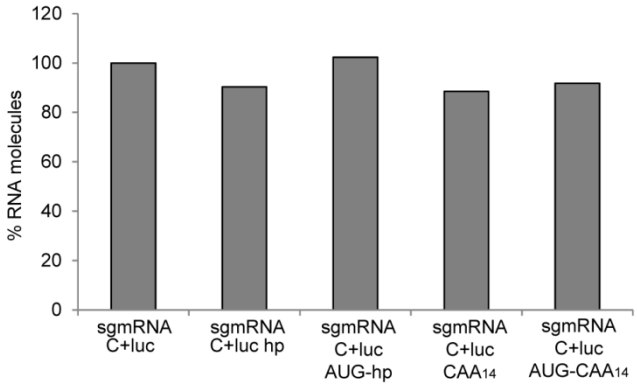

Figure S1. Analysis of SINV sgmRNA C+luc levels by qRT-PCR. BHK cells were transfected with Lipofectamine 2000 and *in vitro* prepared SINV sgmRNAs. Total RNA was isolated at 2 hpt and used as template to quantify the amount of SINV sgmRNA C+Luc by qRT-PCR as described in *Materials and Methods*. The relative RNA levels of SINV sgmRNA C+luc variants are represented with respect to SINV sgmRNA C+luc wt.

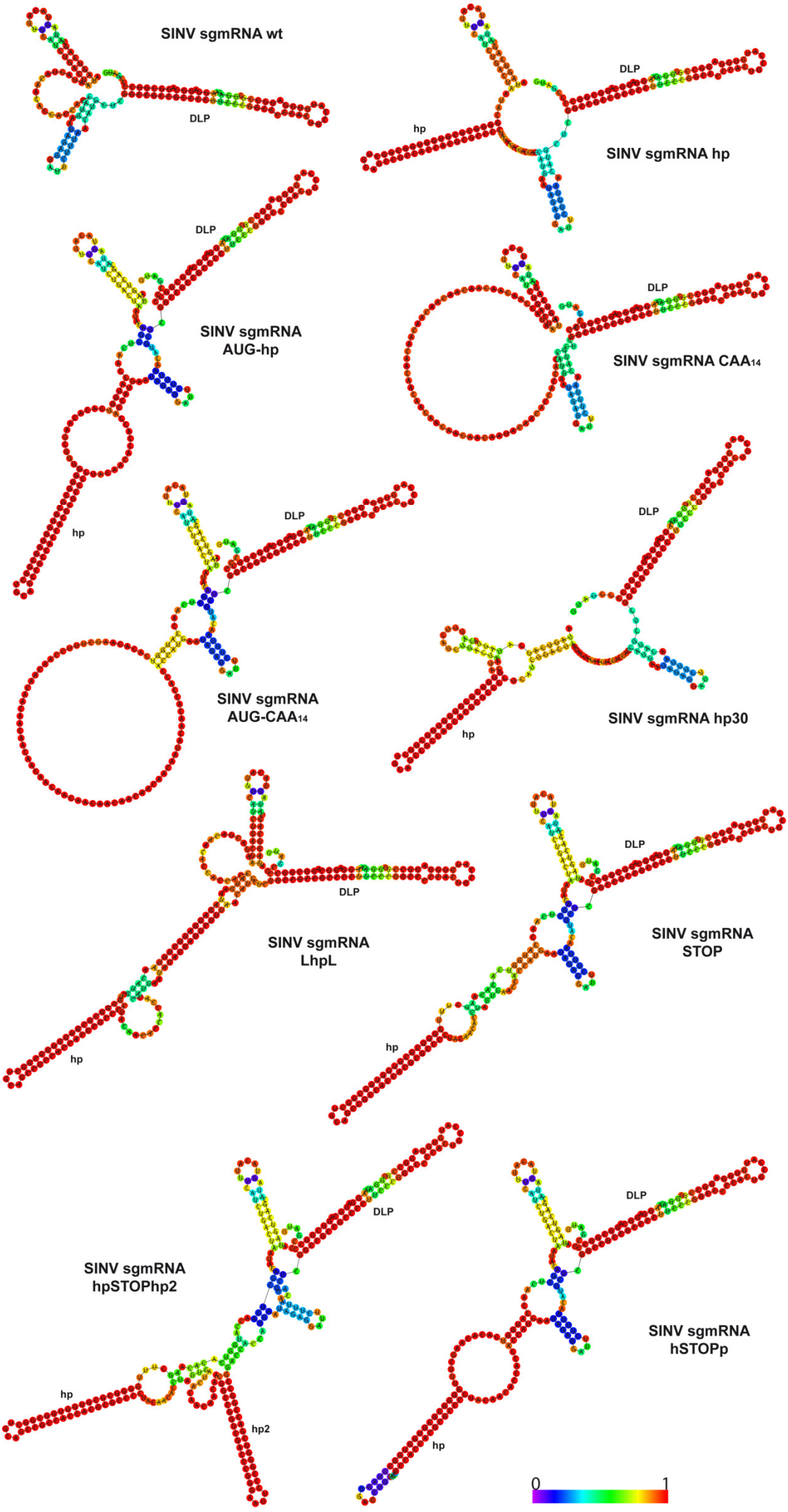
SINV sgmRNA wt
DLP
SINV sgmRNA hp
hp
DLP
SINV sgmRNA AUG-hp
DLP
SINV sgmRNA CAA14
DLP
hp
SINV sgmRNA AUG-CAA14
DLP
SINV sgmRNA hp30
DLP
hp
SINV sgmRNA LhpL
DLP
hp
SINV sgmRNA STOP
DLP
hp
SINV sgmRNA hpSTOPhp2
DLP
hp
hp2
SINV sgmRNA hSTOPp
DLP
hp
0
1

Figure S2. Base-pairing probabilities of the 5' end of different SINV sgmRNAs. Minimum free energy secondary structure was generated using the RNAfold program for each of the SINV sgmRNA leader sequences described in this work. The predicted structures are colored by base-pairing probabilities. Unpaired bases are colored according to the probability of being unpaired.

Table S1. Nomenclature of plasmids, replicons, sgmRNAs and mRNAs.

Plasmid	replicon/gmRNA	sgmRNA	Reference	Product obtained by *in vitro* transcription
pT7 rep C+luc	rep C+luc	C+luc	(1)	SINV replicon bearing the C protein followed by the luc gene.
pT7 rep C+luc hp	rep C+luc hp	C+luc hp		SINV replicon bearing the C protein followed by the luc gene. Contains a hairpin structure inside the L26S.
pT7 rep C+luc AUG-hp	rep C+luc AUG-hp	C+luc AUG-hp		SINV replicon bearing the C protein followed by the luc gene. Contains an AUG sequence in good context for translation before a hairpin structure inserted inside the L26S.
pT7 rep C+luc CAA_{14}	rep C+luc CAA_{14}	C+luc CAA_{14}		SINV replicon bearing the C protein followed by the luc gene. Contains an unstructured insert inside the L26S.
pT7 rep C+luc AUG-CAA_{14}	rep C+luc AUG-CAA_{14}	C+luc AUG-CAA_{14}		SINV replicon bearing the C protein followed by the luc gene. Contains an AUG sequence in good context for translation before an unstructured sequence inserted inside the L26S.
pT7 rep C+luc LhpL	rep C+luc LhpL	C+luc LhpL		SINV replicon bearing the C protein followed by the luc gene. Contains two similar sequences corresponding to nucleotides 35-75 of wt L26S (including the AUG start codon), before and after a hairpin structure inserted inside the L26S.
pT7 rep C+luc STOP	rep C+luc STOP	C+luc STOP		SINV replicon bearing the C protein followed by the luc gene. Contains an AUG sequence in good context for translation before a hairpin structure inserted inside the L26S, and two in-frame stop codons downstream of this hairpin.
pT7 rep C+luc hpSTOPhp2	rep C+luc hpSTOPhp2	C+luc hpSTOPhp2		SINV replicon bearing the C protein followed by the luc gene. Contains an AUG sequence in good context for translation before a hairpin structure inserted inside the L26S, two in-frame stop codons downstream of this hairpin, and a second hairpin structure following both stop codons.
pT7 rep C+luc hSTOPp	rep C+luc hSTOPp	C+luc hSTOPp		SINV replicon bearing the C protein followed by the luc gene. Contains inside the L26S an AUG sequence in good context for translation before a hairpin structure that contains two in-frame stop codons.
pT7 rep C+luc hp30	rep C+luc hp30	C+luc hp30		SINV replicon bearing the C protein followed by the luc gene. Contains a hairpin structure inside the L26S, separated 30nts from the initiator AUG.
pT7 C+luc		C+luc	(1)	SINV sgmRNA bearing the C protein followed by the luc gene. This mRNA is unable to replicate.
pT7 C+luc hp		C+luc hp		SINV sgmRNA bearing the C protein followed by the luc gene. Contains a hairpin structure inside the L26S. This mRNA is unable to replicate.

pT7 C+luc AUG-hp		C+luc AUG-hp		SINV sgmRNA bearing the C protein followed by the luc gene. Contains an AUG sequence in good context for translation before a hairpin structure inserted inside the L26S. This mRNA is unable to replicate.
pT7 C+luc CAA_{14}		C+luc CAA_{14}		SINV sgmRNA bearing the C protein followed by the luc gene. Contains an unstructured insert inside the L26S. This mRNA is unable to replicate.
pT7 C+luc AUG-CAA_{14}		C+luc AUG-CAA_{14}		SINV sgmRNA bearing the C protein followed by the luc gene. Contains an AUG sequence in good context for translation before an unstructured sequence inserted inside the L26S. This mRNA is unable to replicate.
pT7 SVwt	wt	wt	(2)	SINV genome containing all the replicative and structural proteins.
pT7 SV AUG-CAA14	AUG-CAA_{14}	AUG-CAA_{14}		SINV genome containing all the replicative and structural proteins. Includes an AUG sequence in good context for translation before an unstructured sequence inserted inside the L26S.
Abbreviations: *gmRNA,* genomic mRNA; *sgmRNA,* subgenomic mRNA; *SINV,* Sindbis Virus; *nsPs,* non-structural proteins; *L26S,* leader sequence of SINV sgmRNA; *luc,* luciferase; s*Ps,* structural proteins; *C,* capsid protein.				

References

1. **Sanz MA, Castello A, Carrasco L.** 2007. Viral translation is coupled to transcription in Sindbis virus-infected cells. J Virol **81:**7061-7068.
2. **Sanz MA, Carrasco L.** 2001. Sindbis virus variant with a deletion in the 6K gene shows defects in glycoprotein processing and trafficking: lack of complementation by a wild-type 6K gene in trans. J Virol **75:**7778-7784.

Table S2. List of primers used in this work.

Mutant	Primer name	5'- 3' sequence
hp	5'hp	CATCTGACTAATACGGGGCGCGTGGTGGCGGCTGCAGCCGCC ACCACGCGCCCCTACAACACCACCACCATGAATAG
	3'hp	GTGGTGGTGTTGTAGGGGCGCGTGGTGGCGGCTGCAGCCGCC ACCACGCGCCCCGTATTAGTCAGATGAAATG
AUG-hp	5'AUG-hp	CATCTGACTAATACAGCTCAACCATGGTCACACAAGCTTTGGGG CGCGTGGTGGCGGC
	3'AUG-hp	CCACCACGCGCCCCAAAGCTTGTGTGACCATGGTTGAGCTGTA TTAGTCAGATGAAATG
CAA_{14}	5'CAA_{14}	CATCTGACTAATACCAACAACAACAACAACAACAACAACAACAAC AACAACAACAATACAACACCACCACCATGAATAG
	3'CAA_{14}	GTGGTGGTGTTGTATTGTTGTTGTTGTTGTTGTTGTTGTTGT TGTTGTTGTTGGTATTAGTCAGATGAAATG
$AUG\text{-}CAA_{14}$	5'$AUG\text{-}CAA_{14}$	AGCTCAACCATGGTCACACAAGCTTTCCAACAACAACAACAACA ACAACAACAACAACAACAACAACAATACAAC
	3'$AUG\text{-}CAA_{14}$	GTTGTTGTTGTTGTTGTTGGAAAGCTTGTGTGACCATGGTTGAG CTGTATTAGTCAGATG
LhpL	3'LhpL	GGCGGCTGCAGCCGCCACCACGCGCCCCAGCATGTTAAAGAAT CCTCTATTCATGGTGGTGGTGTTGTAGTATTAGTCAGATGAAATG
STOP	5'STOPAUG-hp	GGCGGCTGCAGCCGCCACCACGCGCCCCTACAACACCTAGTGA ACCACCATGAATAG
hpSTOPhp2	5'hpSTOPhp2	GGCGGCTGCAGCCGCCACCACGCGCCCCTACAACACCTAGTGA TCACAACAACAACGGCCGGTCGACCCGGGAATTCCCGGGTCGA CCGGCCGGACCACCATGAATAGAG
hSTOPp	3'hSTOPp	GGCGGCTGCAGCTCACTACGCCACCACGCGCCCCAAAGCTTGT GTGACCATGGTTG
hp30	5'hp30	GGCGGCTGCAGCCGCCACCACGCGCCCCTCATCTGACTAATACT ACAACACCACCACCATGAATAGAGG
	5' Hpa	GCTATGGCGTTAACCGGTCTG
	3' Aat	CGTTCTTGACGTCGAAC
	5'SacI-T7prom	GCGCGCGAGCTCTAATACGACTCACTATAGATAGTCAGCATAGT
	Left luc2	AGGTCTTCCCGACGATGA
	Right luc2	GTCTTTCCGTGCTCCAAAAC
	Left Act2	CTCTCCCTCATGCCATCCT
	Right Act2	GTAGCCACGCTCGGTCAG