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Loop model: Mechanism to explain partial gene duplications in segmented dsRNA viruses

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Abstract

Gene rearrangements in a head-to-tail fashion have been described several times for gene segments of the rota-, phytoreo-, and orbiviruses. Several mechanisms have been proposed to explain the occurrence of partial duplications, however, none of these models has been fully satisfactory to explain the occurrence of all the observed duplicated genes. Based on recently available structural data about the λ3 RNA-dependent-RNA-polymerase of reoviruses, we propose the 'loop model' as a plausible explanation for the occurrence of partial gene duplications in dsRNA viruses.

Keywords: Loop model; RNA duplication; Reoviridae; Rotavirus; Phytoreovirus; Orbivirus

Rotaviruses, phytoreoviruses, and orbiviruses belong to the family of *Reoviridae*, which are characterized by a segmented double-stranded (ds)RNA genome [1,2]. Their respective genomes consist of 11, 12, and 10 capped segments [1]. The rota-, phytoreo-, and orbivirus particles are composed of three concentric layers, with a central core, a middle protein layer and an outer protein layer [3,4]. The central core contains the RNA-dependent-RNA-polymerase (RdRp) and other enzymes responsible for the replication steps [1,5,6]. *Reoviridae* display a very wide sequence diversity mainly generated through: (a) mutations, resulting from misincorporations by the error-prone RdRp; (b) gene reassortment, a rather common event due to the segmented nature of their genome; and (c) gene rearrangement in which sudden genomic changes can occur in gene segments due to deletions, insertions, and/or (partial) duplications [7,8]. The rather frequent occurrence of these partial gene duplications in the rotaviral genes NSP1, VP6, NSP3, NSP4, and NSP5 [9–12], the S12 gene of phytoreoviruses, and RNA9 and RNA10 of the orbiviruses [13,14] is intriguing (Table 1).

Results

Recently, the three-dimensional structure of the reovirus λ3 RdRp has been determined. Close structural resemblances were found with other RdRps such as the rotavirus RdRp VP1 (S.C. Harrison, personal communication), the poliovirus RdRp, the HCV RdRp, the bacteriophage Φ6 RdRp, and the HIV-1 reverse transcriptase [15]. This cage like λ3 RdRp contains four channels, leading to the central catalytic core, at the "left," "rear," "front," and "bottom" sites of the protein, with the following functions: template entrance, substrate entrance, template exit, and mRNA exit, in case of transcription (Fig. 1). A CAP-binding site is also present between the template entrance and exit (Fig. 1). Tao and colleagues suggested that this CAP-binding site keeps the 5′-capped end of the positive strand in close proximity to the polymerase, and renders the 3′-end of the negative

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Table 1
Rearrangements found in members of the *Reoviridae* family

Origin/strain	Genus	Species	Breakpoints Duplicated region		Length duplication	Gene	Random nucleotides between copies	original ORF intact	Reference
Po/C60	Rotavirus	Rotavirus A	78	620	542	NSP5	TCTT	Yes	[35]
Bo/C7/183	Rotavirus	Rotavirus A	40	615	575	NSP5	None	Yes	[37]
La/Ala	Rotavirus	Rotavirus A	169	623	454	NSP5	None	Yes	[36]
Bo/VMRI	Rotavirus	Rotavirus A	328	628	300	NSP5	None	Yes	[7]
Hu/H57	Rotavirus	Rotavirus A	50	972	922	NSP3	None	Yes	[32]
Hu/A64	Rotavirus	Rotavirus A	81	572	491	NSP4	None	Yes	[33]
Bo/BrvE	Rotavirus	Rotavirus A	92	808	716	NSP1	None	No	[8]
Bo/BrvA	Rotavirus	Rotavirus A	340	1452	1112	NSP1	None	No	[29]
Lamb/Lp14	Rotavirus	Rotavirus A	768	1241	473	VP6	None	Yes	[31]
Hu/Mc345	Rotavirus	Rotavirus A	108	625	517	NSP5	None	Yes	[38]
Po/CC86	Rotavirus	Rotavirus A	293	621	328	NSP5	TTTTTTCGTC	Yes	[34]
Hu/Z10262	Rotavirus	Rotavirus A	44	616	572	NSP5	None	Yes	[11]
Hu/IGV-S,-F	Rotavirus	Rotavirus A	282	1501	1219	NSP1	None	Yes	[10]
Hu/IGV-S	Rotavirus	Rotavirus A	283	1005	722	NSP3	None	Yes	[10]
Hu/IGV-F	Rotavirus	Rotavirus A	333	986	653	NSP3	None	Yes	[10]
Hu/M2	Rotavirus	Rotavirus A	42	614	572	NSP5	None	Yes	[9]
Hu/M1	Rotavirus	Rotavirus A	6	963	957	NSP3	None	Yes	[9]
RDV-P	Phytoreovirus	Rice Dwarf virus	193	277	84	S12	None	No	[14]
RDV-S-6	Phytoreovirus	Rice Dwarf virus	605	726	121	S12	None	No	[14]
CS154[a]	Orbivirus	Bluetongue virus	nk[b]	nk	nk	RNA10	nk	Yes	[13]
CS184[a]	Orbivirus	Bunyip Creek virus	nk	nk	nk	RNA9	nk	Yes	[13]

[a] The analysis of the rearrangements in the Bluetongue virus and Bunyip Creek virus strains CS154 and CS184, was done using T1 RNase mapping and in vitro translation. Therefore, the exact location of the breakpoints is unknown.
[b] nk, not known.

strand free and single-stranded in close proximity to the template entrance, which is a necessary feature for fast, multiple rounds of transcription [15]. In case of replication (negative strand synthesis), the 3′-end of the positive strand is pulled through the polymerase, and the newly formed double-stranded RNA is caught by the CAP-binding site. With this knowledge about the transcription and replication events in λ3, and most likely in the RdRp's of other *Reoviridae*, we propose a 'loop model' to explain the occurrence of partially duplicated genes in dsRNA viruses. This model suggests that duplications occur at the transcription step. When the 3′-end of the negative strand has passed through the polymerase catalytic core, it reattaches with the positive capped strand, still attached to the CAP-binding site, leaving the 3′-end of the negative strand free for the next round of transcription [15] (Figs. 1B and C). Our loop model is based on the assumption that this 3′-end of the negative strand is inserted again in the template entrance before the complete negative strand is transcribed, forming a loop (Fig. 1D). When the 3′-end of the negative strand reaches the center of the polymerase, the catalytic core sooner or later makes a mistake and switches template (Fig. 1E), transcribing again a more upstream portion of the negative strand, this way forming the duplication (Figs. 1F and G). This mechanism of forming a partial duplication is highly unlikely to occur at the replication step because: (a) the 3′-end of the positive strand is not in close proximity of the template entrance, as is the case for the 3′-end of the negative strand during transcription, (b) replication (negative strand synthesis) occurs only once, opposed to transcription (mRNA synthesis) which occurs multiple times in a row, (c) the synthesis of the negative strand with the positive strand as a template results in dsRNA, which would probably be too voluminous to be inserted into the template entrance in addition to the positive strand (three strands, two positive, strands and one minus strand), and (d) partial duplication at the replication step, after packaging in core like structures, would create a partially unpaired segmental structure, as has been suggested by Kojima et al. [16]. Taking the dimensions of the duplications described in the literature (at least 84 nucleotides, Table 1), the size of dsRNA (0.28 nm/nucleotide) [17], and the overall diameter of the RdRp (6.5 nm, $R = 3.25$ nm) [15] into account, the physical plausibility of this loop model can be calculated. When we make the conservative assumption that the duplication (loop) must be at least as long as one circumference of the RdRp ($2\pi R = 2 \times 3.14 \times 3.25$ nm $= 20.4$ nm), the duplication must be at least 20.4 nm long. The length of the smallest duplication is 84 nucleotides and these will span 23.52 nm (84 nucleotides $\times$ 0.28 nm/nucleotide). These conservative calculations show that the overall dimensions of the involved molecules physically allow the loop phenomenon to occur.

Discussion

Viable rotavirus strains with aberrant electropherotypes have been isolated from a variety of host species, including humans, calves, pigs, rabbits, and lambs [18–

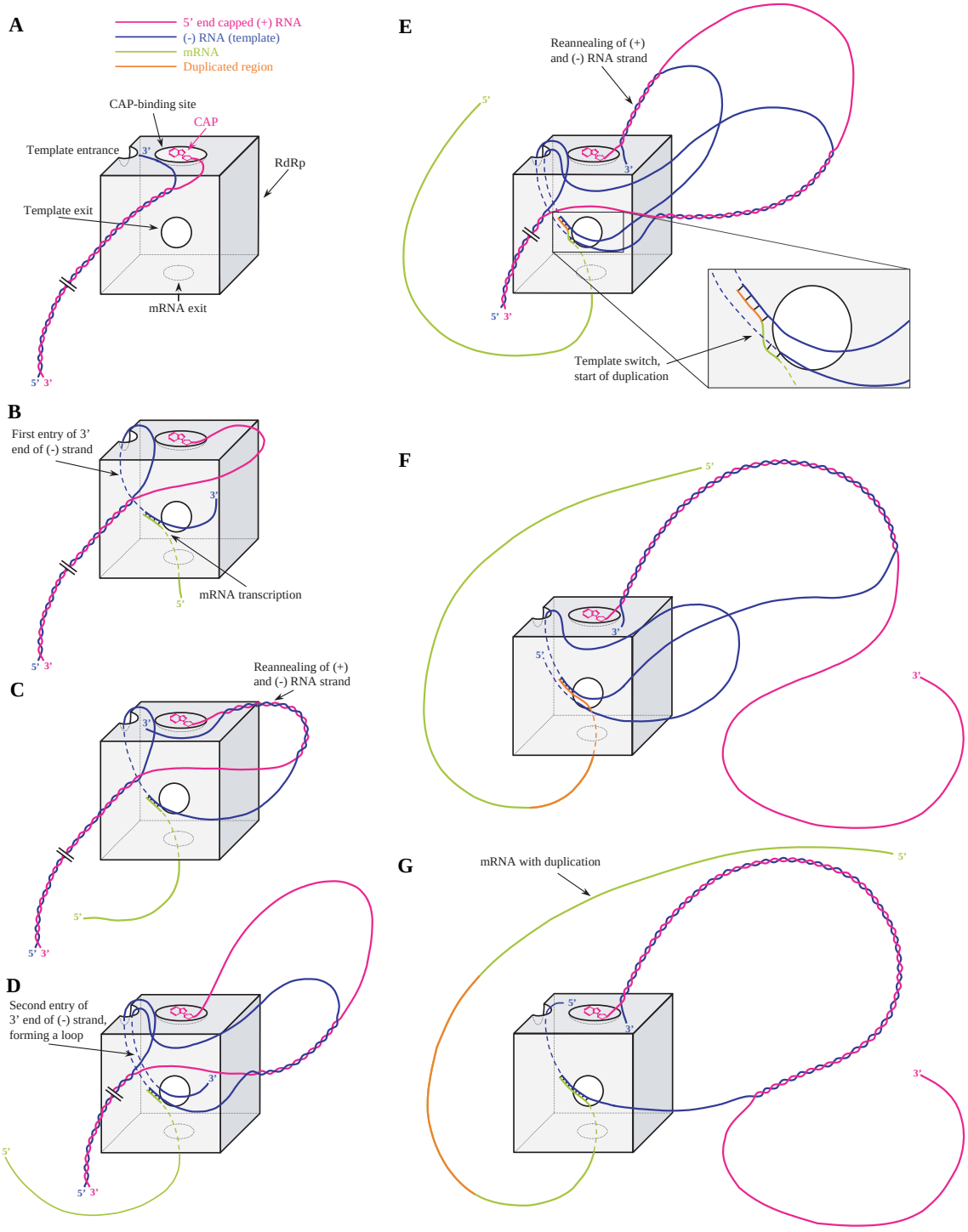

Fig. 1. The 'Loop Model' to explain the occurrence of partial duplications in genes of members of the *Reoviridae*, based on the conserved nature of the λ3 RNA-dependent-RNA-polymerase of the reovirus. The cage like λ3 polymerase contains four channels at the "left site" (template entrance), "rear site" (substrate entrance, omitted for simplicity), "front site" (template exit), and "bottom site" (mRNA exit in case of transcription) of the protein leading to the central catalytic core (A). A CAP-binding site is present between the template entrance and exit, and keeps the 5′-capped end of the positive strand in close proximity to the polymerase and renders the 3′-end of the minus strand free and single-stranded in close proximity to the template entrance (A). The 'loop model' suggests that duplications occur at the transcription step. After the 3′ end of the negative strand has passed through the polymerase catalytic core, it reattaches with the positive capped strand, still attached to the CAP-binding site, leaving the 3′-end of the negative strand free for the next round of transcription (A, B, and C). The 'loop model' suggests that this 3′-end of the negative strand is inserted again in the template entrance before the complete gene is transcribed, forming a loop (D). When the 3′-end of the negative strand reaches the center of the λ3 polymerase, the catalytic core sooner or later switches template (E), transcribing again a more upstream portion of the negative strand, this way forming the duplication (E, F, and G).

28]. Molecular methods revealed gene rearrangements in a head-to-tail fashion for gene segments encoding NSP1 [10,29,30], VP6 [31], NSP3 [9,10,32], NSP4 [33], and NSP5 [7,9,11,34–37]. Such rearrangements have also been observed for other members of the *Reoviridae* family (Table 1). Head-to-tail duplications have been described in the S12 gene of rice dwarf virus (phytoreovirus) [14], in RNA 10 of bluetongue virus, and in RNA 9 of Bunyip creek virus (orbiviruses) [13]. Personal communication between Desselberger and Joklik also states that genome rearrangements may occur in members of the Orthoreovirus genus [12]. Different mechanisms have been suggested to explain the occurrence of partial duplications in rotaviral genes. In the 'transcriptional slippage' model, it is proposed that the RdRp slips backwards on the template at a certain point and starts transcribing again at a more upstream point proceeding to the 3′-end of the RNA [37]. The 'copy choice' model suggests that the RdRp detaches from the template strand and jumps to another template strand before beginning to transcribe again [37]. Shen et al. [31], and Kojima et al. [10,38] suggested that direct repeats near the break points of the duplications might have played a role in the occurrence of duplications. Gorziglia et al. [36], and Gault et al. [9] proposed that formation of intragenic secondary structures might cause the RdRp to make a mistake and continue at a place more upstream on the template. However, none of these models has been fully satisfactory to explain the occurrence of all the observed duplicated genes. Our loop model is a plausible explanation for the occurrence of duplications in genes of viruses containing a dsRNA genome. Although the template entrance is not meant to fit two negative strands, small changes in the tertiary protein folding structure, or certain amino acid substitutions in the RdRp might allow the 3′-end of the negative strand to be inserted again into the template entrance, and the loop phenomenon to occur. Patton et al. [39] showed that apart from the 5′-and 3′-untranslated regions, the ORFs of rotaviral genes also contain *cis*-acting signals that promote minus strand synthesis during the assembly of new virions. The duplication of these *cis*-acting signals might explain the fact that several rotavirus strains with duplicated genes might coexist with, or even have a replication advantage compared to strains without the duplication [39,40]. This article introduces a new testable model about the occurrence of partial gene duplications in multisegmented dsRNA viruses into the literature, and provides a good starting point for further structural analyses of RdRps from different members of the *Reoviridae* family.

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