**Epstein-Barr virus (EBV) can cause B-cell lymphomas**

* **EBV** is the first human DNA tumor virus discovered more than **50 years ago** and causes ~200,000 cases of cancers every year.
* In primary EBV infection, the virus transits across the oropharyngeal epithelium to reach the B cell compartment. EBV converts primary B cells into activated blasts, which enable EBV to colonize the B cell compartment. Indeed, EBV-transformed lymphoblasts can be seen transiently in patients with infectious mononucleosis, which is caused by primary EBV infection.
* Although T- and NK-cell surveillance eventually contains lymphoblast proliferation, EBV latently infected B cells are the **reservoir** from which the virus establishes **lifelong infection**. With HIV infection, organ transplantation, or primary immunodeficiency, **impaired control of EBV latently infected B cells leads to fatal lymphoproliferative diseases and lymphomas.**

**LCLs is a useful model for studying EBV-mediated B-lymphoid oncogenesis**

* In vitro, EBV transforms primary resting B lymphocytes (RBLs) to continuously proliferating lymphoblastoid cell lines (LCLs). LCLs express the same viral genes as some EBV lymphomas. These viral genes include six Epstein-Barr virus nuclear antigens (EBNAs), three latent membrane proteins (LMPs), and multiple microRNAs.
* LCLs are therefore a useful model for studying EBV-mediated B-lymphoid oncogenesis

*In vitro*, EBV transforms primary human resting B lymphocytes (RBLs) into lymphoblastoid cell lines (LCLs). LCLs express type III EBV latency genes, including EBV nuclear antigens (EBNAs) 1, 2, leader protein (LP), 3A, 3B, and 3C, latent membrane proteins (LMPs) 1 and 2, nonpolyadenylate small RNAs, and microRNAs (miRNAs) ([3](https://pmc.ncbi.nlm.nih.gov/articles/PMC6580941/#B3)).

*In vivo*, cells expressing the type III EBV latency program are efficiently removed by the normal immune system. However, when the normal immune system is impaired, such as in transplant recipients undergoing immune suppressive treatment or in AIDS, type III latency program-expressing B cells can develop into lymphoproliferative diseases or lymphomas.

Thus, LCLs serve as a useful model system to study the roles of EBV in oncogenesis. Understanding the molecular mechanisms of EBV-mediated growth transformation will not only provide insight into EBV pathogenesis but also identify potential therapeutics.

**Chromosome structural features offer insights into EBV-mediated B-lymphoid oncogenesis**

Overview of Hi-C.

Here we report a method named Hi-C that adapts the above approach to enable purification of ligation products followed by massively parallel sequencing. Hi-C allows unbiased identification of chromatin interactions across an entire genome. Briefly: cells are crosslinked with formaldehyde; DNA is digested with a restriction enzyme that leaves a 5′-overhang; the 5′-overhang is filled, including a biotinylated residue; and the resulting blunt-end fragments are ligated under dilute conditions that favor ligation events between the cross-linked DNA fragments. The resulting DNA sample contains ligation products consisting of fragments that were originally in close spatial proximity in the nucleus, marked with biotin at the junction. A Hi-C library is created by shearing the DNA and selecting the biotin-containing fragments with streptavidin beads. The library is then analyzed using massively parallel DNA sequencing, producing a catalog of interacting fragments

1. Cells are cross-linked with formaldehyde, resulting in covalent links between spatially adjacent chromatin segments (DNA fragments: dark blue, red; Proteins, which can mediate such interactions, are shown in light blue and cyan). Chromatin is digested with a restriction enzyme (here, HindIII; restriction site: dashed line, see inset) and the resulting sticky ends are filled in with nucleotides, one of which is biotinylated (purple dot). Ligation is performed under extremely dilute conditions to create chimeric molecules; the HindIII site is lost and a NheI site is created (inset). DNA is purified and sheared. Biotinylated junctions are isolated with streptavidin beads and identified by paired-end sequencing. (**B**) Hi-C produces a genome-wide contact matrix. The submatrix shown here corresponds to intrachromosomal interactions on chromosome 14. Each pixel represents all interactions between a 1Mb locus and another 1Mb locus; intensity corresponds to the total number of reads (0-50). Tick marks appear every 10Mb. (**C**, **D**) We compared the original experiment to a biological repeat using the same restriction enzyme (**C**, range: 0-50 reads) and to results with a different restriction enzyme (**D**, range: 0- 100 reads, NcoI).

**Xiang’s results:**

To explore whether the two spatial compartments correspond to known features of the genome, we compared the compartments identified in our 1 Mb correlation maps to known genetic and epigenetic features. Compartment A correlates strongly with the presence of genes (Spearman’s rho=0.431, p<10−137), higher expression (via genome-wide mRNA expression, Spearman’s rho=0.476, p<10−145 [Fig. S5]), and accessible chromatin (as measured by DNAseI sensitivity, Spearman’s rho=0.651, p negligible) (15,16). Compartment A also shows enrichment for both activating (H3K36 trimethylation, Spearman’s rho=0.601, p<10−296) and repressive (H3K27 trimethylation, Spearman’s rho=0.282, p<10−56) chromatin marks.

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* **Compartment A (Active)**: Enriched for open chromatin, gene-rich regions, high transcriptional activity, and euchromatin. These regions tend to interact more with each other and are associated with accessible, active chromatin states.
* **Compartment B (Inactive)**: Enriched for closed chromatin, gene-poor regions, low transcriptional activity, and heterochromatin. These regions interact more among themselves but less frequently with A compartments.

**How the division works:**

1. **Hi-C captures chromatin interactions**: Hi-C sequencing generates a genome-wide interaction map that shows how frequently different regions of DNA interact.
2. **Correlation analysis**: Principal component analysis (PCA) is often used on the interaction matrix. The first principal component (PC1) helps classify regions into **A and B compartments**.
3. **Compartmentalization**: The sign of PC1 (positive or negative) correlates with known chromatin features. Typically, **positive PC1 values correspond to compartment A (active), while negative PC1 values correspond to compartment B (inactive).**

**Future Direction**

Nature Review Immunology 2003: B cells under influence: transformation of B cells by Epstein–Barr virus: Epstein–Barr virus (EBV) enters the lymphoid tissue of the oropharynx and infects B cells. Molecular analysis of EBV-infected B cells in tonsils of patients with infectious mononucleosis indicated that EBV mainly infects germinal-centre and/or memory B cells, but also some naive B cells. Clonal expansion of infected B cells in the interfollicular region is (largely) restricted to germinal-centre/memory B cells and is not accompanied by somatic hypermutation (SHM). Occasionally, EBV-positive B cells are detected in the germinal centre. These cells also undergo clonal expansion, but they do not participate in a normal germinal-centre reaction. It is unclear whether EBV directly infects germinal-centre B cells, or whether EBV-infected memory B cells enter germinal centres. Members of clones of EBV-positive B cells in the interfollicular region show variation in morphology — small cells, blasts and Hodgkin and Reed–Sternberg (HRS)-like cells — and presumably also EBV-gene expression pattern. Most EBV-positive B cells are eliminated by cytotoxic T cells. A few EBV-positive B cells downregulate the expression of EBV-encoded genes, so that they cannot be detected by T cells, and establish the long-term reservoir of EBV-positive cells. GC, germinal centre.

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