Supporting Information

Determination of the vRNA and cRNA promoter activity by M segment-specific non-coding nucleotides of influenza A virus

by H. Shin *et al*.

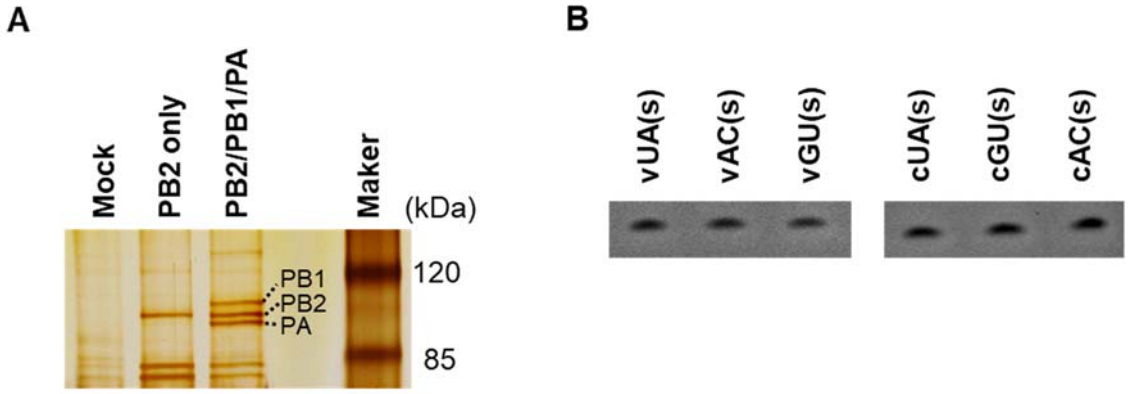

Figure S1. Characterization of purified influenza viral polymerase complex and the synthetic RNA promoters. (A) Silver staining of the purified viral proteins. *In vitro* assembled trimeric PB2-CBP/PB1/PA complex was purified using IgG Sepharose after co-transfection of 293T cells with three plasmids expressing PB2-TAP, PB1 and PA. Mock, no transfected; PB2 only, transfected with the PB2-TAP-expressing plasmid alone; PB2/PB1/PA, transfected with the three different plasmids. Marker sizes are indicated in kDa. (B) The synthetic RNA promoters were electrophoresized on denaturing urea PAGE (20%) and detected by staining with fluorescent nucleic acid dye, GelRed™ (Biotium).

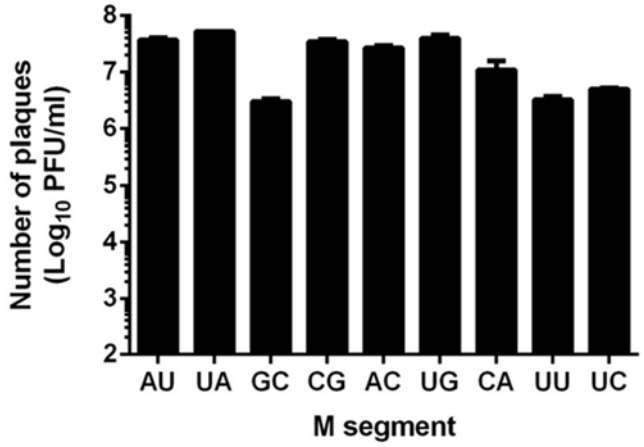

Figure S2. Plaque titration of the recombinant viruses of passage 2 (P2). Recombinant viruses of P1 were infected into MDCK cells for 2 days at an MOI of 10^{-4} [exceptionally 5×10^{-6} for M(UG)]. The number of infectious viral particles (P2) in culture supernatants was quantified by plaque assay and expressed as means ± SEM ($\log_{10}$ PFU/ml) from an experiment in triplicate.

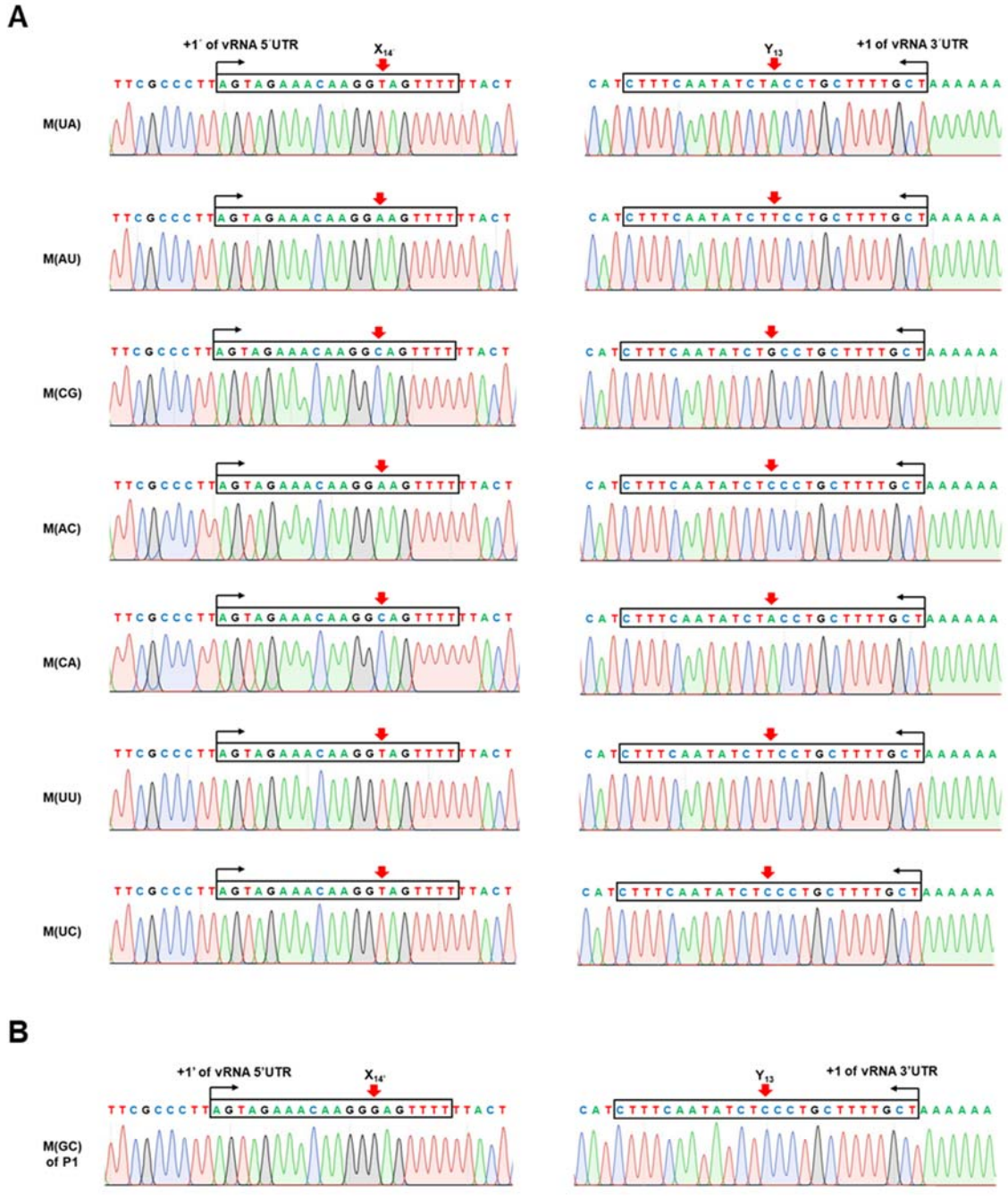

Figure S3. Determination of 5′ and 3′ UTR sequences of the wild-type [M(UA)] and mutant M segments. Terminal nucleotides were identified by 5′ (left) and 3′ (right) RACE with vRNAs of P2 (A) or P1 (B) viruses. Noncoding regions of the M segments are marked in boxes and terminal ends with black arrows. Thick red arrows point the target sequences, X14′ and Y13, of the vRNAs. Recombinant viruses are designated at the left side of chromatograms.

Supplementary Tables

Table S1. Primers for cloning the reporter plasmids

Oligonucleotide	Sequence (5′ to 3′)[a]	Use
vRNA(EGFP) (fwd)	ATAT*CGTCTC*GTATT**AGTAGAAACAAGGTAGTTTTTT** **A**ACTTGTACAGCTCGTCCATGC	Amplification of vRNA(EGFP)[b]
vRNA(EGFP) (rev)	TATT*CGTCTC*AGGG**AGCAAAAGCAGGTAGATATTGA** **AAG**ATGGTGAGCAAGGGCGAGGA	
cRNA(EGFP) (fwd)	AT*CGTCTC*GTATT**AGCAAAAGCAGGTAGATATTG**	Amplification of cRNA(EGFP)[c]
cRNA(EGFP) (rev)	TA*CGTCTC*AGGG**AGTAGAAACAAGGTAGTTTT**	

[a]*Bsm*BI sites in italic; M segment-derived 5′ and 3′ UTRs in bold; start and stop codons in red; EGFP coding sequences underlined.

[b]For PCR amplification of vRNA(EGFP) or vUA, pEGFPLuc (BD Biosciences) was used as a template.

[c]For PCR amplification of cRNA(EGFP) or cUA, pHH21-EGFP-vM was used as a template.

Table S2. Oligonucleotides designed for site-directed mutagenesis

Oligonucleotide	Sequence (5′ to 3′)[a]	Use
A_{14}·(fwd)	AGTAGAAACAAGG**A**AGTTTTTTA	Mutation of U to A at nt 14′ of 5′ UTR of the M vRNA or A to U at nt 14 of 3′ UTR of the M cRNA
A_{14}·(rev)	TAAAAAACT**T**CCTTGTTTCTACT	
G_{14}·(fwd)	AGTAGAAACAAGG**G**AGTTTTTTA	Mutation of U to G at nt 14′ of 5′ UTR of the M vRNA or A to C at nt 14 of 3′ UTR of the M cRNA
G_{14}·(rev)	TAAAAAACT**C**CCTTGTTTCTACT	
C_{14}·(fwd)	AGTAGAAACAAGG**C**AGTTTTTTA	Mutation of U to C at nt 14′ of 5′ UTR of the M vRNA or A to G at nt 14 of 3′ UTR of the M cRNA
C_{14}·(rev)	TAAAAAACT**G**CCTTGTTTCTACT	
U_{13}(fwd)	CATCTTTCAATATCT**T**CCTGCTTTTGCT	Mutation of A to U at nt 13 of 3′ UTR of the M vRNA or U to A at nt 13′ of 5′ UTR of the M cRNA
U_{13}(rev)	AGCAAAAGCAGG**A**AGATATTGAAAGATG	
G_{13}(fwd)	CATCTTTCAATATCT**G**CCTGCTTTTGCT	Mutation of A to G at nt 13 of 3′ UTR of the M vRNA or U to C at nt 13′ of 5′ UTR of the M cRNA
G_{13}(rev)	AGCAAAAGCAGG**C**AGATATTGAAAGATG	
C_{13}(fwd)	CATCTTTCAATATCT**C**CCTGCTTTTGCT	Mutation of A to C at nt 13 of 3′ UTR of the M vRNA or U to G at nt 13′ of 5′ UTR of the M cRNA
C_{13}(rev)	AGCAAAAGCAGG**G**AGATATTGAAAGATG	

[a]Mutation sites are highlighted in bold.

Table S3. Oligonucleotides for 5′ and 3′ RACE of the M segment

Oligonucleotide	Sequence (5′ to 3′)	Use
TSO	ACACTCTTTCCCTACACGACGCTCTTCCGA TCTrGrGrG	Template switching oligonucleotide for 5′ RACE
M5RACE-out(fwd)	CCGTCAGGCCCCCT	cDNA synthesis and nested PCR of M segment cDNA (outer primer set) for 5′ RACE
TSO-out	AATGATACGGCGACCACCGAGATCTACACTCTTT CCCTACACGACGCTCTTCCGATCT	
M5RACE-in(fwd)	AGTAGAAACAAGG	Nested PCR of M segment cDNA (inner primer set) for 5′ RACE
M5RACE-in(rev)	CAAGCAGCAGAGGCCATGGA	
M3RACE dT	GTGGTATCAACGCAGAGTTTTTTTTTTTTTTTT	cDNA synthesis for 3′ RACE
M3RACE-out(fwd)	GACCAGCACTGGAGCTAGGA	Nested PCR of M segment cDNA (outer primer) for 3′ RACE
M3RACE(rev)	GTGGTATCAACGCAGAG	Nested PCR of M segment cDNA for 3′ RACE
M3RACE-in(fwd)	TTCCTATACAGTTTAACTGC	Nested PCR of M segment cDNA (inner primer) for 3′ RACE