOBEC3 cluster, AID is to all intents and purposes an APOBEC3, or vice versa, which species this occurred for it could be argued was solved a long time ago. It is not possible to know which species this occurred for it could be argued that RELIK emerged in another species and crossed over to the rabbit.

Figure 4. Phylogenetic relationships among human APOBEC3 cytidine deamination domains (CDDs). The SplitsTree was made using protein sequences with APOBEC2 (hA2) and AID given as outliers. The CDD domains of the double-domain hA3s have been split and analysed as hA3Bn, hA3Bc, etc. where n and c indicate the N- and C-terminal domains, respectively. Those that edit HPV DNA have been highlighted.

In a large European study, approximately 65 per cent and approximately 6 per cent of cervical cancers were associated with HPV16 and HPV18, respectively (Arbyn & Dillner 2007). In addition, for HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66, there is significant evidence that they can cause cervical cancer (Cogliano et al. 2005). To place ourselves in the best position to detect APOBEC3-edited HPV DNA, we selected the overlapping promoter/origin of replication region, which is located in the long non-coding region of all HPVs, based on the logic that this region would remain single-stranded for a longer period of time compared with other regions of the genome.

3DPCR was used to select for edited HPV DNA. We screened six HPV1a planter warts and found hypermutated DNA in one. Averaging across both strands, the mean cytidine editing frequency was approximately 11 per cent (range 4–61% per clone; figure 5a), which is highly comparable with the degree of editing seen for retroviruses. The dinucleotide context associated with editing was TpC and CpC (figure 5b). Such a preference is typical of APOBEC3 deaminases and animony for AID. To reduce the list of APOBEC3 candidates, transfection experiments were carried out with a full-length cloned HPV1a plasmid. Although there is no in vitro culture system for HPV, the promoter region can open up and support transcription.

It transpired that three out of seven human APOBEC3 deaminases, hA3A, hA3C and hA3H, were capable of editing HPV1a DNA. The mean cytidine editing frequencies in vitro, 25–29% (range 13–60% per clone), were approximately twofold higher than those in vivo, which may reflect stronger APOBEC3 gene expression driven by the powerful cytomegalovirus immediate-early promoter. Importantly, the editing context was almost identical to that found in vivo, supporting the hypothesis that editing arose as a result of an APOBEC3 enzyme (figure 5b).

Yet we were unable to find traits that could help eliminate one or other of the enzymes. Indeed, as hA3A

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and hA3H are expressed in keratinocytes, while hA3C is probably the most widely expressed of all the human A3 deaminases, there is no reason to exclude one or the other.

Although there is no data on APOBEC3 expression in mucosal keratinocytes, the finding of APOBEC3 editing of HPV1a led us to examine total DNA from nine HPV16-positive pre-cancerous cervical biopsies for evidence of hyperediting as opposed to cervical carcinoma tissue. The carcinoma harbours little HPV DNA and what there is is generally integrated. Furthermore, as the carcinoma is mono- or oligoclonal, sampling would be highly restricted. Of two out of nine samples, two showed evidence of hypermutation in the ori/promoter region (figure 5c). The mean editing frequency was approximately 9 per cent (range 5–24%). Once again, the dinucleotide editing context was TpC and CpC (figure 5d) indicating an APOBEC3 deaminase, possibly A3A, A3C and/or A3H.

Additional transfection experiments were performed using human APOBEC1 and 2 (hA1, hA2) as well as the murine deaminases mA1, mA2 and mA3. 3DPCR failed to identify any editing of HPV1a down to a frequency of 10^{-4}. Given the negative result for hA3B, and the fact that hA1 and mA1 traffic between the cytoplasm and nucleus, a nuclear localization per se is insufficient for deamination of HPV1a DNA.

These three human APOBEC3 enzymes encode a single cytidine deamination domain (CDD) as opposed to hA3B, hA3DE, hA3F and hA3G that encode two CDDs. All three can be found within the cytoplasm and nucleus as can hA3B. Human A3DE, hA3F and hA3G are essentially cytoplasmic in location. Furthermore, A3A is phylogenically closely related to the active CDD2 domain of A3B while A3C is related to the active CDD2 domains of A3F and A3G, indicating that rather subtle differences must explain the HPV editing phenomenon by APOBEC3 enzymes (figure 4).

It is curious to note that, similar to hA3A/C/H, AID too has a single CDD, and as mentioned earlier, AID is slightly closer to the APOBEC3 cluster of deaminases than APOBEC1, APOBEC2 or APOBEC4 (figure 4; Vartanian et al. 2008). Can AID edit HPV DNA in vitro? It can indeed, and the dinucleotide context is GpC and ApC just as it is for rearranged immunoglobulin V regions. In view of this, it is probable that AID is not involved in HPV deamination in vivo.

To resume, HPV genomic DNA can be edited by at least four human cytidine deaminases in vitro and possibly one to three in vivo and extends the range of viral targets for APOBEC3 enzymes to include double-stranded DNA viruses. It is a little surprising that the degree of editing can be as high as 61 per cent of potential targets when the whole panoply of DNA mismatch repair enzymes is omnipresent in the nucleus. For example, an edited IgV region with so many edited C residues has never been reported. These contrasting findings might be explained by the fact that

Figure 5. APOBEC3 editing of HPV DNA in vivo. (a) Sample HPV1a.1. A small selection of HPV1a-edited DNA from a planter wart. Only sequence differences are given with respect to the unedited reference sequence. As only a fraction of the sequences are shown, the numbers to the right indicate the total number of mutations per sequence. (b) Dinucleotide context of HPV1a-edited sites where the dot indicates the edited site. The data for A3A, A3C and A3H were derived from transfection experiments. (c) Sample HPV16.29. A small selection of HPV16-edited DNA from a pre-cancerous cervical biopsy. Legend same as figure 5a. The TATA box within the promoter region is highlighted. (d) Dinucleotide context of HPV16-edited sites where the dot indicates the edited site. Black bar, experimental; grey bar, in vivo.
HPV genomes are packaged within the nucleus. It could be that the HPV hypermutants originate from neo-assembled viral cores where they would be sequestered from the mismatch repair machinery. Within the nucleus, G:U mismatch repair, which predominantly yields G:C, will mitigate against the effects of APOBEC3 editing. It is possible that a vestigial C→T transition reflects the opposing forces of APOBEC3 editing and G:U mismatch repair. Accordingly, the real frequency of hA3A/C/H editing should be considerably greater.

6. APOBEC3s AND CANCER

Transcriptionally active HPV genomes are in the form of mini-chromosomes complete with nucleosome. If they are vulnerable to cytidine deamination, say in a subset of cells with overexpressed hA3A/C/H, then it raises the intriguing question as to how chromosomal DNA is protected from these three nuclear APOBEC3 deaminases, which are, when given the chance, hugely efficient at editing ssDNA in vivo and in vitro (figure 5). Given the considerable literature linking ectopic expression of AID in a multitude of tissues to cancer (Kou et al. 2007; Matsumoto et al. 2007; Komori et al. 2008; Marusawa 2008; Morisawa et al. 2008), as well as AID editing outside of rearranged IgV regions, it appears that hA3A, hA3C and hA3H might well be implicated in a similar manner. As the three laws of thermodynamics tell us that no machine is perfect, it is plausible that they too, as AID, may occasionally turn against the human genome. Indeed this would seem to be the null hypothesis. While extremely efficient mutators, their effects are surely countered by highly efficient DNA repair machinery and so it may be extremely difficult to identify hyperedited human genes. As mentioned earlier, HPV hypermutants could well have been sequestered from the repair machinery by the packaging of the deaminated DNA. From this perspective, hyperedited dsDNA genomes could serve as surrogate markers for these powerful mutators. It seems necessary to investigate their promoters and identify the transcription factors involved as well as to establish their interactomes.

It is interesting that edited HPV genomes, whether in vitro or in vivo, represent low-frequency phenomena, and squares with the notion that these events arise from rare situations where control breaks down. That the degree of editing of some HPV genomes in vivo was strictly comparable with those in vivo, where the powerful cytomegalovirus immediate-early gene promoter drives gene expression, suggests that there must be a few cells in vivo where the expression of A3A/C/H must be very elevated indeed. In this context, it has been noted that numerous APOBEC3 genes are upregulated in tumour cell lines (Jarmuz et al. 2002).

It has not escaped the attention of many in the cancer genomics field that there are far more mutations in a cancer genome than hitherto suspected. Furthermore, the majority of mutations are C→T transitions (Greenman et al. 2007). Given the power of mismatch repair, a single C→T transition could reflect the vestige of local AID/APOBEC3 editing. Hyperediting of genomic DNA in sites of chronic inflammation would set up a broad mutant spectrum from which a cancer genome could emerge. Yet hypermutation is unlike the sequential accumulation of point mutations, which is akin to the series WORD→WOKE→GORE→GONE→GENE (Maynard Smith 1970). Hypermutation, even if severely corrected, allows jumps through sequence space that could propel the cell faster along the road to cancer.

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