VIROLOGY

An ultraweak interaction in the intrinsically disordered replication machinery is essential for measles virus function

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Measles virus genome encapsidation is essential for viral replication and is controlled by the intrinsically disordered phosphoprotein (P) maintaining the nucleoprotein in a monomeric form (N) before nucleocapsid assembly. All paramyxoviruses harbor highly disordered amino-terminal domains (PNTD) that are hundreds of amino acids in length and whose function remains unknown. Using nuclear magnetic resonance (NMR) spectroscopy, we describe the structure and dynamics of the 90-kDa N0PNTD complex, comprising 450 disordered amino acids, at atomic resolution. NMR relaxation dispersion reveals the existence of an ultraweak N-interaction motif, hidden within the highly disordered PNTD, that allows PNTD to rapidly associate and dissociate from a specific site on N while tightly bound at the amino terminus, thereby hindering access to the surface of N. Mutation of this linear motif quenches the long-range dynamic coupling between the two interaction sites and completely abolishes viral transcription/replication in cell-based minigenome assays comprising integral viral replication machinery. This description transforms our understanding of intrinsic conformational disorder in paramyxoviral replication. The essential mechanism appears to be conserved across Paramyxoviridae, opening unique new perspectives for drug development against this family of pathogens.

INTRODUCTION

Measles virus (MeV) is a nonsegmented negative-sense RNA virus belonging to the family of Paramyxoviridae that includes a number of emerging human pathogens with dangerously high mortality rates for which there is currently no treatment. In addition to the viral polymerase (L), paramyxoviral replication machinery is composed of the nucleoprotein (N), which encapsidates the viral genome, and the tetrameric phosphoprotein (P) (1–3), a cofactor of L that interacts with N at different stages of the viral replication process.

Paramyxoviral P proteins are essential for replication and transcription, interacting with N at different stages of the viral cycle (4–9) and exhibiting very long intrinsically disordered N-terminal domains (PNTD), whose function is not understood (10, 11). The first 40 amino acids of P bind tightly to N that is maintained in a monomeric state (N0P) (12, 13) before encapsidation of the viral RNA to form the nucleocapsid (NC). X-ray structures of heterodimeric constructs of minigenome assays comprising integral viral replication machinery. This description transforms our understanding of intrinsic conformational disorder in paramyxoviral replication. The essential mechanism appears to be conserved across Paramyxoviridae, opening unique new perspectives for drug development against this family of pathogens.

RESULTS

NMR spectroscopy describes the conformational behavior of PNTD

We characterized the conformational behavior of PNTD at the molecular level using NMR spectroscopy (fig. S2). Protein backbone resonances were assigned, and representative multiconformational ensembles were

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generated on the basis of 1H, 15N, and 13C (CO, Cα, and Cβ) backbone chemical shifts, using a combination of flexible-meccano and the genetic algorithm ASTEROIDS (Fig. 1 and fig. S2) (28, 29). The resulting ensemble faithfully predicted experimental ensemble-averaged residual dipolar couplings (RDCs) that are not used in the description (Fig. 1).

This site-specific description of the conformational sampling of free PNTD reveals that α helices that contained in the first 37 residues (α1 and α2), which are involved in formation of the N0P complex, appear as transient helical structures within the unbound protein, as previously observed for vesicular stomatitis virus P (30). Two additional regions have similar or higher propensities to form helices in the unbound PNTD: residues 87 to 93 (α3) and 189 to 198 (α4; Fig. 1A and fig. S2). Spin relaxation reveals increased rigidity in α2, α3, and the hydrophobic region around position 110 that is implicated in the binding of STAT (signal transducers and activators of transcription) (31), while long, highly dynamic segments link α1/2 to α3 and α3 to α4 (Fig. 1).

The highly disordered N0P complex contains a hidden dynamic interaction site with N

We used NMR to investigate the N0PNTD complex by titrating 15N-labeled P1–304 with unlabeled P1–50N1–525 (Fig. 2). P1–304 is able to displace P1–50 from the P1–50N1–525 complex, supporting the observation that this complex can be purified from a mixture of P1–304 and P1–50N1–525 (figs. S3 and S4A). In addition to decreased peak intensities in α1/2, known to engage in the N0P complex (13–15, 32), a 20-residue sequence is found to interact with N, comprising the α4 helical motif. Small local increases in relaxation rates were also observed, centered on aromatic residues F65 and Y110 to Y113, suggesting the existence of additional transient hydrophobic interactions with N within the complex.

Exchange NMR reveals the detail of the additional N interaction site

To further investigate this additional interaction site, intermediate stoichiometric complexes were formed where 15N-labeled P1–304 is in exchange between bound and unbound forms (Fig. 2). While the bound α1/2 region is in slow exchange, manifest through duplication of the peaks on the edges of the helical region (fig. S4, B to D), the region around α4 exhibits chemical exchange kinetics in the millisecond regime between free and bound PNTD, as evidenced by 15N Carr–Purcell–Meiboom–Gill (CPMG) relaxation dispersion (33) measured at substoichiometric ratios (50 μM P1–304 and 25 μM P1–50N1–525; fig. S5).

Using a shorter construct of PNTD (P140–304) that does not contain the N-terminal interaction site, the α4 site is shown to bind independently from α1/2 (Fig. 3 and fig. S5, C and D). This analysis reveals much more detail concerning the exact nature of the interaction motif, particularly in identifying an additional linear motif (181DVETA185) termed δ immediately preceding helix α4, which also participates in the interaction (Fig. 3B). This motif exhibits no detectable intrinsic propensity or secondary structure in the free form of PNTD. 15N relaxation dispersion experiments measured at two magnetic field strengths (600 and 950 MHz) allowed us to estimate the intrinsic dissociation constant (Kd) for the combined δα4 interaction with N0P as 614 ± 218 μM (Fig. 3D). Both α4 and δ motifs have the same effective Kd and exchange kinetics, indicating that they participate in a concerted interaction with N0P. NMR titration studies using the N-terminal region of PNTD, without the δα4 motif (P140–185), show that the transient hydrophobic interaction sites centered on residues 65 and 110 are still present even in the absence of the second binding motif (fig. S6).

Identification of a conserved interaction motif within PNTD helix α4

To delineate the core interaction site involved in δα4 binding to N, we compared the sequences and secondary structure predictions of PNTD from different paramyxoviruses, revealing that a helix resembling α4 is predicted throughout the family (Fig. 4A). A comparison of 259 non-redundant, curated PNTD MeV sequences also shows clear conservation of sequence identity in α1, α3, and the δα4 motif (Fig. 4B), including two
Fig. 2. P$_{1-304}$ interaction with N. (A) $R_2$ of P$_{1-304}$ (gray bars) and P$_{1-304}N_1-525$ (red lines) as purified from a Superdex 200 column (fig. S3) measured at a $^1$H frequency of 950 MHz. (B) Interaction profile of P$_{1-304}$ with P$_{1-50}N_1-525$. Titration admixtures included 25 (gray), 50 (red), 100 (green), and 150 μM (blue) final concentration of P$_{1-50}N_1-525$ and P$_{1-304}$ at a final concentration of 50 μM. Shown are normalized peak intensities ($I/I_0$). (C) Interaction profile of P$_{1-304}$, HEL→AAAA with P$_{1-50}N_1-525$. Concentrations and colors are the same as in (B). (D) $^1$H-$^1^5$N heteronuclear single-quantum coherence (HSQC) spectrum of P$_{1-304}$ in absence (blue) and presence (red) of P$_{1-50}N_1-525$. ppm, parts per million. (E) $^1$H-$^1^5$N HSQC spectra of P$_{1-304}$ (red), P$_{1-50}N_1-525$ (green), and P$_{1-304}N_1-525$ (blue).

Fig. 3. δα$_4$ interaction with N. (A) Intensity ratios of $^{15}$N P$_{140-304}$ peaks extracted from $^1$H-$^{1^5}$N HSQC spectra in the presence of 5% (gray bars), 10% (red), or 20% (blue) P$_{1-50}N_1-525$ with respect to the unbound P$_{140-304}$. Concentration of P$_{140-304}$ remained constant at 200 μM throughout the titration. (B) $R_1$ of P$_{140-304}$ alone (gray bars) and with 20% P$_{1-50}N_1-525$ (blue) recorded at 16.5 T. (C) CPMG relaxation dispersion of $^{15}$N P$_{140-304}$ (200 μM) in the presence of 20% P$_{1-50}N_1-525$. $\Delta R_{2,\text{eff}}$ was determined at 14 T as the difference in effective $R_2$ at CPMG frequencies of 31 and 1000 Hz. (D) CPMG relaxation dispersion of $^{15}$N P$_{140-304}$ (200 μM) in the presence of 20% P$_{10}N_1$N$_5$ at 22.3 T and 14 T. Data from eight sites throughout δ and α$_4$ were fitted simultaneously, assuming a two-site exchange process giving $k_{ex} = 624 \pm 88$ s and population, 4.7 ± 0.5%. Example sites from residues S180 (δ) and L193 (α$_4$) are shown.

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consecutive bulky hydrophobic residues (isoleucine, leucine, or valine). On the basis of these comparisons, the central interaction motif, 191 HELL 194 was mutated (HELL → AAAA). This mutant no longer interacts with N through δ4 (Fig. 2C and fig. S7), although the helical propensity remains intact (fig. S7C). Comparison of MeV P sequences also underlines the strongly conserved acidic nature of the long dynamic loop connecting residues 125 and 170 (comprising 10 conserved acidic side chains and no conserved basic side chains).

The δ4 motif induces a more compact conformation of N0P
We investigated the influence of the HELL → AAAA mutation on the overall dimensions of the P1–304N1–525 complex using SAXS. Ensemble analysis based on SAXS and NMR data from wild-type (WT) and mutated forms reveals that the P1–304N1–525 ensemble requires a bimodal distribution of radii of gyration (fig. S8, A to D), likely due to interactions between δ4 and N. Mutation of the HELL motif leads to significant quenching of the population of more compact conformations, in agreement with the abrogation of binding around δ4. The presence of the 125–amino acid disordered NTAIL domain potentially masks the impact of compaction of N0P on SAXS data. We therefore carried out a similar analysis on N1–405P1–304 (in the absence of NTAIL) further emphasizing the effect of δ4, exhibiting average radii of gyration of WT and HELL → AAAA forms of N1–405P1–304 of 45 ± 6 and 58 ± 8 Å, respectively (fig. S8, E and F).

![Fig. 4. Conservation and functional impact of the δ4 motif.](image-url)

(A) Alignment of phosphoproteins from different related viruses (respective UniProt identifiers are shown on the left, and morbilliviruses are shown above the dashed line). Full PNTD sequences aligned with MUSCLE (35). Positions of the helical elements predicted using PSIPRED (57) are shown as boxes (red, negatively charged; blue, positively charged). (B) Conservation of MeV sequences of PNTD. Comparison of 259 nonredundant sequences curated in Genbank (60). The position of α helices, additional interaction sites δ and α, STAT interaction site, and the RNA editing site defining the start position of the unique domain of V are indicated. Image obtained using WebLogo software (http://weblogo.berkeley.edu/logo.cgi). (C and D) Functional impact of HELL → AAAA mutation in cellulo. (C) Ability of MeV P constructs to associate with MeV N protein in cultured human cells, as determined by Gaussia luciferase–based protein complementation assay. Gray zone indicates the threshold NLR (normalized luminescence ratio) value. (D) Inability of HELL → AAAA P mutant to support the expression of two reporter genes from either a (+) or (−) strand MeV minigenome when coexpressed with MeV N and L proteins using a reverse genetic assay [relative light units (RLU): luciferase reporter activity]. NanoLuc, nanoluciferase.
**Functional relevance of the HELL interaction site in cellula**

The functional importance of the δα4 N\textsuperscript{NP} interaction site for viral function was determined by measuring polymerase replication in vivo using cell-based MeV genome replication assays exploiting reverse genetics with a dual-luciferase reporter (9). In these assays, the viral polymerase function relies on simultaneously providing a host cell with the antigenomic or genomic RNA, N, P, and the polymerase (L), and polymerase-mediated transcription is determined by the quantification of the luciferase activities. Irrespective of whether antigenomic or genomic RNA was provided, luciferase activity observed with 8VVT was completely suppressed when using P\textsubscript{HELL}AAAA (Fig. 4D). All P variants were expressed (fig. S9A), and N:P binding and P oligomerization was unaffected by mutation (Fig. 4C and fig. S9B), indicating that the abrogation of transcription/replication results unambiguously from the absence of the δα4 motif.

**δα4 binds N on its N-terminal lobe**

Although N\textsubscript{CORE} backbone resonances are not observed in \textsuperscript{1}H-15N correlation spectra of N\textsuperscript{NP}, a truncated construct comprising the N-terminal lobe N\textsubscript{STD} (34) was used to identify the interaction site (fig. S10, A and B) that is located in a contiguous region spanning residues 96 to 127, situated on the opposite side of N to the known N\textsuperscript{NP} binding site. The HELL→AAAA mutant of P\textsubscript{140-304} did not interact with P\textsubscript{1-50N1-525} (fig. S7), and the interaction profile of P\textsubscript{140-304} closely resembles that obtained from P\textsubscript{1-50N1-525}, confirming that N\textsubscript{STD} contains the full interaction site for δα4 (Fig. 5).

**DISCUSSION**

Paramyxoviral phosphoproteins are essential for viral replication and transcription, acting as essential cofactors for the polymerase complex. The architecture of paramyxoviral P proteins is highly conserved over Paramyxoviridae, comprising a tetrameric coiled-coil domain and a long intrinsically disordered P\textsubscript{STD}, whose length varies between 215 and 470 amino acids. Although the first 40 N-terminal amino acids are known to bind N\textsuperscript{2}, the role of the remainder of P\textsubscript{STD} has remained unknown, and no rationale exists for the conserved requirement of these long unfolded chains in all paramyxoviral P proteins.

We used NMR spectroscopy to investigate the conformational behavior of MeV P\textsubscript{STD} in its free state and to describe the structural, dynamic, and kinetic behavior of this highly disordered 90-kDa complex. Although unfolded, P\textsubscript{STD} exhibits transient helical propensities in the N-terminal N binding site (δα1/2), around residues 87 to 93 (α3) and 189 to 198 (α4), linked by long, highly dynamic segments (Fig. 1).

In addition to the known N-terminal binding concerning the first 40 amino acids of P\textsubscript{STD}, a 20-residue sequence, comprising the α4 \textsuperscript{191}HELL\textsuperscript{194} motif, preceded by the additional unstructured motif δ, also interacts with N. The HELL interaction motif, as well as the helix itself, appears to be conserved within morbilliviruses and representative interaCTS with N. The HELL interaction motif, as well as the helix itself, and the presence of two N binding sites on P\textsubscript{STD} helps to rationalize the presence of long intrinsically disordered P\textsubscript{STD} domains (11) throughout the paramyxoviral family. The length of the long dynamic linker connecting the two interaction sites provides sufficient degrees of freedom to sample very different conformations and, in particular, to allow for significant conformational disorder within the complex (Fig. 5), even when both sites are occupied. The relative weakness of the δα4 interaction, therefore, leads to rapid exchange between more voluminous free and more compact bound forms of P\textsubscript{40-300} while δα1/2 remains bound. When simultaneously bound to N, the δα1/2 and δα4 binding sites are positioned at opposite extremities of the N\textsubscript{CORE} structure, so that the conformational fluctuations of the acidic loop between the binding sites on P\textsubscript{STD} are able to maximally frustrate access to the surface of N, potentially inhibiting interaction with RNA and consequent self-assembly with other N monomers as well as interactions with host proteins that may play a role in the immune response (39).

The δα4 interaction is abrogated by mutation of four central residues (\textsuperscript{191}HELL\textsuperscript{194}) of the α4 motif, although its helical propensity is maintained under alanine mutation, demonstrating that its helical nature alone is not sufficient to ensure interaction. Mutation of α4 also removes
the more compact, enwrapped form of N0P from the equilibrium as shown from small-angle scattering. We demonstrated the importance of the $\delta\alpha_4$ interaction site for viral function by measuring polymerase replication in vivo using recombinant minigenome assays. Only the N-genomic RNA NC can be used as a template, so that the observed lack of reporter gene expression from the genomic (− strand) demonstrates that the HELL $\rightarrow$ AAAA mutation prevents assembly of N and RNA into a functional NC and subsequent transcription. The possibility that HELL $\rightarrow$ AAAA specifically targets replication and not transcription is supported by the previous demonstration that a P$_{NTD}$-deleted P protein remains active in transcription in the closely related Sendai paramyxovirus (40).

Despite its weak affinity, the $\delta\alpha_4$ interaction is therefore essential for viral function, reinforcing recent observations of the importance of ultra-

weak interactions for intrinsically disordered protein function, as illustrated for example in the case of linear motifs present in the nuclear pore complex (41). In the case of N0P, the combination of distinct affinities on P$_{NTD}$ regulated by its highly disordered nature, coordinates allosteric coupling between the two interaction sites linking opposite ends of N$_{CORE}$. The bipartite N:P$_{NTD}$ interactions may also facilitate NC assembly or provide the molecular basis of formation of organelles that stimulate viral replication (42). We note that the observations made here in the context of P$_{NTD}$ are potentially equally valid for the genome-edited viral protein V (43–46) that comprises P$_{NTD}$ until the editing site (residue 229), followed by a folded zinc finger domain, which is unique to V.

In summary, an atomic resolution description of the structure and dynamics of the highly disordered 90-kDa N0P complex of MeV, comprising 450 intrinsically disordered amino acids, reveals the presence of
a hitherto unidentified N interaction site, 150 amino acids distant from α₁/₂ that is essential for viral function. The newly identified motif exhibits an intrinsic affinity that is remarkably low but nevertheless specific, constraining the unfolded chain to rapidly interchange between different states while bound at the N-terminal site, thereby affording protection against N-RNA, N-N, or N–host factor interactions. We discover that this linear motif is essential for MeV replication and transcription, opening up new perspectives for drug development against these increasingly important human pathogens. Paramyxoviruses share similar replication machinery with filoviruses, including Ebola, suggesting that the discovery of this essential allosteric mechanism may have yet further-reaching consequences for human health.

**MATERIALS AND METHODS**

**Cloning, protein expression, and purification**

N⁰ constructs comprising full-length P⁰ were generated by purifying a heterodimeric N₁-525P₁-304 complex with a TEV (tobacco etch virus) cleavage site between the two proteins, similar to the production of the shorter P₁-50N₁-525 described previously (20), or by mixing TEV-cleaved P₁-50N₁-525 with P₁-304 at equal concentrations and subsequent purification of the P₁-304N₁-525 complex by size exclusion chromatography (SEC; fig. S3). TEV cleavage was performed before a final SEC (Superdex 200, GE Healthcare) in NMR buffer [50 mM Na-phosphate (pH 6), 150 mM NaCl, and 2 mM dithiothreitol (DTT)]. P₁-304 was cloned into PET41c(+) between Nde I and Xho I sites of the insert, yielding a construct with a C-terminal 8His-tag. P₁-304N₁-525 at 1H frequencies of 600 and 950 MHz under otherwise the same conditions as for P₁-304. Data recorded at both fields were fitted together using ChemEx (https://github.com/gbouvignies/chemex) and a two-state exchange model. The Kᵣ value was calculated from the concentrations of P₁-340 and P₁-50N₁-525 as well as the fraction of bound P₁-340 (pₐ), as obtained from the fit and assuming a 1:1 binding stoichiometry. The error of the Kᵣ value was estimated by error propagation from the fitting error of pₐ and assuming a 5% error in concentration determination of both proteins. Nonoverlapped residues 180, 181, 182, 184, 185, 193, 196, and 199 covering both δ and α₄ were included in the fit.

**Small-angle x-ray scattering**

SAXS experiments were performed on P₁-304N₁-525 complexes purified, as described above. Samples were adjusted to three different concentrations between 0.25 and 3.5 mg/ml and were measured at 20°C on the BM29 beamline at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. Scattering was recorded at a wavelength of 0.992 Å, and samples were exposed 10 times for 2 s. All frames were analyzed for radiation damage and excluded if necessary. All other frames of sample and buffer were averaged respectively, and buffer scattering curves were subtracted from the scattering curves of the samples.

**Ensemble description of P₁-304 and P₁-304N₁-525**

A statistical coil ensemble of P₁-304 comprising 10,000 structures was generated using flexible-mecanno. Two hundred conformations that best described the experimentally obtained N, HN, CA, CB, and CO chemical shifts were selected from the ensemble using the genetic algorithm ASTEROIDS. A new ensemble of 8500 conformers was calculated from the concentrations of P₁-340 and P₁-50N₁-525 as well as the fraction of bound P₁-340 (pₐ), as obtained from the fit and assuming a 1:1 binding stoichiometry. The error of the Kᵣ value was estimated by error propagation from the fitting error of pₐ and assuming a 5% error in concentration determination of both proteins. Nonoverlapped residues 180, 181, 182, 184, 185, 193, 196, and 199 covering both δ and α₄ were included in the fit.

**NMR experiments**

Unless otherwise noted, all experiments were acquired in NMR buffer [50 mM Na-phosphate (pH 6), 150 mM NaCl, and 2 mM DTT] at 25°C. The spectral assignments of the different ¹³C- and ¹⁵N-labeled P constructs were obtained using sets of triple resonance experiments correlating Cα, Cβ, and CO resonances at a ¹H frequency of 600 MHz. N₁-261 was assigned as a ¹³C-, ¹⁵N-, and ²D-labeled sample using band-selective excitation short transient–transverse relaxation–optimized spectroscopy (BEST-TROSY) triple-resonance experiments recorded at a ¹H frequency of 700 MHz (47). The spectra were processed with NMRPipe (48), and automatic assignment was performed with the program MARS (49) and manually verified. Secondary chemical shifts were calculated using the random coil values from refDB (50). For the measurement of RDCs, ¹³C, ¹⁵N-labeled P₁-304 was aligned in polyethylene glycol (PEG) and 1-hexanol, yielding a D₂O splitting of 25 Hz (51). RDCs were measured using BEST-type HNCO experiments that allow for spin-coupling measurements in the ¹³C dimension at a ¹H frequency of 800 MHz (52).

Interactions between different proteins were performed as described in the main text and figures. Peak intensities (I) and ¹⁵N as well as ¹³C chemical shifts were extracted from ¹H-¹⁵N HSQC or ¹H-¹⁵N TROSY spectra (for N₁-261). Combined chemical shift differences (ΔCS) were calculated as

\[
\Delta CS = \sqrt{\left(\delta^{1H} - 6.5\right)^2 + \left(\delta^{15N}\right)^2}
\]

For the measurement of RDCs, ¹³C, ¹⁵N-labeled P₁-304 at 5 to 10 delays between 0 and 1.71 s. ¹⁵N relaxation rates were obtained by sampling the decay of magnetization using 5 to 10 delay times between 0.001 and 0.25 s. The spin-lock field was 1500 Hz, and Rₛ was calculated from R₁ and R₁p, considering the resonance offset (53).

¹⁵N relaxation dispersion was measured at a P₁-304 concentration of 50 μM in the presence of 25 μM unlabeled P₁-50N₁-525 at 600 Hz and using 14 points at CPMG frequencies between 31 and 1000 Hz using a constant-time relaxation of 32 ms (33). ¹⁵N relaxation dispersion of P₁-340 was measured at a concentration of 200 μM in the presence of 40 μM P₁-50N₁-525 at ¹H frequencies of 600 and 950 MHz under otherwise the same conditions as for P₁-304. Data recorded at both fields were fit together using ChemEx (https://github.com/gbouvignies/chemex) and a two-state exchange model. The Kᵣ value was calculated from the concentrations of P₁-340 and P₁-50N₁-525 as well as the fraction of bound P₁-340 (pₐ), as obtained from the fit and assuming a 1:1 binding stoichiometry. The error of the Kᵣ value was estimated by error propagation from the fitting error of pₐ and assuming a 5% error in concentration determination of both proteins. Nonoverlapped residues 180, 181, 182, 184, 185, 193, 196, and 199 covering both δ and α₄ were included in the fit.

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Two days later, the expression of the two-luciferase reporter generated new was the control of T7 promoter were transfected together in BSR-T7 cells. MeV minigenome RNA of (+) or (−) reaction and In-Fusion (Clontech) recombination. Glu1 and glu2 in the C terminus or N terminus of the N-terminal glu1 domain, and N elongated by 324 nucleotides (+) or (−) polarity, N, L, and P protein under the control of T7 promoter were transfected together in BSR-T7 cells. Two days later, the expression of the two-luciferase reporter gene was recorded by luminescence measurement in the presence of their specific substrate. Each test was performed three times with each condition performed in triplicate.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/4/8/eaat7778/DC1

**Fig. S1.** Scheme showing the different domains of N and P proteins referred to in the main text.

**Fig. S2.** NMR of P1–304.

**Fig. S3.** Purification of P1–304N1–525 complexes.

**Fig. S4.** Interaction of N with β523 of P.

**Fig. S5.** Analysis of P interaction dynamics with N1–525.

**Fig. S6.** Interaction of P1–160 with N.

**Fig. S7.** P1–304–305 interaction with P1–304N1–525.

**Fig. S8.** Defining the conformational ensemble of P1–304N1–525 from SAXS curves.

**Fig. S9A.** Expression of MeV P protein constructs used for functional studies.

**Fig. S9B.** Functional assays using MeV minireplicon.

**Fig. S10.** NMR spectroscopy of N-terminal domain of NCORE in interaction with P1–304–305.

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