Rig-I regulates NF-κB activity through binding to *Nf-κb1* 3′-UTR mRNA

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Retinoic acid inducible gene I (RIG-I) senses viral RNAs and triggers innate antiviral responses through induction of type I IFNs and inflammatory cytokines. However, whether RIG-I interacts with host cellular RNA remains undetermined. Here we report that Rig-I interacts with multiple cellular mRNAs, especially *Nf-κb1*. Rig-I is required for NF-κB activity via regulating Nf-κb1 expression at posttranscriptional levels. It interacts with the multiple binding sites within 3′-UTR of *Nf-κb1* mRNA. Further analyses reveal that three distinct tandem motifs enriched in the 3′-UTR fragments can be recognized by Rig-I. The 3′-UTR binding with Rig-I plays a critical role in normal translation of Nf-κb1 by recruiting the ribosomal proteins [ribosomal protein L13 (Rpl13) and Rpl8] and rRNAs (18S and 28S). Down-regulation of Rig-I or Rpl13 significantly reduces Nf-κb1 and 3′-UTR–mediated luciferase expression levels. These findings indicate that Rig-I functions as a positive regulator for NF-κB signaling and is involved in multiple biological processes in addition to host antivirus immunity.

R in i i in i I n I (I -I) h n n- r I r i I r I in ini i in inn n i ir I i n r n nin ir I N , r r i in ifi r r in, I N-β r r i I r 1 (I -1; I n , I , r r i), n i in n r I N r - I r r 3 (I 3) n N -κ in lin (1–5). In r n r, h r h n in n r n in h I -I i- n in ir I N r h I I r N . n r i h i nifi h i r n n n I N i n I r ni I -I, in I in h r I in ini I i i i (I I:), 5′- i h h N , I n N n h n h n I r r r h 5′ n 3′ n n i - n N n (1, 6–12). r, N r h I n h n i I -I i n in in 5′- i h h - N - in n n r (13). r n I , h r I r r n I I -I in i h r i n - r r N - n I i h r r I i r h in r i n I -I i h n n I N i n , n h n r i n I h n n i I n r r N in in n i n I r n i n (14–17). h i h r I n r n r n in h h n- in h in r i n n I -I n i N i n . r, h h r I -I in h I I r N n h h i I i I n i n i h in in r in n in . In , I -I ri in I i nifi in in in h r I i I i I in N 4 in I-*trans* r i- n i i ( )-in I i r n i i n, i I in h I -I n i n n i r n i r in -in I i r ni i n (18). r r I n I i n in ir i n r h I -I i / - / N h i (19). I i I n n h r h / - i h i r n i n in r I in n r i n n I I r r - (20, 21). I i n I , I -I h r hi h n i- I r i i n i r *Caenorhabditis elegans* I . I i i i I r in h n n n in I N, in i in-1, n N ir in i I . I -I i in I in h in i n infl r r

n h in n infl r i i (22). h r - i I r r h i -I– fi i n I I i - i h n i ir r in ni I h -i2 (αi2) r i n n - I i i n (23), r r i r n- I i i h r i n in r r n n n n in in r in (I) r i n n 1 i i n (24, 25), n in r i i i in i n i h *Escherichia coli* i ir -in h i i (26). h r r , I -I r I i I in I in I i I i I i I r in I in r ni i n n n I ir I N .

Results

Rig-I Interacts with Multiple Cellular mRNAs. i ni h - n i I in r i n i -I i h h I I r N , r- r N i n r i i i n (I) in n ni- i ni r h I I I h rin I ni - I I in (1. 4. 6), hi h I r i - r i - i -I. I N r r h I i - r i i - ifi in r I I I (i . 1*A*), n n I in n i I n- 014868 h I n i r rr . In rin N n n n i - n i - i -I r i i n h h i n I in n i i 298 r r h n - r I h in n r I (I r i >2). ri in I , h i h hi h I r i (5.9) i nifi *Nf-κb1/p105* n I r r r r r in 105, hi h n r - i r - in n r h r N -κ 1 ni 50 (i . 1*B* n I 1). r rin n, r h N i h I r i >2, in I in *Nf-κb1/p105*, *Inhibitor of kappaB kinase epsilon* (*Ikbke*), *Irf3*, *Rous sarcoma oncogene* (*Src*), *kinesin family member 24* (*Kif24*), *etc.*, r ifi - in h r i i (i . 1*C*) n in ni– i -I r i i r h I I - n r h *Rig-I*$^{-/-}$ rin r ni fi r I ( ) (i . 1*D*). r h r r , h in r i n i -I i h *Nf-κb1* nfir in ni– i -I r i i r h in i r r i n ifi r in n i -I r in i h I N r r I n (i . 1*A*). i n in r h N -κ – i in i n in (27, 28). I h i I N -κ i i n n h in r i n, h I in i . 1*C* r r i h , I , n n - h h N -κ i i n h n n h in r i n i -I i h *Nf-κb1* N (i . 1*B*). h h i -I in r i h i I i I h I I r N , i I *Nf-κb1/p105.* h r N I I

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The authors declare no conflict of interest.

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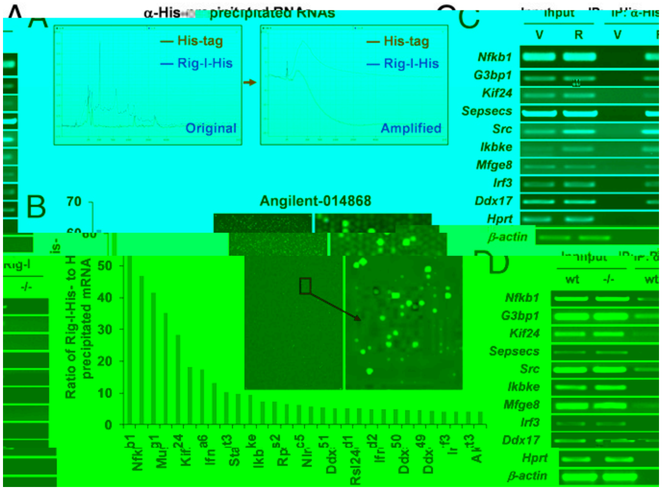

Fig. 1. Multiple cellular RNAs are immunoprecipitated with Rig-I. (*A*) Quantitative or qualitative evaluation for the total RNA extracted from the pooled anti-His precipitates from total cell lysates of 1.B4.B6 cell line stably expressing His-tag or His-tagged Rig-I. The graphs shown are of RNA abundance against molecular size before or after two-round amplification. (*B*) Equal amounts of labeled cRNA samples were analyzed by using Agilent 44k Whole Mouse Genome Microarray (Agilent; 014868). Some of the positive mRNAs were shown in the histogram. The indicated mRNAs were further detected by RT-PCR in either anti-His precipitates from the cell lysates of the 1.B4.B6 cell line stably expressing His-tagged Rig-I (*C*) or anti–Rig-I precipitates from the cell lysates of WT but not from that of *Rig-I*$^{-/-}$ MEFs (*D*). *X-box binding protein 1 (Xbp1)*, *hypoxanthine-guanine phosphoribosyltransferase (Hprt)*, and *β-actin* are shown as negative controls.

… *Interferon alpha-6 precursor* (*Ifna6*), *Interferon gamma receptor 2* (*Ifngr2*), *Interferon alpha B* (*Ifnab*), *NLR family, CARD domain containing 5* (*Nlrc5*), *Src*, *Stat3* … (29). …

Rig-I Interacts with *Nf-κb1* 3′-UTR mRNA and Positively Regulates Nf-κb1 Expression. … Rig-I … *Nf-κb1* … 3′ … (3′- …) … 5′ … *p105* … *p105-* … (Fig. 2*A*). … 5′ 7- … (5′ … 7 …) … 800 … (Fig. 2*A*). … NI … 3 3 … *p105* 3′- … 3 … (*Luc-p105* 3′- …) … *Luc-p105* 3′- … *p105* … *p105-* … (Fig. 2*B*). … Rig-I … 3′- … *p105* … 3 … 40 (… 40) … *Luc-p105* 3′- … 3- … *Rig-I*$^{-/-}$ … *Luc-p105* 3′- …

… in *Rig-I*$^{-/-}$ … (Fig. 2*C*). … *Rig-I*$^{-/-}$ … I– … 3 … *Luc-p105* 3′- … *Luc-* 105 3′- … 3 … (Fig. 2*D*). … *p105* 3′- … Rig-I in … NI … 3 3 … (Fig. 2*B*). … Rig-I … N-κ 1 … 3′- … Rig-I … *p105* 3′- … *p105* 3′- … Fig. 2*C* … 3, … Rig-I … N … *p105* 3′- … Fig. 2*D* … *d* … Rig-I … *a* … *c*. … *p105* 3′- …

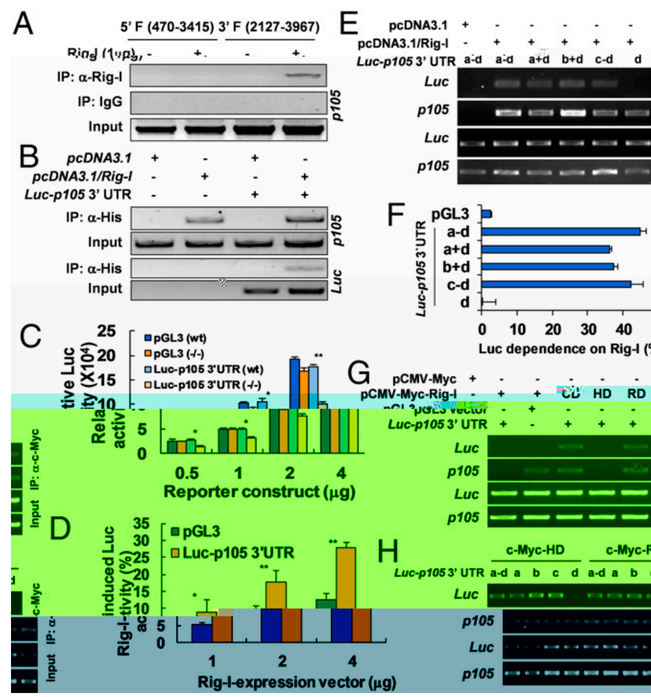

Fig. 2. Rig-I interacts with *Nf-κb1* 3′-UTR mRNA and positively regulates Nf-κb1 expression. (*A*) *Nf-κb1/p105* mRNA fragments as indicated in IgG or anti–Rig-I precipitates from the reactions of Rig-I protein with 5′ or 3′ mRNA fragments are detected by RT-PCR using the primers located within overlapping regions between two fragments. (f esTm(,)TJETETBT/F11Tf1(1h55me)(nt)1(s)-(()TJ

Rig-I Can Recognize Three Distinct Tandem Motifs Enriched in *Nf-κb1* 3′-UTR mRNA.

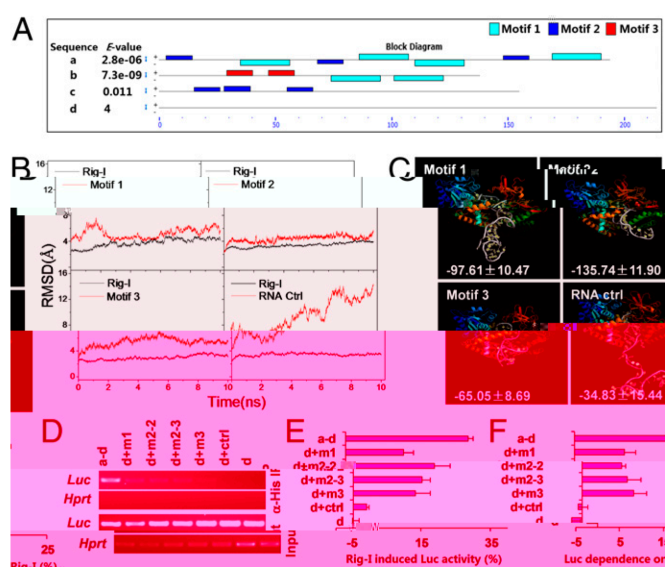

Fig. 3. Structural features of *p105* 3′-UTR mRNA for Rig-I binding. (*A*) The distribution of the three enriched motifs in *p105* 3′-UTR mRNA fragments. (*B*) The C5′ and Cα atom rmsds for dual-tandem motifs or control RNA and Rig-I protein are shown. (*C*) The binding model between Rig-I and dual-tandem motifs or control RNA. The binding-free energy data are labeled in each

Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase (Sepsecs)

Rig-I Regulates Nf-κb1 Translation via Interacting with Ribosomal Components.

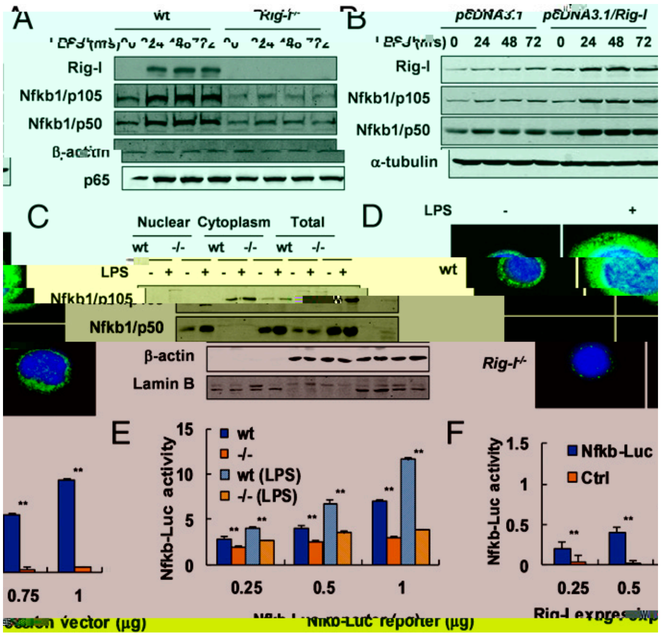

Fig. 4. Rig-I is a positive regulator for NF-κB activity. Western blots for the indicated proteins in splenocytes (*A*) and the 1.B4.B6 cell line (*B*) stably expressing Rig-I. (*C*) Immunoblots of Nf-κb1/p105/p50 in total cell lysate, cytoplasmic and nuclear fractions from splenocytes. (*D*) Confocal images for Nf-κb1/p105/p50 (green) distribution in splenic B lymohocytes with an original magnification of 400×. (

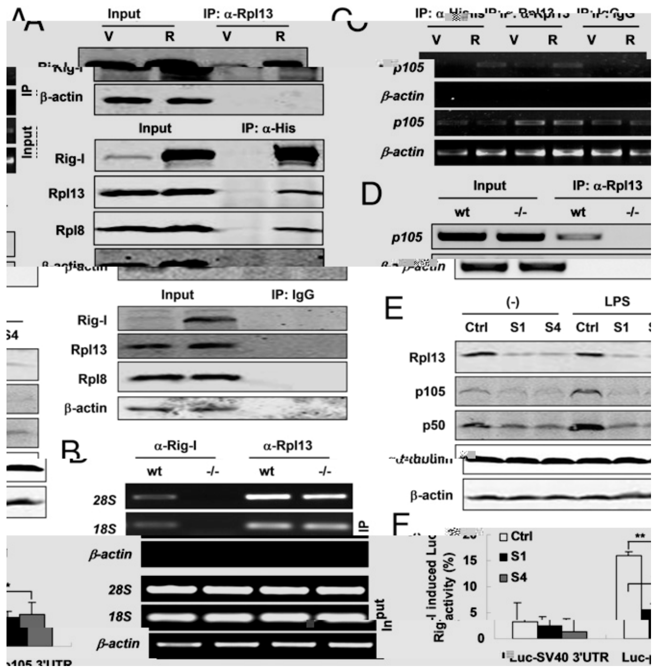

Fig. 5. Rig-I regulates Nf-κb1 translation via interacting with ribosomal components. (*A*) Immunoblots for the indicated proteins in IgG, anti-His, and anti-Rpl13 precipitates from the lysates of 1.B4.B6 cell line stably transfected with empty vector (V) or His-tagged Rig-I expression vector (R). (*B*) RT-PCR for 28S, 18S rRNAs in anti-Rig-I, or anti-Rpl13 precipitates from the lysates of WT or *Rig-I*$^{-/-}$ MEFs. (*C*) *p105* mRNA by RT-PCR in the precipitates under the same experimental settings as in *A*. (*D*) RT-PCR for *p105* mRNA in anti-Rpl13 precipitates as in *B*. (*E*) Western blots for the indicated proteins in NIH 3T3 cells stably expressing *Rpl13* siRNA (S1 and S4) and control siRNA (Ctrl). (*F*) Luc activity in the same cell lines as in *E* but cotransfected with Rig-I expression and *Luc*-reporter constructs with *p105* or SV40 3′-UTR is expressed as a percentage relative to that of Rig-I empty vector. One of three independent experiments is shown as mean ± SD ($n = 3$, $*P < 0.05$; $**P < 0.01$).

Discussion

in r ihiniiin r I 5 (. . i . n . . / - i /), ri - n n h i i l - n iniiin r r h 40 ri l . . ni in 48 iniiin l n ʲ inin 40 n 60 . . ni (39). h , r h I -I rii in h r ri l , N ir l ri i n, r ri r lin . In r hi n i n, 18 n 28 r N , h n n 40 n 60 ri l . . ni , r h n in r i h i -I. r h r i- n r r *Rpl13* il n in ri n . h h h N -κ 1/ 105 r in l . l i nifi n l r i h h i n-r l i n l13. n i n i h hi fin in , *p105* 3′- . – i l i r ii . i r . inNI. 3 3 ll l r in *Rpl13* i N , n in, n r in r i l r l h 3′- in r l in N -κ 1/ 105 r n l i n.

In n l i n, r n r h i -I n i n ii r l r r N -κ i n lin . h r l r i -I i n n n h in r i n i h h l i l i i hin 3′- *Nf-κb1* N hr hi /h li in n - r in l r l r in. h l h li r r r i h h n i n ri h i hin *p105* 3′- i r i l r i -I . in in n r in r n l i n. In i i n, i -I i r . in l in ri - . l , N ir l ri i n, r ri r lin . r r i in

ri l n n hil . in in *p105* 3′- N . i n h i r n r l i -I in h n i ir r n , r l in N -κ ii . , n r in r n l i n, ll h l in r i n n r , hi h h r in hi . , . r h r in n i i n hi " lin " l l r n in . r . I i . li h h r ill r l . n fi i l r . r n r n in . i l i l r n r l n r r h.

Materials and Methods

All of the experiments were processed according to the standard protocols. The detailed materials and methods are provided as *SI Materials and Methods*. Primers used in this study are listed in Table S3.

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