Meta Gene 16 (2018) 57–61

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

Co-evolving pairs of complementary nucleotide sequence regions as candidates for bundling signals in viruses with segmented genomes

Yoshiyuki Suzuki

Graduate School of Natural Sciences, Nagoya City University, 1 Yamanohata, Mizuho-cho, Mizuho-ku, Nagoya-shi, Aichi-ken 467-8501, Japan

ARTICLE INFO

Keywords:
Avian orthoreovirus
Bundling signal
Co-evolution
Complementarity
Pteropine orthoreovirus
Secondary structure

ABSTRACT

Some of RNA viruses with segmented genomes are believed to undergo selective packaging, in which one copy of each genomic segment is incorporated into a virion. The selective packaging is thought to be mediated by supra-molecular complex formation of genomic RNA segments through inter-segmental base pairing of complementary nucleotide sequence regions termed the bundling signal. Here the nucleotide sequences of 10 genomic segments (large: L1, L2, and L3; medium: M1, M2, and M3; and small: S1, S2, S3, and S4) for 29 avian (ARV) and 8 pteropine (PRV) orthoreovirus isolates were analyzed to identify co-evolving pairs of complementary nucleotide sequence regions within and between genomic segments. The co-evolving pairs within and between genomic segments were predicted to be involved in intra-segmental and inter-segmental interactions, respectively. One co-evolving pair each was identified within M1 and S3, and both pairs indeed tended to constitute stems in the intra-segmental RNA secondary structures. In addition, one co-evolving pair each was identified between S3 and S4, L2 and S4, and L3 and M1, and the co-evolving pair between S3 and S4 tended to constitute loops in the intra-segmental RNA secondary structures. The co-evolving pair identified between S3 and S4 was considered as a candidate for the bundling signal in ARV and PRV.

1. Introduction

Genomes of RNA viruses belonging to the families *Arenaviridae*, *Birnaviridae*, *Bromoviridae*, *Bunyaviridae*, *Chrosoviridae*, *Closteroviridae*, *Cystoviridae*, *Orthomyxoviridae*, *Partitiviridae*, *Picobirnaviridae*, and *Reoviridae* are divided into segments (McDonald et al. 2016). By virtue of the segmented nature of the genome, these viruses can generate mutants by means of exchanging genomic segments upon co-infection of a cell, called the reassortment (McDonald et al. 2016). In fact, the species is defined as a group of isolates that can reassort with each other in these viruses (Attoui et al. 2011; McCauley et al. 2011).

Among the RNA viruses with segmented genomes, those belonging to the families *Orthomyxoviridae* and *Reoviridae* possess highly divided genomes comprising 6–8 and 9–12 segments, respectively (Carstens 2011; Condit 2013). These viruses are believed to undergo selective packaging, in which one copy of each genomic segment is incorporated into a virion (Hutchinson et al. 2010; McDonald and Patton 2011). The mechanism of selective packaging is not fully understood. However, it has been postulated that genomic segments of RNA are bundled as a supra-molecular complex through inter-segmental interaction of complementary nucleotide sequence regions, termed the bundling signal (Goto et al. 2013), and that intra-segmental RNA secondary structures facilitate the interaction (Suzuki and Kobayashi 2013; Kobayashi et al. 2016).

Generally, functions of molecules such as RNAs and proteins are exerted through formation of secondary and tertiary structures. Nucleotide or amino acid sequences may evolve neutrally or nearly neutrally as long as the structures of molecules are conserved (Kimura, 1983, 1985). Consequently, structures are sometimes more useful than sequences for clarifying distant evolutionary relationships among molecules (Nasir and Caetano-Anolles 2015; Seligmann and Raoult 2016). Formation of structures in RNAs and proteins is mediated by interactions of nucleotides and amino acids, respectively. Interacting nucleotides and amino acids may co-evolve under the constraint for maintaining structures. Therefore, interacting nucleotides and amino acids may be identified from the analysis of co-evolution (Lin et al., 2012; Rashidipour et al. 2016; Marouzi et al. 2017; Skwark et al. 2017).

The pair of complementary nucleotide sequence regions constituting the bundling signal may co-evolve through compensatory mutations under the constraint for maintaining complementarity (Kimura 1983, 1985). Co-evolution in the bundling signal may suppress reassortment of wild-type and mutant genomic segments and may eventually promote speciation (Hutchinson et al. 2010; McDonald and Patton 2011). Therefore, a bundling signal may be represented as a pair of complementary nucleotide sequence regions between genomic segments with a signature of co-evolution during speciation (Boyce and

E-mail address: yossuzuk@nsc.nagoya-cu.ac.jp.

https://doi.org/10.1016/j.mgene.2018.01.013
Received 7 December 2017; Received in revised form 23 January 2018; Accepted 30 January 2018
Available online 01 February 2018

McCrae 2015; Boyce et al. 2016).

Avian (ARV) and pteropine (PRV) orthoreoviruses are closely related in the family *Reoviridae* but do not appear to reassort with each other (Attoui et al. 2011; Farkas et al. 2016). ARV is an etiological agent of various diseases including arthritis and synovitis in birds worldwide (Day 2009). PRV is an emerging and zoonotic agent causing respiratory diseases in humans transmitted from the reservoir of fruit bats in Southeast Asia (Tan et al. 2017). These viruses possess 10 genomic segments of RNA, which are classified according to the size; i.e., large (L1, L2, and L3), medium (M1, M2, and M3), and small (S1, S2, S3, and S4). It has been reported that supra-molecular complex formation of genomic segments in reoviruses is initiated with bundling of small genomic segments (Sung and Roy 2014; Fajardo et al. 2015, 2017). The purpose of the present study was to search for candidates of bundling signals in ARV and PRV from the analysis of genomic sequences.

2. Materials and methods

2.1. Sequence data

The complete genomic sequences of 29 ARV and 8 PRV isolates were retrieved from the International Nucleotide Sequence Database (INSD) on July 22, 2017 (Supplementary Table S1). Multiple alignment for the total of 37 nucleotide sequences was made for each of 10 genomic segments using the computer program MAFFT (version 7.305b) (Katoh et al. 2002). The 10 multiple alignments obtained were analyzed for identifying the co-evolving pairs of complementary nucleotide sequence regions as described below.

2.2. Data analysis

From the comparison of 10 multiple alignments, pairs of mono-nucleotide positions that were complementary in each of ARV and PRV isolates were identified within and between genomic segments. Here the nucleotide positions containing a gap in any of ARV and PRV isolates were eliminated from the analysis, and only the Watson-Crick base pairs of U and A, and C and G were regarded as complementary, though U and G may form a wobble base pair, to reduce false-positives in prediction of co-evolving pairs of complementary nucleotide sequence regions that were functionally relevant. Among the pairs of complementary mono-nucleotide positions identified above, co-varying pairs were defined as the pairs in which the bases in ARV isolates were different from those in PRV isolates. The proportion of co-varying pairs among all pairs of complementary mono-nucleotide positions was designated p_{cov}.

In the above analysis, pairs of complementary mono-nucleotide positions were identified regardless of whether they were involved in the direct interaction or not. Therefore, the pairs identified above should consist of not only the interacting pairs but also the non-interacting pairs that were complementary by chance and may interact with different mono-nucleotide positions from each other. Compensatory mutations that do not disrupt complementary are more likely to be neutral or nearly neutral and cause co-evolution in the interacting pairs than in the non-interacting pairs. Consequently, the proportion of co-varying pairs among the interacting pairs is expected to be greater than that among the non-interacting pairs, and p_{cov} may represent an intermediate value of these proportions by the law of large numbers. Therefore, a pair of complementary nucleotide sequence regions with the length of 2 or more bases including a greater number of co-varying pairs of complementary mono-nucleotide positions than expected from p_{cov} may be co-evolving and thus interacting (Lin et al., 2012; Rashidipour et al. 2016; Marouzi et al. 2017; Skwark et al. 2017).

Based on this rationale, 10 multiple alignments obtained above were further analyzed for identifying pairs of complementary nucleotide sequence regions with the length of 2 or more bases within and between genomic segments. For each pair with the length of X bases, a statistical test was conducted to examine whether the number of co-varying pairs of complementary mono-nucleotide positions included (Y) was significantly greater than that expected from p_{cov}. Specifically, the binomial probability for the occurrence of Y or more events in X trials was computed with the parameter value of p_{cov}. Correction for multiple testing was conducted with the total number of the pairs with the length of X bases by the methods of Bonferroni (Sokal and Rohlf 1995) and Benjamini and Hochberg (1995) for obtaining 5% family-wise false-positive rate (FW-FPR) and 5% false-discovery rate (FDR), respectively.

3. Results

3.1. Co-evolving pairs of complementary nucleotide sequence regions

Lack of reassortment between ARV and PRV isolates was confirmed by the phylogenetic analysis for each of 10 genomic segments; ARV and PRV isolates always formed distinct clusters in the phylogenetic tree (data not shown). The length of the pairs of complementary nucleotide sequence regions identified within and between genomic segments (Supplementary Table S1) ranged from 1 to 6 bases (Table 1). It should be noted that the pairs listed in Table 1 overlap; i.e., a pair with the length of n bases includes $(n - i + 1)$ pairs with the length of i bases $(1 \le i \le n)$. The p_{cov} value was obtained to be 0.0337 (= 332,720/9,883,373). For each pair of complementary nucleotide sequence regions with a particular length, a statistical test of co-evolution was conducted with the correction for multiple testing. For example, for the pairs with the length of 5 bases, 38 statistical tests were conducted with the correction (Table 1). Overall, 4 pairs of complementary nucleotide sequence regions with the length of 5 or 6 bases were judged as co-evolving with the 5% FW-FPR, and additional 9 pairs with the length of 3 to 6 bases were judged as co-evolving with the 5% FDR (Table 1). The nucleotide sequences of ARV and PRV isolates at the 13 co-evolving pairs are presented in Table 2, in which the positions are numbered according to the isolate S1133 for ARV (Teng et al. 2014) and the isolate Talikud-74 for PRV (Taniguchi et al. 2017). After eliminating overlaps, 5 co-evolving pairs remained; one co-evolving pair each with 6 bases long within M1, 5 bases long within S3, 4 bases long between S3 and S4, 3 bases long between L2 and S4, and 3 bases long between L3 and M1 (Table 2).

It has been reported that the supra-molecular complex formation of genomic segments in reoviruses is initiated with bundling of small genomic segments (Sung and Roy 2014; Fajardo et al. 2015, 2017). Therefore, only the small genomic segments (S1, S2, S3, and S4) were

Table 1
Numbers of pairs of complementary nucleotide sequence regions in ARV and PRV isolates.

		Y[d]					
	X[c]	4	3	2	1	0	Total
All[a]	6	0[e]	***1***	0	0	0	1
	5	***1***	***2***	0	1	34	38
	4	0	*5*	1	6	467	479
	3	N.A.[f]	*4*	14	86	3455	3559
	2	N.A.	N.A.	2213	6018	446,829	455,060
	1	N.A.	N.A.	N.A.	332,720	9,530,653	9,863,373
Small[b]	5	***1***	0	0	0	5	6
	4	0	***3***	0	2	35	40
	3	N.A.	1	6	11	274	292
	2	N.A.	N.A.	108	317	12,173	12,598
	1	N.A.	N.A.	N.A.	15,574	262,064	277,638

[a] All of 10 genomic segments were used for the analysis.
[b] Only 4 small genomic segments (S1, S2, S3, and S4) were used for the analysis.
[c] Length of co-evolving pairs of complementary nucleotide sequence regions.
[d] Number of co-varying pairs of complementary mono-nucleotide positions.
[e] Numbers of pairs of complementary nucleotide sequence regions judged as co-evolving with 5% FW-FPR and 5% FDR are bold-faced and italicized, respectively.
[f] Not applicable.

Table 2
Nucleotide sequences at the co-evolving pairs of complementary nucleotide sequence regions in ARV and PRV isolates.

	X[c]	Y[d]	Genomic segment	Positions in ARV[e]	Sequence in ARV	Positions in PRV[f]	Sequence in PRV	Genomic segment	Positions in ARV	Sequence in ARV	Positions in PRV	Sequence in PRV
All[a]	**6**[g]	**3**	**M1**	**2233-2238**	**5′CCAGGG3′**	**2245–2250**	**5′CUGUGG3′**	**M1**	**2254–2259**	**5′CCCUGG3′**	**2266–2271**	**5′CCACAG3′**
	5	3	M1	2233–2237	5′CCAGG3′	2245–2249	5′CUGUG3′	M1	2255–2259	5′CCUGG3′	2267–2271	5′CACAG3′
	5	3	M1	2234–2238	5′CAGGG3′	2246–2250	5′UGUGG3′	M1	2254–2258	5′CCCUG3′	2266–2270	5′CCACA3′
	4	*3*	*M1*	*2233–2236*	*5′CCAG3′*	*2245–2248*	*5′CUGU3′*	*M1*	*2256–2259*	*5′CUGG3′*	*2268–2271*	*5′ACAG3′*
	4	*3*	*M1*	*2234–2237*	*5′CAGG3′*	*2246–2249*	*5′UGUG3′*	*M1*	*2255–2258*	*5′CCUG3′*	*2267–2270*	*5′CACA3′*
	4	*3*	*M1*	*2234–2236*	*5′CAG3′*	*2246–2248*	*5′UGU3′*	*M1*	*2256–2258*	*5′CUG3′*	*2268–2270*	*5′ACA3′*
	5	**4**	**S3**	**1154–1158**	**5′CACRU3′**	**1136–1140**	**5′GUCCC3′**	**S3**	**1171–1175**	**5′AYGUG3′**	**1153–1157**	**5′GGGAC3′**
	4	*3*	*S3*	*1154–1157*	*5′CACR3′*	*1136–1139*	*5′GUCC3′*	*S3*	*1172–1175*	*5′YGUG3′*	*1154–1157*	*5′GGAC3′*
	4	*3*	*S3*	*1155–1158*	*5′ACRU3′*	*1137–1140*	*5′UCCC3′*	*S3*	*1171–1174*	*5′AYGU3′*	*1153–1156*	*5′GGGA3′*
	4	***3***	***S3***	***41–44***	***5′GUGU3′***	***40–43***	***5′GAAC3′***	***S4***	***31–34***	***5′ACAC3′***	***36–39***	***5′GUUC3′***
	3	*3*	*S3*	*42–44*	*5′UGU3′*	*41–43*	*5′AAC3′*	*S4*	*31–33*	*5′ACA3′*	*36–38*	*5′GUU3′*
	3	***3***	***L2***	***1977–1979***	***5′CAG3′***	***1980–1982***	***5′UCA3′***	***S4***	***181–183***	***5′CUG3′***	***186–188***	***5′UGA3′***
	3	***3***	***L3***	***3921–3923***	***5′CGA3′***	***3916–3918***	***5′GAG3′***	***M1***	***51–53***	***5′UCG3′***	***52–54***	***5′CUC3′***
Small[b]	**5**	**4**	**S3**	**1154-1158**	**5′CACRU3′**	**1136–1140**	**5′GUCCC3′**	**S3**	**1171–1175**	**5′AYGUG3′**	**1153–1157**	**5′GGGAC3′**
	4	3	S3	1154–1157	5′CACR3′	1136–1139	5′GUCC3′	S3	1172–1175	5′YGUG3′	1154–1157	5′GGAC3′
	4	3	S3	1155–1158	5′ACRU3′	1137–1140	5′UCCC3′	S3	1171–1174	5′AYGU3′	1153–1156	5′GGGA3′
	4	**3**	**S3**	**41–44**	**5′GUGU3′**	**40–43**	**5′GAAC3′**	**S4**	**31–34**	**5′ACAC3′**	**36–39**	**5′GUUC3′**

[a] All of 10 genomic segments were used for the analysis.
[b] Only 4 small genomic segments (S1, S2, S3, and S4) were used for the analysis.
[c] Length of co-evolving pairs of complementary nucleotide sequence regions.
[d] Number of co-varying pairs of complementary mono-nucleotide positions.
[e] Positions are numbered according to the isolate S1133 for ARV (Teng et al. 2014).
[f] Positions are numbered according to the isolate Talikud-74 for PRV (Taniguchi et al. 2017).
[g] Pairs of complementary nucleotide sequence regions judged as co-evolving with both 5% FW-FPR and 5% FDR and with only 5% FDR are indicated in the plain-text and italicized, respectively, and those that remained after eliminating overlaps are bold-faced.

analyzed separately for identifying the co-evolving pairs of complementary nucleotide sequence regions in a similar manner as described above. The p_{cov} value for the small genomic segments was obtained to be 0.0561 (= 15,574/277,638), and 4 pairs of complementary nucleotide sequence regions with the length of 4 or 5 bases were judged as co-evolving with both 5% FW-FPR and 5% FDR (Table 1). The nucleotide sequences of ARV and PRV isolates at the 4 co-evolving pairs are presented in Table 2. After eliminating overlaps, 2 co-evolving pairs remained; one co-evolving pair each with 5 bases long within S3 and 4 bases long between S3 and S4, both of which were also identified above (Table 2).

3.2. Secondary structures at co-evolving pairs of complementary nucleotide sequence regions

For the co-evolving pairs of complementary nucleotide sequence regions within and between genomic segments to be functionally relevant, the former and the latter co-evolving pairs are expected to constitute stems and loops, respectively, in the intra-segmental RNA secondary structures. Indeed, in both of the intra-segmental secondary structures of M1 and S3 predicted for each of 29 ARV and 8 PRV isolates using RNAfold (version 2.3.5) (Lorenz et al. 2011), the co-evolving pairs identified within M1 and S3 tended to constitute stems in the 3′ untranslated region, respectively (Fig. 1; Supplementary Table S1). It is interesting to note that the nucleotide sequences 5′GGGUAUGCUG GUA3′ and 5′CCCAC3′ flanked by the co-evolving pairs within M1 and S3, respectively, were conserved among all of ARV and PRV isolates (Fig. 1), indicating the functional importance of these sequences in the loops.

The co-evolving pair with the length of 4 bases identified between S3 and S4 constituted a loop in the 5′ end of protein-coding region in both of the intra-segmental secondary structures of S3 and S4 in 7 PRV isolates (Fig. 1; Supplementary Table S1). Among the total of 9,863,373 pairs of complementary mono-nucleotide positions identified in the analysis of all genomic segments, 2,995,998 pairs were observed to be located in loops in 7 or more of 37 ARV and PRV isolates. Therefore, the probability that the co-evolving pair with the length of 4 bases constituted loops in 7 isolates by chance was $(2{,}995{,}998/9{,}863{,}373)^4 = 0.00851$. Similarly, the probability that the co-evolving pair with the length of 4 bases constituted loops in 7 isolates by chance in the analysis of small genomic segments was $(88{,}063/277{,}638)^4 = 0.0101$. These results suggest that the co-evolving pair identified between S3 and S4 is functionally relevant and interacts with each other as a bundling signal. By contrast, the co-evolving pairs with the length of 3 bases identified between L2 and S4, and L3 and M1 did not constitute loops in the intra-segmental RNA secondary structures in any of ARV and PRV isolates (data not shown), suggesting that they were likely to be false-positives.

4. Discussion

4.1. Co-evolving pairs of complementary nucleotide sequence regions as candidates for bundling signals in viruses with segmented genomes

In the present study, co-evolving pairs of complementary nucleotide sequence regions were identified within M1 and S3 and between S3 and S4, L2 and S4, and L3 and M1 of ARV and PRV isolates, which were predicted to be involved in intra-segmental and inter-segmental interactions, respectively. The predictions were supported for the co-evolving pairs identified within M1 and S3 and between S3 and S4, which tended to constitute stems and loops in the intra-segmental RNA secondary structures, respectively. The co-evolving pair identified between S3 and S4 was considered as a candidate for the bundling signal. It has also been reported that the co-evolving pairs identified within and between genomic segments constituted stems and loops, respectively, in the analyses of mammalian and avian rotavirus A (RVA) (Suzuki 2014) and bluetongue (BTV) and epizootic hemorrhagic disease (EHDV) viruses (Suzuki 2016). These results validate the strategy of predicting co-evolving pairs of complementary nucleotide sequence regions within and between genomic segments to be involved in intra-segmental and inter-segmental interactions, respectively, and identifying the latter co-evolving pairs as candidates for bundling signals in viruses with segmented genomes (Suzuki 2014, 2015, 2016).

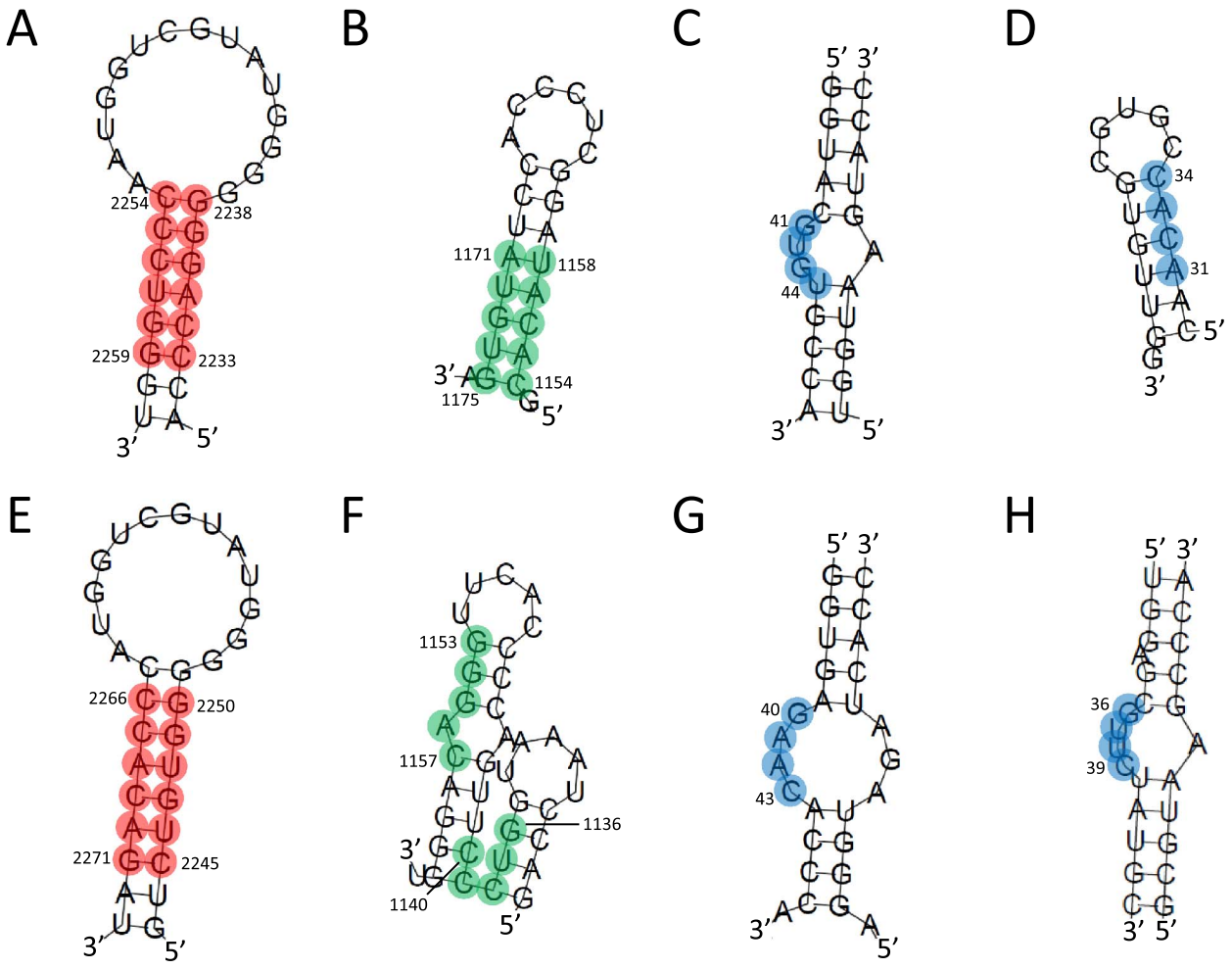

Fig. 1. Local intra-segmental RNA secondary structures of M1 (A), S3 (B and C), and S4 (D) of the isolate S1133 for ARV (Teng et al. 2014) and M1 (E), S3 (F and G), and S4 (H) of the isolate Talikud-74 for PRV (Taniguchi et al. 2017). Intra-segmental RNA secondary structure was predicted for the entire sequence region of each genomic segment using RNAfold (version 2.3.5) (Lorenz et al. 2011). Co-evolving pairs of complementary nucleotide sequence regions identified within M1 (A and E) and S3 (B and F) and between S3 (C and G) and S4 (D and H), whose ends are labeled with position numbers, are colored red, green, and blue, respectively.

4.2. Difficulty in understanding the mechanism of selective packaging

The strategy of predicting bundling signals adopted in the present study was designed based on the notion that a pair of complementary nucleotide sequence regions constituting a bundling signal may co-evolve and suppress reassortment between species. One candidate for the bundling signal was obtained in the analysis of ARV and PRV with 10 genomic segments, as described above. Similarly, one candidate each has been obtained in the analyses of mammalian and avian RVA with 11 genomic segments (Suzuki 2014) and BTV and EHDV with 10 genomic segments (Boyce et al. 2016; Suzuki 2016). It should be noted, however, that at least $(k - 1)$ bundling signals are required for bundling k genomic segments. Although it is still unclear whether all genomic segments are bundled by bundling signals in these viruses (Brooke et al. 2013; Brooke 2014; Suzuki 2016), it is likely that many bundling signals were missed in these analyses, implying that pairs of complementary nucleotide sequence regions constituting bundling signals may not always co-evolve. Some bundling signals may be shared between species, as has been proposed for BTV and EHDV (Boyce et al. 2016; Suzuki 2016). In this case, reassortment may be suppressed between species due to incompatibility in proteins encoded by different genomic segments (Hutchinson et al. 2010; McDonald and Patton 2011). Additionally, some bundling signals may change genomic locations during evolution, as has been proposed for influenza A virus (Gavazzi et al. 2013; Gerber et al. 2014) and RVA (Suzuki 2015). It is important to identify more bundling signals for understanding the mechanism of selective packaging in viruses with segmented genomes.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mgene.2018.01.013.

Acknowledgements

The author thanks two anonymous reviewers for valuable comments. This work was supported by a Grant-in-Aid for Research in Nagoya City University (43) to Y.S.

Disclosure

The author declares no conflict of interest.

References

Attoui, H., Mertens, P.P.C., Becnel, J., Belaganahalli, S., Bergoin, M., Brussaard, C.P., Chappell, J.D., Ciarlet, M., del Vas, M., Dermody, T.S., Dormitzer, P.R., Duncan, R., Fang, Q., Graham, R., Guglielmi, K.M., Harding, R.M., Hillman, B., Makkay, A., Marzachì, C., Matthijnssens, J., Milne, R.G., Mohd Jaafar, F., Mori, H., Noordeloos, A.A., Omura, T., Patton, J.T., Rao, S., Maan, M., Stoltz, D., Suzuki, N., Upadhyaya, N.M., Wei, C., Zhou, H., 2011. Family *Reoviridae*. In: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, Amsterdam, pp. 541–637.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and

powerful approach to multiple testing. J. R. Statist. Soc. B 57, 289–300.
Boyce, M., McCrae, M.A., 2015. Rapid mapping of functional cis-acting RNA elements by recovery of virus from a degenerate RNA population: application to genome segment 10 of bluetongue virus. J. Gen. Virol. 96, 3072–3082.
Boyce, M., McCrae, M.A., Boyce, P., Kim, J.T., 2016. Inter-segment complementarity in orbiviruses: a driver for co-ordinated genome packaging in the Reoviridae? J. Gen. Virol. 97, 1145–1157.
Brooke, C.B., 2014. Biological activities of 'noninfectious' influenza A virus particles. Futur. Virol. 9, 41–51.
Brooke, C.B., Ince, W.L., Wrammert, J., Ahmed, R., Wilson, P.C., Bennink, J.R., Yewdell, J.W., 2013. Most influenza A virions fail to express at least one essential viral protein. J. Virol. 87, 3155–3162.
Carstens, E.B., 2011. Introduction to virus taxonomy. In: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, Amsterdam, pp. 3–20.
Condit, R.C., 2013. Principles of virology. In: Knipe, D.M., Howley, P.M., Cohen, J.I., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B. (Eds.), Fields Virology, Sixth Edition. Lippincott Williams & Wilkins, Philadelphia, pp. 21–51.
Day, J.M., 2009. The diversity of the orthoreoviruses: molecular taxonomy and phylogenetic divides. Infect. Genet. Evol. 9, 390–400.
Fajardo Jr., T., Sung, P.-Y., Roy, P., 2015. Disruption of specific RNA-RNA interactions in a double-stranded RNA virus inhibits genome packaging and virus infectivity. PLoS Pathog. 11, e1005321.
Fajardo Jr., T., Sung, P.-Y., Celma, C.C., Roy, P., 2017. Rotavirus genomic RNA complex forms via specific RNA–RNA interactions: disruption of RNA complex inhibits virus infectivity. Viruses 9, 167.
Farkas, S.L., Marton, S., Dandar, E., Kugler, R., Gal, B., Jakab, F., Balint, A., Kecskemeti, S., Banyai, K., 2016. Lineage diversification, homo- and heterologous reassortment and recombination shape the evolution of chicken orthoreoviruses. Sci. Rep. 6, 36960.
Gavazzi, C., Yver, M., Isel, C., Smyth, R.P., Rosa-Calatrava, M., Lina, B., Moules, V., Marquet, R., 2013. A functional sequence-specific interaction between influenza A virus genomic RNA segments. Proc. Natl. Acad. Sci. U. S. A. 110, 16604–16609.
Gerber, M., Isel, C., Moules, V., Marquet, R., 2014. Selective packaging of the influenza A genome and consequences for genetic reassortment. Trends Microbiol. 22, 446–455.
Goto, H., Muramoto, Y., Noda, T., Kawaoka, Y., 2013. The genome-packaging signal of the influenza A virus genome comprises a genome incorporation signal and a genome-bundling signal. J. Virol. 87, 11316–11322.
Hutchinson, E.C., von Kirchbach, J.C., Gog, J.R., Digard, P., 2010. Genome packaging in influenza A virus. J. Gen. Virol. 91, 313–328.
Katoh, K., Misawa, K., Kuma, K.-i., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066.
Kimura, M., 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge, New York, Melbourne.
Kimura, M., 1985. The role of compensatory neutral mutations in molecular evolution. J. Genet. 64, 7–19.
Kobayashi, Y., Dadonaite, B., van Doremalan, N., Suzuki, Y., Barclay, W.S., Pybus, O.G., 2016. Computational and molecular analysis of conserved influenza A virus RNA secondary structures involved in infectious virion production. RNA Biol. 13, 883–894.
Lin, X., Hong, T., Mu, Y., Torres, J., 2012. Identification of residues involved in water versus glycerol selectivity in aquaporins by differential residue pair co-evolution. Biochim. Biophys. Acta 1818, 907–914.
Lorenz, R., Bernhart, S.H., Honer zu Siederdissen, C., Tafer, H., Flamm, C., Stadler, P.F., Hofacker, I.L., 2011. ViennaRNA Package 2.0. Algorithms Mol. Biol. 6, 26.
Marouzi, S., Rad, A.S., Beigoli, S., Baghaee, P.T., Darban, R.A., Chamani, J., 2017. Study on effect of lomefloxacin on human holo-transferrin in the presence of essential and nonessential amino acids: spectroscopic and molecular modeling approaches. Int. J. Biol. Macromol. 97, 688–699.
McCauley, J.W., Hongo, S., Kaverin, N.V., Kochs, G., Lamb, R.A., Matrosovich, M.N., Perez, D.R., Palese, P., Presti, R.M., Rimstad, E., Smith, G.J.D., 2011. Family *Orthomyxoviridae*. In: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, Amsterdam, pp. 749–761.
McDonald, S.M., Patton, J.T., 2011. Assortment and packaging of the segmented rotavirus genome. Trends Microbiol. 19, 136–144.
McDonald, S.M., Nelson, M.I., Turner, P.E., Patton, J.T., 2016. Reassortment in segmented RNA viruses: mechanisms and outcomes. Nat. Rev. Microbiol. 14, 448–460.
Nasir, A., Caetano-Anolles, G., 2015. A phylogenomic data-driven exploration of viral origins and evolution. Sci. Adv. 1, e1500527.
Rashidipour, S., Naeeminejad, S., Chamani, J., 2016. Study of the interaction between DNP and DIDS with human hemoglobin as binary and ternary systems: spectroscopic and molecular modeling investigation. J. Biomol. Struct. Dyn. 34, 57–77.
Seligmann, H., Raoult, D., 2016. Unifying view of stem-loop hairpin RNA as origin of current and ancient parasitic and non-parasitic RNAs, including in giant viruses. Curr. Opin. Microbiol. 31, 1–8.
Skwark, M.J., Croucher, N.J., Puranen, S., Chewapreecha, C., Pesonen, M., Xu, Y.Y., Turner, P., Harris, S.R., Beres, S.B., Musser, J.M., Parkhill, J., Bentley, S.D., Aurell, E., Corander, J., 2017. Interacting networks of resistance, virulence and core machinery genes identified by genome-wide epistasis analysis. PLoS Genet. 13, e1006508.
Sokal, R.R., Rohlf, F.J., 1995. Biometry, 3rd edition. W.H. Freeman and Company, New York.
Sung, P.-Y., Roy, P., 2014. Sequential packaging of RNA genomic segments during the assembly of bluetongue virus. Nucleic Acids Res. 42, 13824–13838.
Suzuki, Y., 2014. A possible packaging signal in the rotavirus genome. Genes Genet. Syst. 89, 81–86.
Suzuki, Y., 2015. A candidate packaging signal of human rotavirus differentiating Wa-like and DS-1-like genomic constellations. Microbiol. Immunol. 59, 567–571.
Suzuki, Y., 2016. Co-evolution in a putative bundling signal of bluetongue and epizootic hemorrhagic disease viruses. Genes Genet. Syst. 91, 283–288.
Suzuki, Y., Kobayashi, Y., 2013. Evolution of complementary nucleotides in 5′ and 3′ untranslated regions of influenza A virus genomic segments. Infect. Genet. Evol. 13, 175–179.
Tan, Y.F., Teng, C.L., Chua, K.B., Voon, K., 2017. *Pteropine orthoreovirus*: an important emerging virus causing infectious disease in the tropics? J. Infect. Dev. Ctries. 11, 215–219.
Taniguchi, S., Maeda, K., Horimoto, T., Masangkay, J.S., Puentespina Jr., R., Alvarez, J., Eres, E., Cosico, E., Nagata, N., Egawa, K., Singh, H., Fukuma, A., Yoshikawa, T., Tani, H., Fukushi, S., Tsuchiaka, S., Omatsu, T., Mizutani, T., Une, Y., Yoshikawa, Y., Shimojima, M., Saijo, M., Kyuwa, S., 2017. First isolation and characterization of pteropine orthoreoviruses in fruit bats in the Philippines. Arch. Virol. 162, 1529–1539.
Teng, L., Xie, Z., Xie, L., Liu, J., Pang, Y., Deng, X., Xie, Z., Fan, Q., Luo, S., Feng, J., Khan, M.I., 2014. Sequencing and phylogenetic analysis of an avian reovirus genome. Virus Genes 48, 381–386.