Conserved NP amino acids control genome packaging of influenza A viruses

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Introduction

The segmented RNA genome of influenza A viruses (IAVs) is complexed by the polymerase and nucleoproteins (NP) to form eight unique viral ribonucleoproteins (vRNPs). Selective pack-aging of these eight vRNPs into a new virion is directed by packaging sequences located in the RNA component of each vRNP[1,2]. Previous mutational work revealed that specific amino acid residues in NP play a similar role in genome packaging of an H7N7 virus (SC35M)[3]. In particular, the introduction of seven head domain residues from the distantly related bat-H17N10-NP impaired packaging of four vRNPs into virions. Since all seven NP residues are found in virtually all conventional IAV subtypes (H1N1-H16N9), we assume a preserved function in genome packaging across different subtypes.

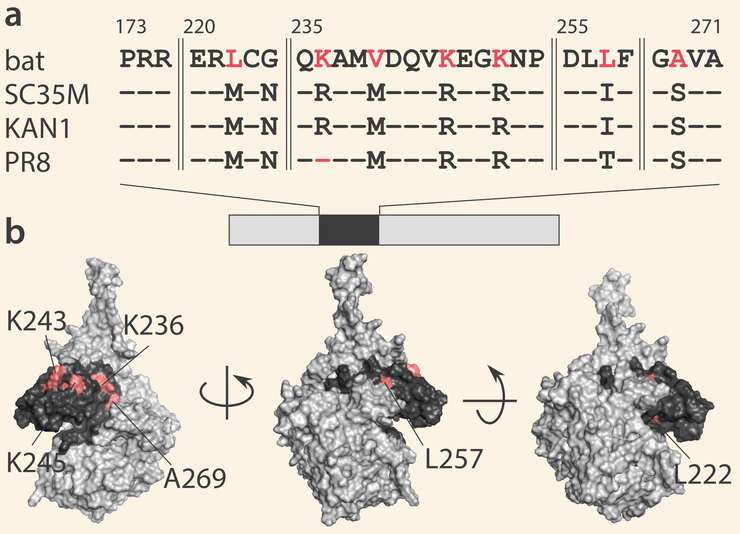
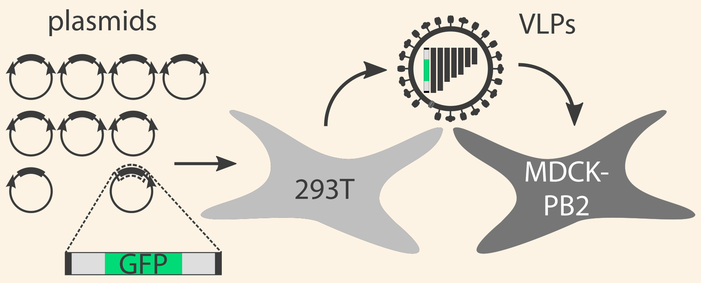


Fig. 1 | NP7. (**a**) alignment of NP head domain sequences. (**b**) NP structure model showing the seven H17N10-specific residues (red) in the head domain (dark grey).

Methods

Virus rescue. Recombinant IAVs of the H1N1 and H5N1 subtypes harboring NP segments with bat-specific amino acid substitutions were generated using the eight-plasmid (pHW2000) transfection system.

Virus growth kinetics and quantification of genome segments in virions. MDCK cells were infected at an MOI of 0.001 and released virion populations were analyzed for infectious virions (PFU) and genome segments by RT-qPCR. Each genome segment in WT virions was set to 1 and the highest abundant genome segment (PB2) in mutant virions was normalized to 1.



8-genome-segment packaging assay. 293T cells were transfected with nine plasmids (7x pHW2000, 1x pCAGGS, 1x pPolI) to constitute VLPs containing a GFP-minigenome (including H1N1-PB2-packaging sequences) and seven full-length segments. The polymerase activity was estimated based on the GFP expression in 293T cells at 36 h post-transfection. VLPs were titrated in MDCK-cells expressing PB2 protein.

Results

An H1N1-IAV with 7 bat-NP specific amino acids cannot be generated

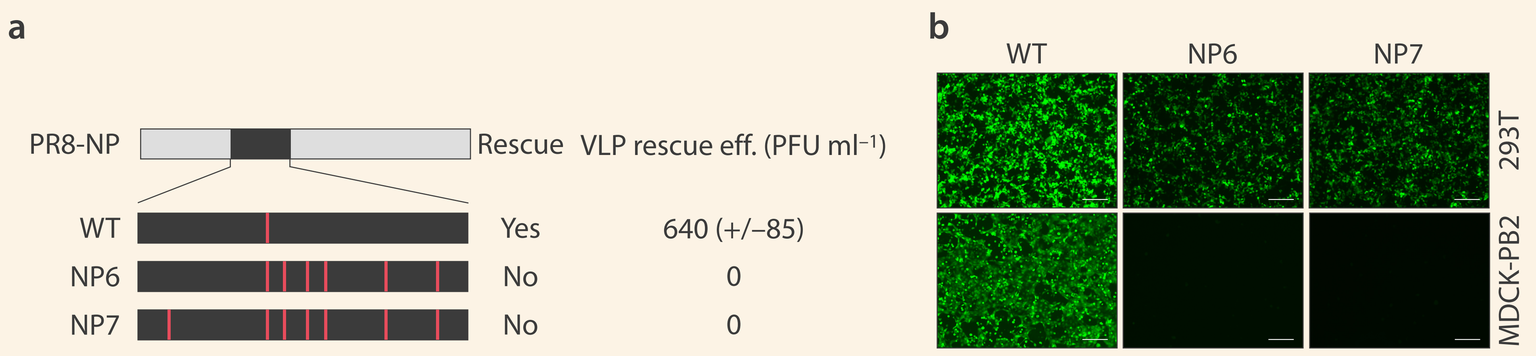


Fig. 2 | PR8-NP7. Seven bat-specific amino acid substitutions in PR8-NP impair polymerase activity and prevent the formation of VLPs and recombinant IAVs (**a**). Micrographs of transfected 293T cells and VLP-infected MDCK-PB2 cells are shown (**b**). Scale bar, 200 µM; eff., efficiency.

An H5N1-NP7 IAV fails to efficiently package several vRNPs

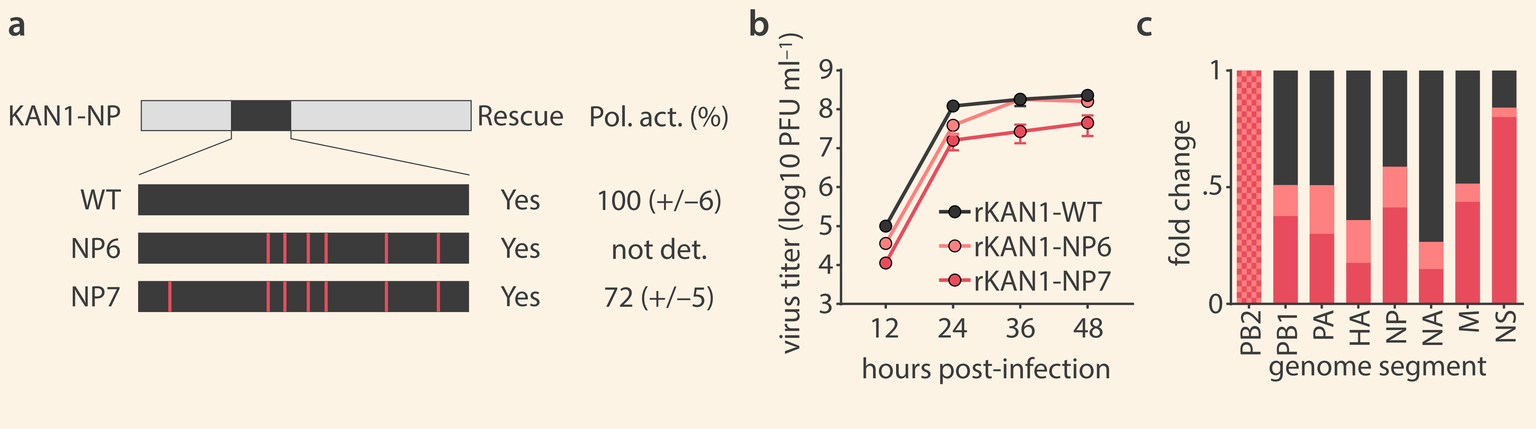
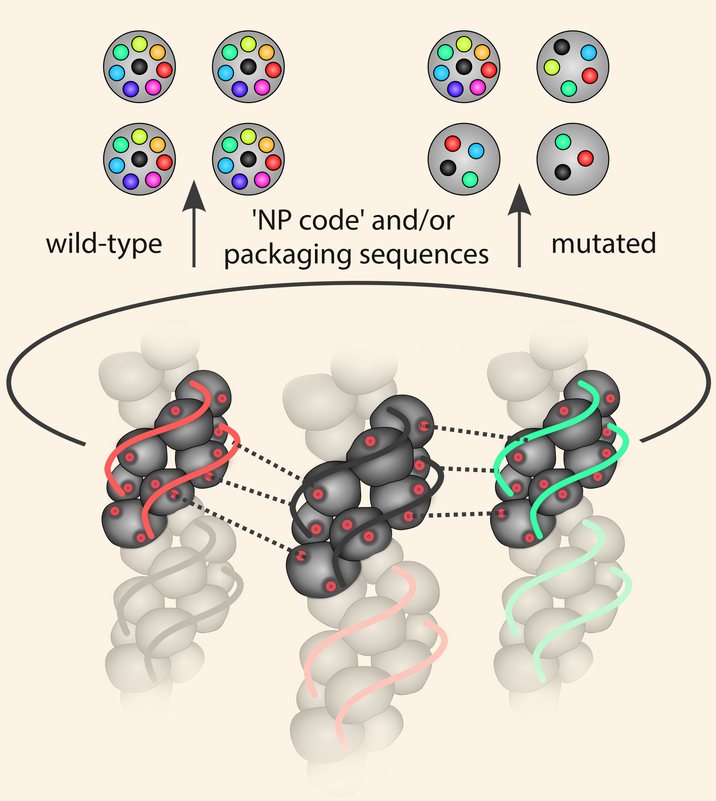
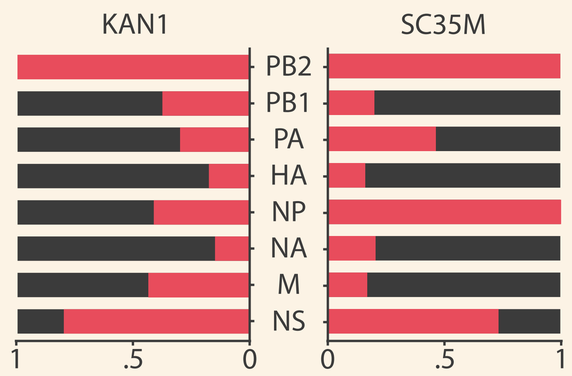


Fig. 3 | KAN1-NP7. Seven bat-specific amino acid substitutions in KAN1-NP do not impair polymerase activity (**a**) but reduce the formation of infectious virions (**b**). In comparison to the WT virus, packaging of several genome segments into virions is affected at 24 hours post-infection (**c**). Segment levels are shown as fold change compared to WT and normalized to the PB2 segment.

Conclusion

A conserved 'NP amino acid code' coordinates genome packaging





We show a functional role of conserved NP amino acids in genome packaging of different IAV subtypes. This 'NP code' (depicted as red dots) likely acts in concert with genome packaging sequences (i.e. genome bundling sequences) to coordinate assembly of eight distinct vRNPs. Differences in the bundling sequences and/or additional NP code residues between the investigated subtypes might explain the observed phenotypic differences.

Acknowledgments and References

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1 | Gerber *et al*. (2014), Trends Microbiol.
2 | Giese, Bolte, Schwemmle (2016), Viruses.
3 | Moreira *et al*. (2016), Nat. Commun.