Supporting Information

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SI Text

Cell Culture, Virus Infection, Cell Transfections, and Plasmid Constructions. Human HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 1% PENSTREP (Invitrogen) at 37 °C and infected with Sendai virus as described in ref. 1. For ChIP experiments, HeLa cells plated in 150-mm plates were transfected by the calcium phosphate method; the transfection mixture contained 5 μg of reporter plasmid and 20 μg of empty vector. Stable transfectants were generated by transfecting HeLa cells with 5 μg of reporter plasmid together with 1 μg of pCDNA3 by calcium phosphate; 24 h later the cells were replated and selected in the presence of G418 to select for stable cell lines. CAT and luciferase assays were performed as described in ref. 1. β-galactosidase was used as internal control in all transfection experiments.

To generate the constructs in which the IFN-β enhancer was placed at increasing distances from the core promoter, we inserted the corresponding DNA fragments taken from bacteriophage λ at the -40 position of the IFN-β promoter. The Distal and Proximal templates were constructed by inserting the IFN-β enhancer (-50 to -105) at the SmaI and BamHI sites in the pBLCAT2 vector, respectively (2). The Sp1 and C/EBP sites of the TK promoter (DistalΔSp1) were deleted by site-directed mutagenesis. The Distal p50 construct was generated by cloning an oligonucleotide containing 2 copies of a p50 homodimer-specific sequence (3) upstream of the TK core promoter in the DistalΔSp1 plasmid. To construct the Distal+Sp1, and Distal+TKcore constructs we cloned oligonucleotides bearing an Sp1 site or the TK core promoter at the AatII site (670 bp upstream of the TK promoter) of the pBLCAT2 backbone (Distal). The Distal+TKfull construct was generated by cloning a restriction fragment bearing the TK full promoter (-105 to $+51$) at the AatII site as described above. The λ templates were generated by cloning an oligonucleotide containing λ operator sites upstream of the TK promoter (Sal/XbaI) and/or next to the IFN-β enhancer (SacI/KpnI) in the Distal template. The λ cI expression plasmid was generated by cloning the cI coding sequence from pAC-λ cI.1 (4) into pCMV Myc Nuc (Invitrogen).

Chromatin Immunoprecipitation. Chromatin preparation and immunoprecipitation experiments were carried out as described in ref. 5. The products were analyzed in 5% acrylamide gel. The antibodies for p65 (sc-372), a-Sp1 (sc-59), IRF3 (sc-9082), TBP (sc-273), CBP (sc-369), GCN5 (sc-6303), and Pol II (sc-9001) were obtained from Santa Cruz, and those for anti-acetyl histone 4 (06–866) and anti-acetyl histone 3 (06–599) were obtained from Upstate Biotechnology.

Primers

RT-PCR primers:

CAT-5: 5′-ATA AAC CCT TTA GGG AAA TAG GCC AGG-3′

CAT-3: 5′-CTG GAT ATA CCA CCG TTG ATA TAC CC-3′

GAPDH 5′: 5′- TTA GCA CCC CTG GCC AAG G-3′

GAPDH 3′: 5′- CTT ACT CCT TGG AGG CCA TG-3′

ChIP primers:

Distal primers:

VIEN TK 3′: 5′-GTG GTT TGT CCA AAC TCA TCA ATG TAT CTT-3′

VIEN TK 5′: 5′-CTC ACT CAT TAG GCA CCC CAG GCT TTA CAC-3′

Proximal primers:

TK CORE 5′: 5′-AGC TTC CTT AGC TCC TGA AAA TCT CGC CAA-3′

TK CORE: 3′: 5′-CGA CGG CCA GTG CCA AGC TTG CAT GCC TGC-3′

Intervening primers:

VIENCATacet-5: CTGCTCCCATTCATCAGTTCCATAG-GTTGG

VIENCATacet-3: CAGTTATTGGTGCCCTTAAACGCC-TGGTGCT

TKVIENacet-5: GGCAACAACGTTGCGCAAACTATTA-ACTGG

TKVIENacet-3: AGGCACCTATCTCAGCGATCTGTCT-ATTTC

Aat II SENSE: 5′-GAG ACG AAA GGG CCT CGT GAT ACG-3′

Aat II REV: 5′-TCA GGG TTA TTG TCT CAT GAG CGG ATA C-3′

Intervening primers encompassing the SP1 binding site at Distal+SP1 construct:

Aat II REV: 5′-TCA GGG TTA TTG TCT CAT GAG CG-G ATA C-3′

A2a: 5′-CAG CGT CTT GTC ATT GGC GAA T-3′

Intervening primers encompassing the TK core promoter at Distal+TK core promoter construct:

Aat II REV: 5′-TCA GGG TTA TTG TCT CAT GAG CG-G ATA C-3′

CORE FW primer: 5′-GAG GTC CAC TTC GCA TAT TA-A GGT G-3′

3C primers:

Direct PCR primers for enhancer/promoter circular intramolecular ligation product (245-bp product):

E1 :5′-ATT CCT CTG AAT AGA GAG AGG-3′

P3: 5′-AAT GTA CCT ATA ACC AGA CCG-3′

Inverse PCR primers for enhancer/SP1 circular intramolecular ligation product (295-bp product):

E1 :5′-ATT CCT CTG AAT AGA GAG AGG-3′

E2: 5′-ACT TTC ACT TCT CCC TTT CAG-3′

Oligos

p50 homo sense:

5′-GACCGGTGACGGGGAGGCCCCCATATCGAAGC-GGGGAGGCCCCCATAT CGAAGCT-3′

p50 homo antisense:

5′-CTAGACGTTCGATATGGGGGCCTCCCCGCTTCG-ATATGGGGGCCTCCCCG TCACCG-3′

VIEN Kpn/Sac operator (distant) sense:

5′-CTATCACCGCCAGTGGTAATTCCCAATTCCACAT-GCAACCATTATCACCG CCGGTGATAGAGCT-3′

VIEN Kpn/Sac operator (distant) antisense:

5′-CTATCACCGGCGGTGATAATGGTTGCATGTGGA-ATTGGGAATTAC CACTGGCGGTGATAGGTAC-3′

TK core Xba/Sal operator (local) sense:

5′-CTAGATATCACCGCCAGTGGTAATTCCCAATTC-CACATGCAA CCATTATCACCGCCGGTGATAG-3′

TK core Xba/Sal operator (local) antisense:

5′-TCGACTATCACCGGCGGTGATAATGGTTGCATG-TGGAATTGGGAAT TACCACTGGCGGTGATAT-3′

Aat II Sp1 sense:

5′-CTCTAGAGGATCCGGCCCCGCCCAGCGTCTTGT-CATTG GCGAATTCGAACACGCAGATGCAGTCGGGG-CGGCGCGGACGT-3′

Aat II Sp1 antisense:

5′-CCGCGCCGCCCCGACTGCATCTGCGTGTTCGAA-

TTCGCCAA TGACAAGACGCTGGGCGGGGCCGGATC-CTCTAGAGACGT-3′

Aat II core sense:

5′-CCCGAGGTCCACTTCGCATATTAAGGTGACGCG-TGTGGCCTCGAACACCG AGGACGT-3′

Aat II core antisense:

5′-CCTCGGTGTTCGAGGCCACACGCGTCACCTTAA-TATGCGAAGTGGA CTCGGGACGT-3′

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2. Luckow B, Schutz G (1987) CAT constructions with multiple unique restriction sites for the functional analysis of eukaryotic promoters and regulatory elements. *Nucleic Acids Res* 15:5490.
3. Kunsch C, Ruben SM, Rosen CA (1992) Selection of optimal kappa B/Rel DNA-binding motifs: Interaction of both subunits of NF-κ B with DNA is required for transcriptional activation. *Mol Cell Biol* 12:4412–4421.
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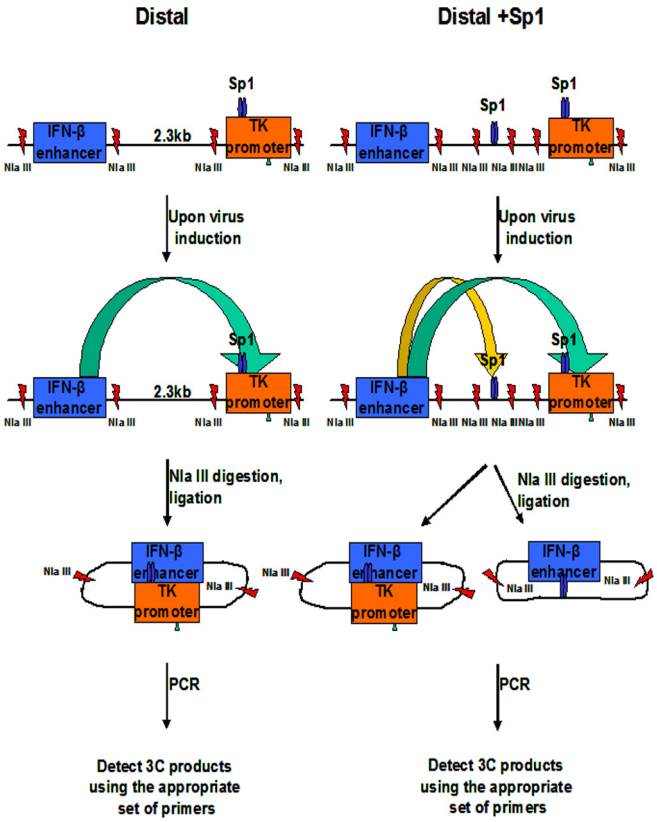

Fig. S1. Steps followed in the 3C experiments shown in Figs. 2 and 5. The Distal and Distal+Sp1 constructs are shown on the top depicting the position of the IFN-b enhancer and TK promoter (blue and orange rectangles, respectively). The NIaIII sites are shown as red arrowheads. The green and yellow arrows indicate the interactions between the IFN-b enhancer and the TK promoter or the IFN-b enhancer and the Sp1 site, respectively, as detected by the 3C method.