RNA duplexes might explain why NPH-II effectively displaces TRAP from its 53-nucleotide-long binding site, whereas DED1, which is not a processive RNA helicase, fails to displace TRAP. For EJC displacement, processive action may not be necessary, because the EJC-binding site comprises only about 10 nucleotides and therefore both DED1 and NPH-II can effectively displace it. Alternatively, because DED1 has been shown to be present in spliceosomal complexes (*28*), displacement of the EJC by DED1 might be potentiated by proteins that co-purify with the EJC preparation but would not be present with the TRAP complex.

The different rates at which NPH-II remodels the TRAP- and the EJC-RNA complexes, and the even starker differences in the rates of TRAP and EJC remodeling observed with DED1, suggest that the properties of a given RNP might also affect the rate at which it can be remodeled by DExH/D proteins. Differences in thermal fraying of nucleotides in the respective protein-binding sites might explain (i) the different rates observed for EJC and TRAP remodeling by NPH-II, (ii) the similarity of rates observed for EJC remodeling with both DED1 and NPH-II, and (iii) the differences between the rate constants for EJC displacement and duplex unwinding. It is thus tempting to speculate that the nature of a given RNP might contribute to the specificity of DExH/D proteins in vivo. Adaptation to the different features of their target RNPs might provide DExH/D proteins with a much greater built-in biochemical specificity toward their biological substrates than was previously concluded on the basis of the largely nonspecific RNA helicase activity of these enzymes.

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31. NPH-II forms, in an ATP-dependent manner, a tight complex with the RNA from which TRAP has been displaced. This complex could not be disrupted without destruction of the TRAP-RNA complex. The NPH-II–RNA complex co-migrated with the TRAP-RNA complex under all separation conditions tested.

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Supporting Online Material

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Materials and Methods

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Identification of Virus-Encoded MicroRNAs

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RNA silencing processes are guided by small RNAs that are derived from double-stranded RNA. To probe for function of RNA silencing during infection of human cells by a DNA virus, we recorded the small RNA profile of cells infected by Epstein-Barr virus (EBV). We show that EBV expresses several microRNA (miRNA) genes. Given that miRNAs function in RNA silencing pathways either by targeting messenger RNAs for degradation or by repressing translation, we identified viral regulators of host and/or viral gene expression.

RNA silencing is part of a primitive immune system against viruses in plants (*1*) and insects (*2*). However, its role in viral infection in human cells has not been investigated. EBV is a large DNA virus of the Herpes family that preferentially infects human B cells (*3*). We cloned the small RNAs from a Burkitt's lymphoma cell line latently infected with EBV (*4*). Four percent of the cloned small RNAs originated from EBV (tables S1 and S2). Most of the EBV sequences were cloned more than once, and the analysis of the genomic sequence flanking the cloned RNAs suggested fold-back structures characteristic of miRNA genes (*5*, *6*). The EBV miRNAs originated from five different double-stranded RNA (dsRNA) precursors that are clustered in two regions of the EBV genome (Fig. 1, A and B). The EBV miRNAs were all readily detectable by Northern blotting, including the ~60-nt fold-back precursor for three of the five miRNAs (Fig. 2A). The first miRNA cluster is located within the mRNA of the *BHRF1* (Bam HI fragment H rightward open reading frame 1) gene encoding a distant Bcl-2 homolog (miR-BHRF1-1 to miR-BHRF1-3). miR-BHRF1-1 is located in the 5′ UTR (untranslated region) and miR-BHRF1-2 and -3 are positioned in the 3′ UTR of the *BHRF1* mRNA. The other EBV miRNAs cluster in intronic regions of the *BART* (Bam HI-A region rightward transcript) gene, whose function remains unknown (*7*) (miR-BART1 and miR-BART2).

EBV latently infected cells can be found in three different latent stages (I to III, Fig.

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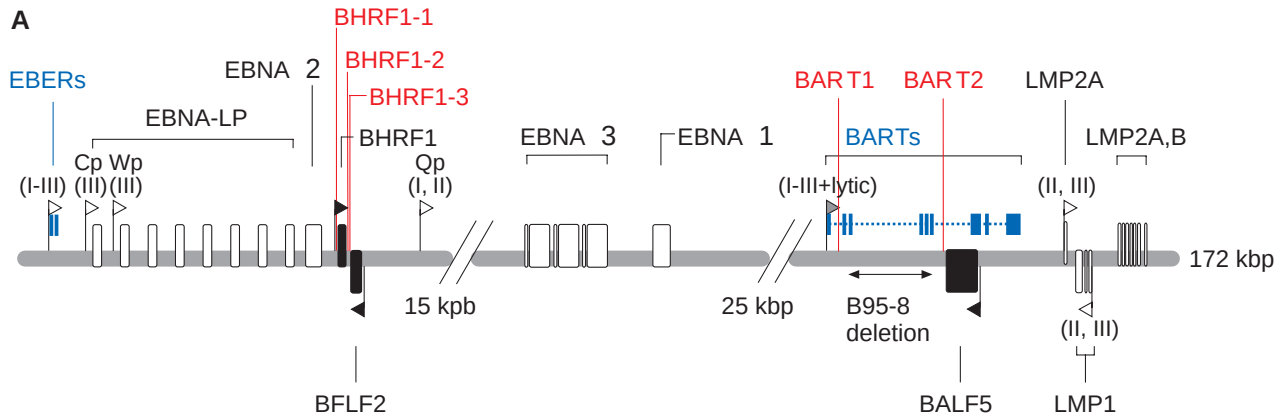
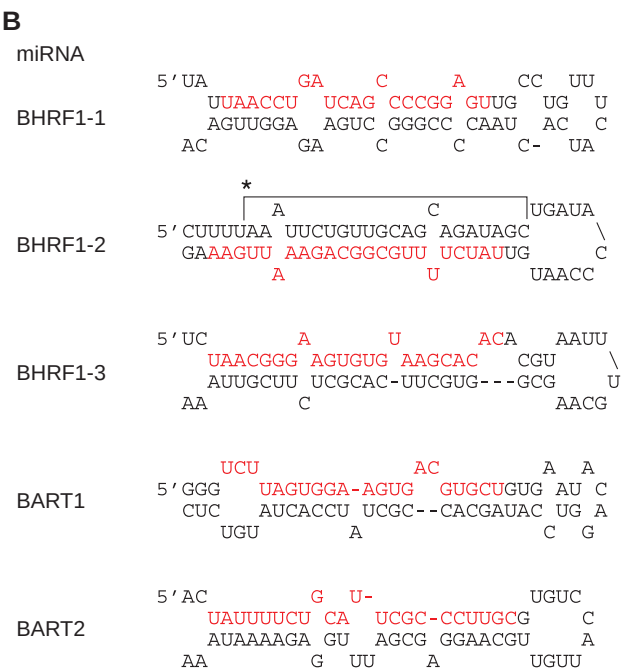
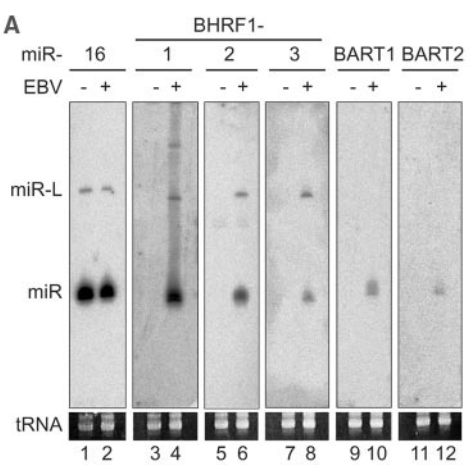


Fig. 1. Genomic positions and secondary structures of EBV miRNAs. (**A**) Diagram of the miRNA-containing segments of the EBV genome. Latent genes are indicated with white boxes, lytic genes with black boxes, previously known noncoding RNAs with blue, and newly identified miRNAs with red. Promoters active at latent stages (I, II, or III) are illustrated as white pennants, those active at lytic stage as black pennants, and those active at all stages as grey pennants. The intronic segments within the BARTs region are indicated as dashed lines, the exonic segments with bold bars. (**B**) Predicted fold-back precursors of the EBV miRNAs. The mature miRNA is highlighted in red. An asterisk is used to denote a low-abundance small RNA that was cloned from the strand opposite to miR-BHRF1-2 strand. LMP, latent membrane protein of EBV.





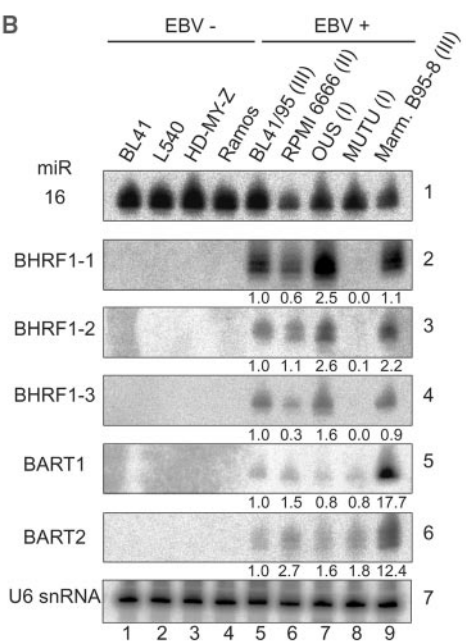


Fig. 2. Expression profile of EBV miRNAs. (**A**) Northern blots for EBV miRNAs made from total RNA isolated from uninfected BL-41 (–) and EBV-infected BL41/95 (+) cells. The expression of human miR-16 was also examined for reference. The position of migration of the mature miRNAs (miR) and its fold-back precursors (miR-L) are indicated. Equal loading of the gel before transfer to the membrane was monitored by ethidium bromide staining of the tRNA band. (**B**) Northern blots for EBV miRNAs made from total RNA isolated from various Hodgkin's and Burkitt's lymphoma cell lines. The latency stage for EBV-positive lines is indicated in parentheses. The numbers below the miR signals indicate relative signal intensity with respect to BL41/95 signals after normalizing for gel loading by using the U6 snRNA signal.

1A) that are characterized by the expression of various subsets of the latent genes. We isolated our small RNAs from a latent stage–III EBV cell line that expresses all latent genes (*8*). To find out whether the expression of the EBV miRNAs is coupled with a specific latent stage, we probed for their expression in cell lines in different stages of latency (Fig. 2B). BART miRNAs were detected in all latent stages, consistent with the reported expression of BART during every stage of EBV infection (*9*). However, BART miRNAs' expression was elevated by ~10-fold in the marmoset cell line B95-8, which contains a higher proportion of cells in the lytic stage (Fig. 2B, lane 9, rows 5 and 6). The expression pattern of BHRF1 miRNAs is dependent on the EBV latency stage. Although cell lines in stage II and III expressed BHRF1 miRNAs (Fig. 2B, lanes 5 and 6), only one of the two stage I cell lines expressed BHRF1 miRNAs (Fig. 2B, lanes 7 and 8). Latency I cell lines are thought to express only the product of the EBV latent gene EBNA1, the small EBV-encoded RNAs (EBERs), and the BARTs (*3*). A new subdivision of latency I stages may have to be introduced to distinguish between BHRF1 miRNA–expressing cell lines in latency I. Although BHRF1 is a lytic protein, latent stage EBV transcripts encompassing the BHRF1 region were observed previously (*10*, *11*). It is likely that the miRNAs BHRF1-1 to BHRF1-3 are also expressed during the lytic stage along with the BHRF1 protein, but high-level transcription of BHRF1 during the lytic cycle may exceed the cellular miRNA processing capacity, and unprocessed transcripts could then be translated.

To identify targets for EBV miRNAs, we used a computational method recently developed for prediction of *Drosophila* miRNA targets (*12*). The top scoring hits for which a gene function annotation was available are listed in tables S3 and S4. The majority of predicted host cell targets have more than one binding site for the viral miRNA, and ~50% of these are additionally targeted by one or several host cell miRNAs. Predicted viral miRNA targets include regulators of cell proliferation and apoptosis, B cell–specific chemokines and cytokines, transcriptional regulators and components of signal transduction pathways.

The miRNA miR-BART2 is capable of targeting the virally encoded DNA polymerase BALF5 for degradation (fig. S1). miR-BART2 is transcribed antisense to the BALF5 transcript and is therefore perfectly complementary to the BALF5 3′ UTR and able to subject this mRNA for degradation (*13*). Evidence for targeted mRNA cleavage of BALF5 comes from a study that identified and characterized a 3.7-kb processing product of the full-length 5.0-kb BALF5 mRNA and mapped the 3′ terminus of the shorter form precisely to our predicted miR-BART2–guided cleavage site (*14*) (fig. S1).

In conclusion, EBV, and probably other members of the herpesvirus family and large genome DNA viruses, contain miRNAs and exploit RNA silencing as a convenient method for gene regulation of host and viral genes in a nonimmunogenic manner.

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Supporting Online Material

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Materials and Methods
Fig. S1
Tables S1 to S4

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Predictive Thresholds for Plague in Kazakhstan

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In Kazakhstan and elsewhere in central Asia, the bacterium *Yersinia pestis* circulates in natural populations of gerbils, which are the source of human cases of bubonic plague. Our analysis of field data collected between 1955 and 1996 shows that plague invades, fades out, and reinvades in response to fluctuations in the abundance of its main reservoir host, the great gerbil (*Rhombomys opimus*). This is a rare empirical example of the two types of abundance thresholds for infectious disease—invasion and persistence—operating in a single wildlife population. We parameterized predictive models that should reduce the costs of plague surveillance in central Asia and thereby encourage its continuance.

Plague (*Yersinia pestis* infection) has had devastating effects on human populations in the past and has become an epithet for outbreaks of infectious disease. It remains endemic in natural populations of rodents and also a medical threat throughout central Asia, as well as parts of Africa, the United States, and South America (*1*). A critical host abundance that must be exceeded for infection to invade a susceptible population is a common outcome of epidemiological models (*2–4*) in which the rate of contacts between individuals (of the type that enables transmission) increases with abundance. For populations having previous exposure to the infection, the invasion threshold is an abundance of susceptible individuals rather than total abundance. A second abundance threshold, sometimes referred to as the critical community size (*5*), may determine whether new susceptibles are recruited at a high enough rate for the infection to persist. This is qualitatively different from the invasion threshold and is always a total population size rather than a number of susceptible hosts. Quantifying either threshold has applied benefits for the management of infectious wildlife disease,

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