(TADs) — chromosomal regions in which genes and the regulatory sequences that control their activity (called promoters and enhancers) interact regularly, thus modulating gene expression. The team compared the skate genome with those of several other vertebrates, and found that, during the little skate's evolution, genome rearrangements had altered the structure of TADs that encompassed genes involved in a cell-signalling system known as the planar cell polarity (PCP) pathway.

The PCP pathway emerged early in animal evolution, and is known[4] to drive the establishment of cell shape and orientation — including in flattened sheets of cells — during development. However, its involvement in skate-fin development had not previously been described. Marlétaz and colleagues confirmed, using several experimental approaches, that the PCP pathway is involved in development and expansion of the skate's anterior pectoral fins. For instance, they showed that the PCP gene *Prickle1* is expressed to a greater degree in the anterior portion of the pectoral fin than in the posterior portion. Addition of a PCP-pathway inhibitor to the water in which young skates were being kept, or implantation of inhibitor-soaked beads directly into the pectoral fins, led to changes in fin development in the anterior region. The authors also corroborated the involvement of this pathway using a modified version of Hi-C known as HiChIP (which detects chromosome looping that participates specifically in gene regulation).

The involvement of PCP in skate-wing development was unexpected, but might have been predicted, because the way in which the flattened pectoral fin grows and expands is reminiscent of the development of other cell sheets regulated by the PCP pathway, such as fly wings[4]. Notably, the authors found scant *Prickle1* expression in the embryonic pectoral fin of the chain catshark (*Scyliorhinus retifer*), which, unlike the batoids, does not undergo an anterior expansion. This further supports the idea that the skate-specific expression pattern of *Prickle1* contributes to the wing-like fin.

Finally, Marlétaz *et al.* examined open chromatin — regions of the genome in which DNA is loosely packaged and so available for transcription factors to bind to genes and so modulate gene expression. Analysis of the open chromatin regions associated with genes that were differentially upregulated or downregulated in the anterior and posterior pectoral fin identified an enhancer involved in the regulation of several *HoxA* genes in the anterior fin. Genes of the *HoxA* cluster direct development of the body plan and limbs in all vertebrates[5]. Their divergent expression in the anterior pectoral fins, driven by the skate *HoxA* enhancer, probably contributes to expansion of this extra growth zone in the batoid wing.

Taken together, Marlétaz and colleagues' results support the idea that the anterior region of the developing skate pectoral fins is under different genetic control from that of the posterior region. Their work suggests that genomic events in the ancestor of the batoids might have facilitated the emergence of this evolutionary novelty.

There is much to celebrate in this paper. The authors have generated data resources for the little-skate genome, and have used these to address an evolutionary conundrum. Their use of TADs and TAD structure to make inferences about the relationship between evolution and development is especially exciting, because it provides a means of identifying key genomic changes that occurred deep in evolutionary time. However, caution should be exercised when interpreting these complex sequence-based data sets[6]. Moreover, the findings still need to be validated using conventional developmental-biology approaches, because the magnitude of a change does not necessarily reflect its importance — small mutations can have large effects on gene regulation, whereas bigger changes can be of little consequence.

The approaches used by Marlétaz and colleagues can also be adopted to address other evo-devo questions in batoids. For instance, what major genetic and structural changes led to the evolution of walking-type behaviour in skates[7], or oscillatory locomotion (a type of underwater flight suited to life in the open sea) in a subset of batoids, including manta rays and bat rays[8]? A combination of these approaches, alongside gene-editing techniques such as CRISPR and single-cell analyses, will enable many other fascinating natural-history stories to be investigated.

**Chris Amemiya** is in the Department of Molecular and Cell Biology, University of California-Merced, Merced, California 95343, USA.
e-mail: camemiya@ucmerced.edu

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Cancer

# Viral agent of genomic instability

**Lori Frappier**

A protein from Epstein–Barr virus called EBNA1 has been shown to bind to and break human chromosome 11, producing instability in the genome that might cause a predisposition to cancer. **See p.504**

Epstein–Barr virus (EBV) is a common herpesvirus that is also associated with several types of cancer, including lymphomas, nasopharyngeal carcinomas and gastric carcinomas[1]. How exactly the virus affects the development of these cancers is unclear, but tumour cells are known to be derived from infected cells that express a small subset of EBV proteins. In fact, the only viral protein that is expressed in all types of EBV-related cancer is Epstein–Barr nuclear antigen 1 (EBNA1), which binds to specific sequences in the viral DNA, allowing it to persist and replicate. The possibility that this protein contributes directly to cancer has long been proposed and disputed[2]. Li *et al.*[3] suggest on page 504 how it might do so — by binding to a specific region on human chromosome 11 and triggering its breakage, thereby inducing genomic instability.

EBV persists in human cells for a lifetime, and promotes cell proliferation in a form of infection referred to as latency. EBNA1 is expressed in the dividing cells, and binds to multiple repeated sequences in the EBV genome. Biochemical and structural studies have defined how the DNA-binding domain of EBNA1 interacts with an 18-base-pair (bp) palindromic DNA sequence[4–6]. The ability of this protein to bind to viral DNA has prompted investigations into whether it also recognizes specific sequences in host DNA, and previous studies of EBV-infected immune cells called lymphocytes have identified numerous possible target sequences, but with no clear consensus[7]. Among these is a cluster of high-affinity EBNA1-binding sites on chromosome 11,

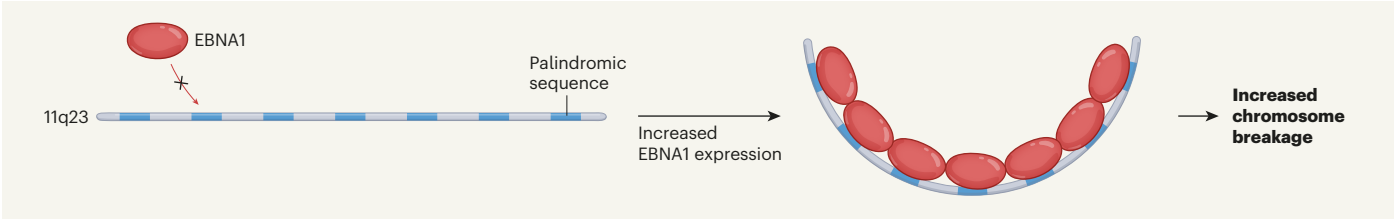


**Figure 1 | Model for binding and breakage of human chromosome 11 by the protein EBNA1.** Li *et al.*[3] suggest that the Epstein–Barr virus (EBV) protein EBNA1 could induce genomic instability, and therefore potentially contribute to EBV-associated cancers, as follows. A DNA region called chromosome 11q23 contains multiple repeated palindromic DNA sequences. EBNA1 can bind to these sequences, but only once its expression reaches a threshold level. Given past observations of similar repeated sites in the EBV genome, EBNA1 bound to the sites could be expected to interact with itself and so bend the DNA. EBNA1-induced changes in DNA and in the structure of chromatin (the complex in which DNA is packaged in the nucleus) could, in some as-yet-undefined way, then promote chromosome breakage at this fragile site, potentially predisposing cells to cancer.

between the *FAM55D* and *FAM55B* genes[8].

Li and colleagues used microscopy to visualize EBNA1 after expressing it in several EBV-negative cell lines, including primary cell lines (those newly extracted from tissues) and cancer cell lines. They found that EBNA1 was highly enriched at two distinct foci in the nuclei of both primary and cancer cell lines. Formation of these foci required the protein's DNA-binding domain, suggesting that it was recognizing a specific sequence in the host DNA.

The authors mapped the EBNA1-bound sites to a 21-kilobase repetitive region of chromosome 11 (11q23). This region contains more than 300 copies of an 18-bp sequence that is remarkably similar to the palindromic EBNA1-binding sites in the EBV genome (Fig. 1). The authors verified the importance of these sequences for EBNA1 recruitment by showing that disrupting these sites abolished the formation of EBNA1 foci.

When Li and colleagues visualized the EBNA1-associated region in chromosomes of dividing cells, they identified aberrant structures characteristic of inherently fragile DNA. They found that chromosome breakage in this region was induced by EBNA1 in a dose-dependent manner, and was triggered in EBV-infected lymphocytes by increasing the protein's levels by as little as twofold. EBNA1-induced chromosomal breaks occurred quickly (within one day of induced EBNA1 expression) and efficiently (with roughly 40% of cells exhibiting breakage at 11q23). The EBNA1-binding region in 11q23 occurs between genes encoding the tumour suppressor ATM (frequently altered in cancers) and the proto-oncogene MLL (often rearranged in leukaemias). One consequence of the breakage induced by EBNA1 was an alteration in the number of copies of these genes in daughter cells after cell division; this might contribute to the induction of cancer.

As expected, the chromosome breakage depended on EBNA1's DNA-binding domain. In addition, another region of the protein — located between amino acids 410 and 460 — greatly stimulated breakage. The region contains a sequence that interacts with a cellular enzyme known as ubiquitin-specific protease 7 (USP7; ref. 9). This enzyme stimulates the DNA-binding activity of EBNA1, which, in turn, recruits USP7 to EBNA1-recognition sites in the EBV genome[8]. There, USP7 can remove particular modifications (known as ubiquitin) from proteins that form nucleosomes[10] — complexes of DNA and protein that exist in cell nuclei and form the basis of the chromosomal material known as chromatin. Therefore, the involvement of this region of EBNA1 in 11q23 breakage might reflect a role for USP7 — either in stimulating EBNA1's binding to these cellular sites, or in altering chromatin structure when recruited by EBNA1.

A key question is whether 11q23 breakage is commonly observed in EBV-associated cancers. To investigate this, Li *et al.* evaluated the frequency of chromosome 11 aberrations in human nasopharyngeal carcinomas, using previously generated whole-genome sequencing data from 78 EBV-positive nasopharyngeal carcinomas. Remarkably, 81% of these tumour samples exhibited structural variations at chromosome 11. A comparison with EBV-negative nasopharyngeal carcinomas would have been ideal here, but the strong association of EBV with this type of cancer makes it hard to find EBV-negative samples.

The authors also analysed whole-genome sequencing data from more than 2,000 cancers of various types, detecting an increase in structural variations on chromosome 11 in tumours categorized as EBV-positive compared with those that were EBV-negative. However, the group also often detected such rearrangements in cancers that were EBV-negative, suggesting that this fragile site can be broken by other mechanisms. Li *et al.* suggest that some of these tumours might have been infected by EBV but then lost the virus, in a 'hit and run' event, although it is not clear whether this happens. It will be interesting to compare 11q23 breakage and rearrangement events in tumours such as gastric carcinoma and Hodgkin's lymphoma — which can be positive or negative for EBV — to obtain a clearer picture of the impact of viral infection (and EBNA1) on this genomic instability.

Overall, Li and colleagues' study suggests that, in lymphocytes latently infected with EBV, expression of EBNA1 might be maintained at a level just below that needed to induce breakage of chromosome 11. Small increases in EBNA1 levels — which could occur after various stimuli — might trigger 11q23 breakage and, through the resulting genetic instability, contribute to the development of cancer. Interestingly, EBNA1 levels increase when the virus is reactivated to the 'lytic' phase of infection[11]. EBV-positive tumours typically show raised expression of specific EBV lytic proteins, raising the possibility that increased EBNA1 expression in these cells led to chromosome 11 aberrations.

The question remains as to how binding of EBNA1 to chromosome 11 induces breakage. It could result from the structural changes that the DNA-binding domain invokes in bound DNA[6,7,12]. Alternatively, it could be due to changes in chromatin structure, because EBNA1 is known to destabilize nucleosomes at some of its binding sites[13] and, as already mentioned, can recruit USP7 (and perhaps other cellular factors) that modulate components of chromatin[10]. Whatever the mechanism, Li and colleagues' study supports the contention that EBNA1 contributes directly to the development of cancer.

**Lori Frappier** is in the Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5G 1M1, Canada.
e-mail: lori.frappier@utoronto.ca

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