Supplementary Methods

Details of the conserved stem loop prediction

A window of appropriate length $L$ is moved stepwise by an amount $L$ across the full genome of the considered virus. At each position, we determined the minimal free-energy secondary structure of the sequence segment contained inside the window using the RNAfold program of the Vienna package\(^1\), and stored the pairing pattern. The conserved stem loops are reconstructed by grouping the pairs that are present in more than 90% of the windows that contain two partner nucleotides. To avoid the influence of both boundary effects and small sequence size on the pair statistics, the window length $L$ must be much larger than a typical miRNA precursor stem loop, which is about 70 nucleotides long. Good statistics also require a sufficiently large number of distinct windows in which every nucleotide appears. To fulfill these requirements, we used two combinations of ($L$, $L$) values, namely (500, 25) and (1000, 50), and then took the intersection of the sets of predicted conserved stems. Finally, we filtered out those stems whose length $l_s$ was too short to be containing a miRNA, or whose closing hairpin loop $l_h$ was too large relative to those observed in miRNA precursors. The analysis of known miRNAs in Rfam4.0 indicates that reasonable values for these parameters $l_s$ and $l_h$ are 15 and 20, respectively.

The first part of the prediction method ends with the re-calculation of the secondary structure of the conserved stem loops.

Cell lines and viruses

The human adrenal small carcinoma cell line SW13 (ATCC CCL-105) was grown in MEM (Gibco) containing 10% heat-inactivated fetal bovine serum (FBS) (Gibco). Five 15 cm dishes at a cell density of $3 \times 10^6$ cells/plate were infected for 1h with YFV-17D in MEM/2% FBS at a multiplicity of infection (MOI) of 10. The inoculum was removed and
cells were cultured in standard growth medium for 24 h at 37 °C. The cells were then harvested and total RNA was isolated.

Huh-7.5 cells were maintained in DMEM (Gibco) supplemented with non-essential amino acids and 10% FBS. HCV-Con1 cells were maintained in the above media plus 0.5 mg/ml G418. Huh-7.5 cells are a sub-line derived from Huh-7 hepatoma cells\(^3\). HCV-Con1 is a Huh-7.5 cell population containing the full-length HCV genotype 1b replicon with the highly adaptive serine to isoleucine substitution at amino acid 2204 of the polypeptide. It was also referred to as Con1/Fl-neo(S2204)\(^7\).

HeLa cells that stably express CD4 and CXCR4 (HeLa-T4+) as well as HeLa cells that additionally produce high levels of all HIV1 viral proteins (HeLa/LAV) were obtained from Drs. R. Axel, and J. Berg and M. Wabl respectively, through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAIS, NIH\(^3\). Both cell lines were maintained in DMEM supplemented with 10% FBS. The concentration of HIV1 Gag capsid (p24) was quantified in cell lysates and culture supernatants using a commercially available HIV1 Gag p24 ELISA (Coulter). HeLa/LAV but not the parental HeLa-T4+ generated HIV1 particles as monitored by Gag p24 production.

The KSHV positive non-adherent BCBL1 cell line\(^4\) and the KSHV negative BJAB cell line\(^5\) were grown in RPMI 1640 medium (GIBCO), supplemented with 10% FCS, Na-pyruvate and 40 U/ml penicillin, 50μg/ml streptomycin sulphate.

The S11 tumor cells\(^6\) and A20 lymphoma cells (ATCC TIB-208) were maintained in RPMI 1640 medium (GIBCO) supplemented with 2 mM L-glutamine, 50 μM β-mercaptoethanol, and 10% FBS (HyClone). Cells were cultured at 1-4 million cells per milliliter, and RNA was extracted from 10\(^9\) cells as described.

Primary human foreskin fibroblasts were cultured in MEM (GIBCO) supplemented with 10% FCS, 10 U/ml moronal, and 10 μg/ml neomycin sulphate. Cells at 90%
confluency were infected with HCMV strain VR1814 at 5 PFU/cell and harvested when a strong cytopathic effect was visible, usually at about 4-5 days post-infection.

**Induction of KSHV replication**

A total of $5 \times 10^6$ BCBL1 cells were induced with 20 ng of phorbol-12-tetradecanoate-13-acetate (TPA)/ml and RNA was isolated 24, 48 and 72 h after TPA treatment.

**Northern blot analysis**

Northern blot analysis was performed as described loading 15 µg of total RNA per lane and using 5' $^{32}$P-radiolabelled oligodeoxynucleotides complementary to the miRNA sequence or to the first 20 nt of MHV68 tRNA sequences.

**Lamin A/C RNAi**

Huh-7.5 and HCV-Con1 cells were electroporated with 75 pmoles of a control siRNA (Ctr), or with siRNA or hairpin RNA (hpRNA) against lamin A (Lam) as described previously. The cells were the plated and maintained for four days prior to analysis. Protein lysates were analyzed by Western blot probed with anti-lamin A antiserum (Cell signaling) and secondary goat anti-rabbit IgG conjugated to horseradish peroxidase. The nitrocellulose was then reacted with mouse anti-actin antibody (Sigma) as a loading control. siRNA and hpRNA sequences are Ctr, 5' GGCGCUUGUGGACAUUCUGTT, 5' CAGAAUGUCCACAAGCGCCTT; Lam siRNA, 5' GAAGGAGCUGGAGAAGACUTT, 5' AGUCUUCUCCAGCUCUUCUTT, Lam hpRNA, 5' GAAGGAGCUGGAGAAGACUUUCGAGUCUUCUCCAGCUCUUCCUU
References


