The EBNA3 proteins

Robert E. White†

† Corresponding Author: Dr Rob White, Section of Virology, Department of Infectious Disease, Imperial College London, London SW7 2AZ, UK. robert.e.white@imperial.ac.uk

Abstract

Epstein-Barr virus is a master manipulator of B cell biology, provoking the activation and proliferation of infected B cells, while ensuring their survival. Central to this is the epigenetic re-configuration of the B cell genome and transcriptome, for which the EBV nuclear antigens (EBNAs) are crucial. This review focuses on the role of the EBNA3 family of proteins – EBNA3A, EBNA3B and EBNA3C – in EBV's manipulation of B cell biology, building on the previous incarnation of this review (Allday *et al.* 2015). In particular, the review discusses the genomic and transcriptomic context of EBNA3 gene expression, mechanisms by which they regulate host genes, and how they impact B cell survival, differentiation and function, alongside distinct insights from mouse models, that reveal both pro- and anti-oncogenic roles of EBNA3 proteins. The review finishes by considering new insights and proposing key unresolved questions about the roles and regulatory mechanisms of EBNA3 proteins.

1 Introduction to EBV and the EBNA3 proteins

Epstein Barr-virus (EBV) is a ubiquitous human virus, that establishes lifelong persistence in the B cells of the vast majority of humans. In a small proportion of individuals EBV is responsible for the development of lymphomas or carcinomas (Farrell 2019). More insidiously, EBV is increasingly epidemiologically associated with the development of diverse autoimmune diseases (most strikingly multiple sclerosis (Bjornevik *et al.* 2022)), and perhaps even diseases of immunological intolerance such as inflammatory bowel disease (Zhang *et al.* 2022). The natural biology of EBV separates into two distinct phases: in differentiated epithelia the virus undergoes productive replication shedding into biological fluids. In B cells it enters a persistent state, where it manipulates the B cell to establish a lifelong site of persistence in its infected host. It is in this process of establishing latency that the EBNA3 proteins are produced, although their effects are felt for the lifetime of EBV's infection of the B cell.

1.1 The B cell biology of EBV

The widespread association of EBV with immunological defects points to its biology in B cells as the key to many of these pathogenic outcomes. In vitro EBV infection of resting B cells triggers them to activate, differentiate and proliferate. For transforming strains (B95-8 being the lab workhorse strain) this produces a lymphoblastoid cell line that (other than two in vitro selection bottlenecks around 1 month (Heath *et al.* 2012) and ≈3-5 months (Counter *et al.* 1994, Sugimoto *et al.* 1999) after infection) are transformed and immortal.

Our best model of EBV biology *in vivo* is the Germinal Centre (GC) model (Figure 1), proposed about 25 years ago by the late David Thorley-Lawson and colleagues (Babcock *et al.* 1999), which has been broadly supported by subsequent observations (reviewed in detail in (Thorley-Lawson 2015). Briefly, the in vivo model reflects normal B cell biology, whereby the B cell is activated and proliferates, and then migrates to (or forms de novo) a germinal

centre in lymphoid tissues. Here the B cell can cycle between the light zone (LZ) where its B cell receptor (BCR) binds antigen and receives survival signals associated with T cell help if its BCR is among the best antigen-binders, and then moves to the dark zone (DZ), where it mutates its BCR gene variable regions and proliferates, before returning to light zone to begin again, with its differentiation state oscillating between LZ- and DZ-associated phenotypes (Young and Brink 2021). At some point, the B cell may receive additional cytokine signals persuading it to switch the class (isotype) of its heavy chain locus, from IgM to (most commonly) one of the IgA or IgG isotypes. Eventually the B cell will leave the germinal centre, differentiating to become either a plasma cell, or a memory B cell.

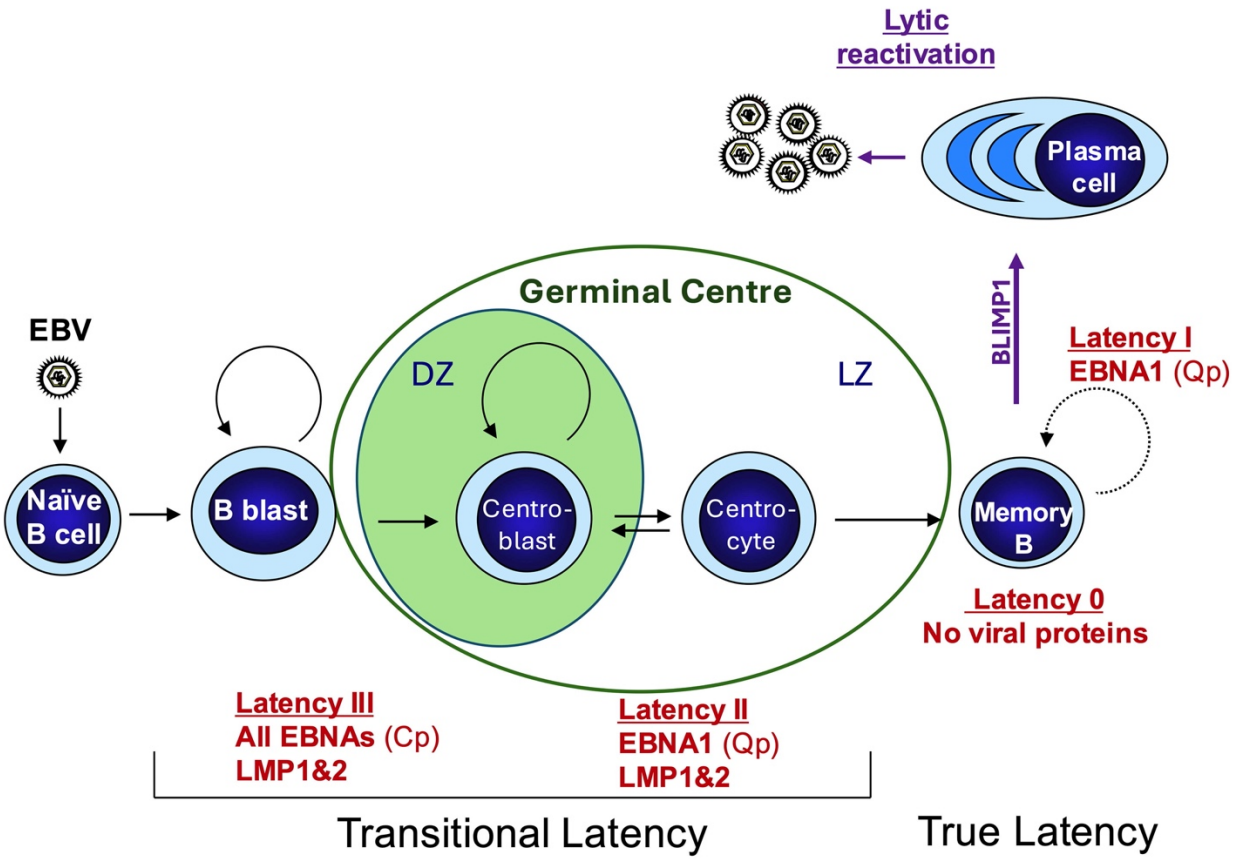

Figure 1. The Germinal centre model of Epstein-Barr virus biology in B cells. Schematic shows B cell differentiation states associated with conventional B cell activation (white text in blue cells) and the germinal centre (LZ= light zone; DZ = dark zone) in green. The various EBV protein-expression profiles are indicated (Latency states III, II, 0 and I) in red text. Plasma cell differentiation and virus reactivation indicated in purple. Near-circular arrows indicate cell states that are proliferating.

EBV manipulates this B cell differentiation process, promoting the activation, hyper-proliferation and survival of the B cells, largely through the "B cell transcription unit" that produces 7 proteins (6 EBV nuclear antigens - EBNAs and the Bcl2 homologue BHRF1), 3 miRNAs, and additional non-coding RNAs. In the germinal centre, the B cell transcription unit is shut down (Roughan and Thorley-Lawson 2009), leaving the latency membrane proteins (LMPs) to supply GC survival signals before the EBV-infected cell leaves as a resting IgD-negative memory B cell (Joseph *et al.* 2000, Souza *et al.* 2007) expressing no viral proteins (Babcock *et al.* 2000, Hochberg *et al.* 2004), except for EBNA1, which is transiently turned on when the cell divides (Hochberg *et al.* 2004). This state of quiescence

in memory B cells, making few or no viral proteins, is the long-term site of EBV latency, undetectable by the cytotoxic T cell response. EBV emerges from this state when the cell is triggered to differentiate into a plasma cell, where the expression of plasma cell-defining transcription factor Blimp1 (*PRDM1*) helps activate the EBV immediate early genes BZLF1 and BRLF1 and initiates virus productive replication.

These lifecycle changes are coupled to changes in viral transcription centred around the B cell transcription unit, whereby virus transcription initiates from one or more copies of a repeated promoter (Wp), before mainly using the BamC promoter (Cp) just upstream. Thus after infection, EBV produces EBNA-LP and EBNA2, followed by BHRF1, the three EBNA3 proteins and EBNA1. EBNA2 subsequently activates LMP promoters (and probably also the BARTs), facilitated by the inhibition of innate repressive factors by EBNA-LP (Cable *et al.* 2024) and tegument proteins including BNRF1 (Tsai *et al.* 2011), establishing the "latency III" transcriptional profile. Once the B cell enters the GC, Cp/Wp are shut-down, switching to the BamQ promoter (Qp) to generate EBNA1, which is produced alongside the LMPs - the latency II transcription state. Emerging as a memory B cell into circulation, EBV makes no viral proteins ("latency 0"), turning on EBNA1 when the cell divides (Latency I) (Roughan and Thorley-Lawson 2009). These latency states, and B cell differentiation states broadly match EBV-associated lymphomas, with diffuse large B cell lymphoma (DLBCL), Hodgkin lymphoma (HL) and Burkitt lymphoma (BL) characterised by latency states III, II and I respectively.

1.2 Overview of EBNA3 functions.

The three EBNA3 proteins – EBNA3A, -3B and -3C – share a common structure, and (as will be detailed in the rest of this review) are transcriptional regulators that are capable of reorganising genetic loci to alter the regulation of host genes. EBNA3-mediated regulation is often slow to be established, and slow to be lost, including some changes in regulation that are epigenetically stable, raising the idea of "hit-and-run" oncogenesis, whereby past EBNA3 expression may be sufficient for them to exert an oncogenic function. There is also considerable overlap between EBNA3 and EBNA2 binding sites on the human genome, that may represent co-operation or antagonism (or both, depending on context) in the regulation of target genes. EBNA3A and EBNA3C are primarily characterised as oncogenes, repressing pro-apoptotic genes and helping to facilitate in vitro establishment of LCLs. In contrast, EBNA3B is dispensable for transformation, and can be mutated in some cancers, casting it as a viral tumour suppressor. The rest of this review will explore what is known about how EBNA3s function, and the role of these functions in B cell biology and the development of EBV-associated cancers, before discussing some remaining unanswered questions.

2 EBNA3 genetics: Diversity and transcriptome complexity

2.1 The genetics and transcriptomics of EBNA3 proteins.

EBV is a member of the lymphocryptovirus (LCV) genus (also called gamma-1-herpesviruses), which are found only in primates (Ehlers *et al.* 2010). LCV genomes are most readily distinguished from the Kaposi sarcoma-associated herpesvirus (KSHV)-like gammaherpesviruses found in all mammals by the presence of the large internal repeat (IR1) which houses Wp and repeated EBNA-LP exons, and by the long alternatively spliced and polyadenylated transcripts that generate the EBNAs, including the EBNA3 proteins. EBV, and the other "old world" LCVs have essentially the same genome organisation, where the EBNA3 proteins are grouped together in the genome, and share the same genetic structure: a shorter 5' exon encoding around 100 aa of the protein, and longer second exon encoding around 900 aa, separated by a short (<100 nucleotide) intron (Figure 2). At the amino-acid level, the EBNA3s have diverged from each other substantially, excepting a region of 20-30% identity (the "homology domain") comprising the last 24 aa encoded by exon 1 and the first ≈200 aa of exon 2 of each EBNA3 (Le Roux *et al.* 1994). All three genes also contain repetitive elements, either imperfect repeats of domains in EBNA3A (Hennessy *et al.* 1986), or internal repeats (in EBNA3B and EBNA3C) that are long enough to complicate short-read genome sequence assembly (Palser *et al.* 2015).

The structural similarities and adjacent genome locations of the EBNA3 genes suggest that they arose from a historic gene duplication event. This probably occurred after the geographical separation of Africa and Europe from the Americas, as the marmoset LCV Callitrichine herpesvirus 3 – the only "New world" LCV sequenced to date – has only one EBNA3 gene, expressed from similarly long alternatively spliced transcripts (Rivailler *et al.* 2002). It is tempting to speculate that understanding the functions of this single protein, alongside identification and study of EBNA3 proteins from a wider range of LCVs, could help identify the most fundamental functions and interactions of human EBNA3 proteins - those that are most likely to represent potential therapeutic targets.

All identified EBNA3 mRNAs arise from alternative splicing of long transcripts initiated at Cp or Wp exclusively during the early (latency III) phases of EBV B cell infection. These transcripts we previously dubbed the "B cell transcription unit" of EBV (Allday *et al.* 2015) as shown in Figure 2. Translation of EBNA3s (and EBNA1) is thought to be driven by an IRES in the “U exon” (Isaksson *et al.* 2003), and transcripts polyadenylate after EBNA3A or EBNA3C (such that EBNA3B and EBNA3C share a polyadenylation site). However, there is no topological reason why transcripts initiated at Qp in latency II could not also give rise to EBNA3 proteins, so future RNA-seq studies assessing EBV transcription in other cell types and cancers and should remain open to this possibility. Our recent direct RNA-seq analysis of EBV transcripts in LCLs revealed a hierarchy in EBNA3 transcript abundance, with EBNA3C transcripts more abundant than EBNA3B, which were more abundant than EBNA3A transcripts (Mamane-Logsdon *et al.* 2025), as indicated in Figure 2. It is not clear whether this difference correlates to protein abundance, as EBNA3 proteins have a low degradation rate in LCLs (Touitou *et al.* 2005), perhaps due to their close association with the nuclear matrix (Petti *et al.* 1990), while their translation efficiency and RNA turnover rates are not known, although EBNA3 transcripts are m_6A methylated by METTL14, which stabilises their transcripts, and interacts with EBNA3C, perhaps as part of a feedback loop to fine-tune EBNA3 expression (Lang *et al.* 2019).

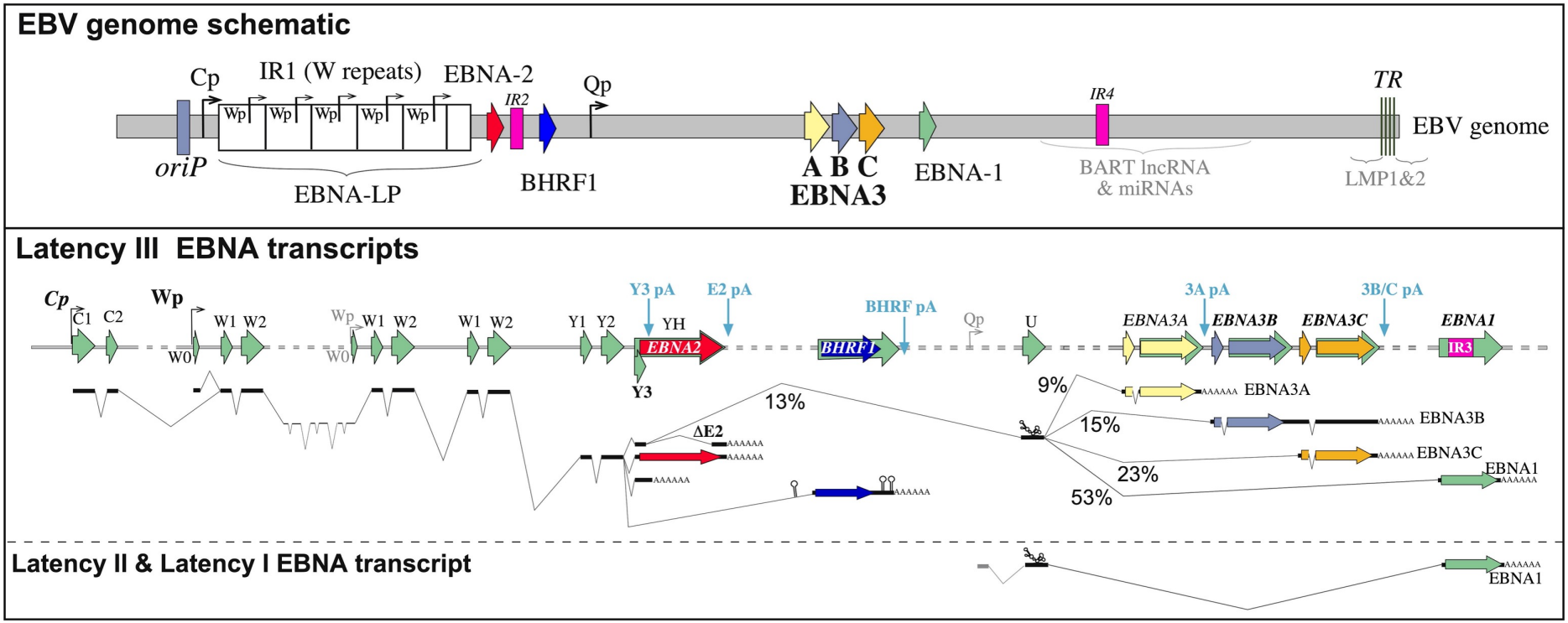

Figure 2. Generation of EBNA3 transcripts. Top schematic shows the EBV genome locations where the main latency gene products are produced. Major internal repeats (IR) and terminal repeats (TR), and latency replication origin (oriP) are labelled. Magenta boxes indicate positions of lytic replication origins (IR2 and IR4). Wp, Cp and Qp promoters are indicated by bent arrows. Latency III schematic shows the genome lay-out above the alternative splicing of transcripts originating at Cp or Wp ("EBNA transcripts). Green arrows and corresponding fat black lines in the latency III schematic indicate exons, with superimposed colour arrows indicating the coding regions of the protein made from each transcript (EBNA-LP is omitted since it could possibly be made from any of the transcripts, depending on which W1 splice acceptor is used; extra repeats of the W1-W2 exon pairs are shown in miniature). Thinner angled lines indicate splice junctions. Hairpins indicate BHRF1 miRNAs. Structured region in U exon represents IRES present in this sequence. Runs of A indicate transcript polyadenylation (pA sites marked above in pale blue). Percentage values indicate the proportion of the upstream splice variants that use the associated splice junction in B95-8 LCLs, based on Mamane-Logsdon et al 2025. Thick lines represent exons and thinner angled lines are splicing events. Latency II/I schematic shows the Qp-derived EBNA transcript.

Evolution and diversity of EBNA3 proteins.

Even within EBV, the EBNA3 proteins exhibit considerable diversity: alongside EBNA2, the EBNA3 proteins are the most variable coding region of the EBV genome. EBV is classified into two main subtypes (type 1 and type 2), almost entirely defined by the EBNA2 and EBNA3 gene sequences (Palser *et al.* 2015, Correia *et al.* 2018). Type 2 EBNA3s are around 90% identical to type 1 EBNA3s (nearer 80% for EBNA3C and EBNA3B due to the greater variation in the internal repeats), and while type 2 EBNA3 sequences are fairly consistent, whereas the type 1 EBNA3 sequences become more diverse with evolutionary distance from Africa (Palser *et al.* 2015). Thus Indonesian sequences are most diversified from African and European sequences (Bridges *et al.* 2019), and a partial sequence of EBNA3B from a Polynesian individual's lymphoma being more diverse still (White *et al.* 2012). Despite the 50kb separation between EBNAs and the EBNA3s, it is rare to find a virus with mixture of type 1 and type 2 EBNAs, even though the region in between does not show this linkage, suggesting a functional dependency between EBNA2 and EBNA3s of the same type (Farrell and White 2021).

A more intriguing question is what are the evolutionary pressures that drive this diversification, and what are the implications of this diversity. As we have learned from longitudinal study of RNA viruses like influenza and SARS-CoV2, key drivers are adaptation to the host, and immune evasion. Since EBNA3 proteins are made only in Latency III, their epitopes are immunodominant in the immune control of the lymphoproliferative phase of the EBV lifecycle (Murray *et al.* 1992) (Gavioli *et al.* 1992) (Ning *et al.* 2011) (Hislop *et al.* 2007). Failure to detect lymphoproliferation risks killing the host, which does not benefit a persistent virus like EBV, so there are potentially short-term evolutionary pressures to lose T cell epitopes, but long-term pressures to retain them. Despite the high diversity of the EBNA3 proteins, most type 1 strains contain most of the well characterised EBNA3 T cell epitopes, and a quarter of these are retained in type 2 EBV (Palser *et al.* 2015). In fact, Latency III proteins (EBNA2, EBNA3s and EBNA-LP) are less likely to have polymorphisms disrupting T cell epitopes than other virus genes, particularly for $CD4^+$ T cell epitopes (Cirac *et al.* 2021), suggesting that there is some selection pressure for EBNA3s to retain (rather than lose) their T cell epitopes. However, analyses correlating changes in epitopes with the HLA of the geographically matched population will be needed to determine whether EBNA3 sequences are evolving to be detected by – or hidden from – CTL responses.

It is also not known how the diversity of EBNA3 proteins may influence their biology: Type 1 and 2 EBNA2s have different binding ability to the transcription factor BS69 (Ponnusamy *et al.* 2019), and regulate different host genes (Lucchesi *et al.* 2008, Singh *et al.* 2022) but whether similar differences exist for EBNA3s remains unreported. But some aspect of EBNA2 or EBNA3 diversity is likely to explain why type 2 EBV (unlike type 1) is so much more common in equatorial regions than at high latitudes.

3 Strategies for studying EBNA3 proteins

There are several distinct approaches that have been used to explore EBNA3 biology, and understanding the shortcomings of different approaches allows critical assessment of the biology these approaches have revealed. In principle, the simplest is to express the proteins in isolation in EBV-negative cells, albeit with risks of overexpression artefacts. However, since the EBNA3 proteins can interact with each other, co-regulate the same genes, (White *et al.* 2010), and often bind the same genomic sites as each other and EBNA2 (McClellan *et al.* 2013, Schmidt *et al.* 2015, Wang *et al.* 2015, Paschos *et al.* 2017), studying the EBNA3

proteins in isolation from other EBV proteins will give at best a limited view of their function, and at worst a deceptive one.

A more informative approach has been to use recombinant viruses. Early protocols using EBNA2-driven survival after recombination in P3HR1 was only able to generate EBNA3B knockouts (Tomkinson *et al.* 1992, Tomkinson *et al.* 1993), and left a type mismatch between the complementing type 1 EBNA2 and the type 2 EBNA3s of P3HR1. Most studies have relied on engineering knockouts, mutations or tags into the BAC clones of B95-8 or Akata. The size and complexity of the 200 kb EBV genome (and the complexity of generating virus-producing cell clones) leaves a risk of artefacts from undetected mutations. This can be mitigated by either using either duplicate recombinant BAC clones or revertant viruses, or using protein fusions – particularly estrogen receptor (ER) fusions – that allow conditional inactivation of EBNA3 functions (Maruo *et al.* 2005, Maruo *et al.* 2006, Skalska *et al.* 2010). While these approaches have been effective for studying EBNA3A and EBNA3C functions, this has been much more challenging to achieve for EBNA3B, as deletions in EBNA3B and fusions at either its N- or C-terminus have been observed to disrupt the splicing and expression of EBNA3C or EBNA3B ((Chen *et al.* 2006) our unpublished observations).

These recombinant viruses have been used to infect either already immortal EBV-negative BL cell lines, or to transform resting B cells into lymphoblastoid cell lines. It is important to note that – probably due to their different differentiation state and perhaps mutational backgrounds – these different cell platforms can give substantially different results in gene expression profiling: for instance, only 10% of genes differentially regulated by an EBNA3B knockout (3BKO) virus in BL31 cells were also differentially regulated in LCLs (White *et al.* 2010). Conversely, where recombinant viruses exhibit defects in transformation (which is the case for EBNA3A and EBNA3C mutant viruses), there is strong selective pressure in culture for mutations to complement the absence of the mutated protein. For instance, this was observed in a conditional EBNA3C-HT LCL that failed to arrest after withdrawal of 4-hydroxytamoxifen, due to a loss of Rb in this cell line (Skalska *et al.* 2010), and such mutations risk concealing the key transforming functions of EBNA3 proteins.

Nevertheless, replication and comparison between experiments has helped identify the most robust studies and data sets. In particular I have found that the two ChiP seq data sets analysing EBNA3-binding genomic loci where EBNA3s were expressed from EBV (one using Flag-tagged fusions in LCLs (Paschos *et al.* 2017), the other a cross-reacting EBNA3A antibody in Mutu III (McClellan *et al.* 2013) are in closer agreement than with data from LCls overexpressing FLAG-tagged EBNA3s (Jiang *et al.* 2014, Schmidt *et al.* 2015). Similarly, studies of EBNA3C-ER fusions in different CDKN2A mutant backgrounds (to avoid selection pressures) identified similar sets of differentially regulated genes, despite one using whole exome microarrays, and the other RNA-seq (Skalska *et al.* 2013, Ohashi *et al.* 2021), and exhibited more disagreements – even in direction of U133 microarray defined gene regulation – with an EBNA3C-ER fusion in CDKN2A wild-type cells (Zhao *et al.* 2011), emphasising the risk of artefacts from mutational selection in cell culture.

A wide range of large scale studies have been conducted to both identify the genes regulated by EBNA3 proteins, and analyse the genomic context of that regulation. Some of these datasets have been made accessible to non-informaticians in a searchable form at ebv.org.uk (maintained by the author). Some observations of regulated genes and EBNA3 binding peaks made in this review include specific interrogation of these data, that may not be otherwise published, citing the paper that generated the data. The caveats in this section should be borne in mind when assessing the value of those observations.

4 EBNA3 Protein structure and key interactions

The previous issue of this review (Allday *et al.* 2015) catalogued the range of interactions between EBNA3s and cellular proteins, so here we will restrict discussion to those interactions that are well characterised or functionally interesting. Some new interactors with EBNA3A have been proposed in a recent proximity biotinylation study (Landman *et al.* 2024), but these are only discussed where functionally relevant.

4.1 Interaction and co-operation between EBNA3s and other EBV latency proteins.

Identifying specific interactions is complicated by the fact that the EBNA3 proteins exhibit extensive co-operation: they interact with each other (Calderwood *et al.* 2007, Paschos *et al.* 2012), co-operate in gene regulation (White *et al.* 2010), and are bound at many of the same genomic loci as both each other and as EBNA2 (McClellan *et al.* 2012, Schmidt *et al.* 2015, Paschos *et al.* 2017). Notably, all these data agree that EBNA3C interacts with and functions with EBNA3A and EBNA3B (and sometimes both), but EBNA3A and EBNA3B do not interact or co-localise in the absence of EBNA3C (White *et al.* 2010, Paschos *et al.* 2017).

There is also considerable evidence of EBNA2 sitting at the same genomic loci as EBNA3s, but evidence of their interaction is limited. EBNA2 interaction with EBNA3A was suggested in a yeast-based interactome screen (Calderwood *et al.* 2007), but has not been confirmed in a B cell context, with a ChIP-reChIP assay unable to find evidence of simultaneous EBNA3A-EBNA2 binding (McClellan *et al.* 2013). Additionally, around a quarter of EBNA2 binding sites on the genome are also occupied by EBNA-LP (Wang *et al.* 2018, Maestri *et al.* 2025), although there is no analysis of whether these are the subset of EBNA2 sites that include or exclude EBNA3 occupancy.

4.2 EBNA3 protein structure.

Currently no structures of EBNA3 proteins (or even regions of EBNA3) that have been solved, so mutagenesis of interaction interfaces is generally blind. The majority of the protein are predicted to be intrinsically disordered, with only the Homology Domain (and a putative leucine zipper in EBNA3C) previously predicted to have secondary structure, as can be seen from the structural modelling at herpesfolds.org (Soh *et al.* 2024). In my hands, AlphaFold3 predicts a lot more structure when EBNA3s are modelled as homo- or hetero-dimers, but these structures have very low confidence scores, and my attempts fail to predict the well established interaction between the short linear motif (SLiM) PLDLS (or similar) on EBNA3A and 3C and the cellular repressor C-terminal binding protein (CtBP), which is the only sequence-specific interaction that has been validated for EBNA3 proteins (Touitou *et al.* 2001, Hickabottom *et al.* 2002, Skalska *et al.* 2010). More than a cursory AlphaFold3 analysis is therefore required to define the detail of interactions between EBNA3 proteins and their key cellular partners.

4.3 Binding to RBPJ directs EBNA3s to DNA: Parallels with Notch Signalling.

Our current model of how EBNA3 proteins (and EBNA2) are directed to DNA is through binding to the DNA binding protein RBPJ (for recombination signal binding protein for Ig kappa J region). RBPJ is also called CBF1 (for Cp binding factor) as there is an RBPJ binding site that recruits EBNA2 to activate Cp after EBV infection (Strobl *et al.* 1997). This RBPJ site can also mediate EBNA3A- or 3C-driven repression of Cp in reporter assays (Waltzer *et al.* 1996, Radkov *et al.* 1997) but has not been demonstrated in the context of virus infection. RBPJ is the canonical DNA-binding protein of the Notch signalling pathway, whereby – in drosophila – a transactivating protein Notch (intracellular domain) is recruited to DNA through binding the RBPJ homologue suppressor of hairless (SuH). This activation is opposed by the activity of Hairless, which competes with Notch for SuH binding, and recruits repressor complexes including C-terminal binding protein (CtBP) to inhibit gene transcription. The activating Notch, and repressive Hairless, both recruited by RBPJ, are parallelled by activating EBNA2 and repressive EBNA3 proteins, whose binding to RBPJ is mutually exclusive (Johannsen *et al.* 1996). However, in mammals, no direct Hairless homologue is known. Instead, repressive factors recruited to RBPJ in mammalian cells (reviewed in (Giaimo *et al.* 2021) include the SHARP complex, L3MBTL3, KyoT2/3 (isoforms B and C of *FHL1*) and BEND6 (Dai *et al.* 2013). Some parallels between EBNA3s and these factors are discussed in Section 4.4.

RBPJ binding to interaction partners is typically mediated by a short ϕWϕP motif: EBNA2 contains PWWP, while EBNA3C contains VWTP (or VWIP in type 2 EBV). However, EBNA3B and EBNA3C lack anything resembling this motif, having VQ[LI]P and [VI][VL]TP respectively in the homologous position to the EBNA3C VW[TI]P. Nevertheless, all of the EBNA3 proteins can bind RBPJ though their homology domain (Robertson *et al.* 1996, Zhao *et al.* 1996, Calderwood *et al.* 2011).

The binding of EBNA3s to RBPJ has been mapped to the homology domain. Of the homology domain's most conserved motifs (MGY; ATϕGC and TϕSA), only mutation of ATϕGC disrupted the ability of EBNA3A to suppress gene activation by EBNA2 (Dalbiès-Tran *et al.* 2001). Mutating this motif reduces binding to RBPJ, as well as ability to sustain LCL transformation and to repress target genes by both EBNA3A (Dalbiès-Tran *et al.* 2001, Maruo *et al.* 2005, Calderwood *et al.* 2011) and EBNA3C (Lee *et al.* 2009, Kalchschmidt *et al.* 2016, Gillman *et al.* 2018). However, these mutants retain some RBPJ binding ability, which (for EBNA3C) is completely lost when the ϕWϕP motif is also mutated (W227S) (Gillman *et al.* 2018). Thus the binding of EBNA3s to RBPJ through their ϕWϕP motif appears essential for their gene regulatory function, despite some residual RBPJ binding capability remaining. Conversely there is little impact of the W227S mutation on the transcriptome during B cell outgrowth (Gillman, White and Allday; unpublished - browsable at www.ebv.org.uk).

4.4 EBNA3A and EBNA3C require binding to CtBP for regulating some genes.

C-terminal binding protein (CtBP) is named for its ability to bind to the C terminus of the adenovirus E1A protein (Schaeper *et al.* 1995). In normal cell biology, CtBP contributes to the repressor functions of drosophila Hairless (Morel *et al.* 2001), which made CtBP an early candidate for the EBNA3 proteins' repressive mechanism. Proteins interact with CtBP through a SLiM that varies around a PLDLSx[K/R/H] consensus (Morel *et al.* 2001). EBNA3C contains a single PLDLSLH motif, while EBNA3A contains two non-consensus motifs (**A**LDLSIH and **V**LDLSIH) 30 amino acids apart. These motifs allow EBNA3A and EBNA3C to interact with CtBP1 (with EBNA3A mediating stronger binding in vitro, perhaps

due to the pair of sites), whereas EBNA3B, which lacks an appropriate SLiM, does not interact (Touitou *et al.* 2001, Hickabottom *et al.* 2002). Mutagenesis of the CtBP SLiM in EBNA3A and/or EBNA3C in the viral genome leads to the loss of regulation by EBNA3s of a number of cell genes (Skalska *et al.* 2010, Ohashi *et al.* 2021). One of these CtBP-dependent targets is CDKN2A, whose repression (specifically of $p16^{INK4A}$ function) is required for LCL outgrowth (Maruo *et al.* 2006, Skalska *et al.* 2010, Skalska *et al.* 2013, Ohashi *et al.* 2021), such that transcriptomic analyses are skewed (presumably by clonal selection) where $p16^{INK4A}$ is active (Ohashi *et al.* 2021). Trans-complementing EBNA3C knockout (3CKO) EBV with over-expressed EBNA3C mutants demonstrated that the binding of EBNA3C to RBPJ makes a larger contribution to EBNA3C's gene regulation function than its binding to CtBP does (Ohashi *et al.* 2021).

Vertebrates have two homologues (CtBP1 and 2) that can bind via PLDLS motifs, so it is notable that EBNA3A contributes to the absence of CtBP2 protein in LCLs (Hertle *et al.* 2009, White *et al.* 2010): EBNA3A binds to an intronic enhancer, and disrupts that enhancer's connection to the CTBP2 promoter (McClellan *et al.* 2013). As a result of the repression of CTBP2, it is likely that the functions of EBNA3s are mediated largely or entirely by CtBP1. It remains an open question whether this lack of CtBP2 changes the targets and mechanisms of EBNA3 gene regulation, compared to the regulation of genes by CtBP during normal B cell biology.

In normal cell biology, there is still a connection between RBPJ and CtBP that may reflect some aspects of EBNA3A and EBNA3C protein functions: first the RBPJ-binding protein SHARP requires CtBP for is repressive activity (Oswald *et al.* 2005); second, CtBP bound to a PLDLS motif can form a core complex with histone deacetylases (HDACs) and the lysine demethylase LSD1 (also called KDM1A) (Kuppuswamy *et al.* 2008). Additionally, LSD1 can also be recruited to RBPJ by L3MBTL3 (Xu *et al.* 2017). Notably, LSD1 and HDAC1 are able to immunoprecipitate both EBNA3A and EBNA3C, but not EBNA3B, and when the CtBP binding sites in either EBNA3C or EBNA3A were mutated, the immunoprecipitation of EBNA3C by LSD1 or HDAC1 were reduced (Skalska 2012), suggesting that EBNA3A binding to CtBP contributes to recruiting LSD1 to EBNA3C. This raises the question of whether the CtBP bound to EBNA3C-binding loci in the absence of EBNA3C (Ohashi *et al.* 2021), might be established by EBNA3A, rather than having existed prior to EBV infection. Nevertheless, further consideration of these cellular RBPJ- and CtBP-interacting complexes might provide insight into EBNA3A- and EBNA3C-mediated gene regulation.

5 Mechanisms of Modulation of host gene expression by EBNA3 proteins.

ChIP-seq analyses have consistently shown that – while some genomic sites occupied by EBNA3C are associated with promoters – the vast majority of genome-binding sites occupied by EBNA3 proteins are distal enhancers, be they upstream, downstream or within introns of the gene that they are regulating (McClellan *et al.* 2012, McClellan *et al.* 2013, Jiang *et al.* 2014, Paschos *et al.* 2017). This separation of promoter and EBNA3 binding sites has both informed mechanisms of regulation, with genomic reorganisation being identified as an important element of EBNA3 function, and the separation of promoter proximal and distal events associated with EBNA3 binding helping us to dissect how cellular factors and processes contribute to regulation by EBNA3s.

5.1 EBNA3 proteins use polycomb repressor complex (PRC) proteins to regulate genes.

Changes in gene expression are typically associated with changes in the epigenetic marks of histones – particularly the N terminal 'tail' of histone 3 (H3) – across gene bodies, promoters and other regulatory region. EBNA3-repressed genes are commonly marked by trimethylation of H3 lysine 27 (H3K27Me3), in place of the activating marks H3K27 acetylation (H3K27Ac) and H3K4Me3 (Paschos *et al.* 2009, McClellan *et al.* 2012, Harth-Hertle *et al.* 2013, Jiang *et al.* 2014). H3K27 is typically trimethylated by the polycomb repressor complex 2 (PRC2), which comprises a histone binding protein (RBBP4/7), a methyltransferase enzyme (EZH1/2), a histone deacetylase (EED) and a zinc finger protein (SUZ12). These epigenetic marks can be subsequently stabilised – including by DNA methylation – by PRC1, which includes an E3 ubiquitin ligase (RING1/2) that mono-ubiquitinates K119 on histone 2A (H2K119-Ub – another repressive chromatin modification), and a ring finger protein (one of PCGF1-6) that directs PRC1 to repressive marks H3K9Me3 or H3K27Me3.

The current model of PRC2 function is that it imposes epigenetic silencing marks after the repression of the target gene by other methods (Riising *et al.* 2014). This has been reported for several EBNA3A-repressed genes (Harth-Hertle *et al.* 2013), but PRC2 epigenetic repression is required prior to repression for the STK39 gene where EBNA3A is acting without EBNA3C (Bazot *et al.* 2018). In LCLs and latency III BL cells, SUZ12 (and thus likely PRC2) only occasionally co-localises with EBNA3 binding sites or EBNA3C-repressed promoters (McClellan *et al.* 2013, Kalchschmidt *et al.* 2016, Paschos *et al.* 2017, Paschos *et al.* 2019), but rather maps across broader regions of H3K27Me3 chromatin of the gene body. SUZ12 immunoprecipitation does not pull down EBNA3C (Kalchschmidt *et al.* 2016, Gillman *et al.* 2018), and EBNA3s regulate genes effectively when SUZ12 is depleted (Paschos *et al.* 2019), whereas EZH2 inhibitors can disrupt EBNA3-mediated gene repression (Jiang *et al.* 2017), separating the functional roles of these two PRC2 components. In contrast to SUZ12, the PRC1 component PCGF4 (better known as Bmi1) commonly localises to sites of EBNA3 binding to the genome – particularly those with both EBNA3A and EBNA3C present – and a Bmi1 antibody was able to co-immunoprecipitate EBNA3C (but not other EBNA3s) (Paschos *et al.* 2019). Bmi1 is particularly important for EBNA3C's ability to activate gene expression, but Bmi1 knockdown did not impact gene repression by EBNA3C (Paschos *et al.* 2019). While the PRC1-associated H2AK119Ub status at these EBNA3C/Bmi1 peaks is unknown, EBNA3C can bind the H2AK119 deubiqutinase Aurora B kinase (AURKB) (Jha *et al.* 2013),

suggesting recruitment and/or activity of Bmi1 could be occurring in the absence of H2K119 ubiquitination.

The epigenetic modulation of target genes, and the recruitment of epigenetic modulators are linked to the motifs required by EBNA3C to bind to RBPJ. Wild-type EBNA3C can bind the histone demethylase KDM2B, but mutation of the TFGC motif that binds RBPJ (Section 4.3) also disrupts KDM2B binding, and is correlated with an inability of the virus to remove activating H3K4Me3 marks from EBNA3C-repressed promoters (Gillman *et al.* 2018). In contrast, Bmi1 binding to EBNA3C is lost only alongside complete loss of RBPJ binding (mutation of both TFGC and W227), and virus with this EBNA3C mutant is unable to replace H3K27Ac with H3K27Me3 marks (Gillman *et al.* 2018). However the TFGC mutation alone is sufficient to disrupt the regulation of host genes by EBV (Maruo *et al.* 2005, Lee *et al.* 2009, Gillman *et al.* 2018), ebv.org.uk), although how much this is due to failure to interact with KDM2B, RBPJ or some other factor(s) remains to be demonstrated.

The importance of KDM2B is reinforced by temporal evidence suggesting that when EBNA3C and RBPJ both accumulate at EBNA3C binding sites on the genome – typically at enhancers distant from gene promoters – activating epigenetic marks are removed from the promoter (e.g. by KDM2B), before the deposition of H3K27Me3 repressive marks (Kalchschmidt *et al.* 2016). This is accompanied by SUZ12 recruitment to promoters, and Bmi1 recruitment to enhancers alongside EBNA3C, but of these, only Bmi1 is essential for the establishment of H3K27Me3 repressive mark (Paschos *et al.* 2019), so the mechanism by which this regulation is imposed remains unclear, as it deviates from our current understanding of PRC function.

5.2 EBNA3s promote 3D reorganisation of genetic loci to regulate genes.

It is becoming increasingly apparent that all of the EBNA proteins incorporate the reorganisation of genetic loci as a core part of their functions (Wang *et al.* 2023, Lieberman and Tempera 2025). Chromatin is organised into transcriptional domains (TADs) within which DNA loops are anchored by cohesin complexes and CTCF. For instance, EBNA1 is best known for its ability to link the EBV genome's latency replication origin (*oriP*) to cellular chromatin to facilitate EBV genome segregation during mitosis (Frappier 2025); EBNA2 can act on enhancers and promoters, including Myc where it induces links from upstream enhancers to the promoter, rather than the downstream enhancers normally used during B cell differentiation (Wood *et al.* 2016); even EBNA-LP is now appreciated to alter host genome interactions between enhancers and promoters, likely through its interactions with YY1 (Cable *et al.* 2024, Maestri *et al.* 2025) and/or EP300 (Wang *et al.* 2018). The regulation and reorganisation of the viral and cellular genomic loci is discussed elsewhere in this volume (Lieberman and Tempera 2025). However, details of EBNA-driven chromatin reorganisation are most well characterised for the MYC gene (by EBNA2) (Wood *et al.* 2016), and a selection of EBNA3-regulated genes, particularly the CDNK2A/B locus, ADAM28/ADAMDEC1; CTBP2; AICDA; BCL2L11; CXCL9-11 (McClellan *et al.* 2013, Wood *et al.* 2016, Wang *et al.* 2023).

Various comparisons of how reorganisation of these loci demonstrate that there is not yet a well-defined coherent mechanism by which EBNA proteins use reorganisation of chromatin domains and promoter-enhancer interactions to regulate genes: EBNA3 binding to an enhancer region can cause its dissociation from or association with a promoter, which can lead to either up-regulation or down-regulation of a promoter (McClellan *et al.* 2013, Allday *et al.* 2015), and these examples will not be re-stated here. In addition, there is considerable

overlap in the genome binding sites of EBNA2 and EBNA3s, and it remains unclear whether these sites are simultaneously bound by both EBNA2 and EBNA3 proteins. However, conditional (estrogen receptor) systems have shown that EBNA2 rapidly (in 48 hours or less) alters expression of its target genes (Spender *et al.* 2001, Maier *et al.* 2006), whereas EBNA3s typically take days to weeks to fully impose changes in gene transcription (Maruo *et al.* 2006, Kalchschmidt *et al.* 2016), suggesting a clear mechanistic difference between how EBNA2 and EBNA3s regulate host genes: a requirement for genome re-configuration to enact EBNA3-mediated regulation could explain this difference.

Global analyses have identified some characteristics of how EBNA3s modulate genes through enhancer-promoter interactions. While EBNA3A and EBNA3C can both activate and repress genes, EBNA3B is probably only able to directly repress genes (Paschos *et al.* 2017). Additionally, where chromatin domains contain several genes differentially regulated by EBNA3s, they are always regulated in the same direction, suggesting that EBNA3s regulate genes at the level of chromatin domains, rather than simple promoter-enhancer interactions (Paschos *et al.* 2017).

Chromatin loops and domains and chromatin loops are typically formed through the activity of CTCF and cohesin complexes (Lieberman and Tempera 2025). Notably, conditional inactivation of EBNA3A reduced both CTCF localisation to certain sites and more widely reduces the localisation of RAD21 (a component of cohesin complexes) to the genome (Wang *et al.* 2023), suggesting that EBNA3A contributes to the formation and/or stabilisation of promoter-enhancer interactions, and TAD boundaries. For the CDKN2A/B locus, this inactivation of EBNA3A or EBNA3C resulted in more promoter-promoter interactions, and fewer associations with distal enhancers, correlating with elevated expression from these promoters (Jiang *et al.* 2017, Wang *et al.* 2023). Importantly, disruption of a CTCF site downstream of the AICDA promoter, can restore the EBNA3C-induced upregulation of AICDA (Wang *et al.* 2023), suggesting that changes in CTCF-dependent loops (or insulator functions) are fundamental to the regulation of at least some genes by EBNA3s. Nevertheless, there is not currently a unified mechanism for how EBNA3s use looping to regulate cell genes.

5.3 EBNA3-modulated gene expression can be epigenetically stable.

As we have discussed, the complex genome rearrangements and changes in transcription induced by EBNA3 proteins are often relatively slow to establish, and (for EBNA3-ER fusions in LCLs) slow to reverse. At least in some contexts, for some genes, these regulatory changes are epigenetically stable, even when the EBNA3s are no longer present. For instance, deactivating and reactivating a virus with dual estrogen receptor fusions of EBNA3A and EBNA3C in LCLs demonstrates the reversible repression of pro-apoptotic genes CDKN2A/B and BCL2L11 (Bim) by EBNA3A and EBNA3C, whereas in contrast, the PRC2-dependent repression of plasma cell differentiation factors BLIMP1 and CDKN2C ($p18^{INK4c}$) by EBNA3A and EBNA3C is not reversed when ER-fusions of EBNA3A and EBNA3C are inactivated in LCLs: indeed this repression appears to be stable in the absence of EBNA3s from 12 days post infection (Styles *et al.* 2017).

Even genes that are reversibly repressed in LCLs can be become more stably shut-down. For instance, in EBV-positive Burkitt Lymphoma cell lines (which do not produce EBNA3s), the reversibly repressed EBNA3A/C targets Bim and CDKN2A ($p16^{INK4A}$) are repressed exclusively by DNA methylation of their promoters, whereas EBV-negative Burkitt lymphoma cell lines typically have mutations in some combination of Bim, $p16^{INK4A}$ and p53 that shuts

down these pathways (Paschos *et al.* 2009). Further, EBNA3C can bind to DNA methyltransferase DNMT3a, and - when overexpressed - mediate methylation of some target genes (Zhang *et al.* 2019). This is a potential mechanism to mediate promoter methylation of EBNA3 target genes in BL, although LCLs tend to lack promoter methylation at EBNA3-regulated promoters. The relationship between polycomb-regulated genes and DNA methylation is complex: H3K27Me3 is normally mutually exclusive with DNA methylation. However, cells that lose activity of PRC2 can see increases in DNA methylation at PRC2-repressed loci (Thornton *et al.* 2014, Richard Albert *et al.* 2025) which could explain the promoter methylation in EBV-positive BL cells. Alternatively, DNA methylation can direct the recruitment of polycomb complexes (McLaughlin *et al.* 2019). The context and interaction of these processes in EBNA3-mediated gene regulation remains unresolved.

Nevertheless, the idea that the impact of EBNA3-mediated gene regulation (particularly repression) can be sustained long after EBNA3 proteins are no longer produced is important when assessing causal processes underlying both EBV-induced cancers and the normal establishment and nature of EBV latency: EBNA3 functions should not be disregarded in the study of HL and BL development, where their impact may be felt even though they are no longer expressed in these tumours: a kind of "hit-and-run” oncogenesis.

6 EBNA3-mediated Gene regulation alters B cell biology & transformation

We have already discussed mechanisms by which EBNA3 proteins can regulate host genes. In this section we will discuss the significance of some of the genes that are targeted by EBNA3 proteins. Most of these targets have been studied by bulk methods - microarray, RNA-seq or RT-qPCR - using either LCLs or Burkitt lymphoma cell lines. However, these observations should now be reconsidered since the recent observation that LCLs reflect a mixture of several distinct differentiation states (SoRelle *et al.* 2021, SoRelle *et al.* 2022). Thus any gene regulation changes could reflect changes in the proportions of these differentiation states as well as direct or indirect gene regulation.

6.1 EBNA3 proteins alter B cell differentiation states.

BCL6 is the master regulator of and classical marker for germinal centre B cells (McLachlan *et al.* 2022). Perhaps a major reason that it took 50 years to discover that LCLs included germinal centre B cells is the complete lack of BCL6 gene expression in LCLs (SoRelle *et al.* 2023). Considerable evidence suggests that EBNA3 proteins are responsible for the repression of BCL6. First, EBNA3 knockout and CtBP mutants in EBV-infected BL31 cells exhibited 10-fold increased BCL6 expression ((White *et al.* 2010); ebv.org.uk), as did one of two EBNA3A knockouts (3AKO) in LCLs ((Harth-Hertle *et al.* 2013); ebv.org.uk). Second, BCL6 expression is lower in Wp-restricted (i.e. EBNA2-mutant but EBNA3-expressing) BL cell lines than in latency I BL, and lower still in LCLs (Kelly *et al.* 2013), and similarly an EBNA2 knockout EBV combined with constitutive Myc expression induced BCL6-low Burkitt Lymphoma-like tumours in cord blood-humanised mice (Bristol *et al.* 2024). Yet despite the lack of BCL6 expression, LCLs contain sub-populations of cells with DZ, LZ, and post-GC chromatin features (SoRelle *et al.* 2023). The mechanism of BCL6 repression is less clear: BCL6 transcripts are higher early after infection with EBNA3C mutant EBV ((Pei *et al.* 2017), ebv.org.uk). There are also EBNA3C/3B and EBNA3C/3A binding loci 75 and 550 kb upstream of BCL6 (based on ChiP-seq data from (Paschos *et al.* 2017)) although their relevance to BCL6 regulation is not clear. EBNA3C is also proposed to promote the proteasomal degradation of BCL6, through the N-terminal region of EBNA3C binding to FBXO11 (Sun *et al.* 2024), and the central region of EBNA3C binding BCL6 (Pei *et al.* 2017).

The concerted effort by EBV to repress BCL6 but still (if the GC model of EBV latency is correct) progress the infected B cells through germinal centre differentiation to become memory B cells, suggests a more sophisticated manipulation of B cell differentiation than simply using the normal BCL6-driven process. Indeed, EBNA3 proteins regulate many regulators and markers of B cell differentiation (summarised in Table 1). EBV infection of BL31 cells – a latency III infection of cells with some characteristics of a germinal centre B cell – changes the transcript level of most transcription factors whose levels change during germinal centre differentiation, most dramatically repressing NFATC2 and BACH2 (White *et al.* 2010). Both BL31 and LCL or primary infection microarrays support a role for EBNA3A in repressing NOTCH2 in a CtBP-dependent manner (Skalska *et al.* 2010) and activating STAT3 (Hertle *et al.* 2009) and for EBNA3C in repressing EBF1 and BACH2 (Kalchschmidt *et al.* 2016).

In mice, NOTCH2 is essential for marginal zone B cells and formation of IgM memory B cells outside germinal centres (Saito *et al.* 2003, Xu *et al.* 2024). NOTCH2 is upregulated by BCR signalling, and in turn boosts levels of BCR signalling components (enhancing BCR

signalling strength) and upregulates the complement receptor (and EBV receptor) CD21 (Xu *et al.* 2024). Conversely, NOTCH2 is not required for differentiation of germinal centre B cells towards memory or plasma cells (Xu *et al.* 2024). Thus (assuming the roles of human NOTCH2 are the same as in mice) the repression of NOTCH2 by EBNA3A may both promote germinal centre differentiation (over extrafollicular activation) and perhaps – by controlling CD21 levels – reduce EBV superinfection.

Beyond transcription factors, EBNA3B represses a number of genes (in LCLs) that are also repressed between naïve and germinal centre B cells (White *et al.* 2010), suggesting EBNA3B is promoting GC differentiation. EBNA3C also modulates some key players in GC biology, increasing transcription of the AICDA gene encoding the mutagenic protein activation-induced cytidine deaminase (AID) that is responsible for the hypermutation of immunoglobulin loci (Kalchschmidt *et al.* 2016). Conversely, AID is repressed by EBNA2 (Tobollik *et al.* 2006), and strikingly lymphomas induced in cord blood cells by infection with the EBNA2-null P3HR1 strain results in class switch recombination in the resulting lymphoma cells (Li *et al.* 2020), suggesting that the balance of EBNA3C and EBNA2 modulation of AID (and perhaps other genes) may control class switch recombination in EBV-infected cells.

It is also becoming apparent that the differentiation state of the infected cell can impact how EBV manages its transformation. For instance, EBNA-LP knockout EBV is unable to sustain transformation of naïve or cord blood B cells, and a 3AKO is similarly deficient in transformation of cord blood B cells (Romero-Masters *et al.* 2020), although it is unresolved whether this extends to naïve B cells, or what underlies this deficit.

However, because many of these genes change in germinal centre B cells, but then revert in memory, we cannot conclude whether these EBNA3 effects act to supress the development of a GC phenotype, to promote exit from a GC phenotype into a memory cell, or to manage transitions within the GC. Additionally the existing transcriptomic studies have not separated the impact of EBV infection on naïve vs memory B cells, and vary in their use of B cells from blood or lymph nodes as infection models. It is not clear whether these experimental differences influence our observations and deductions.

6.2 EBNA3A and EBNA3C suppress plasma cell differentiation

As mentioned in section 1.1, plasma cell differentiation is linked to the reactivation of EBV into its productive (“lytic”) replication cycle. Plasma cell differentiation is normally suppressed by BCL6 and BACH2 (Ochiai *et al.* 2008). While doing this BACH2 promotes CSR, directly opposed by BLIMP1 (Muto *et al.* 2010). EBV Reactivation is initiated by the activity of the plasma cell differentiation factors BLIMP1 (gene name: PRDM1) and the stress response protein XBP1 (Bhende *et al.* 2007, Sun and Thorley-Lawson 2007, Reusch *et al.* 2015). There is transcriptomic evidence that each of the EBNA3s may contribute to the regulation of BLIMP1 (Hertle *et al.* 2009, White *et al.* 2010). Both *PRDM1* and the *CDKN1C* gene encoding the cyclin-dependent kinase inhibitor $p18^{INK4C}$ that is required for plasma cell differenation (Tourigny *et al.* 2002), are both directly repressed by the combination of EBNA3A and EBNA3C: simultaneous inactivation of conditional EBNA3A and EBNA3C during transformation resulted in increased markers of plasma cell differentiation, although this was not accompanied by clear-cut evidence of BZLF1 reactivation (Styles *et al.* 2017). Notably, EBV has additional controls on plasma cell differentiation, with EBV-mirBHRF1-2 (Ma *et al.* 2015) and LMP1 (Vrzalikova *et al.* 2011) also contributing to the repression of BLIMP1, so the full scope of viral reactivation triggers during plasma cell differentiation remains to be defined.

Table 1. EBNA3-influenced factors important for normal GC differentiation

Gene	Normal Expression pattern	EBNA3 regulation	Citations
GPR183 (EBI2)	Promotes migration to GC; prevents entry to GC	Activated by EBNA2 Repressed by EBNA3B and EBNA3C	(White *et al.* 2010), (Ohashi *et al.* 2021)
BACH2	Upregulated in GC B cells; suppresses Plasma cell differentiation	Low in LCLs. EBNA3B&C-dependent repression in BL31 (like BCL6).	(White *et al.* 2010)
BCL6	GC master transcriptional regulator	Repressed by EBNA3C&EBNA3B). Degraded by EBNA3C	(White *et al.* 2010) (Pei *et al.* 2017)
IRF8	Modulates BCL6 and AICDA; enables LZ/DZ organisation	Degraded by EBNA3C	(Banerjee *et al.* 2013)
ST3GAL1 (PNA)	GC glycan marker: High Gal-β(1-3)-GalNAc on GC B cells bound by PNA, modified by ST3GAL1.	ST3GAL1 repressed by EBNA3B	(White *et al.* 2010)
EPHB1	High in GC	Repressed by 3A; CtBP dependent	(Hertle *et al.* 2009, Skalska *et al.* 2010)
NOTCH2	Increases BCR sensitivity. Increased during Germinal centre exit (to memory). Required for Extrafollicular memory / Marginal zone B cells.	Repressed by EBNA3A	(Hertle *et al.* 2009, White *et al.* 2010)
FOXO1	TF promoting DZ formation	EBNA3A-repressed	(Hertle *et al.* 2009, Skalska *et al.* 2010)
CXCR4	Upregulation Required for Dark zone formation	EBNA3B-repressed	(Chen *et al.* 2006)
STAT3	Crucial for LZ/DZ cycling	May avoid silencing via EBNA3B repression of INPP5F	(White *et al.* 2010)
AICDA	Mediates hypermutation of Ig loci in dark zone.	EBNA3C-upregulated	(Skalska *et al.* 2013, Kalchschmidt *et al.* 2016, Ohashi *et al.* 2021)
CD83	Surface marker of Light Zone B cells	None observed	
CD86	Surface marker of Light Zone B cells	Upregulated in by EBNA3C and EBNA3A perhaps. (EBNA3C in BL31, one of two EBNA3A mutant LCLs)	(Hertle *et al.* 2009, White *et al.* 2010)
PRDM1 (BLIMP1)	Transcription factor driving plasma cell differentation	Repressed by EBNA3A and EBNA3C (but Elevated in Wp-restricted BL)	(Styles *et al.* 2017) (Kelly *et al.* 2013)
CDKN2C ($p18^{INK4C}$)	Cyclin-dependent kinase inhibitor	Repressed by EBNA3A and EBNA3C	(Styles *et al.* 2017)
IRF4	Required for CSR; Higher in Plasma cells – opposes BCL6 and supports BLIMP1.	EBNA3C binds to IRF4 at genomic loci	(Banerjee *et al.* 2013, Jiang *et al.* 2014)

6.3 EBNA3A and EBNA3C prevent apoptosis and senescence of proliferating and differentiating B cells.

A key element of the normal B cell differentiation process is the programmed cell death of B cells whose BCR lacks specificity for an appropriate antigen. As with any cell, apoptotic signals might arise either when the proliferative state of the B cell leads to DNA damage or replicative stress. Additionally, germinal centre B cells are programmed to arrest or die when they do not receive suitable signals from their BCR and from helper T cells that recognise peptides derived from the BCR-bound antigen.

Signals of cell stress often trigger cell cycle arrest though the upregulation of cyclin-dependent kinase inhibitors (CDKis), which stop cell cycle progression by preventing cyclin phosphorylation. The most demonstrably important of these for blocking B cell transformation by EBV is $p16^{INK4A}$ (encoded alongside $p14^{ARF}$ by the *CDKN2A* gene). Both promoters of the CDKN2A gene are inhibited by EBNA3C, with contributions from EBNA3A, and without this inhibition, infected B cells die or senesce around 2 weeks post infection, and pre-established LCLs senesce when $p16^{INK4A}$ is derepressed by silencing EBNA3C or EBNA3A (Maruo *et al.* 2006, Skalska *et al.* 2010). Genetic mutations that disrupt $p16^{INK4A}$ rescue EBNA3C-deficient LCL outgrowth from cell cycle arrest (Skalska *et al.* 2013, Ohashi *et al.* 2021).

Between them, EBNA3A and/or EBNA3C have been shown to repress all of the CDKi genes, either directly or indirectly (Figure 3): *CDKN2B* encoding $p15^{INK4b}$ lies adjacent to *CDKN2A*, and is probably regulated in the same TAD, but is also more directly repressed by EBNA3A binding to transcription factor MIZ1 (Bazot *et al.* 2014) that can otherwise activate CDKN2B; CDKN2C encoding $p18^{INK4C}$ requires either EBNA3A or EBNA3C for its repression, and is epigenetically stable (Styles *et al.* 2017); EBNA3A and EBNA3C combine to upregulate the mir221/222 cluster that repress $p57^{KIP2}$ and (more modestly) $p27^{KIP1}$ translation (Bazot *et al.* 2015); and EBNA3C can promote the degradation of $p21^{WAF1/CIP1}$ via the kinase PIM1 (Banerjee *et al.* 2014), although EBNA3C expression did not prevent $p21^{WAF1/CIP1}$ activation in BL cell lines (Hui *et al.* 2018), so the extent of $p21^{WAF1/CIP1}$ repression is not clear. Thus the EBNA3 proteins can reduce or eliminate the activity of all the major cyclin-dependent kinase inhibitors.

A second key role of the EBNA3s is to prevent the death of the infected B cell in the face of the Germinal Centre selection process. The key survival signals for EBV-infected germinal centre B cells are thought to come from the LMPs, which provide signals that might otherwise come from BCR activation and the CD40 co-receptor activated by T cell help (Kieser 2025). Without BCR and CD40 signals, or with BCR signalling alone, GC B cells are much more prone to apoptosis through the intrinsic (mitochondrial) pathway governed by the balance between anti-apoptotic BCL2 family members and corresponding BH3-containing pro-apoptotic proteins (Young and Brink 2021, Victora and Nussenzweig 2022). However, deprivation of CD40 signalling alone is not sufficient to trigger the death of LZ B cells (Mayer *et al.* 2017), and instead Fas "death receptor" signalling from other cells in the GC (and perhaps other mechanisms) can eliminate self-reactive or constitutively proliferating B cells (Butt *et al.* 2015, Razzaghi *et al.* 2021, Young and Brink 2021). While there is not clear evidence of EBNA3 proteins regulating Fas, the TRAIL receptor 1 and TRAIL decoy receptor 4 genes (TNFRSF10A and 10D) are strongly repressed by EBNA3B (White *et al.* 2010). Since one promotes, and the other blocks TRAIL-mediated cell death, it is unclear whether this may also contribute to GC survival.

The key mediator of intrinsic apoptosis of GC cells lacking BCR signalling is Bim (BCL2L11) (Gao *et al.* 2012). EBV uses both EBNA3A and EBNA3C to comprehensively silence BIM transcription (Anderton *et al.* 2008, McClellan *et al.* 2013, Wood *et al.* 2016), promoting long term epigenetic silencing (Paschos *et al.* 2009), which presumably provides a second level of resistance to GC-related apoptosis (alongside constitutive LMP signalling). EBNA3A also changes the behaviour of other BCL2 proteins. Early after infection, EBV-transformed B cells are protected from apoptosis by both MCL-1 and BCL2 activity. However, early after infection, EBV-infected cells lacking EBNA3A are no longer protected by MCL-1, which becomes localised away from mitochondria, and later EBNA3A also upregulates the BCL2A1 gene encoding another Bcl2 protein - BFL-1 to boost protection from apoptosis (Price *et al.* 2017).

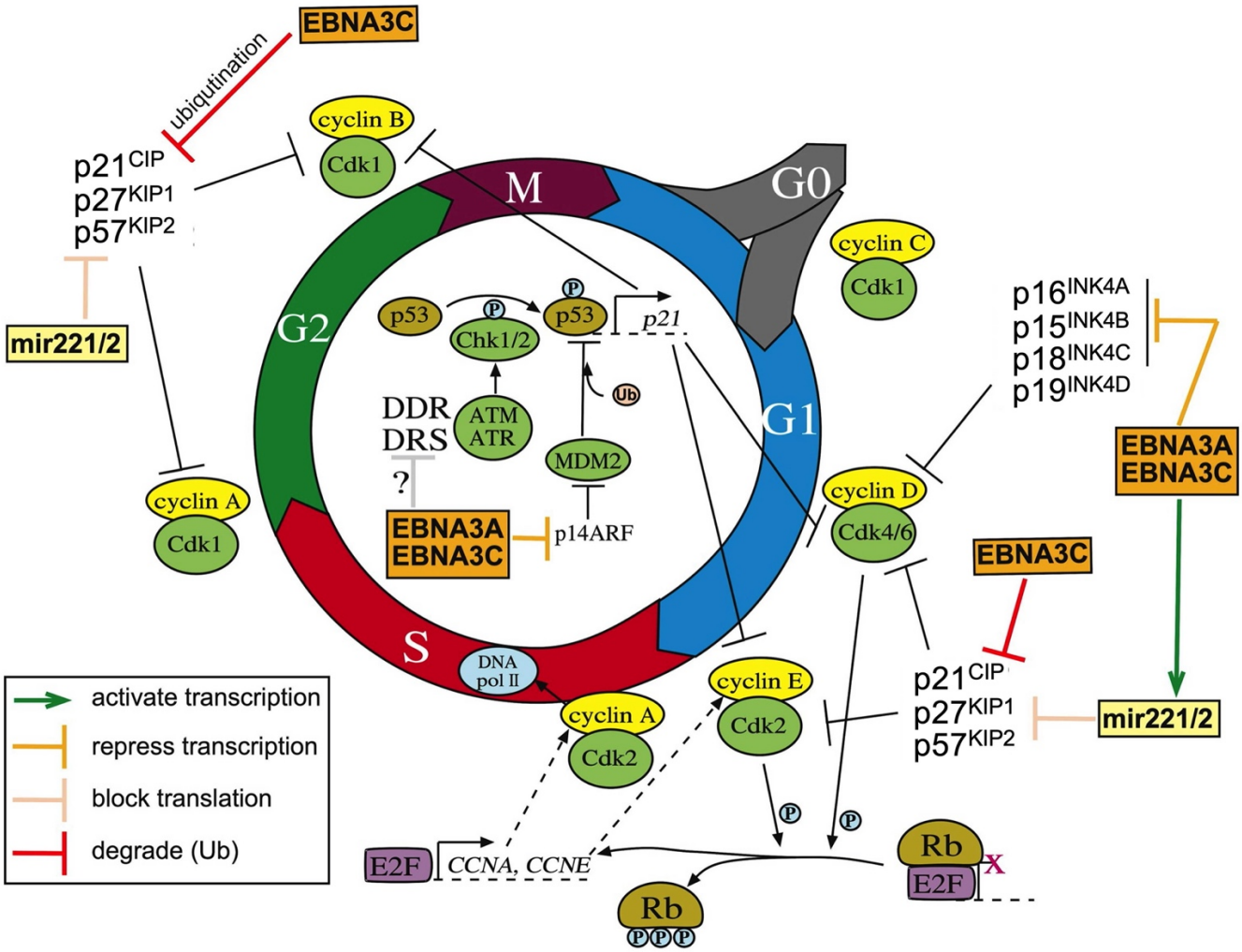

Figure 3. EBNA3 proteins prevent apoptosis and senescence by blocking CDKi activity. Central circle shows phases of the cell cycle, with the cyclin/CDK complexes that control passage between these phases, and the cyclin-dependent kinase inhibitors (CDKi) are indicated by their protein names in black text whose activities are marked by black inhibitory signs. Arrows representing inhibition or activation as a result of EBNA3 activity are colour coded with the grey inhibition indicator representing the unknown mechanism by which EBNA3s alter DNA damage responses (DDR) and/or DNA replicative stress response (DRS).

6.4 EBNA3C and EBNA3A mediate protection from DNA damage and/or replicative stress.

There is also some indication that EBNA3 proteins modulate the DNA damage response (DDR), another key driver of senescence and cell death. EBV infection of B cells induces a robust DNA damage response during its hyperproliferative phase, 4-8 days post infection, signified by γH2AX foci and ATM phosphorylation, and induction of a number of DDR-associated genes including p53 (Nikitin *et al.* 2010, Shukla *et al.* 2016, Pich *et al.* 2019). In the first week after B cell infection, an EBNA3C mutant EBV exhibited reduced p53 levels (Shukla *et al.* 2016) while EBNA3A/EBNA3C double knockout (3ACKO)-infected cells proliferated slightly more rapidly, but had very little p53 protein detected, in contrast with elevated p53 in wild-type EBV infected cells (Pich *et al.* 2019). This is somewhat at odds with the suggestion that EBNA3C mediates the degradation of p53 (Saha *et al.* 2009, Yi *et al.* 2009).
It has variously been suggested that the γH2AX signal is caused by the DNA damage response (driven by ATM phosphorylation by Chk2 kinase (Nikitin *et al.* 2010)) or is a DNA replication stress response, triggered by ATR phosphorylation by CHK1, with no indication of ATM-induced DDR (Mordasini *et al.* 2017, Pich *et al.* 2019). In the former study, a 3CKO showed increased proportion of γH2AX-positive proliferated cells, while there was also a slight elevation of γH2AX level in 3ACKO cells, albeit perhaps only matching the increased proliferation of these cells (Pich *et al.* 2019). Additionally, overexpressed EBNA3C can interact with and reduce expression of H2AX (Jha *et al.* 2014) and protect cells from apoptosis induced by the DNA damage agent etoposide (Saha *et al.* 2012). Additionally, a recent interaction screen suggests that EBNA3A can interact with MRE11 - a component of the DNA break repair complex - and PML, which accumulates at sites of DNA damage (Landman *et al.* 2024). However, as yet there is no detailed mechanistic exploration of how the DNA damage response is altered by EBV.

6.5 EBNA3 proteins change the B cell's signalling landscape.

EBNA3 proteins (working alongside EBNA2) appears to have a substantial impact on genes involved with BCR signalling. BCR activation triggers three main signalling pathways - MAP kinase activation, phosphoinositol-3-phosphate (PIP_3) signalling and the phospholipase-C pathway (reviewed in (Wen *et al.* 2019)). BCR/Phospholipase C signalling produces diacyl glycerol as a second messenger that activates (among others) Ras GDP-releasing proteins (RASGRPs) to activate Ras/ERK signalling. RASGRP3 is essential for BCR signalling to allow B cell activation, while RASGRP1 can mediate BCR-triggered apoptosis in certain contexts (Guilbault and Kay 2004, Coughlin *et al.* 2006). EBNA3C can regulate both of these genes, binding at the RASGRP1 promoter and repressing it in both 3CHT LCLs and BL31 cells (Skalska *et al.* 2010, White *et al.* 2010, Ohashi *et al.* 2021), and repressing RASGRP3 in BL31 cells (but not in 3CHT-conditional LCLs) (Khasnis *et al.* 2022). Since LCLs tend to select for reduced RASGRP3 expression in culture (Lee *et al.* 2010), it remains possible that EBNA3C-modulation of RASGRP3 in LCLs is masked. Conversely the regulation might be indirect, as there are not clearcut EBNA3C-binding peaks near to either gene in our ChIP-seq data, although RASGRP1 promoter binding is supported by ChIP-qPCR (Skalska *et al.* 2013).

EBNA3 proteins have a more wide-ranging impact on genes modulating PI3 kinase/Akt signalling. BCR signalling results in the formation of phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) that activates Akt signalling. PIP_3 is generated by a PI3 kinase, which comprises a regulatory subunit (*PIK3R* genes) and p110 catalytic kinase subunit (PIK3C

genes). In addition, PIP_3 is also turned over by the action of phosphatases (INPP5 genes), typically removing the 5' phosphate to convert PIP_3 into PIP_2. EBV infection in BL31 cells results in the transcriptional upregulation of several of the regulatory subunits (PIK3R1, 3, 5, and 6).In particular, PIK3R1 in EBV-infected BL31 cells exhibit EBNA3C-dependent upregulation (Khasnis *et al.* 2022), with a promoter proximal EBNA3 ChIP-seq signal in Mutu III BL cells (McClellan *et al.* 2013) seen as a predominantly EBNA3C peak in LCLs (Paschos *et al.* 2017), while PIK3R3 shows some evidence of regulation by EBNA3A (Khasnis *et al.* 2022). Of the catalytic PI3K subunits, EBNA3C contributes to boosting transcription of the gamma subunit PIK3CG in LCLs (Skalska *et al.* 2013, Ohashi *et al.* 2021), with EBNA3C and EBNA3B binding at the promoter, and EBNA3A and EBNA3C binding to an intronic site (Paschos *et al.* 2017).

The negative regulators of PIP3 signalling known to impact BCR signalling are INPP5D/SHIP1 (Pauls and Marshall 2017) and more recently proposed INPP5B (Droubi *et al.* 2022). There is no clear evidence that either of these is regulated by the EBNA3 proteins, although INPP5D is repressed by the EBV-BHRF1-2 miRNA which supports LCL transformation (Chen *et al.* 2019), and perhaps also by EBNA2 (Khasnis *et al.* 2022). However, the related INPP5F is profoundly silenced by EBNA3B, with all three EBNA3s binding 20kb upstream of its promoter (White *et al.* 2010, Paschos *et al.* 2017). There is no indication whether INPP5F might suppress BCR signalling, but it can silence STAT3 (Kim *et al.* 2014), and STAT3 is crucial for helping B cells maintain light and dark zone cycling (Fike *et al.* 2023).

The functional significance of these signalling changes have not been investigated. The changes in BCR signalling most likely help control the B cell's navigation of the germinal centre environment, perhaps creating a cellular environment where LMP2 signalling sustains B cell survival regardless of whether or not the BCR is bound to antigen. While the changes in integrin signalling may correspondingly alter the responses to interaction of the B cell with the T cells, follicular dendritic cells and other stromal cells of the lymph node, creating an intracellular signalling profile that epigenetically sets up EBV-infected B cell to survive (and perhaps ignore) any combination of signals from nearby cells.

7 In vivo biology of the EBNA3 proteins in latency and lymphomagenesis.

The contributions of EBNA3s to EBV's manipulation of B cell biology has been most effectively studied in vivo using recombinant EBVs in mice reconstituted with human immune stem cells, from either foetal liver (White *et al.* 2012, Murer *et al.* 2018) or cord blood (Romero-Masters *et al.* 2018, Romero-Masters *et al.* 2020), with additional insights gained from overexpressing EBV proteins in transgenic mice (Sommermann *et al.* 2020).

7.1 EBNA3B functions as a tumour suppressor gene.

EBNA3B is the least studied of the EBNA3 proteins. EBNA3B mutant viruses are fully competent in B cell transformation (Tomkinson *et al.* 1992, Chen *et al.* 2005), and manipulations of the EBNA3B gene (particularly insertions) often disrupts downstream EBNA3C mRNA splicing and/or protein levels (Chen *et al.* 2006) and our unpublished observations). A 3BKO EBV induced monomorphic B cell tumours in the spleen that resemble activated B cell diffuse large B cell lymphoma, compared to the polymorphic PTLD phenotype of wild-type EBV-infected mice (White *et al.* 2012). Importantly, EBV-positive lymphomas in patients can also have mutations in EBNA3B, including nonsense mutations in transplant or HIV-associated DLBCLs (Gottschalk *et al.* 2001), and small in-frame deletions in BL and HL (White *et al.* 2012). However, while one each of the ≈10 random BL, HL and ABC-DLBCLs tested exhibited an unambiguous deletion or frame-shift, the extremely polymorphic nature of EBNA3 proteins has made it difficult to estimate of EBNA3B mutation frequency in lymphomas versus its natural polymorphism frequency. These observations of oncogenesis by EBNA3B-mutant EBVs in animal model, and EBNA3B mutation in natural tumours argue that EBNA3B has characteristics of a tumour suppressor. Additional examples of EBV mutation in NK/T lymphomas in particular (Okuno *et al.* 2019, Wongwiwat *et al.* 2022), and also Wp-restricted BLs (Kelly *et al.* 2005) suggest that EBV mutation is a common step in lymphomagenesis.

Mechanistically, it is unclear what EBNA3B functions or targets prevent the development of lymphomas. It could be that loss of EBNA3B reduces T cell surveillance, as seen from reduced homing of T cells towards 3BKO LCLs, in part due to the reduced production of CXCL10 (White *et al.* 2012), although there are a number of other potential cytokines and T cell-regulating surface ligands and receptors differentially expressed in 3BKO LCLs (White *et al.* 2010). Alternatively, since EBNA3B promotes GC differentiation of infected B cells (Section 6.1), it is possible that EBNA3B mutation locks the cell into a more proliferative stage of the B cell lifecycle. EBNA3B also directly represses a number of reported oncogenes, the most obvious of which is the protein component of telomerase, TERT (White *et al.* 2010). Notably, the selection bottleneck 3-5 months post infection requires upregulation of TERT (Sugimoto *et al.* 1999) (or the alt telomere pathway) and we never observed a slow-down of 3BKO LCL growth at this point, but did for revertant or wild-type LCLs (my unpublished observations), reinforcing the importance of TERT upregulation for true LCL immortalisation.

Conversely, 3BKO EBV may be less able to induce pathogenicity in multiple sclerosis: A recent study (Laderach *et al.* 2025) showed that EBV-infected B cells can home to the brain in mice, and recruit inflammatory T cells there via CXCR3 as a potential mechanism for initiation of MS autoimmunity. 3BKO-infected B cells express less CXCL9 and CXCL10 (CXCR3 ligands), and – perhaps for the same reason that 3BKO-induced tumours have less

T cell infiltration (White *et al.* 2012) – promoted less inflammatory T cell recruitment to the brain. Future studies will have to confirm whether the altered differentiation state of 3BKO B cells (reflected in vivo in the monomorphic nature of the 3BKO lymphomas) underlies both reduced migration to the brain and the altered CXCL9/10 expression that causes this.

However, there is not yet any indication of which EBNA3B-repressed gene or genes are the key contributors to lymphomagenesis caused by EBNA3B mutation. There is also the oddity that EBNA3B mutations might promote lymphomagenesis in BL and HL – two tumour types thought to not normally produce EBNA3 proteins (excepting Wp-restricted BL). It remains an open question whether EBNA3s are produced in these lymphomas more than is currently appreciated, or if the dysregulation caused by EBNA3B mutation may be epigenetically locked, even after the EBNA3s are no longer expressed.

7.2 EBNA3A and EBNA3C biology in mouse models.

The contributions of EBNA3s to EBV's manipulation of B cell biology has been most effectively studied in vivo using recombinant EBVs in mice reconstituted with human immune stem cells, from either foetal liver (Murer *et al.* 2018) or cord blood (Romero-Masters *et al.* 2018, Romero-Masters *et al.* 2020). Like their behaviour in vitro, EBNA3A and EBNA3C-mutant EBVs in vivo show reduced transformation, in terms of both EBV-positive cell number and reduced tumour formation: either no tumour (Murer *et al.* 2018), or delayed and monoclonal tumour outgrowth compared to polyclonal tumours for wild-type EBV (Romero-Masters *et al.* 2018). Again, consistent with in vitro observations, 3CKO-infected cells exhibited elevated expression of $p16^{INK4A}$, as did the 3AKO in one study (Murer *et al.* 2018). The absence of $p16^{INK4A}$ induction in the other study (Romero-Masters *et al.* 2020) may be explained by a residual level of EBNA3A expression from a cryptic start site in that knockout.

Nevertheless, the numbers of 3AKO and 3CKO-infected B cells observed in vivo are considerably higher than might be expected from their very poor transformation efficiency in vitro. The 3AKO- and 3CKO-infected cells in vivo also had lower expression of LMPs, which is not normally seen in steady-state EBNA3-mutant LCLs (other than the early post-infection Latency IIb state (Price and Luftig 2015)), but regulation of LMP promoters by EBNA3C has been reported in other contexts (Allday *et al.* 1993, Zhao and Sample 2000, Jimenez-Ramirez *et al.* 2006). Perhaps due to this reduced LMP1 expression, regions of 3CKO-infected cells had higher levels of $CD4^+$ T cell infiltration than wild-type EBV, likely due to their increased production of T cell chemokines (Romero-Masters *et al.* 2018). It seems likely that these 3CKO tumours rely on T cells to provide CD40 ligand (and perhaps other ligands) to help survival of the infected B cell, a phenomenon also seen for LMP1-knockout EBV in vivo (Ma *et al.* 2015).

Perhaps most intriguing is the detection of 3AKO and 3CKO-infected cells in mouse spleens 12 weeks after infection that express EBERs, but not other EBNAs or LMPs (Murer *et al.* 2018), suggesting that absence of EBNA3s may promote transition of EBV transcription to the latency 0 profile. It is still not clear whether the corresponding wild-type EBV also generates latency 0 cells, but they are lost to the analysis amongst the abundant presence of latency III cells. Thus, it is possible that the upregulation of $p16^{INK4A}$ (and/or other factors upregulated to provoke cell cycle arrest in EBNA3 mutants) may promote transition to a resting cell phenotype and corresponding Latency 0 transcription state. Alternatively, the failure of EBNA3s to protect cells from proliferative arrest (Section 6.4) might mean that the only EBNA3-knockout cells to survive are those that have shut-down EBNA2 (and thus Myc) expression, as they transition through latency II to latency 0.

Contradicting this idea, the P3HR1 virus, which lacks EBNA2 (and has a truncated EBNA-LP) remains able to induce monoclonal tumours from cord blood infection in NSG mice. These tumours contained different foci with both of the following states: either 1) an EBNA-LP/EBNA3-postive state [the classic Wp-restricted version of latency III seen in the P3HR1 BL cell line], or 2) an EBNA-LP/ LMP1-high latency II state probably using Qp, found in cells whose morphology resembles the Reed-Sternberg cells commonly seen in HL. Interestingly, these lymphomas were also class-switched to IgG (unlike the EBNA2 expressing EBV controls), suggesting that (unlike all other experimental contexts) EBV was able to induce class switching in these cells in mice (Li *et al.* 2020). It is particularly striking that HL is characterised by elevated IgG responses to EBNA-LP and EBNA3A (Sarathkumara *et al.* 2024), which could imply that our understanding of EBV gene expression in HL in vivo remains incomplete.

In tumour cell lines from a T cell-deficient mouse clone transgenically expressing LMPs in B cells, EBNA3A (but not EBNA3B or EBNA3C) was found to improve outgrowth (Sommermann *et al.* 2020). Additional CD19-dependent expression of EBNA3A enhanced LMP1-associated lymphomagenesis, and blocked plasma cell differentiation (which EBNA3C did not) and reduced CDKN2A expression. This enhancement of lymphomagenesis by EBNA3A was prevented by BLIMP1 complementation, but not by CDKN2A knockout, implying that disrupting plasma cell differentiation is the main driver of lymphomagenesis caused by EBNA3A, at least in this model (Sommermann *et al.* 2020). Nevertheless, dysregulation of B cell differentiation emerges as a potential theme in the development of EBV associated lymphoma, in the context of both loss of EBNA3B and gain of EBNA3A function.

8 Summary, unanswered questions and challenges

EBV's contribution to the regulation of host genes and the development of cancer are both complex, multilayered biological processes, and the EBNA3 proteins lie at the heart of both. EBV is highly adapted to prosper in humans for life, and only rarely does this lead to severe disease. This highly attuned biology of EBV to humans is observed in the complexity of genes regulated by the EBNAs – particularly in managing to navigate GC B cell differentiation despite silencing BCL6, the supposed "master regulator" of germinal centre B cells: perhaps this title should be passed to EBV! EBNA3s achieve this through complex manipulations of histone modifications and 3D genome topology that can be epigenetically stable, potentially sustained even after the EBNA3s are shut down. Thus disruptions to the balance between EBNA3 biology and B cell differentiation – such as through mutation of virus or host – may be fundamental to EBV lymphomagenesis, even for lymphomas where the EBNA3s are no longer expressed. Nevertheless, there remain many unanswered questions about the core functions of EBNA3 proteins in B cell and EBV biology:

- To what extent is our current understanding or EBNA3-associated changes in cells distorted by changes in B cell differentiation in our model systems? Are the changes we have observed in bulk RNA-seq due to changes in B cell differentiation states, or are EBNA3-driven changes observable only in a certain differentiation state?

- To what extent do the levels or functions of EBNA3s change in different B cell states or subsets? For instance, in transforming naïve vs memory B cells, or during transitions from activated B cells, through germinal centre states and into memory.

- Are there functional parallels between EBNA3s and cellular RBPJ-mediated gene regulatory complexes (Notch/Hairless; SHARP; L3MBTL3; KyoT2/3) that teach us about their mechanisms of action?

- What are the relative importances of 3A and 3C in leveraging CtBP for gene regulation? What are the connections between RBPJ, CtBP, LSD1, HDACs, KDM2A and Bmi1, and their requirements for gene regulation by the EBNA3s?

- How do EBNA3 functions interact with EBNA2? Is this competition or co-operation, or variable in a locus-specific manner? Or do their roles and interactions change with B cell differentiation?

- What is responsible for the H3K27 trimethylation at EBNA3C-repressed genes, since it is not SUZ12-associated? And how does the conventionally repressive Bmi1 facilitate gene activation by EBNA3C?

- What is the epigenetic stability of different EBNA3 gene targets, and why are they different? What are the implications of this for EBV-associated disease? Could reversing these epigenetic changes represent a legitimate therapeutic strategy in EBV-associated cancers that no longer express EBNA3s?

- What are the most fundamental biological functions/targets of the EBNA3s? Studying evolutionarily distant EBNA3 homologues – particularly the single EBNA3 homologue in New World LCVs – has potential to distinguish core targets or functions of EBNA3s that have since diversified into the distinct EBNA3s: is it essential for managing B cell survival, or differentiation, cell signalling or T cell interactions, or some other currently unexplored functions?

- Are the functions of EBNA3s the same in vivo as in vitro? How do their functions (and their regulation) interact with the complex cellular and cytokine environment of the lymphoid organs?

The abilities of the EBNA3s to oversee the reprogramming and survival of human B cells is fundamental to all EBV B cell diseases, both dysregulation in lymphomas and the reprogramming of controls in the germinal centre that could risk autoimmunity. Addressing these questions about the fundamentals of EBNA3 functions in EBV biology promises new understandings of fundamental B cell biology and associated disease.

9 References

Allday, M. J., Q. Bazot and R. E. White (2015). "The EBNA3 Family: Two Oncoproteins and a Tumour Suppressor that Are Central to the Biology of EBV in B Cells." Current topics in microbiology and immunology **391**: 61-117.

Allday, M. J., D. H. Crawford and J. A. Thomas (1993). "Epstein-Barr virus (EBV) nuclear antigen 6 induces expression of the EBV latent membrane protein and an activated phenotype in Raji cells." The Journal of general virology **74 (Pt 3)**(3): 361-369.

Anderton, E., J. Yee, P. Smith, T. Crook, R. E. White and M. J. Allday (2008). "Two Epstein-Barr virus (EBV) oncoproteins cooperate to repress expression of the proapoptotic tumour-suppressor Bim: clues to the pathogenesis of Burkitt's lymphoma." Oncogene **27**(4): 421-433.

Babcock, G. J., L. L. Decker, R. B. Freeman and D. A. Thorley-Lawson (1999). "Epstein-barr virus-infected resting memory B cells, not proliferating lymphoblasts, accumulate in the peripheral blood of immunosuppressed patients." The Journal of Experimental Medicine **190**(4): 567-576.

Babcock, G. J., D. Hochberg and A. D. Thorley-Lawson (2000). "The expression pattern of Epstein-Barr virus latent genes in vivo is dependent upon the differentiation stage of the infected B cell." Immunity **13**(4): 497-506.

Banerjee, S., J. Lu, Q. Cai, A. Saha, H. C. Jha, R. K. Dzeng and E. S. Robertson (2013). "The EBV Latent Antigen 3C Inhibits Apoptosis through Targeted Regulation of Interferon Regulatory Factors 4 and 8." PLoS Pathog **9**(5): e1003314.

Banerjee, S., J. Lu, Q. Cai, Z. Sun, H. C. Jha and E. S. Robertson (2014). "EBNA3C Augments Pim-1 Mediated Phosphorylation and Degradation of p21 to Promote B-Cell Proliferation." PLOS Pathogens **10**(8): e1004304.

Bazot, Q., T. Deschamps, L. Tafforeau, M. Siouda, P. Leblanc, M. L. Harth-Hertle, C. Rabourdin-Combe, V. Lotteau, B. Kempkes, M. Tommasino, H. Gruffat and E. Manet (2014). "Epstein-Barr virus nuclear antigen 3A protein regulates CDKN2B transcription via interaction with MIZ-1." Nucleic Acids Res **42**(15): 9700-9716.

Bazot, Q., K. Paschos and M. J. Allday (2018). "Epstein-Barr Virus (EBV) Latent Protein EBNA3A Directly Targets and Silences the STK39 Gene in B Cells Infected by EBV." Journal of virology **92**(7): e01918-01917.

Bazot, Q., K. Paschos, L. Skalska, J. S. Kalchschmidt, G. A. Parker and M. J. Allday (2015). "Epstein-Barr Virus Proteins EBNA3A and EBNA3C Together Induce Expression of the Oncogenic MicroRNA Cluster miR-221/miR-222 and Ablate Expression of Its Target p57KIP2." PLOS Pathogens **11**(7): e1005031.

Bhende, P. M., S. J. Dickerson, X. Sun, W.-h. Feng and S. C. Kenney (2007). "X-box-binding protein 1 activates lytic Epstein-Barr virus gene expression in combination with protein kinase D." The Journal of Virology **81**(14): 7363-7370.

Bjornevik, K., M. Cortese, B. C. Healy, J. Kuhle, M. J. Mina, Y. Leng, S. J. Elledge, D. W. Niebuhr, A. I. Scher, K. L. Munger and A. Ascherio (2022). "Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis." Science **375**(6578): 296-301.

Bridges, R., S. Correia, F. Wegner, C. Venturini, A. Palser, R. E. White, P. Kellam, J. Breuer and P. J. Farrell (2019). "Essential role of inverted repeat in Epstein-Barr virus IR-1 in B cell transformation; geographical variation of the viral genome." Philos Trans R Soc Lond B Biol Sci **374**(1773): 20180299.

Bristol, J. A., S. E. Nelson, M. Ohashi, A. Casco, M. Hayes, E. A. Ranheim, A. S. Pawelski, D. R. Singh, D. J. Hodson, E. C. Johannsen and S. C. Kenney (2024). "Latent Epstein-Barr virus infection collaborates with Myc over-expression in normal human B cells to induce Burkitt-like Lymphomas in mice." PLoS Pathog **20**(4): e1012132.

Butt, D., T. D. Chan, K. Bourne, J. R. Hermes, A. Nguyen, A. Statham, L. A. O'Reilly, A. Strasser, S. Price, P. Schofield, D. Christ, A. Basten, C. S. Ma, S. G. Tangye, T. G. Phan, V. K. Rao and R. Brink (2015). "FAS Inactivation Releases Unconventional Germinal Center B Cells that Escape Antigen Control and Drive IgE and Autoantibody Production." Immunity **42**(5): 890-902.

Cable, J. M., N. M. Reinoso-Vizcaino, R. E. White and M. A. Luftig (2024). "Epstein-Barr virus protein EBNA-LP engages YY1 through leucine-rich motifs to promote naive B cell transformation." PLoS Pathog **20**(7): e1011950.
Cable, J. M., W. Wongwiwat, J. C. Grabowski, R. E. White and M. A. Luftig (2024). "Sp140L Is a Novel Herpesvirus Restriction Factor." bioRxiv: 2024.2012.2013.628399.
Calderwood, M. A., S. Lee, A. M. Holthaus, S. C. Blacklow, E. Kieff and E. Johannsen (2011). "Epstein-Barr virus nuclear protein 3C binds to the N-terminal (NTD) and beta trefoil domains (BTD) of RBP/CSL; only the NTD interaction is essential for lymphoblastoid cell growth." Virology **414**(1): 19-25.
Calderwood, M. A., K. Venkatesan, L. Xing, M. R. Chase, A. Vazquez, A. M. Holthaus, A. E. Ewence, N. Li, T. Hirozane-Kishikawa, D. E. Hill, M. Vidal, E. Kieff and E. Johannsen (2007). "Epstein-Barr virus and virus human protein interaction maps." Proceedings of the National Academy of Sciences **104**(18): 7606-7611.
Chen, A., M. Divisconte, X. Jiang, C. Quink and F. Wang (2005). "Epstein-Barr virus with the latent infection nuclear antigen 3B completely deleted is still competent for B-cell growth transformation in vitro." The Journal of Virology **79**(7): 4506-4509.
Chen, A., B. Zhao, E. Kieff, J. C. Aster and F. Wang (2006). "EBNA-3B- and EBNA-3C-regulated cellular genes in Epstein-Barr virus-immortalized lymphoblastoid cell lines." J Virol **80**(20): 10139-10150.
Chen, Y., D. Fachko, N. S. Ivanov, C. M. Skinner and R. L. Skalsky (2019). "Epstein-Barr virus microRNAs regulate B cell receptor signal transduction and lytic reactivation." PLoS Pathog **15**(1): e1007535.
Cirac, A., R. Poirey, M. Dieckmeyer, K. Witter, H. J. Delecluse, U. Behrends and J. Mautner (2021). "Immunoinformatic Analysis Reveals Antigenic Heterogeneity of Epstein-Barr Virus Is Immune-Driven." Front Immunol **12**: 796379.
Correia, S., R. Bridges, F. Wegner, C. Venturini, A. Palser, J. M. Middeldorp, J. I. Cohen, M. A. Lorenzetti, I. Bassano, R. E. White, P. Kellam, J. Breuer and P. J. Farrell (2018). "Sequence Variation of Epstein-Barr Virus: Viral Types, Geography, Codon Usage, and Diseases." J Virol **92**(22): 1487.
Coughlin, J. J., S. L. Stang, N. A. Dower and J. C. Stone (2006). "The role of RasGRPs in regulation of lymphocyte proliferation." Immunology letters **105**(1): 77-82.
Counter, C. M., F. M. Botelho, P. Wang, C. B. Harley and S. Bacchetti (1994). "Stabilization of short telomeres and telomerase activity accompany immortalization of Epstein-Barr virus-transformed human B lymphocytes." The Journal of Virology **68**(5): 3410-3414.
Dai, Q., C. Andreu-Agullo, R. Insolera, L. C. Wong, S. H. Shi and E. C. Lai (2013). "BEND6 is a nuclear antagonist of Notch signaling during self-renewal of neural stem cells." Development **140**(9): 1892-1902.
Dalbiès-Tran, R., E. Stigger-Rosser, T. Dotson and C. E. Sample (2001). "Amino acids of Epstein-Barr virus nuclear antigen 3A essential for repression of Jkappa-mediated transcription and their evolutionary conservation." The Journal of Virology **75**(1): 90-99.
Droubi, A., C. Wallis, K. E. Anderson, S. Rahman, A. de Sa, T. Rahman, L. R. Stephens, P. T. Hawkins and M. Lowe (2022). "The inositol 5-phosphatase INPP5B regulates B cell receptor clustering and signaling." J Cell Biol **221**(9).
Ehlers, B., K. Spieß, F. Leendertz, D. Gatherer and D. J. McGeoch (2010). "Lymphocryptovirus phylogeny and the origins of Epstein-Barr virus." Journal of General Virology **91**(Pt 3): 630-642.
Farrell, P. J. (2019). "Epstein-Barr Virus and Cancer." Annual review of pathology **14**: 29-53.
Farrell, P. J. and R. E. White (2021). "Do Epstein-Barr Virus Mutations and Natural Genome Sequence Variations Contribute to Disease?" Biomolecules **12**(1).
Fike, A. J., S. B. Chodisetti, N. E. Wright, K. N. Bricker, P. P. Domeier, M. Maienschein-Cline, A. M. Rosenfeld, S. A. Luckenbill, J. L. Weber, N. M. Choi, E. T. Luning Prak, M. Mandal, M. R. Clark and Z. S. M. Rahman (2023). "STAT3 signaling in B cells controls germinal center zone organization and recycling." Cell Rep **42**(5): 112512.
Frappier, L. (2025). "EBNA1." Curr Top Microbiol Immunol.
Gao, Y., H. Kazama and S. Yonehara (2012). "Bim regulates B-cell receptor-mediated apoptosis in the presence of CD40 signaling in CD40-pre-activated splenic B cells differentiating into plasma cells." Int Immunol **24**(5): 283-292.

Gavioli, R., P. O. De Campos-Lima, M. G. Kurilla, E. Kieff, G. Klein and M. G. Masucci (1992). "Recognition of the Epstein-Barr virus-encoded nuclear antigens EBNA-4 and EBNA-6 by HLA-A11-restricted cytotoxic T lymphocytes: implications for down-regulation of HLA-A11 in Burkitt lymphoma." Proc Natl Acad Sci U S A **89**(13): 5862-5866.
Giaimo, B. D., E. K. Gagliani, R. A. Kovall and T. Borggrefe (2021). "Transcription Factor RBPJ as a Molecular Switch in Regulating the Notch Response." Adv Exp Med Biol **1287**: 9-30.
Gillman, A. C. T., G. Parker, M. J. Allday and Q. Bazot (2018). "Epstein-Barr Virus (EBV) nuclear antigen 3C inhibits expression of COBLL1 and the ADAM28-ADAMDEC1 locus via interaction with the histone lysine demethylase KDM2B." The Journal of Virology: JVI.01362-01318.
Gottschalk, S., C. Y. Ng, M. Perez, C. A. Smith, C. Sample, M. K. Brenner, H. E. Heslop and C. M. Rooney (2001). "An Epstein-Barr virus deletion mutant associated with fatal lymphoproliferative disease unresponsive to therapy with virus-specific CTLs." Blood **97**(4): 835-843.
Guilbault, B. and R. J. Kay (2004). "RasGRP1 sensitizes an immature B cell line to antigen receptor-induced apoptosis." J Biol Chem **279**(19): 19523-19530.
Harth-Hertle, M. L., B. A. Scholz, F. Erhard, L. V. Glaser, L. Dölken, R. Zimmer and B. Kempkes (2013). "Inactivation of intergenic enhancers by EBNA3A initiates and maintains polycomb signatures across a chromatin domain encoding CXCL10 and CXCL9." PLOS Pathogens **9**(9): e1003638.
Heath, E., N. Begue-Pastor, S. Chaganti, D. Croom-Carter, C. Shannon-Lowe, D. Kube, R. Feederle, H.-J. Delecluse, A. B. Rickinson and A. I. Bell (2012). "Epstein-Barr virus infection of naïve B cells in vitro frequently selects clones with mutated immunoglobulin genotypes: implications for virus biology." PLOS Pathogens **8**(5): e1002697.
Hennessy, K., F. Wang, E. W. Bushman and E. Kieff (1986). "Definitive identification of a member of the Epstein-Barr virus nuclear protein 3 family." Proceedings of the National Academy of Sciences **83**(15): 5693-5697.
Hertle, M. L., C. Popp, S. Petermann, S. Maier, E. Kremmer, R. Lang, J. Mages and B. Kempkes (2009). "Differential gene expression patterns of EBV infected EBNA-3A positive and negative human B lymphocytes." PLOS Pathogens **5**(7): e1000506.
Hickabottom, M., G. A. Parker, P. Freemont, T. Crook and M. J. Allday (2002). "Two nonconsensus sites in the Epstein-Barr virus oncoprotein EBNA3A cooperate to bind the co-repressor carboxyl-terminal-binding protein (CtBP)." Journal of Biological Chemistry **277**(49): 47197-47204.
Hislop, A. D., G. S. Taylor, D. Sauce and A. B. Rickinson (2007). "Cellular responses to viral infection in humans: lessons from Epstein-Barr virus." Annu Rev Immunol **25**: 587-617.
Hochberg, D., J. M. Middeldorp, M. Catalina, J. L. Sullivan, K. Luzuriaga and D. A. Thorley-Lawson (2004). "Demonstration of the Burkitt's lymphoma Epstein-Barr virus phenotype in dividing latently infected memory cells in vivo." Proceedings of the National Academy of Sciences **101**(1): 239-244.
Hochberg, D., T. Souza, M. Catalina, J. L. Sullivan, K. Luzuriaga and D. A. Thorley-Lawson (2004). "Acute infection with Epstein-Barr virus targets and overwhelms the peripheral memory B-cell compartment with resting, latently infected cells." J Virol **78**(10): 5194-5204.
Hui, K. F., P. L. Yeung, K. P. Tam and A. K. S. Chiang (2018). "Counteracting survival functions of EBNA3C in Epstein-Barr virus (EBV)-driven lymphoproliferative diseases by combination of SAHA and bortezomib." Oncotarget **9**(38): 25101-25114.
Isaksson, A., M. Berggren and A. Ricksten (2003). "Epstein-Barr virus U leader exon contains an internal ribosome entry site." Oncogene **22**(4): 572-581.
Jha, H. C., M. P. A J, A. Saha, S. Banerjee, J. Lu and E. S. Robertson (2014). "Epstein-Barr virus essential antigen EBNA3C attenuates H2AX expression." Journal of virology **88**(7): 3776-3788.
Jha, H. C., Q. Cai, M. A. J. Prasad and E. S. Robertson (2013). "EBNA3C-mediated regulation of aurora kinase B contributes to Epstein-Barr virus-induced B-cell proliferation through modulation of the activities of the retinoblastoma protein and apoptotic caspases." Journal of virology **87**(22): 12121-12138.
Jiang, S., B. Willox, H. Zhou, A. M. Holthaus, A. Wang, T. T. Shi, S. Maruo, P. V. Kharchenko, E. C. Johannsen, E. Kieff and B. Zhao (2014). "Epstein-Barr virus nuclear antigen 3C binds to BATF/IRF4 or SPI1/IRF4 composite sites and recruits Sin3A to repress CDKN2A." Proc Natl Acad Sci U S A **111**(1): 421-426.

Jiang, S., H. Zhou, J. Liang, C. Gerdt, C. Wang, L. Ke, S. C. S. Schmidt, Y. Narita, Y. Ma, S. Wang, T. Colson, B. Gewurz, G. Li, E. Kieff and B. Zhao (2017). "The Epstein-Barr Virus Regulome in Lymphoblastoid Cells." Cell Host Microbe **22**(4): 561-573 e564.
Jimenez-Ramirez, C., A. J. Brooks, L. P. Forshell, K. Yakimchuk, B. Zhao, T. Z. Fulgham and C. E. Sample (2006). "Epstein-Barr virus EBNA-3C is targeted to and regulates expression from the bidirectional LMP-1/2B promoter." J Virol **80**(22): 11200-11208.
Johannsen, E., C. L. Miller, S. R. Grossman and E. Kieff (1996). "EBNA-2 and EBNA-3C extensively and mutually exclusively associate with RBPJkappa in Epstein-Barr virus-transformed B lymphocytes." The Journal of Virology **70**(6): 4179-4183.
Joseph, A. M., G. J. Babcock and D. A. Thorley-Lawson (2000). "EBV persistence involves strict selection of latently infected B cells." Journal of immunology (Baltimore, Md : 1950) **165**(6): 2975-2981.
Kalchschmidt, J. S., R. Bashford-Rogers, K. Paschos, A. C. T. Gillman, C. T. Styles, P. Kellam and M. J. Allday (2016). "Epstein-Barr virus nuclear protein EBNA3C directly induces expression of AID and somatic mutations in B cells." The Journal of Experimental Medicine **213**(6): 921-928.
Kalchschmidt, J. S., A. C. T. Gillman, K. Paschos, Q. Bazot, B. Kempkes and M. J. Allday (2016). "EBNA3C Directs Recruitment of RBPJ (CBF1) to Chromatin during the Process of Gene Repression in EBV Infected B Cells." PLOS Pathogens **12**(1): e1005383.
Kelly, G. L., A. E. Milner, R. J. Tierney, D. S. G. Croom-Carter, M. Altmann, W. Hammerschmidt and A. I. Bell (2005). "Epstein-Barr virus nuclear antigen 2 (EBNA2) gene deletion is consistently linked with EBNA3A, -3B, and -3C expression in Burkitt's lymphoma cells and with increased resistance to apoptosis." The Journal of Virology **79**(16): 10709-10717.
Kelly, G. L., J. Stylianou, J. Rasaiyaah, W. Wei, W. Thomas, D. Croom-Carter, C. Kohler, R. Spang, C. Woodman, P. Kellam, A. B. Rickinson and A. I. Bell (2013). "Different patterns of Epstein-Barr virus latency in endemic Burkitt lymphoma (BL) lead to distinct variants within the BL-associated gene expression signature." J Virol **87**(5): 2882-2894.
Khasnis, S., H. Veenstra, M. J. McClellan, O. Ojeniyi, C. D. Wood and M. J. West (2022). "Regulation of B cell receptor signalling by Epstein-Barr virus nuclear antigens." Biochem J **479**(23): 2395-2417.
Kieser, A. (2025). "The Latent Membrane Protein 1 (LMP1): Biological Functions and Molecular Mechanisms." Curr Top Microbiol Immunol.
Kim, H. S., A. Li, S. Ahn, H. Song and W. Zhang (2014). "Inositol Polyphosphate-5-Phosphatase F (INPP5F) inhibits STAT3 activity and suppresses gliomas tumorigenicity." Sci Rep **4**: 7330.
Kuppuswamy, M., S. Vijayalingam, L. J. Zhao, Y. Zhou, T. Subramanian, J. Ryerse and G. Chinnadurai (2008). "Role of the PLDLS-binding cleft region of CtBP1 in recruitment of core and auxiliary components of the corepressor complex." Mol Cell Biol **28**(1): 269-281.
Laderach, F., I. Piteros, E. Fennell, E. Bremer, M. Last, S. Schmid, L. Rieble, C. Campbell, I. Ludwig-Portugall, L. Bornemann, A. Gruhl, K. Eulitz, P. Gueguen, J. Mietz, A. Muller, G. Pezzino, J. Schmitz, G. Ferlazzo, J. Mautner and C. Munz (2025). "EBV induces CNS homing of B cells attracting inflammatory T cells." Nature.
Landman, S. L., M. E. Ressing, A. M. Gram, R. T. N. Tjokrodirijo, P. A. van Veelen, J. Neefjes, R. C. Hoeben, A. G. van der Veen and I. Berlin (2024). "Epstein-Barr virus nuclear antigen EBNA3A modulates IRF3-dependent IFNbeta expression." J Biol Chem **300**(9): 107645.
Lang, F., R. K. Singh, Y. Pei, S. Zhang, K. Sun and E. S. Robertson (2019). "EBV epitranscriptome reprogramming by METTL14 is critical for viral-associated tumorigenesis." PLOS Pathogens **15**(6): e1007796.
Le Roux, A., B. Kerdiles, D. Walls, J. F. Dedieu and M. Perricaudet (1994). "The Epstein-Barr virus determined nuclear antigens EBNA-3A, -3B, and -3C repress EBNA-2-mediated transactivation of the viral terminal protein 1 gene promoter." Virology **205**(2): 596-602.
Lee, J. E., H. Y. Nam, S. M. Shim, G. R. Bae, B. G. Han and J. P. Jeon (2010). "Expression phenotype changes of EBV-transformed lymphoblastoid cell lines during long-term subculture and its clinical significance." Cell Prolif **43**(4): 378-384.
Lee, S., S. Sakakibara, S. Maruo, B. Zhao, M. A. Calderwood, A. M. Holthaus, C. Y. Lai, K. Takada, E. Kieff and E. Johannsen (2009). "Epstein-Barr virus nuclear protein 3C domains necessary for

lymphoblastoid cell growth: interaction with RBP-Jkappa regulates TCL1." J Virol **83**(23): 12368-12377.
Li, C., J. C. Romero-Masters, S. Huebner, M. Ohashi, M. Hayes, J. A. Bristol, S. E. Nelson, M. R. Eichelberg, N. Van Sciver, E. A. Ranheim, R. S. Scott, E. C. Johannsen and S. C. Kenney (2020). "EBNA2-deleted Epstein-Barr virus (EBV) isolate, P3HR1, causes Hodgkin-like lymphomas and diffuse large B cell lymphomas with type II and Wp-restricted latency types in humanized mice." PLoS Pathog **16**(6): e1008590.
Lieberman, P. M. and I. Tempera (2025). "Chromatin Control of EBV Infection and Latency." Curr Top Microbiol Immunol.
Lucchesi, W., G. Brady, O. Dittrich-Breiholz, M. Kracht, R. Russ and P. J. Farrell (2008). "Differential gene regulation by Epstein-Barr virus type 1 and type 2 EBNA2." Journal of virology **82**(15): 7456-7466.
Ma, J., N. Kui, D. Redmond, Y. Liu, O. Elemento, D. M. Knowles and W. Tam (2015). "EBV-miR-BHRF1-2 targets PRDM1/Blimp1: potential role in EBV lymphomagenesis." Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, UK: -.
Ma, S.-D., X. Xu, J. Plowshay, E. A. Ranheim, W. J. Burlingham, J. L. Jensen, F. Asimakopoulos, W. Tang, M. L. Gulley, E. Cesarman, J. E. Gumperz and S. C. Kenney (2015). "LMP1-deficient Epstein-Barr virus mutant requires T cells for lymphomagenesis." The Journal of clinical investigation **125**(1): 304-315.
Maestri, D., L. B. Caruso, J. M. Cable, R. Sklutuis, S. Preston-Alp, R. E. White, M. A. Luftig and I. Tempera (2025). "EBNA leader protein orchestrates chromatin architecture remodeling during Epstein-Barr virus-induced B cell transformation." Nucleic Acids Res **53**(12).
Maier, S., G. Staffler, A. Hartmann, J. Höck, K. Henning, K. Grabusic, R. Mailhammer, R. Hoffmann, M. Wilmanns, R. Lang, J. Mages and B. Kempkes (2006). "Cellular target genes of Epstein-Barr virus nuclear antigen 2." The Journal of Virology **80**(19): 9761-9771.
Mamane-Logsdon, A., I. Zane, J. S. Chong, O. H. I. Chou, J. Huang, M. Rawal, A. C. Gillman, W. Wongwiwat, M. Saleban, I. Donovan-Banfield, D. A. Matthews and R. E. White (2025). "A direct RNA-seq-based EBV latency transcriptome offers insights into the biogenesis of EBV gene products." J Gen Virol **106**(8).
Maruo, S., E. Johannsen, D. Illanes, A. Cooper, B. Zhao and E. Kieff (2005). "Epstein-Barr virus nuclear protein 3A domains essential for growth of lymphoblasts: transcriptional regulation through RBP-Jkappa/CBF1 is critical." The Journal of Virology **79**(16): 10171-10179.
Maruo, S., Y. Wu, S. Ishikawa, T. Kanda, D. Iwakiri and K. Takada (2006). "Epstein-Barr virus nuclear protein EBNA3C is required for cell cycle progression and growth maintenance of lymphoblastoid cells." Proceedings of the National Academy of Sciences **103**(51): 19500-19505.
Mayer, C. T., A. Gazumyan, E. E. Kara, A. D. Gitlin, J. Golijanin, C. Viant, J. Pai, T. Y. Oliveira, Q. Wang, A. Escolano, M. Medina-Ramirez, R. W. Sanders and M. C. Nussenzweig (2017). "The microanatomic segregation of selection by apoptosis in the germinal center." Science **358**(6360).
McClellan, M. J., S. Khasnis, C. D. Wood, R. D. Palermo, S. N. Schlick, A. S. Kanhere, R. G. Jenner and M. J. West (2012). "Downregulation of integrin receptor-signaling genes by Epstein-Barr virus EBNA 3C via promoter-proximal and -distal binding elements." Journal of virology **86**(9): 5165-5178.
McClellan, M. J., C. D. Wood, O. Ojeniyi, T. J. Cooper, A. Kanhere, A. Arvey, H. M. Webb, R. D. Palermo, M. L. Harth-Hertle, B. Kempkes, R. G. Jenner and M. J. West (2013). "Modulation of enhancer looping and differential gene targeting by epstein-barr virus transcription factors directs cellular reprogramming." PLOS Pathogens **9**(9): e1003636.
McLachlan, T., W. C. Matthews, E. R. Jackson, D. E. Staudt, A. M. Douglas, I. J. Findlay, M. L. Persson, R. J. Duchatel, A. Mannan, Z. P. Germon and M. D. Dun (2022). "B-cell Lymphoma 6 (BCL6): From Master Regulator of Humoral Immunity to Oncogenic Driver in Pediatric Cancers." Mol Cancer Res **20**(12): 1711-1723.
McLaughlin, K., I. M. Flyamer, J. P. Thomson, H. K. Mjoseng, R. Shukla, I. Williamson, G. R. Grimes, R. S. Illingworth, I. R. Adams, S. Pennings, R. R. Meehan and W. A. Bickmore (2019). "DNA Methylation Directs Polycomb-Dependent 3D Genome Re-organization in Naive Pluripotency." Cell Rep **29**(7): 1974-1985 e1976.

Mordasini, V., S. Ueda, R. Aslandogmus, C. Berger, C. Gysin, D. Huhn, A. A. Sartori, M. Bernasconi and D. Nadal (2017). "Activation of ATR-Chk1 pathway facilitates EBV-mediated transformation of primary tonsillar B-cells." Oncotarget **8**(4): 6461-6474.
Morel, V., M. Lecourtois, O. Massiani, D. Maier, A. Preiss and F. Schweisguth (2001). "Transcriptional repression by suppressor of hairless involves the binding of a hairless-dCtBP complex in Drosophila." Curr Biol **11**(10): 789-792.
Murer, A., D. McHugh, N. Caduff, J. Kalchschmidt, M. Barros, A. Zbinden, R. Capaul, G. Niedobitek, O. Chijioke and C. Münz (2018). "EBV persistence without its EBNA3A and 3C oncogenes in vivo." PLOS Pathogens **14**(4): e1007039.
Murray, R. J., M. G. Kurilla, J. M. Brooks, W. A. Thomas, M. Rowe, E. Kieff and A. B. Rickinson (1992). "Identification of target antigens for the human cytotoxic T cell response to Epstein-Barr virus (EBV): implications for the immune control of EBV-positive malignancies." J Exp Med **176**(1): 157-168.
Muto, A., K. Ochiai, Y. Kimura, A. Itoh-Nakadai, K. L. Calame, D. Ikebe, S. Tashiro and K. Igarashi (2010). "Bach2 represses plasma cell gene regulatory network in B cells to promote antibody class switch." The EMBO Journal **29**(23): 4048.
Nikitin, P. A., C. M. Yan, E. Forte, A. Bocedi, J. P. Tourigny, R. E. White, M. J. Allday, A. Patel, S. S. Dave, W. Kim, K. Hu, J. Guo, D. Tainter, E. Rusyn and M. A. Luftig (2010). "An ATM/Chk2-mediated DNA damage-responsive signaling pathway suppresses Epstein-Barr virus transformation of primary human B cells." Cell Host Microbe **8**(6): 510-522.
Ning, R. J., X. Q. Xu, K. H. Chan and A. K. Chiang (2011). "Long-term carriers generate Epstein-Barr virus (EBV)-specific CD4(+) and CD8(+) polyfunctional T-cell responses which show immunodominance hierarchies of EBV proteins." Immunology **134**(2): 161-171.
Ochiai, K., A. Muto, H. Tanaka, S. Takahashi and K. Igarashi (2008). "Regulation of the plasma cell transcription factor Blimp-1 gene by Bach2 and Bcl6." International immunology **20**(3): 453-460.
Ohashi, M., M. Hayes, K. McChesney and E. Johannsen (2021). "Epstein-Barr virus nuclear antigen 3C (EBNA3C) interacts with the metabolism sensing C-terminal binding protein (CtBP) repressor to upregulate host genes." PLoS Pathog **17**(3): e1009419.
Okuno, Y., T. Murata, Y. Sato, H. Muramatsu, Y. Ito, T. Watanabe, T. Okuno, N. Murakami, K. Yoshida, A. Sawada, M. Inoue, K. Kawa, M. Seto, K. Ohshima, Y. Shiraishi, K. Chiba, H. Tanaka, S. Miyano, Y. Narita, M. Yoshida, F. Goshima, J.-I. Kawada, T. Nishida, H. Kiyoi, S. Kato, S. Nakamura, S. Morishima, T. Yoshikawa, S. Fujiwara, N. Shimizu, Y. Isobe, M. Noguchi, A. Kikuta, K. Iwatsuki, Y. Takahashi, S. Kojima, S. Ogawa and H. Kimura (2019). "Defective Epstein-Barr virus in chronic active infection and haematological malignancy." Nature microbiology **20**: 1472.
Oswald, F., M. Winkler, Y. Cao, K. Astrahantseff, S. Bourteele, W. Knochel and T. Borggrefe (2005). "RBP-Jkappa/SHARP recruits CtIP/CtBP corepressors to silence Notch target genes." Mol Cell Biol **25**(23): 10379-10390.
Palser, A. L., N. E. Grayson, R. E. White, C. Corton, S. Correia, M. M. Ba Abdullah, S. J. Watson, M. Cotten, J. R. Arrand, P. G. Murray, M. J. Allday, A. B. Rickinson, L. S. Young, P. J. Farrell and P. Kellam (2015). "Genome diversity of Epstein-Barr virus from multiple tumor types and normal infection." J Virol **89**(10): 5222-5237.
Paschos, K., Q. Bazot, G. Ho, G. A. Parker, J. Lees, G. Barton and M. J. Allday (2017). "Core binding factor (CBF) is required for Epstein-Barr virus EBNA3 proteins to regulate target gene expression." Nucleic Acids Res **45**(5): 2368-2383.
Paschos, K., Q. Bazot, J. Lees, P. J. Farrell and M. J. Allday (2019). "Requirement for PRC1 subunit BMI1 in host gene activation by Epstein-Barr virus protein EBNA3C." Nucleic Acids Res **47**(6): 2807-2821.
Paschos, K., G. A. Parker, E. Watanatanasup, R. E. White and M. J. Allday (2012). "BIM promoter directly targeted by EBNA3C in polycomb-mediated repression by EBV." Nucleic Acids Research **40**(15): 7233-7246.
Paschos, K., P. Smith, E. Anderton, J. M. Middeldorp, R. E. White and M. J. Allday (2009). "Epstein-barr virus latency in B cells leads to epigenetic repression and CpG methylation of the tumour suppressor gene Bim." PLOS Pathogens **5**(6): e1000492.
Pauls, S. D. and A. J. Marshall (2017). "Regulation of immune cell signaling by SHIP1: A phosphatase, scaffold protein, and potential therapeutic target." Eur J Immunol **47**(6): 932-945.

Pei, Y., S. Banerjee, H. C. Jha, Z. Sun and E. S. Robertson (2017). "An essential EBV latent antigen 3C binds Bcl6 for targeted degradation and cell proliferation." PLOS Pathogens **13**(7): e1006500.
Petti, L., C. Sample and E. Kieff (1990). "Subnuclear localization and phosphorylation of Epstein-Barr virus latent infection nuclear proteins." Virology **176**(2): 563-574.
Pich, D., P. Mrozek-Gorska, M. Bouvet, A. Sugimoto, E. Akidil, A. Grundhoff, S. Hamperl, P. D. Ling, W. Hammerschmidt, R. F. Ambinder and D. E. Griffin (2019). "First Days in the Life of Naive Human B Lymphocytes Infected with Epstein-Barr Virus." mBio **10**(5): e01723-01719.
Ponnusamy, R., R. Khatri, P. B. Correia, C. D. Wood, E. J. Mancini, P. J. Farrell and M. J. West (2019). "Increased association between Epstein-Barr virus EBNA2 from type 2 strains and the transcriptional repressor BS69 restricts EBNA2 activity." PLOS Pathogens **15**(7): e1007458.
Price, A. M., J. Dai, Q. Bazot, L. Patel, P. A. Nikitin, R. Djavadian, P. S. Winter, C. A. Salinas, A. P. Barry, K. C. Wood, E. C. Johannsen, A. Letai, M. J. Allday and M. A. Luftig (2017). "Epstein-Barr virus ensures B cell survival by uniquely modulating apoptosis at early and late times after infection." eLife **6**: e22509.
Price, A. M. and M. A. Luftig (2015). "To Be or Not IIb: A Multi-Step Process for Epstein-Barr Virus Latency Establishment and Consequences for B Cell Tumorigenesis." PLOS Pathogens **11**(3): e1004656.
Radkov, S. A., M. Bain, P. J. Farrell, M. West, M. Rowe and M. J. Allday (1997). "Epstein-Barr virus EBNA3C represses Cp, the major promoter for EBNA expression, but has no effect on the promoter of the cell gene CD21." The Journal of Virology **71**(11): 8552-8562.
Razzaghi, R., S. Agarwal, N. Kotlov, O. Plotnikova, K. Nomie, D. W. Huang, G. W. Wright, G. A. Smith, M. Li, K. Takata, M. Yamadi, C. Yao, J. J. O'Shea, J. D. Phelan, S. Pittaluga, D. W. Scott and J. R. Muppidi (2021). "Compromised counterselection by FAS creates an aggressive subtype of germinal center lymphoma." J Exp Med **218**(3).
Reusch, J. A., D. M. Nawandar, K. L. Wright, S. C. Kenney and J. E. Mertz (2015). "Cellular differentiation regulator BLIMP1 induces Epstein-Barr virus lytic reactivation in epithelial and B cells by activating transcription from both the R and Z promoters." J Virol **89**(3): 1731-1743.
Richard Albert, J., T. Urli, A. Monteagudo-Sanchez, A. Le Breton, A. Sultanova, A. David, M. Scarpa, M. Schulz and M. V. C. Greenberg (2025). "DNA methylation shapes the Polycomb landscape during the exit from naive pluripotency." Nat Struct Mol Biol **32**(2): 346-357.
Riising, E. M., I. Comet, B. Leblanc, X. Wu, J. V. Johansen and K. Helin (2014). "Gene silencing triggers polycomb repressive complex 2 recruitment to CpG islands genome wide." Mol Cell **55**(3): 347-360.
Rivailler, P., Y. G. Cho and F. Wang (2002). "Complete genomic sequence of an Epstein-Barr virus-related herpesvirus naturally infecting a new world primate: a defining point in the evolution of oncogenic lymphocryptoviruses." J Virol **76**(23): 12055-12068.
Robertson, E. S., J. Lin and E. Kieff (1996). "The amino-terminal domains of Epstein-Barr virus nuclear proteins 3A, 3B, and 3C interact with RBPJ(kappa)." J Virol **70**(5): 3068-3074.
Romero-Masters, J. C., M. Ohashi, R. Djavadian, M. R. Eichelberg, M. Hayes, J. A. Bristol, S. Ma, E. A. Ranheim, J. Gumperz, E. C. Johannsen and S. C. Kenney (2018). "An EBNA3C-deleted Epstein-Barr virus (EBV) mutant causes B-cell lymphomas with delayed onset in a cord blood-humanized mouse model." PLOS Pathogens **14**(8): e1007221.
Romero-Masters, J. C., M. Ohashi, R. Djavadian, M. R. Eichelberg, M. Hayes, N. A. Zumwalde, J. A. Bristol, S. E. Nelson, S. Ma, E. A. Ranheim, J. E. Gumperz, E. C. Johannsen and S. C. Kenney (2020). "An EBNA3A-Mutated Epstein-Barr Virus Retains the Capacity for Lymphomagenesis in a Cord Blood-Humanized Mouse Model." J Virol **94**(10).
Roughan, J. E. and D. A. Thorley-Lawson (2009). "The intersection of Epstein-Barr virus with the germinal center." Journal of virology **83**(8): 3968-3976.
Saha, A., J. Lu, L. Morizur, S. K. Upadhyay, M. P. Aj and E. S. Robertson (2012). "E2F1 mediated apoptosis induced by the DNA damage response is blocked by EBV nuclear antigen 3C in lymphoblastoid cells." PLOS Pathogens **8**(3): e1002573.
Saha, A., M. Murakami, P. Kumar, B. Bajaj, K. Sims and E. S. Robertson (2009). "Epstein-Barr virus nuclear antigen 3C augments Mdm2-mediated p53 ubiquitination and degradation by deubiquitinating Mdm2." J Virol **83**(9): 4652-4669.

Saito, T., S. Chiba, M. Ichikawa, A. Kunisato, T. Asai, K. Shimizu, T. Yamaguchi, G. Yamamoto, S. Seo, K. Kumano, E. Nakagami-Yamaguchi, Y. Hamada, S. Aizawa and H. Hirai (2003). "Notch2 is preferentially expressed in mature B cells and indispensable for marginal zone B lineage development." Immunity **18**(5): 675-685.
Sarathkumara, Y. D., R. R. Xian, Z. Liu, K. J. Yu, J. K. C. Chan, Y. L. Kwong, T. H. Lam, R. Liang, B. Chiu, J. Xu, W. Hu, B. T. Ji, A. E. Coghill, A. M. Kelly, R. M. Pfeiffer, N. Rothman, R. F. Ambinder, A. Hildesheim, Q. Lan, C. Proietti and D. L. Doolan (2024). "A proteome-wide analysis unveils a core Epstein-Barr virus antibody signature of classic Hodgkin lymphoma across ethnically diverse populations." Int J Cancer **155**(8): 1476-1486.
Schaeper, U., J. M. Boyd, S. Verma, E. Uhlmann, T. Subramanian and G. Chinnadurai (1995). "Molecular cloning and characterization of a cellular phosphoprotein that interacts with a conserved C-terminal domain of adenovirus E1A involved in negative modulation of oncogenic transformation." Proc Natl Acad Sci U S A **92**(23): 10467-10471.
Schmidt, S. C. S., S. Jiang, H. Zhou, B. Willox, A. M. Holthaus, P. V. Kharchenko, E. C. Johannsen, E. Kieff and B. Zhao (2015). "Epstein-Barr virus nuclear antigen 3A partially coincides with EBNA3C genome-wide and is tethered to DNA through BATF complexes." Proceedings of the National Academy of Sciences **112**(2): 554-559.
Shukla, S. K., H. C. Jha, D. W. El-Naccache and E. S. Robertson (2016). "An EBV recombinant deleted for residues 130-159 in EBNA3C can deregulate p53/Mdm2 and Cyclin D1/CDK6 which results in apoptosis and reduced cell proliferation." Oncotarget **7**(14): 18116-18134.
Singh, D. R., S. E. Nelson, A. S. Pawelski, J. A. Cantres-Velez, A. S. Kansra, N. P. Pauly, J. A. Bristol, M. Hayes, M. Ohashi, A. Casco, D. Lee, S. A. Fogarty, P. F. Lambert, E. C. Johannsen and S. C. Kenney (2022). "Type 1 and Type 2 Epstein-Barr viruses induce proliferation, and inhibit differentiation, in infected telomerase-immortalized normal oral keratinocytes." PLoS Pathog **18**(10): e1010868.
Skalska, L. (2012). Deregulation of the Cell Cycle by EBV Nuclear Antigens EBNA3A and EBNA3C. Doctor of Philosophy (PhD) Dissertation, Imperial College London.
Skalska, L., R. E. White, M. Franz, M. Ruhmann and M. J. Allday (2010). "Epigenetic repression of p16(INK4A) by latent Epstein-Barr virus requires the interaction of EBNA3A and EBNA3C with CtBP." PLOS Pathogens **6**(6): e1000951.
Skalska, L., R. E. White, G. A. Parker, E. Turro, A. J. Sinclair, K. Paschos and M. J. Allday (2013). "Induction of p16(INK4a) Is the Major Barrier to Proliferation when Epstein-Barr Virus (EBV) Transforms Primary B Cells into Lymphoblastoid Cell Lines." PLOS Pathogens **9**(2): e1003187.
Soh, T. K., S. Ognibene, S. Sanders, R. Schaper, B. B. Kaufer and J. B. Bosse (2024). "A proteome-wide structural systems approach reveals insights into protein families of all human herpesviruses." Nat Commun **15**(1): 10230.
Sommermann, T., T. Yasuda, J. Ronen, T. Wirtz, T. Weber, U. Sack, R. Caeser, J. Zhang, X. Li, V. T. Chu, A. Jauch, K. Unger, D. J. Hodson, A. Akalin and K. Rajewsky (2020). "Functional interplay of Epstein-Barr virus oncoproteins in a mouse model of B cell lymphomagenesis." Proc Natl Acad Sci U S A **117**(25): 14421-14432.
SoRelle, E. D., J. Dai, E. N. Bonglack, E. M. Heckenberg, J. Y. Zhou, S. N. Giamberardino, J. A. Bailey, S. G. Gregory, C. Chan and M. A. Luftig (2021). "Single-cell RNA-seq reveals transcriptomic heterogeneity mediated by host-pathogen dynamics in lymphoblastoid cell lines." Elife **10**.
SoRelle, E. D., N. M. Reinoso-Vizcaino, J. Dai, A. P. Barry, C. Chan and M. A. Luftig (2023). "Epstein-Barr virus evades restrictive host chromatin closure by subverting B cell activation and germinal center regulatory loci." Cell Rep **42**(8): 112958.
SoRelle, E. D., N. M. Reinoso-Vizcaino, G. Q. Horn and M. A. Luftig (2022). "Epstein-Barr virus perpetuates B cell germinal center dynamics and generation of autoimmune-associated phenotypes in vitro." Front Immunol **13**: 1001145.
Souza, T. A., B. D. Stollar, J. L. Sullivan, K. Luzuriaga and D. A. Thorley-Lawson (2007). "Influence of EBV on the peripheral blood memory B cell compartment." Journal of immunology (Baltimore, Md : 1950) **179**(5): 3153-3160.
Spender, L. C., G. H. Cornish, B. Rowland, B. Kempkes and P. J. Farrell (2001). "Direct and indirect regulation of cytokine and cell cycle proteins by EBNA-2 during Epstein-Barr virus infection." The Journal of Virology **75**(8): 3537-3546.

Strobl, L. J., H. Höfelmayr, C. Stein, G. Marschall, M. Brielmeier, G. Laux, G. W. Bornkamm and U. Zimber-Strobl (1997). "Both Epstein-Barr viral nuclear antigen 2 (EBNA2) and activated Notch1 transactivate genes by interacting with the cellular protein RBP-J kappa." Immunobiology **198**(1-3): 299-306.
Styles, C. T., Q. Bazot, G. A. Parker, R. E. White, K. Paschos and M. J. Allday (2017). "EBV epigenetically suppresses the B cell-to-plasma cell differentiation pathway while establishing long-term latency." PLoS biology **15**(8): e2001992.
Sugimoto, M., T. Ide, M. Goto and Y. Furuichi (1999). "Reconsideration of senescence, immortalization and telomere maintenance of Epstein-Barr virus-transformed human B-lymphoblastoid cell lines." Mechanisms of ageing and development **107**(1): 51-60.
Sun, C. C. and D. A. Thorley-Lawson (2007). "Plasma cell-specific transcription factor XBP-1s binds to and transactivates the Epstein-Barr virus BZLF1 promoter." J Virol **81**(24): 13566-13577.
Sun, K., D. Bose, R. K. Singh, Y. Pei and E. S. Robertson (2024). "The F-box E3 ligase protein FBXO11 regulates EBNA3C-associated degradation of BCL6." J Virol **98**(7): e0054824.
Thorley-Lawson, D. A. (2015). "EBV Persistence--Introducing the Virus." Curr Top Microbiol Immunol **390**(Pt 1): 151-209.
Thornton, S. R., V. L. Butty, S. S. Levine and L. A. Boyer (2014). "Polycomb Repressive Complex 2 regulates lineage fidelity during embryonic stem cell differentiation." PLoS One **9**(10): e110498.
Tobollik, S., L. Meyer, M. Buettner, S. Klemmer, B. Kempkes, E. Kremmer, G. Niedobitek and B. Jungnickel (2006). "Epstein-Barr virus nuclear antigen 2 inhibits AID expression during EBV-driven B-cell growth." Blood **108**(12): 3859-3864.
Tomkinson, B., E. Kieff and E. Kieff (1992). "Use of second-site homologous recombination to demonstrate that Epstein-Barr virus nuclear protein 3B is not important for lymphocyte infection or growth transformation in vitro." The Journal of Virology **66**(5): 2893-2903.
Tomkinson, B., E. Robertson and E. Kieff (1993). "Epstein-Barr virus nuclear proteins EBNA-3A and EBNA-3C are essential for B-lymphocyte growth transformation." The Journal of Virology **67**(4): 2014-2025.
Touitou, R., M. Hickabottom, G. Parker, T. Crook and M. J. Allday (2001). "Physical and functional interactions between the corepressor CtBP and the Epstein-Barr virus nuclear antigen EBNA3C." The Journal of Virology **75**(16): 7749-7755.
Touitou, R., J. O'Nions, J. Heaney and M. J. Allday (2005). "Epstein-Barr virus EBNA3 proteins bind to the C8/alpha7 subunit of the 20S proteasome and are degraded by 20S proteasomes in vitro, but are very stable in latently infected B cells." The Journal of general virology **86**(Pt 5): 1269-1277.
Tourigny, M. R., J. Ursini-Siegel, H. Lee, K. M. Toellner, A. F. Cunningham, D. S. Franklin, S. Ely, M. Chen, X. F. Qin, Y. Xiong, I. C. MacLennan and S. Chen-Kiang (2002). "CDK inhibitor p18(INK4c) is required for the generation of functional plasma cells." Immunity **17**(2): 179-189.
Tsai, K., N. Thikmyanova, J. A. Wojcechowskyj, H.-J. Delecluse and P. M. Lieberman (2011). "EBV tegument protein BNRF1 disrupts DAXX-ATRX to activate viral early gene transcription." PLOS Pathogens **7**(11): e1002376.
Victora, G. D. and M. C. Nussenzweig (2022). "Germinal Centers." Annu Rev Immunol **40**: 413-442.
Vrzalikova, K., M. Vockerodt, S. Leonard, A. Bell, W. Wei, A. Schrader, K. L. Wright, D. Kube, M. Rowe, C. B. Woodman and P. G. Murray (2011). "Down-regulation of BLIMP1α by the EBV oncogene, LMP-1, disrupts the plasma cell differentiation program and prevents viral replication in B cells: implications for the pathogenesis of EBV-associated B-cell lymphomas." Blood **117**(22): 5907-5917.
Waltzer, L., M. Perricaudet, A. Sergeant and E. Manet (1996). "Epstein-Barr virus EBNA3A and EBNA3C proteins both repress RBP-J kappa-EBNA2-activated transcription by inhibiting the binding of RBP-J kappa to DNA." J Virol **70**(9): 5909-5915.
Wang, A., R. Welch, B. Zhao, T. Ta, S. Keles and E. Johannsen (2015). "Epstein-Barr Virus Nuclear Antigen 3 (EBNA3) Proteins Regulate EBNA2 Binding to Distinct RBPJ Genomic Sites." J Virol **90**(6): 2906-2919.
Wang, C., X. Liu, J. Liang, Y. Narita, W. Ding, D. Li, L. Zhang, H. Wang, M. M. L. Leong, I. Hou, C. Gerdt, C. Jiang, Q. Zhong, Z. Tang, C. Forney, L. Kottyan, M. T. Weirauch, B. E. Gewurz, M. S. Zeng, S. Jiang, M. Teng and B. Zhao (2023). "A DNA tumor virus globally reprograms host 3D genome architecture to achieve immortal growth." Nat Commun **14**(1): 1598.

Wang, C., H. Zhou, Y. Xue, J. Liang, Y. Narita, C. Gerdt, A. Y. Zheng, R. Jiang, S. Trudeau, C.-W. Peng, B. E. Gewurz and B. Zhao (2018). "Epstein-Barr Virus Nuclear Antigen Leader Protein Coactivates EP300." The Journal of Virology **92**(9): e02155-02117.
Wen, Y., Y. Jing, L. Yang, D. Kang, P. Jiang, N. Li, J. Cheng, J. Li, X. Li, Z. Peng, X. Sun, H. Miller, Z. Sui, Q. Gong, B. Ren, W. Yin and C. Liu (2019). "The regulators of BCR signaling during B cell activation." Blood Sci **1**(2): 119-129.
White, R. E., I. J. Groves, E. Turro, J. Yee, E. Kremmer and M. J. Allday (2010). "Extensive co-operation between the Epstein-Barr virus EBNA3 proteins in the manipulation of host gene expression and epigenetic chromatin modification." PLoS ONE **5**(11): e13979.
White, R. E., P. C. Rämer, K. N. Naresh, S. Meixlsperger, L. Pinaud, C. Rooney, B. Savoldo, R. Coutinho, C. Bödör, J. Gribben, H. A. Ibrahim, M. Bower, J. P. Nourse, M. K. Gandhi, J. Middeldorp, F. Z. Cader, P. Murray, C. Münz and M. J. Allday (2012). "EBNA3B-deficient EBV promotes B cell lymphomagenesis in humanized mice and is found in human tumors." The Journal of clinical investigation **122**(4): 1487-1502.
Wongwiwat, W., B. Fournier, I. Bassano, A. Bayoumy, C. Elgueta Karstegl, C. Styles, R. Bridges, C. Lenoir, D. BoutBoul, D. Moshous, B. Neven, T. Kanda, R. G. Morgan, R. E. White, S. Latour and P. J. Farrell (2022). "Epstein-Barr Virus Genome Deletions in Epstein-Barr Virus-Positive T/NK Cell Lymphoproliferative Diseases." J Virol **96**(12): e0039422.
Wood, C. D., H. Veenstra, S. Khasnis, A. Gunnell, H. M. Webb, C. Shannon-Lowe, S. Andrews, C. S. Osborne and M. J. West (2016). "MYC activation and BCL2L11 silencing by a tumour virus through the large-scale reconfiguration of enhancer-promoter hubs." eLife **5**: 9742.
Xu, T., S. S. Park, B. D. Giaimo, D. Hall, F. Ferrante, D. M. Ho, K. Hori, L. Anhezini, I. Ertl, M. Bartkuhn, H. Zhang, E. Milon, K. Ha, K. P. Conlon, R. Kuick, B. Govindarajoo, Y. Zhang, Y. Sun, Y. Dou, V. Basrur, K. S. Elenitoba-Johnson, A. I. Nesvizhskii, J. Ceron, C. Y. Lee, T. Borggrefe, R. A. Kovall and J. F. Rual (2017). "RBPJ/CBF1 interacts with L3MBTL3/MBT1 to promote repression of Notch signaling via histone demethylase KDM1A/LSD1." EMBO J **36**(21): 3232-3249.
Xu, T., T. Zhang, C. Xu, F. Yang, W. Zhang and C. Huang (2024). "Notch2 signaling governs activated B cells to form memory B cells." Cell Rep **43**(7): 114454.
Yi, F., A. Saha, M. Murakami, P. Kumar, J. S. Knight, Q. Cai, T. Choudhuri and E. S. Robertson (2009). "Epstein-Barr virus nuclear antigen 3C targets p53 and modulates its transcriptional and apoptotic activities." Virology **388**(2): 236-247.
Young, C. and R. Brink (2021). "The unique biology of germinal center B cells." Immunity **54**(8): 1652-1664.
Zhang, H., S. Zhao and Z. Cao (2022). "Impact of Epstein-Barr virus infection in patients with inflammatory bowel disease." Front Immunol **13**: 1001055.
Zhang, S., Y. Pei, F. Lang, K. Sun, R. K. Singh, Z. L. Lamplugh, A. Saha and E. S. Robertson (2019). "EBNA3C facilitates RASSF1A downregulation through ubiquitin-mediated degradation and promoter hypermethylation to drive B-cell proliferation." PLoS Pathog **15**(1): e1007514.
Zhao, B., J. C. Mar, S. Maruo, S. Lee, B. E. Gewurz, E. Johannsen, K. Holton, R. Rubio, K. Takada, J. Quackenbush and E. Kieff (2011). "Epstein-Barr virus nuclear antigen 3C regulated genes in lymphoblastoid cell lines." Proceedings of the National Academy of Sciences **108**(1): 337-342.
Zhao, B., D. R. Marshall and C. E. Sample (1996). "A conserved domain of the Epstein-Barr virus nuclear antigens 3A and 3C binds to a discrete domain of Jkappa." The Journal of Virology **70**(7): 4228-4236.
Zhao, B. and C. E. Sample (2000). "Epstein-barr virus nuclear antigen 3C activates the latent membrane protein 1 promoter in the presence of Epstein-Barr virus nuclear antigen 2 through sequences encompassing an spi-1/Spi-B binding site." J Virol **74**(11): 5151-5160.