NCp7 activates HIV-1RNA dimerization by converting a transient loop-loop complex into a stable dimer*

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Nucleocapsid protein 7 (NCp7), the human immunodeficiency virus type 1 (HIV-1) nucleocapsid protein, was shown to strongly potentiate the dimerization of the retroviral genomic RNA. This process involves the interaction of two retroviral RNA monomer subunits near their 5′-ends. A region located upstream from the splice donor site was recently identified as being responsible for the formation of dimeric HIV-1 RNA. This region appeared to be confined within a stem-loop structure, with an autocomplementary sequence in the loop. In an in vitro study of spontaneous dimer formation, we reported that the 77–402 RNA transcript forms two distinct dimers differing in their thermostability: D37 and D55. We identified D37 as a “kissing” complex structure, formed via a loop-loop interaction between the two monomers, and D55 as a double stranded structure involving all nucleotides of the stem-loop via canonical base pairing. In this report, we have characterized the role of NCp7 in the HIV-1Lai RNA dimerization process by using in vitro dimerization assays with RNA transcripts of different lengths and dimer thermal dissociation. Our results show that the nucleocapsid protein NCp7 activates RNA dimerization very likely in interaction with the kissing complex and converts it into a stable dimer. Furthermore, this NCp7-promoted conversion only occurs if the 240–280 stem-loop structure is present in HIV-1Lai RNA molecules and contains the autocomplementary C258CGCGC262 sequence. This study suggests that, under physiological conditions, an NCp7-mediated RNA conformational change is involved in the maturation of the HIV-1 RNA dimer.

Retrovirus virions carry a diploid genome consisting of an RNA complex formed by the association of two identical unspliced viral RNA molecules (1). In mature virions, RNA molecules are tightly associated with viral finger protein molecules, nucleocapsid proteins (NCps)‡ (2, 3). Retroviral NCp is generated once the gag gene product, the structural Pr55gag protein, has been processed by the viral protease (4) and is highly conserved among all known retroviruses (5). The HIV-1 NC protein (NCp7) is a small basic protein with two zinc fingers of the C2XCX3HXC4 form, flanked by regions rich in basic residues (5). Zinc fingers were found to bind zinc with high affinity (6) through a tetracoordinated complex involving the three cysteine residues and the histidine residue (7). Nuclear magnetic resonance revealed the three-dimensional structure of NCp7 (8, 9), and the zinc motifs were found to be in close proximity due to the presence of the histidine residue and to the conformation of the basic linker PRKKG35 (10–12). Point mutation of the conserved cysteine and histidine residues resulted in a drastic reduction of genomic RNA packaging (13–15). Moreover, mutation affecting NCp7 folding impairs the co-assembly of Gag and Gag-pol precursors (10, 16) as well as the protection of genomic RNA (11), and this results in a drastic decrease in viral infectivity. In vivo, NCp7 was shown to interact, at the 5′-end of genomic RNA, with a sequence referred to as the ψ sequence, located by directed mutagenesis between the primer binding site (PBS) and AUG of the gag gene (14). Chimeric viruses containing a nonhomologous ψ sequence and an NC-coding gene were noninfectious, suggesting a highly specific recognition process (17).

Previous works have reported in vitro studies on NCp7 and have revealed its nucleic acid binding—especially to the ψ sequence (18)—and annealing activities (2, 19–26). NCp binds preferentially to single-stranded nucleic acids with a high affinity for viral RNA (1). Thus, NCp seems to play a key role in several steps of the viral life cycle. During reverse transcription, NCp stimulates RNA primer annealing to the PBS at the 5′ end of the viral RNA (19–21) and also participates in the initial strand transfer event required for long terminal repeat synthesis (22–24). NC protein has also been shown to activate retroviral HIV-1 RNA dimerization in vitro (2, 21, 27), which results in the formation of the dimer linkage structure. The dimer linkage structure, defined by electron microscopy, is an RNA-RNA interaction site located in the ψ sequence close to the 5′-ends of viral RNA molecules (28, 29). The nature of the base-pairing interactions of the dimer linkage structure has been studied by analyzing spontaneous RNA transcript dimerization in vitro (2, 27). Primary results were in favor of the hypothesis that HIV-1 RNA dimerization involves purine-rich sequences, which should form noncanonical base pairs (30–32). However, additional studies indicated that such sequences are dispensable for RNA dimerization of HIV-2 (33), HIV-1 (34, 35), and Moloney murine leukemia virus (36). Another model was thus proposed to explain the dimerization process of viral RNA. HIV-1 (35, 37–39), Moloney murine leukemia virus (36), and avian leukosis virus RNA transcripts, which contain an autocomplementary sequence in a stem-loop structure, located downstream from the PBS and upstream from the splice donor site, are able to dimerize spontaneously in vitro. It has been proposed that HIV-1 RNA dimer formation is initiated by the annealing of these autocomplementary sequences via a loop-

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1 The abbreviations used are: NCp, nucleocapsid protein; HIV, human immunodeficiency virus; Tm, melting temperature; nt, nucleotide; DEL, deletion.

2 P. Fossé, unpublished observations.
loop interaction between the two RNA molecules, resulting in the formation of a “kissing” complex at 37 °C (39). This intermediary complex should spontaneously evolve at 55 °C toward a more stable dimer involving all the nucleotides of the stem-loop structure in canonical base pairs (39). However, the role of the NC protein in this process has not yet been investigated.

The ability of the NC protein to destabilize nucleic acid structures (41) and to promote nucleic acid annealing and renaturation (42) should be taken into account when explaining RNA dimerization in the presence of the protein. In this report, we analyze the role of a synthetic NCp7 in the in vitro dimerization process of HIV-1RNA transcripts.

MATERIALS AND METHODS

Molecular Clones—Details of the pDM2, pDM3, pDM6, and pDM7 plasmid constructions are given elsewhere (35).

RNA Transcripts 77–402, 224–402, 224–296, 296–402, and 224–402Del—HaeIII and RsaI restriction enzymes were obtained from New England Biolabs. The pDM3 plasmid was digested by HaeIII and transcribed by T7 RNA polymerase and gave rise to a transcript starting from position 77 of the genomic HIV-1RNA sequence and ending at position 402. This transcript will be referred to as RNA 77–402 (Fig. 5).

The pDM2 plasmid was digested by either HaeIII or RsaI and transcribed by T7 RNA polymerase and gave rise to transcripts starting from position 296 of the genomic HIV-1RNA sequence and ending at positions 402 and 296, respectively. These transcripts will be referred to as RNA 224–402 and RNA 224–296 transcripts (Fig. 5).

The pDM6 plasmid was digested by HaeIII and transcribed by T7 RNA polymerase and gave rise to transcripts starting from position 296 of the genomic HIV-1RNA sequence and ending at positions 402. This transcript will be referred to as RNA 296–402 (Fig. 5).

The pDM7 plasmid, first digested by HaeIII, was transcribed with T7 RNA polymerase and gave rise to RNA transcript 224–402Del, devoid of nucleotides 257–266 (Fig. 5). In Vitro RNA Synthesis and Purification—Experimental procedures for the HIV-1RNA transcript production and purification are given elsewhere (35, 39).

Synthetic Nucleoside Protein—Solid phase synthesis of HIV-1 NCp7 protein, consisting of 72 amino acids, was performed as described previously (43).

Stock solutions of NCp7 were conserved at 10−3 M and 4 °C in Milli-Q water (Millipore) containing three equivalent molar concentrations of ZnSO4.

HIV-1 RNA Transcript Dimerization Assays in the Presence of the NCp7—In a standard dimerization assay, RNA in 14 μl of Milli-Q water was heated for 2 min at 90 °C, chilled on ice for 2 min, and adjusted to 4 μl with 4 μl of 5 × NC buffer containing 100 mM Tris-HCl, pH 7.5, 250 mM NaCl, 25 mM dithiothreitol, and 1 mM MgCl2 and 2 μl of 10 × NCp7 solution, i.e. 17.5 protein molecules/RNA strand, (or poly-l-lysine) or polyethylene glycol 8000 or 2 μl of Milli-Q water). Poly-l-lysine and polyethylene glycol 8000 were purchased from Sigma.

The samples were incubated for 15 min at 37 °C or at temperatures ranging from 10 to 60 °C. At the end of incubation, all the samples were cooled on ice for 2 min, placed for 2 min at room temperature, and then mixed with 10 μl of water and 3 μl of 5% SDS for 5 min more at room temperature. The RNA transcripts were phenol extracted for deproteinization with 30 μl of phenol saturated by 40 μl Tris-HCl, pH 6.5, 0.1% SDS, and 2.5 mM EDTA. Three μl of loading buffer (50% (w/v) glycerol and 0.025% (w/v) tracking dyes) were then added, and the samples were loaded on a 1.5% Sea Kem agarose gel and electrophoresed at 5 V/cm and 4°C in buffer containing 50 mM Tris-borate, pH 8.3, 1 mM EDTA, and 0.2 μg/ml ethidium bromide. Monomeric RNA transcripts were obtained at 20°C in a buffer containing 10 mM Tris-HCl, pH 7.5.

Melting Temperature (Tm) Determination of Dimer Dissociation—After denaturation at 90 °C for 2 min in nuclease-free water, the RNA was incubated in a buffer containing 50 mM Tris-HCl, pH 7.5, and 100 mM NaCl (dimer buffer) at a strand concentration of 0.8 μM at either 37 °C or 55 °C for 90 min for optimal dimerization (35). The resulting dimer, D37 (unstable dimer) and D55 (stable dimer), were then dialyzed (Millipore V6 filters, 0.025 μm) for 2 h at 4°C against 1 × NC buffer containing 20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 5 mM dithiothreitol, and 0.2 mM MgCl2. Unstable dimer was then incubated in the presence of NCp7 (i.e. 1 molecule of the protein per 20 nt of RNA 77–402) for 15 min at 37°C. Both dimers were then treated with SDS-phenol, as described above, for deproteinization, and 20-μl aliquots of each sample were then incubated for 5 min at varying temperatures and from 10 to 65°C and electrophoresed as described above.

After fluorescent scanning of the gels using a Biozprofiler apparatus (Vilber Lourmat, Marne La Vallee, France), the percentage of dimer and monomer was estimated. The percentage of the dimer was defined as the area of the dimer peak divided by the sum of the areas of monomer and dimer peaks. The Tm was estimated from the plot of the amount of the dimer as a function of temperature.

RESULTS

NCp7 Activates HIV-1 RNA Dimerization—In vitro dimerization of the HIV-1RNA 77–402 transcript was performed in the presence of varying amounts of NCp7. After dimerization, the dimer was deproteinized and analyzed by agarose gel electrophoresis. Fig. 1A shows that although the RNA 77–402 transcript incubated alone migrates mainly as a monomer (lane D, around 15–20% of dimer), in the presence of NCp7, the monomer disappears and the RNA dimer together with a small amount of higher order multimers (lanes 1/20, 1/10, 1/5, and 1/2). It should be noted, for purposes of comparison, that the dimer samples obtained in the presence or absence of the protein were submitted to the same deproteinization step. Under these conditions, NCp7 activates RNA 77–402 dimerization, and this activation is almost complete for an NCp7 to RNA ratio of at least 1 NCp7 molecule/20 nt (Fig. 1A, lane 1/20). This result is in accordance with several previous works in which retroviral NC was found to activate retroviral RNA dimerization in vitro (2, 21, 27).

The specificity of this activation process was tested by using poly-l-lysine instead of NCp7 in the same dimerization assay. This peptide, a highly basic polypeptide, was unable to activate RNA 77–402 dimerization efficiently (Fig. 1B, lanes 1/20, 1/10, and 1/5); the percentage of dimer remained the same as in the RNA control (Fig. 1B, lane D). However, multimers were induced by poly-l-lysine (Fig. 1B, lanes 1/20, 1/10, and 1/5). This could result from electrostatic binding of the basic polypeptides to the RNA molecules. In the case of NCp7, at ratios higher than 1/50 protein/nt, such a multimerization occurs (Fig. 1A, lanes 1/20, 1/10, 1/5, and 1/2), but we observed a difference between the migration of the multimers formed in the presence of NCp7 and the migration of those formed in the presence of poly-l-lysine. This difference could be due to the fact that in the presence of poly-l-lysine, we observed an aggregation process leading to trimers, tetramers, pentamers, etc., whereas in the presence of NCp7, the multimers seem to correspond to multimerization of dimers leading to tetramers and even higher order structures, i.e. hexamers and octamers.
Monomer, dimer, and multimers of RNA 77–402 are indicated. The kissing complex, resulting from the loop-loop interaction of the RNA, was termed D37 and identified as an unstable process (39), and, as previously shown, one of these dimers, characterized the spontaneous polyethylene glycol 8000 (PEG8000) in the samples. RNA 77–402 can be seen in a monomeric form at 20 °C in a buffer containing 10 mM Tris-HCl, pH 7.5 (lanes M). Samples, after treatment with SDS-phenol, were analyzed by ethidium bromide-stained 1.5% agarose gel electrophoresis. Monomer, dimer, and multimers of RNA 77–402 are indicated.

Also, we observed a decrease of multimer content (at least from ratio 1/20 to 1/5; Fig. 1A), which may be due to the fact that as the concentration of NCp7 increases, the protein coats the RNA, and the interaction between RNA molecules may not be as efficient. We also tested the influence of polyethylene glycol on the dimerization process, since polyethylene glycol is known to enhance the rate of hybridization of nucleic acids (46). Increasing amounts of polyethylene glycol did not have a detectable effect on RNA 77–402 dimerization (Fig. 1C). In the presence of NCp7, dimerization was complete after 5 min at 37 °C, and no kinetic profile was observed whatever the protein concentration. These results are in accordance with those presented by Darlix and co-workers (2) with the NCp15 protein and indicate that NCp7 probably does not function in a catalytic manner.

Effect of the NCp7 on the Dimerization Process as a Function of the Temperature—We analyzed the effect of the temperature on spontaneous in vitro dimerization of HIV-1_Lai RNA 77–402 (Fig. 2A). On incubation in the NC buffer, spontaneous dimerization of RNA 77–402 occurred. Up to 37 °C, about 60% of the RNA was dimeric (Fig. 2A, lanes 20, 30, and 37), whereas between 45 and 50 °C, this dimer dissociated (Fig. 2A, lanes 45 and 50). This dissociation led to the formation of two monomeric conformers (m and m'), the nature of which remains unknown. For temperatures higher than 50 °C, some dimer reappeared when m' disappeared (Fig. 2A, lanes 55 and 60). The same results were observed in dimer buffer while characterizing the spontaneous in vitro RNA 77–402 dimerization process (39), and, as previously shown, one of these dimers, formed at 37 °C, was termed D37 and identified as an unstable kissing complex, resulting from the loop-loop interaction of the complementary sequence (39). The other dimer, formed at 55 °C, was termed D55 and corresponded to a more stable double-stranded structure.

Samples required SDS-phenol treatment, before gel analysis, in the presence of the NCp7 protein. Thus, RNA 77–402 dimerization was investigated as a function of the temperature in the absence of NCp7 but in NC buffer followed by SDS-phenol treatment (Fig. 2B). Under these conditions, dimerization, observed without SDS-phenol treatment between 20 and 37 °C (Fig. 2A), no longer occurred (Fig. 2B, lanes 20 and 37). This suggests that the kissing complex was not stable enough to resist such treatment and that only the dimer formed at 55 °C was stable enough to remain intact (Fig. 2B, lanes 55 and 60).

Then, the experiment was performed in the presence of the NCp7 nucleocapsid protein to observe the effect of this protein on dimer formation (Fig. 2C). NCp7 promoted complete RNA 77–402 dimerization at temperatures ranging from 30 to 60 °C (Fig. 2C). Dimer instability, triggered with SDS-phenol treatment (Fig. 2B) and occurring in the 45–50 °C range (Fig. 2A), had disappeared in the presence of the protein (Fig. 2C). This could signify that NCp7 promotes the formation of a unique dimer able to support depolymerization (Fig. 2C, lanes 20–60) and strongly suggests that the dimer formed in the presence of NCp7 is the same as the one observed at 55 °C in its absence. We were eager to check whether NCp7 was capable of converting the unstable kissing complex into the stable dimer.

NCp7 Converts an Unstable Dimer into a Stable One—The thermal stability of the dimer formed at 55 °C, in the absence of the protein, was compared with that of the kissing complex submitted to NCp7 activity (Fig. 2). The unstable dimer, D37, and the stable dimer, D55, were formed in the dimer buffer and then dialyzed against the NC buffer so that experiments in the presence of the protein could be performed. The unstable dimer, D37, was incubated with NCp7 for 15 min at 37 °C, but not the stable one. Both dimers were then submitted to depolymerization by SDS-phenol treatment before the thermal denaturation experiment. Agarose gels corresponding to these experiments.
NCp7 activates HIV-1 RNA dimerization in vitro. The NCp7 protein (Fig. 4) interacts with RNA transcripts to promote dimerization. When RNA transcripts 224–402 and 224–296 were tested for dimer formation in the absence or presence of NCp7, the autocomplementary sequence in the monomeric form (Fig. 4A, lane 257B) and oligomer 257M were identified. The results show that NCp7 activates HIV-1 RNA dimerization in vitro. The NCp7 protein is involved in activating HIV-1 RNA dimerization in vitro.

DISCUSSION

This work aimed at elucidating the role of NCp7 in the in vitro dimerization process of HIV-1 RNA. We have previously proposed a model of spontaneous in vitro dimerization of transcript RNA 77–402 under conditions of low ionic strength (39). This model, which has been confirmed by another recent study (49), suggests the formation of a kissing complex at 37 °C, which is converted into a stable dimer only if the temperature is increased to 55 °C. Those two entities, D37 and D55, are involved in activating HIV-1 RNA dimer formation, and we have proposed NCp7 as the trigger provoking the conformational change that causes the kissing complex to be transformed at 37 °C into the stable double stranded dimer (Fig. 6).

We report here that NCp7 was capable of activating RNA 77–402 transcript dimerization (Fig. 1), within a 20 to 60 °C range, at 50 mM NaCl, pH 7.5 (Fig. 2C). These results confirm that NC protein from HIV-1 activates HIV-1 RNA dimerization.
in vitro (2, 21) at NC/RNA ratios equal to or higher than 1/50. When the NC/RNA ratio reaches 1/20, NCp7-promoted dimerization is complete (Fig. 1A). The fact that NCp7 was inactive at temperatures lower than 20°C (Fig. 2C, lanes 10 and 20) could be due to the very strong stability of the stems in the kissing complex, suggesting that the protein would be incapable of destabilizing these stems at temperatures lower than 20°C.

More important is the finding that, at 37°C, NCp7 is able to convert the unstable dimer, corresponding to the kissing complex previously described (39), into a stable dimer (Fig. 3), the stability of which corresponds to that of D55. This suggests that NCp7 interacts with the kissing complex to allow this conversion, which does not spontaneously occur at 37°C without the protein. The recognition of such a loop-loop complex by NCp7 is a process comparable with that found in the Rom protein. This bacterial protein facilitates sense-antisense RNA pairing by binding to the transiently formed hairpin pairs of RNA I and complementary RNA II (50, 51).

NCp7-dependent dimerization of HIV-1 RNA has already been investigated. Sakagushi et al. (27) described a specific nucleotide region required for NCp7 binding and RNA dimerization, which is the same as the one previously described by Darlix et al. (2). However, the model proposed by Sakagushi et al. (27) is not in accordance with the data presented here, since RNA 296–402, which corresponds to RNA 311–415 of HIV-1\textsubscript{Mal} described by Darlix et al. (2) and which contains the 44-nt RNA described by Sakagushi et al. (27), is unable to dimerize significantly with or without NCp7 (Fig. 5). In contrast, dimerization mediated by NCp7 of HIV-1\textsubscript{Mal} RNA 1–415, described by De Rocquigny et al. (21), is in accordance with our results. This RNA transcript contains the entire sequence of the corresponding HIV-1\textsubscript{Lai} RNA 77–402 transcript.

We found that the autocomplementary G\textsuperscript{297}CGCG\textsuperscript{202} sequence in stem-loop structure 240–280 (Fig. 6, Monomer), is necessary for HIV-1\textsubscript{Lai} RNA dimer formation in the presence of NCp7 (Fig. 5), and that NCp7 is able to promote RNA 77–402 dimerization at 50°C, whereas in the absence of the protein, no dimer is formed (Fig. 2). Because the RNA annealing between the two monomers happens spontaneously without NCp7 (Fig. 2A), these results are more in favor of a specific action of NCp7 on the kissing complex to convert it into a stable dimer rather than in promoting the loop-loop interaction driven by the GCGCG sequence and the stem-loop structure of the RNA molecule. However, considering our results, we cannot definitively exclude that Ncp7 might promote the formation of the stable dimer directly from the monomer (Fig. 6, Monomer → D55). To determine whether NCp7 would specifically recognize the 240–280 stem-loop structure, we tried to identify a site on the protein exhibiting a high affinity for the 240–280 stem-loop
structure by gel retardation assay of NCp7 binding to the HIV-1 RNA transcript. No significant difference was found in NCp7 binding affinity to RNA 224–402 and RNA 224–402DEL (data not shown). However, both RNA transcripts had the NC binding site described by Sakagushi et al. (27) in their sequences. At the present time, experiments with shorter or mutated RNA transcripts are under way with gel retardation assays.

NC protein was reported to possess nucleic acid unwinding (41, 52) and renaturation (42) activities. Thus, RNA-RNA recognition would first occur via the autocomplementary G\textsuperscript{527}CGGGC\textsuperscript{529} sequence to form the kissing complex, and then, once bound to this transient complex, NCp7 would open both stems of the complex to permit the formation of the double stranded dimer (Fig. 6). Both activities of the protein are required to explain a RNA dimerization specifically activated by NCp7, since no other proteins, at least those known for their nucleic acid-annealing activity, are able to promote this process (2, 26). As described previously (39), in the absence of the protein, conversion of D37 into D55 is only possible when the temperature is increased to above 55°C. In contrast, at 37°C, NCp7 alone is capable of overcoming this major energy barrier and converting the kissing complex into the stable dimer. In light of these findings, we can propose the model described in Fig. 6.

As already reported, an autocomplementary sequence located in the loop of such a stem-loop structure has already been found downstream from the PBS of several other retroviral RNA genomes (36, 53). This strongly supports the concept that all retroviruses could have the involvement of this RNA structure in their dimerization process in common. If this were true, NC proteins of other retroviruses could be proposed as having the same mechanism of action as NCp7 does on HIV-1\textsubscript{Lai} RNA dimerization. Girard et al. (54) have just reported that NCp10 activates in vitro dimerization of Moloney murine leukemia virus RNA transcripts, which contain the autocomplementary stem-loop structure 283–298. Furthermore, the nucleocapsid proteins of various species could be exchangeable for this process, since NCp10 of Moloney murine leukemia virus and NCp12 of Rous sarcoma virus are capable of promoting HIV-1 RNA dimerization in vitro (2).

It has been suggested that the dimerization and encapsidation processes are linked, since encapsidation of the retroviral genome is governed by specific interactions between the NC domain of gag and the encapsidation-dimerization sequence (for review, see Ref. 3). It is noteworthy that the 240–280 RNA stem-loop structure is located within the 5′-RNA-packaging signal of HIV-1 described recently by Clever et al. (40). Given our model, an appealing concept is that the NC protein could be a link between dimerization and encapsidation via the 240–280 RNA stem-loop structure. This structure could be a site for NC binding, among others described (2, 17, 18, 27), and perhaps a determinant for the encapsidation signal.

Our results also suggest that NCp7 may control HIV-1 RNA dimer maturation, and this would extend the role of the NC protein in vitro to one of its functions in the retroviral life cycle. Indeed, it has been reported that the genomic RNA dimer appears to be initially encapsidated in an extended conformation and then adopts a condensed structure (34, 3, and references therein). The authors suggested that dimeric RNA of HIV-1 (34) undergoes a change in conformation, which they termed a maturation event, after releasing the virus from the cell. Such a RNA maturation step would correlate with the cleavage of Gag precursor molecules by the viral protease and therefore with the presence of the processed nucleocapsid protein (34). The same authors found that an immature dimeric RNA isolated from protease-negative mutant virions dissociates into monomers at a lower temperature than the mature wild-type dimer. It has also been reported that the linkage between subunits of B77 sarcoma virus RNA is stabilized as a function of time during extracellular virion maturation (48). In this case, RNA monomers are linked by unstable base pair regions in the immature dimer, which are disrupted during RNA phenol extraction. This observation is reminiscent of that found with the kissing complex, an unstable dimer that dissociates after SDS-phenol treatment and could correspond to such an immature dimer. These results are consistent with our in vitro model of the HIV-1 RNA dimerization process. In the absence of the protein, the HIV-1 RNA transcript forms an unstable dimer, namely the one that totally dissociates at room temperature during phenol extraction (Fig. 2B). Under the effect of NCp7, this dimer then undergoes a change in conformation to become more stable (Fig. 3). This change can be considered the first step of the dimer maturation event if indeed this step corresponds to a stabilization of the 240–280-base pair region (Fig. 6). In this manner, NCp7 would also promote the stabilization of other RNA-RNA interactions occurring along the genomic RNA, which certainly exist during nucleocapsid maturation, once the virus has been released from the cell. Our results lead to a simple model system in which the molecular and structural aspects of the RNA dimerization process and NCp7-mediated RNA maturation can be studied in detail.

However, when we propose that NCp7 plays the same role in vivo as in vitro, we should be aware that we cannot exclude the possibility that in vivo there may be some other accessory factors that could aid in the formation of this stable dimer.

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REFERENCES

1. Coffin, J. M. (1984) in RNA Tumor Viruses (Weiss, R., Teich, N., Varmus, H., Coffin, J., eds) Vol. 1, pp. 261–368, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2. Darlix, J.-L., Gabus, C., Nugeyre, M.-T., Clavel, F., and Barre-Sinoussi, F. (1990) J. Mol. Biol. 216, 689–699
3. Darlix, J.-L., Lapadat-Tapiez, M., de Roqueyng, H., and Roques, B. P. (1995) J. Mol. Biol. 254, 523–537
4. Dickson, C., Eisenman, R., Fan, H., Hunter, E., and Reich, N. (1985) in RNA Tumor Viruses (Weiss, R., Teich, N., Varmus, H., Coffin, J., eds) Part II, pp. 512–648, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
5. Covey, S. N. (1986) Nucleic Acids Res. 14, 623–633
6. Mély, Y., Cornille, F., Fourré-Zaluski, M. C., Darlix, J.-L., Roques, B. P., and Gérard, D. (1991) Biopolymers 31, 899–906
7. Berg, J. (1866) Science 232, 485–487
8. South, T. L., and Summers, M. F. (1983) Protein Sci. 2, 3–19
9. Morellet, N., Julian, N., De Roqueyng, H., Maigret, S., Darlix, J.-L., and Roques, B. P. (1992) EMBO J. 11, 3095–3096
10. Morellet, N., De Roqueyng, H., Mély, Y., Julian, N., Déménez, H., Ottmann, M., Gérard, D., Darlix, J.-L., Fourré-Zaluski, M.-C., and Roques, B. P. (1994) J. Mol. Biol. 235, 287–301
11. Déménez, H., Dong, C. Z., Ottmann, M., Rouyez, M. C., Julian, N., Morellet, N., Mély, Y., Darlix, J. L., Fourré-Zaluski, M. C., Saragosti, S., and Roques, B. P. (1994) J. Mol. Biol. 235, 1174–1176
12. Mély, Y., Julian, N., Morellet, N., De Roqueyng, H., Dong, C. Z., Piemont, E., Roques, B. P., and Gérard, D. (1994) Biochemistry 33, 12085–12091
13. Meric, C., Goilloud, E., and Spahr, M. P. F. (1988) J. Virol. 62, 3328–3333
14. Aldovini, A., and Young, R. A. (1990) J. Virol. 64, 1920–1926
15. Gereckel, R. J., Nigida, S. M. Jr., Bers, J. W., Jr., Arthur, L. O., Henderson, L. E., and Rein, A. (1990) J. Virol. 64, 5207–5211
16. Ottmann, M., Gabus, C., and Darlix, J.-L. (1995) J. Virol. 69, 1778–1784
17. Berkowitz, R. D., Ohagen, A., Hsglund, S., and Goff, S. P. (1995) J. Virol. 69, 6445–6466
18. Damour, J., Buruvoy, A., Jung, G., and Moelling, K. (1994) EMBO J. 13, 1525–1533
19. Prats, A.-C., Sarthi, L., Gabus, C., Litvak, S., Keith, G., and Darlix, J.-L. (1988) J. Mol. Biol. 217, 1777–1783
20. Barat, C., Lullien, V., Schatz, O., Keith, G., Nugeyre, M. T., Grüninger-Leicht, F., Barre-Sinoussi, F., Le Grice, S. F. J., and Darlix J.-L. (1989) EMBO J. 8, 3279–3285
21. De Roqueyng, H., Gabus, C., Vincent, A., Fourré-Zaluski, M.-C., Roques, B., and Darlix, J.-L. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6472–6476
22. Darlix, J.-L., Vincent, A., Gabus, C., De Roqueyng, H., and Roques, B. (1993) C. R. Acad. Sci. Paris 316, 763–771
23. Lapadat-Tapolsky, M., De Rocquigny, H., Van Gent, D., Roques, B., Plasterk, R., and Darlix, J.-L. (1993) *Nucleic Acids Res.* **21**, 831–839
24. You, J. C., and McHenry, C. S. (1994) *J. Biol. Chem.* **269**, 31491–31495
25. Tsuchihashi, Z., and Brown, P. O. (1994) *J. Virol.* **68**, 5863–5870
26. Lapadat-Tapolsky, M., Pernelle, C., Borie, C., and Darlix, J.-L. (1995) *Nucleic Acids Res.* **23**, 2434–2441
27. Sakagushi, K., Zambrano, N., Baldwin, E. T., Shapiro, B. A., Erickson, J. E., Omichinski, J. G., Clare, G. M., Grouenhorn, A. M., and Appela, E. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5219–5223
28. Bender, W., Chien, Y. H., Chattopadhyay, S., Vogt, P. K., Gardner, M. R., and Davidson, N. (1978) *J. Virol.* **25**, 888–896
29. Murti, K. G., Bondurant, M., and Tereba, A. (1981) *J. Virol.* **25**, 888–896
30. Marquet, R., Baudin, F., Gabus, C., Darlix, J. L., Mougel, M., Ehresmann, C., and Ehresmann, B. (1991) *Nucleic Acids Res.* **18**, 2349–2357
31. Awang, G., and Sen, D. (1993) *Biochemistry* **32**, 11453–11457
32. Sundquist, W., and Heaphy, S. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5393–5397
33. Berkout, B., Oude Essink, B. B., and Schoneveld I. (1993) *FASEB J.* **7**, 181–187
34. Fu, W., Gerelick, R. J., and Rein, A. (1994) *J. Virol.* **68**, 5013–5018
35. Muriaux, D., Girard, P.-M., Bonnet-Mathonière, B., and Paoletti, J. (1995) *J. Biol. Chem.* **270**, 8209–8216
36. Girard, P.-M., Bonnet-Mathonière, B., Muriaux, D., and Paoletti, J. (1995) *Biochemistry* **34**, 9785–9794
37. Skripkin, E., Paillart, J. C., Marquet, R., Ehresmann, B., and Ehresmann, C. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 4945–4949
38. Laughrea, M., and Jette, L. (1994) *Biochemistry* **33**, 13464–13474
39. Muriaux, D., Fosse, P., and Paoletti, J. (1996) *Biochemistry* **35**, 5075–5082
40. Clever, J., Sassetti, C., and Parslow, T. G. (1995) *J. Virol.* **69**, 2101–2109
41. Khan, R., and Giedroc, D. P. (1992) *J. Biol. Chem.* **267**, 6689–6695
42. Dib-Hajj, F., Khan, R., and Giedroc, D. P. (1993) *Protein Sci.* **2**, 231–243
43. De Rocquigny, H., Ficheux, D., Gabus, C., Fournié-Zaluski, M.-C., Darlix, J.-L., and Roques, B. (1991) *Biochem. Biophys. Res. Commun.* **180**, 1010–1018
44. Laughrea, M., and Jette, L. (1994) *Biochemistry* **33**, 13464–13474
45. Priel, E., Aflalo, E., Seri, I., Henderson, L. E., Arthur, L. O., Aboud, M., Segal, S., and Blair, D. G. (1995) *FEBS Lett.* **362**, 59–64
46. Feng, Y., Fu, W., Winter A. J., Levin, J. G., and Rein, A. (1995) *J. Virol.* **69**, 2486–2490
47. Laughrea, M., and Jette, L. (1996) *Biochemistry* **35**, 8705–8714