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Review

Structural disorder within paramyxoviral nucleoproteins

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ABSTRACT

In this review I summarize available data pointing to the abundance of structural disorder within the nucleoprotein (N) from three paramyxoviruses, namely the measles (MeV), Nipah (NiV) and Hendra (HeV) viruses. I provide a detailed description of the molecular mechanisms that govern the disorder-to-order transition that the intrinsically disordered C-terminal domain (N_{TAIL}) of their N proteins undergoes upon binding to the C-terminal X domain (XD) of the homologous phosphoproteins. I also show that a significant flexibility persists within N_{TAIL}–XD complexes, which makes them illustrative examples of "fuzziness". Finally, I discuss the functional implications of structural disorder for viral transcription and replication in light of the promiscuity of disordered regions and of the considerable reach they confer to the components of the replicative machinery.

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1. Introduction

The measles (MeV), Nipah (NiV) and Hendra (HeV) viruses belong all to the *Paramyxovirinae* sub-family within the *Paramyxoviridae* family of the *Mononegavirales* order. MeV is a *Morbillivirus* member, while NiV and HeV have been classified in the *Henipavirus* genus. *Paramyxovirinae* possess a non-segmented, negative-stranded RNA genome that encodes six proteins: the nucleoprotein (N), the phosphoprotein (P), the matrix protein, the F and H glycoproteins and the RNA-dependent RNA polymerase (RdRp) or "large" protein (L). The genome of *Paramyxoviridae* is encapsidated by a regular array of multiple copies of the N protein forming a helical nucleocapsid that serves as template for both transcription and replication. These activities are ensured by the RdRp that is made of the L and P proteins, with P serving as an essential polymerase factor allowing recruitment of L onto the nucleocapsid (Fig. 1). This ribonucleoprotein complex made of RNA and of the N, P and L proteins constitutes the replication machinery of *Paramyxoviridae*.

In paramyxoviruses, N exists in a soluble, monomeric form (N°) and in a nucleocapsid assembled form. Once N is synthesized, a chaperone is required to maintain it in a soluble and monomeric form. This role is played by the P protein, whose association simultaneously prevents illegitimate self-assembly of N. This soluble N°–P complex is used as the substrate for the encapsidation of the nascent genomic RNA chain during replication. The assembled form of N also forms complexes with either isolated P or P bound to L, which are both essential to RNA synthesis by the viral polymerase (see [1–5] for reviews on transcription and replication).

In the past decade, a wealth of bioinformatics and experimental evidence has been gathered showing that paramyxoviral N proteins possess long intrinsically disordered regions (IDRs) (for reviews see [5–12]). Intrinsically disordered proteins (IDPs) and IDRs are ubiquitous proteins/regions lacking stable secondary and tertiary structures under physiological conditions of pH and salinity in the absence of their biological partner and thus exist as dynamic ensembles of interconverting conformers [13]. IDPs/IDRs are functional while being either fully or partly disordered, and they complement the functional repertoire of folded proteins, being able to interact with several partners thus exerting multiple biological functions (see [14] and references cited therein).

The abundance of IDRs in paramyxoviral N proteins and the difficulty of obtaining homogenous polymers of N suitable for X-ray analysis explain the relative paucity of structural data obtained so far by X-ray crystallography. However, the combined use of techniques such as circular dichroism (CD), nuclear magnetic resonance (NMR), small angle X-ray scattering (SAXS), electron microscopy (EM) and site-directed spin labelling (SDSL) coupled with

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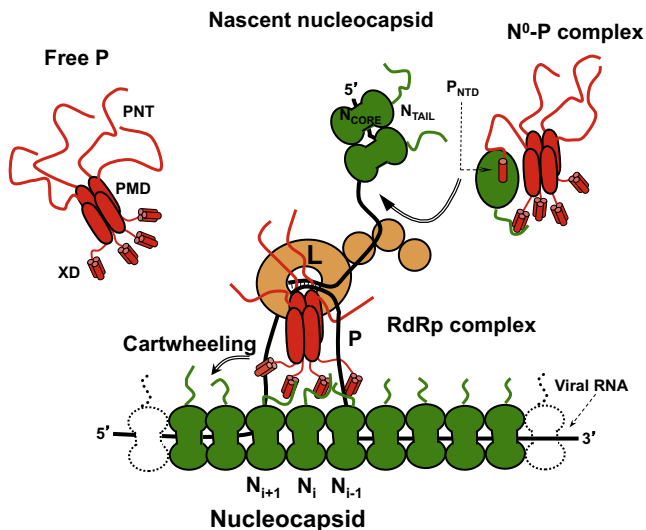 *E-mail address:* Sonia.Longhi@afmb.univ-mrs.fr

Fig. 1. Schematic illustration of the *Paramyxoviridae* replicative complex. Scheme of the replicative complex with the RNA represented by a black line. The neo-synthetized RNA is shown already partially encapsidated. The N and P intrinsically disordered regions are symbolized by lines. The extended conformation of the disordered regions would allow the formation of a tripartite complex between N°, P and L required for nucleocapsid assembly. The P/L complex forms the RNA-dependent RNA polymerase (RdRp) complex, which cartwheels onto the nucleo-capsid complex via the XD domain of P. P is shown as a tetramer to reflect the prevalence of this oligomeric state in paramyxoviral P proteins. The N protein has been drawn with a peanut-shape according to available structural data.

electron paramagnetic resonance (EPR) have shed light onto the molecular features of these proteins and have provided a quite accurate description of their conformational behaviour.

In this review I will summarize all the available molecular information on the N proteins of three representative *Paramyxovirinae* members, namely MeV, NiV and HeV, and I will focus on their disordered regions and the interactions they establish with their partners. Finally, I will discuss the functional implications of disorder for transcription and replication.

2. Structural organization of N

In *Paramyxovirinae*, N binds to exactly six nucleotides [3], a property that dictates the so-called "rule of six", i.e. the requirement for the viral genome to be a multiple of six in order to ensure efficient transcription and replication. By encapsidating the viral genome the N protein not only protects it from degradation, but it also renders it competent for transcription and replication. Indeed, the viral polymerase has a very poor processivity and hence cannot transcribe nor replicate RNA when the latter is not encapsidated by the N protein [15].

Paramyxovirinae nucleoproteins consist of two regions: a structured N-terminal moiety, N_{CORE}, and a C-terminal domain, N_{TAIL} (Fig. 2A). While N_{CORE} contains all the regions necessary for self-assembly and RNA-binding, as well as for interaction with the N-terminal, disordered region of P (PNT) within the N°–P complex (Fig. 1), N_{TAIL} is responsible for interaction with the C-terminal X domain (XD) of the P protein (Fig. 2A) (see [5–12] for reviews). The structures of MeV and HeV XD have been solved and shown to consist of a triple α-helical bundle [16–19] (Fig. 2A). High-resolution structural data is also available for the X domains of the closely related Sendai (SeV) and mumps virus (MuV) [20,21]. Contrary to all other paramyxoviral X domains investigated so far, MuV XD does not however interact with the C-terminal region of N but rather with the N_{CORE} region [22]. Interestingly, while in the majority of *Paramyxovirinae* members the C-terminal

nucleocapsid-binding region of P adopts a stably folded, compact conformation, it is disordered in respiratory syncytial virus (RSV), a member of the *Pneumovirinae* subfamily [23]. Similarly, the X domains from *Rubulavirus* members were found to span a structural continuum ranging from stable α-helical bundles to largely disordered forms in solution [20,24].

N_{TAIL} domains from MeV, NiV and HeV possess features that are hallmarks of intrinsic disorder: (i) they are hyper-sensitive to proteolysis [25,26], (ii) they cannot be visualized in cryo-EM reconstructions of nucleocapsids [27], (iii) they have an amino acid sequence that is highly variable amongst phylogenetically related members [28], and (iv) they are predicted to be mainly (if not fully) disordered by the secondary structure and disorder predictors implemented within the MeDor metaserver [29]. The disordered nature of these N_{TAIL} domains has been confirmed experimentally [19,28,30–34].

As for all N proteins of the *Mononegavirales* family, and with the sole exception of the N protein from the Borna disease virus [35], MeV and *Henipavirus* N proteins self-assemble to form large helical nucleocapsid-like particles with a broad size distribution when expressed in heterologous systems [19,25,36–38]. MeV nucleocapsids, as visualized by negative stain transmission EM, have a typical herringbone-like appearance [25,27,30,37,39]. EM studies by two independent groups led to real-space helical reconstruction of MeV nucleocapsids [27,39] (Fig. 2B). These EM studies [27,30,39,40], along with recent solid-state NMR studies [41], showed that the removal of the disordered N_{TAIL} domain, which protrudes from the globular body of N_{CORE}, leads to more ordered and more rigid nucleocapsids, with significant changes in both pitch and twist.

High-resolution structural data on *Paramyxoviridae* N is limited. So far, the only crystal structures of N proteins that have been solved are those of the N protein from RSV and from parainfluenza virus 5 (PIV5, a *Rubulavirus* member) [42,43]. In both cases, the N proteins are in the form of N:RNA rings, where these latter correspond to one turn of a nucleocapsid helix. In the case of PIV5, the N protein was subjected to limited proteolysis to remove the N_{TAIL} disordered region prior to crystallization [43], whereas in the case of RSV, it is the full-length form that was crystallized [42], in line with the fact that the RSV N protein is shorter as compared to its *Paramyxovirinae* counterparts and devoid of the disordered N_{TAIL} region. In both RSV and PIV5, the nucleoprotein consists of two lobes (NTD and CTD) separated by a hinge that accommodates the RNA. The RNA is tightly packed between the two N lobes, being located on the external face of N:RNA rings [42,43]. Each N protomer contacts 6 (PIV5) or 7 (RSV) nucleotides. For both RSV and PIV5, each N subunit possesses an extended N-terminal and C-terminal arm (NTD-arm, CTD-arm) that makes contacts with the preceding (N_{i-1}) and following (N_{i+1}) protomer, respectively. Using the structure of RSV N:RNA rings as template, a model of MeV N:RNA was built and docked within the electron density map of MeV nucleocapsids [40]. Although the disordered N_{TAIL} domain could not be resolved in the reconstruction of the nucleocapsid, the fit suggests that N_{TAIL} would point towards the interior of the helical nucleocapsid [40].

The crystal structure of a monomeric, RNA-free form of the NiV N protein devoid of the NTD-arm and of N_{TAIL} (N_{32-383}) in complex with the N-terminal N°-binding region of P (P_{NTD}, aa 1–50 of P) has also been solved [44]. P_{NTD} binds to CTD and interferes with the binding of the CTD-arm from the N_{i+1} protomer and the NTD-arm from the N_{i-1} protomer thereby providing a structural explanation for the ability of PNT to prevent N self-assembly [44]. A notable structural difference between NiV N from one hand and PIV5 and RSV N from the other hand is the angle between NTD and CTD formed at the hinge region. In the NiV N°$_{CORE}$-P_{NTD} structure, the putative RNA-binding groove is open, with NTD bowing down by
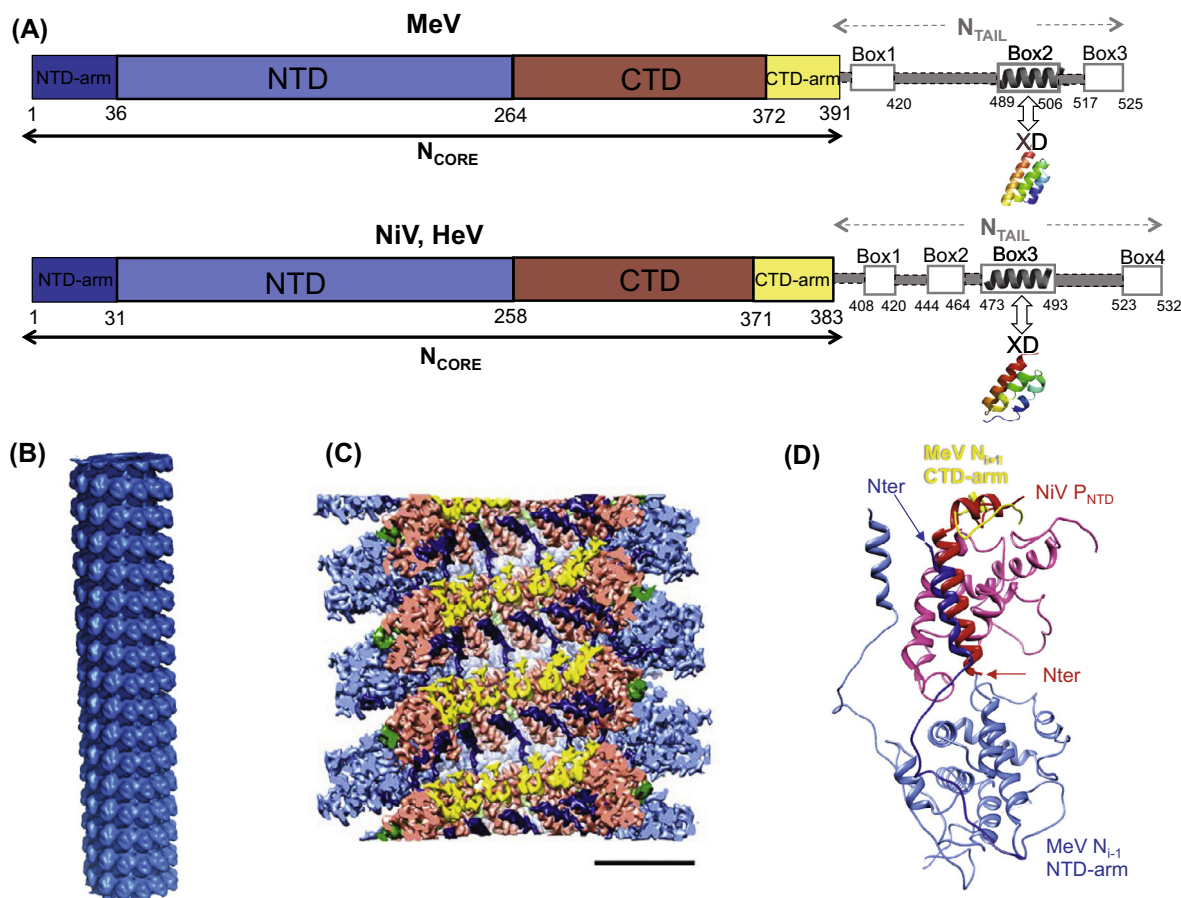
Fig. 2. Organization of the MeV, NiV and HeV nucleoproteins. (A) Modular organization of N from MeV and henipaviruses showing that N is composed of a folded domain, N_{CORE}, and a C-terminal disordered region, N_{TAIL}. The various boxes, corresponding to putative or experimentally proven MoREs, are shown, as is the α-MoRE (see grey helix). The box that interacts with XD is indicated by an arrow. The crystal structure of MeV and HeV XD is shown (PDB code 1OKS and 4HEO, respectively) [16,19]. (B) Cryo-electron microscopy reconstruction of the MeV nucleocapsid [27,62]. (C) Surface representation of the cryo-EM 3D reconstruction of the MeV trypsin-digested, helical nucleocapsid (cut away view). The colour code is the same as in panel A. The RNA is shown in green. The scale bar corresponds to 50 Å. Reproduced with permission from [45]. (D) Ribbon representation of a protomer of MeV N as observed in trypsin-digested MeV nucleocapsids [45]. The NTD-arm of the preceding N protomer (N_{i-1}) (blue) and the CTD-arm of the following N protomer (N_{i+1}) (yellow) are also shown with ribbon representation. The structure of NiV P_{NTD}, as observed in the $N°_{CORE}$–P_{NTD} complex [44] and then docked at the surface of N, is shown as a red ribbon.

about 30° from the CTD. Modelling the open conformation of PIV5 N onto its nucleocapsid-ring structure shows a rotation of its CTD towards the centre of the ring cavity, thereby exposing the RNA in the RNA-binding groove and making it accessible for the viral polymerase during genome transcription and replication [43].

Recently, elegant cryo-EM studies led to near-atomic resolution of the MeV helical nucleocapsid formed by the folded N_{CORE} domain [45]. Combined with the atomic structures of RSV N and NiV $N°_{CORE}$-P_{NTD}, 3D reconstruction of MeV helical nucleocapsid allowed building a reliable pseudo-atomic model of the MeV N_{CORE}–RNA helix. Those studies confirmed the role of the NTD-arm and CTD-arm in maintaining the cohesion of N protomers (Fig. 2C). Like in the structure of PIV5 nucleocapsid, and contrary to the structure of the RSV nucleocapsid [42], the N_{i-1} and N_{i+1} subunits in the MeV N_{CORE}–RNA helix do not interact directly. Beyond ensuring cohesion between adjacent N protomers, the NTD-arm and CTD-arm also play a critical role in rigidifying the CTD thus keeping N in a closed conformation allowing the RNA to be trapped between the NTD and the CTD chaws. Four aromatic residues (Phe11, Phe269, Tyr303 and Phe324), conserved in *Paramyxovirinae* members [44], stack together thereby fixing the α-helix of the NTD-arm. In addition, the MeV N_{CORE} structure provides a structural basis explaining how P_{NTD} can prevent N self-assembly. Indeed, when the structure of the RNA-free,

monomeric form of NiV N_{CORE} in complex with P_{NTD} is superimposed onto the MeV N_{CORE} structure, the α-helix of the NTD-arm of the MeV N_{i-1} protomer perfectly superimposes onto the helix α1 of P_{NTD} (Pα1) from the NiV $N°_{CORE}$–P_{NTD} structure, while the loop of the CTD-arm of the MeV N_{i+1} protomer overlaps with helix α2 of P_{NTD} (Pα2) (Fig. 2D). Thus Pα1 competes with the NTD-arm of the N_{i-1} protomer, and Pα2 with the CTD-arm of the N_{i+1} protomer, as already proposed [44]. The first α-helix of P_{NTD} (Pα1) and the α-helix of the NTD-arm of N appear to play similar roles: they lock the CTD and the NTD–CTD junction in a stable conformation that can be either open or closed depending on the presence of the RNA embedded in the interdomain cleft.

All those studies showed that in *Paramyxoviridae* nucleocapsids the RNA is not accessible to the solvent, and has to be partially released from N to become accessible to the polymerase. Therefore, a conformational change must occur within N to allow exposure of the RNA. The disordered N_{TAIL} domain is thought to play a major role in this conformational change (see Section 7).

3. Residual order within N_{TAIL} domains

Although HeV, NiV and MeV N_{TAIL} domains are mostly disordered, they possess a certain amount of residual structure

[28,30,31]. In line with this, bioinformatics analyses indicated the presence of short order-prone regions, corresponding to putative Molecular Recognition Elements (MoREs) [28,31]. MoREs are short, order-prone regions within IDPs that have a certain propensity to bind to a partner and to undergo induced folding (i.e. a disorder-to-order transition) [46,47].

In the case of MeV, one MoRE of α-helical nature was predicted to occur within one (i.e. Box2, aa 489–506) out of three regions (i.e. Box1–3) conserved within members of the *Morbillivirus* genus [48] (Fig. 2A). While Box1 (aa 401–420) interacts with a yet unidentified nucleoprotein receptor (NR) expressed at the surface of dendritic cells of lymphoid origin [49] and of T and B lymphocytes [50], Box2 is the region responsible for interaction with XD [16,31,51,52]. Analysis of the Cα chemical shifts of N_{TAIL} and of the mobility of spin labels grafted within Box2 showed that the α-MoRE of MeV N_{TAIL} is partly preconfigured as an α-helix in the absence of XD [18,53,54]. In addition, an atomic-resolution ensemble description of the α-MoRE of MeV N_{TAIL} was obtained by combining residual dipolar coupling measurements and ensemble optimization methods [55,56]. The α-MoRE was shown to exist in a rapidly interconverting conformational equilibrium between an unfolded form and four discrete α-helical conformers of different length encompassing the XD binding site [57]. Spectroscopic approaches unveiled that binding of XD triggers stable α-helical folding of the MoRE [16,18,31,51,53,54,57,58]. Similar studies carried out on SeV N_{TAIL} unveiled a similar conformational behaviour of the free form, although in that case the α-MoRE was shown to sample an extended conformation and only three helical conformers [56,59].

Henipavirus N_{TAIL} domains contain four predicted MoREs [28], with Box3 having been shown to undergo α-helical induced folding upon binding to XD [19,26,32,33,60] (Fig. 2A). Interestingly, SDSL EPR spectroscopy studies unveiled a considerable conformational heterogeneity within Box3 consistent with the occurrence of multiple helical conformers of different length [32]. In agreement, analysis of the Cα chemical shifts of the free form of both HeV and NiV N_{TAIL} domains showed that Box3 is at least transiently populated as an α-helix [19,33].

While Box1, Box2 and Box4 are not involved in binding to XD, they influence to some extent the α-helical folding of Box3 as well as the compaction properties of N_{TAIL}. In particular, Box1, which is devoid of α-helical propensities, was found to be a major determinant of protein compaction, a finding that incidentally also indicates that the ability to adopt a collapsed state does not depend on the content in regular secondary structure [60]. Interestingly, subtle differences between NiV and HeV N_{TAIL} were observed [32,33,60]. For example, a NiV N_{TAIL} truncated form devoid of Box1 and Box2 was found to possess a more extended conformation than its HeV counterpart [60]. This difference in the extent of compaction might arise from slight differences in sequence polarity between the two proteins. Indeed, they slightly differ in their k value, where the latter reflects the linear distribution of oppositely charged residues [61]. Proteins with low k values are well-mixed sequences in which intrachain electrostatic repulsions and attractions are counterbalanced, leading to conformations that resemble either self-avoiding random walks or generic Flory random coils [61].

NMR studies further confirmed that NiV and HeV N_{TAIL} proteins have distinct features in spite of their high sequence identity (74%) [33]. In particular, the NiV N_{TAIL} Box2 region experiences a slightly higher extent of α-helical preconfiguration and seemingly plays a role in binding to XD, while HeV N_{TAIL} Box2 does not. A close inspection of the Box2 region reveals a notable difference at position 457, where the Asp of HeV N_{TAIL} is replaced by an Asn in NiV N_{TAIL}. In light of the proposed role of charges in pre-orienting Box3 at the HeV XD surface [19] (see Section 5), it is tempting to

speculate that this substitution could be responsible for the observed difference in the role of Box2 during binding for the two viruses.

Notably, the subtle differences observed between NiV and HeV N_{TAIL} domains do not affect XD-binding abilities, with Box3 being functionally interchangeable between the two viruses. However, there are no cross-interactions between proteins from HeV or NiV and proteins from MeV (unpublished data).

4. Structural state of N_{TAIL} domains within nucleocapsid-like particles

Beyond being disordered in isolation, the MeV and *Henipavirus* N_{TAIL} domains are also disordered within full-length N proteins from nucleocapsid-like particles, as judged from NMR studies carried out on ^{15}N-labeled nucleocapsids [19,33,57] (Fig. 3A–C). In those studies, both MeV and *Henipavirus* N_{TAIL} were found to retain their disordered state *in situ*, i.e. when appended to nucleocapsids. For the three viruses, experimental evidence was obtained supporting a model in which the first 50 disordered amino acids of N_{TAIL} are conformationally restricted. In the case of MeV, by combining those data with previous modelling data [40], a model was proposed where N_{TAIL} escapes from the inner channel to the outside of the nucleocapsid *via* the interstitial space between successive N_{CORE} helical turns [19,57] (Fig. 3D). Notably, this model provides a plausible explanation for the increased rigidity of nucleocapsids in which the flexible N_{TAIL} region has been cleaved off [30,39,45]. The inherent flexibility of intact nucleocapsids likely confers at least partial accessibility to the N-terminal region of N_{TAIL}, thereby accounting for the ability of the Box1 region to bind to NR in the context of nucleocapsids released in the extracellular compartment [49,50]. The flexibility of the N_{TAIL} region sandwiched between successive turns of the nucleocapsid may be the basis for variations in pitch and twist that may be related to switches between transcription and replication [62] (see Section 7).

5. Molecular mechanisms of N_{TAIL}–XD complex formation

The N_{TAIL} domains of MeV, NiV and HeV were shown to bind to XD with an equilibrium dissociation constant (K_D) in the μM range [26,63]. As already mentioned, binding to XD triggers α-helical folding of N_{TAIL} [16,26]. Interestingly, while NMR titration experiments with ^{15}N-labeled N_{TAIL} indicated an α-helical transition within MeV N_{TAIL} upon addition of the homologous X domain, as judged from the appearance of new peaks in the α-helical region of the N_{TAIL} spectra [18,26,51], no such peaks were observed in the HeV and NiV N_{TAIL} spectra even with saturating amounts of XD [19,33].

In the case of MeV, a model of the interaction in which the α-MoRE of N_{TAIL} adopts an α-helical conformation and is embedded in a large hydrophobic cleft delimited by helices α2 and α3 of XD has been proposed [16] and successively validated by Kingston and co-workers, who solved the crystal structure of a chimeric construct made of XD and the N_{TAIL} region encompassing residues 486–504 [17] (Fig. 4A). Those studies unveiled that Box2 is tightly packed at the binding interface. The residues involved in the interaction of the two partners are mainly hydrophobic, involving Leu481, Leu484, Ile488, Phe497, Met500 and Ile504 from XD, and Ser491, Ala494, Leu495, Leu498 and Met501 from N_{TAIL} (Fig. 4A). In addition, site directed mutagenesis studies revealed that Ala502 is also involved in the interaction with XD, as deduced from the 30-fold increase in the K_D observed with an N_{TAIL} variant bearing an Asp at this position [64]. Recently, random mutagenesis studies confirmed the crucial role of N_{TAIL} residue Ser491 in
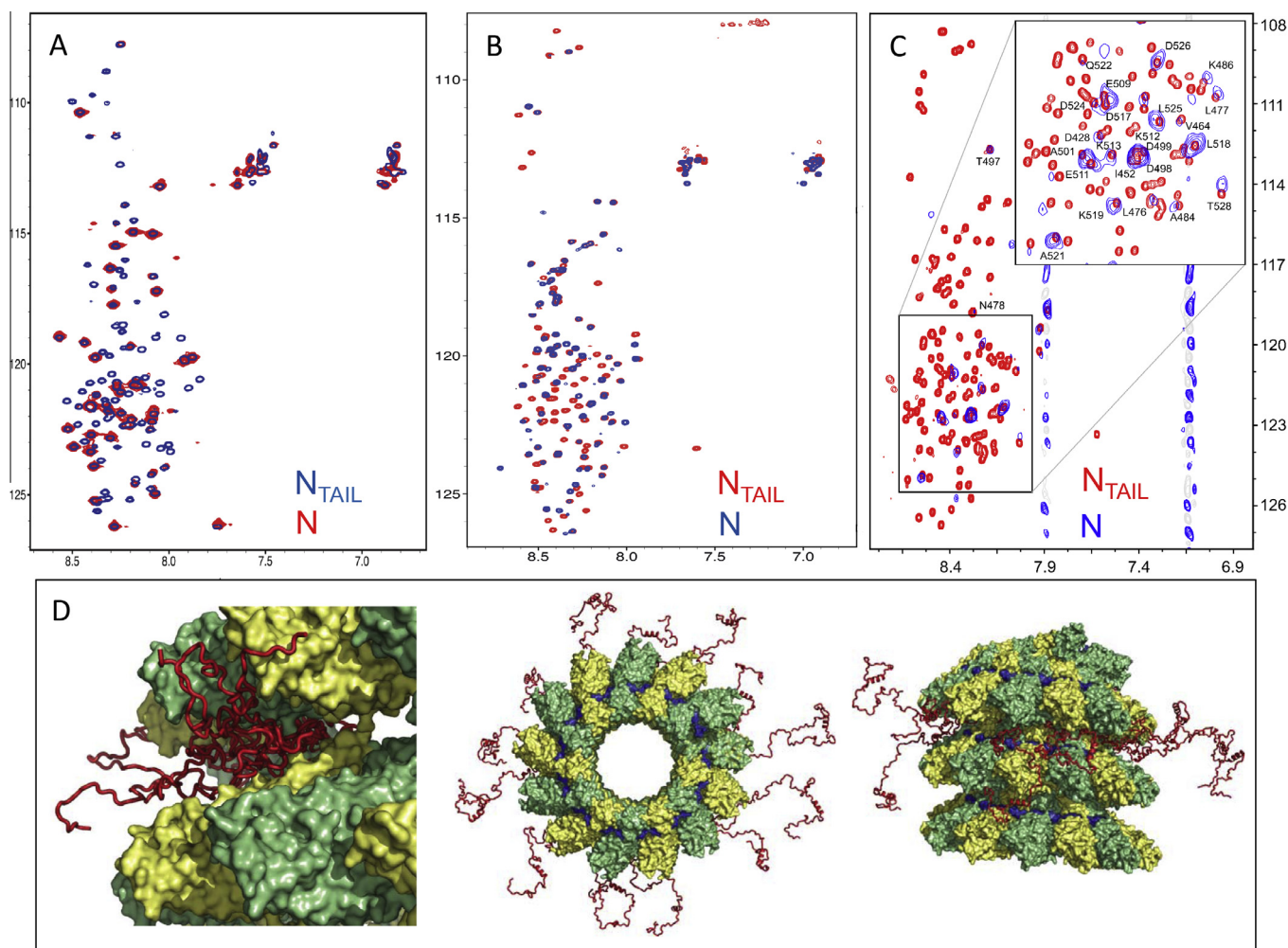
Fig. 3. Structural state of N_{TAIL} in the context of nucleocapsid-like particles. Superimposition of the ^1H–^{15}N HSQC spectrum of N_{TAIL} either in isolation or in the context of nucleocapsid-like particles in MeV (A), HeV (B) and NiV (C). The x and the y axis correspond to ^1H and ^{15}N frequencies (in ppm), respectively. Data shown in (A) were taken from [57]. Data shown in (B) and (C) were taken from [19] and [33], respectively. (D) Proposed model of the location of N_{TAIL} (red) in intact nucleocapsids where successive N_{CORE} monomers are coloured green and yellow and RNA in blue. Left: representation of the conformational sampling of N_{TAIL} from a single N protomer in the capsid. Different copies of N_{TAIL} (red) are shown to indicate the available volume sampling of the chain. Only the first 50 amino acids of N_{TAIL} are shown. Middle and right panels: representation of the 13 N_{TAIL} conformers from a single turn of the nucleocapsid shown across (middle) or along (right) the axis of the nucleocapsid. Modified from [57].

complex formation [65]. Those studies also unveiled a previously unnoticed role for residue Arg497, whose side chain points out of the binding surface. In spite of its orientation towards the solvent, the side chain of Arg497 is at bonding distance from the OH group of Tyr480 of XD (Fig. 4A). Through generation and characterization of a "mirror" XD variant bearing the Y480F substitution, the crucial role of the Arg497–Tyr480 interaction in stabilizing the N_{TAIL}–XD complex was confirmed [65].

ITC studies revealed that *Henipavirus* N_{TAIL}–XD complexes are stable under NaCl concentrations as high as 1 M, suggesting that the interaction does not rely on polar contacts [26], in line with an interaction driven by the burying of apolar residues of N_{TAIL} at the XD surface, as already observed in the case of MeV [16,22]. Strikingly, while in the case of NiV, N_{TAIL}/XD complex formation could be documented by size-exclusion chromatography, it could not in the case of HeV [26]. This suggests that the NiV complex is tighter than its HeV counterpart, consistent with NMR studies that pointed out a unique, additional role for Box2 in binding to XD in the case of NiV [33].

While the crystal structure of HeV XD is available [19], no structural data of the *Henipavirus* N_{TAIL}–XD complex is available so far. Based on spectroscopic data showing that Box3 undergoes

α-helical folding [19,26,32,33], the more hydrophobic side of the amphipathic α-MoRE located within the Box3 region of *Henipavirus* N_{TAIL} was modelled at the hydrophobic surface delimited by helices α2 and α3 of XD using the MeV N_{TAIL}–XD structure as a template (Fig. 4B and C). In all complexes, the interface is hydrophobic, in agreement with findings by Meszaros and co-workers, who reported that the binding interfaces of protein complexes involving IDPs are often enriched in hydrophobic residues [66]. In striking contrast, in the SeV N_{TAIL}–XD complex, the binding interface is dominated by charged residues [67].

Although direct structural data on *Henipavirus* N_{TAIL}–XD complexes are still lacking, recent NMR studies provided the first clues on the structure of the HeV complex. In particular, analysis of chemical shift perturbations in reciprocal titration studies allowed residues involved in the interaction to be identified [19]. The availability of the crystal structure of HeV XD allowed mapping at the XD surface the residues involved in binding to N_{TAIL} [19]. Although the binding interface is made of hydrophobic residues, the binding pocket of XD is surrounded by charged residues that may establish electrostatic interactions with basic residues of Box3 (Fig. 4B and C). This observation suggests that the HeV N_{TAIL}–XD interaction could be controlled by a combination of
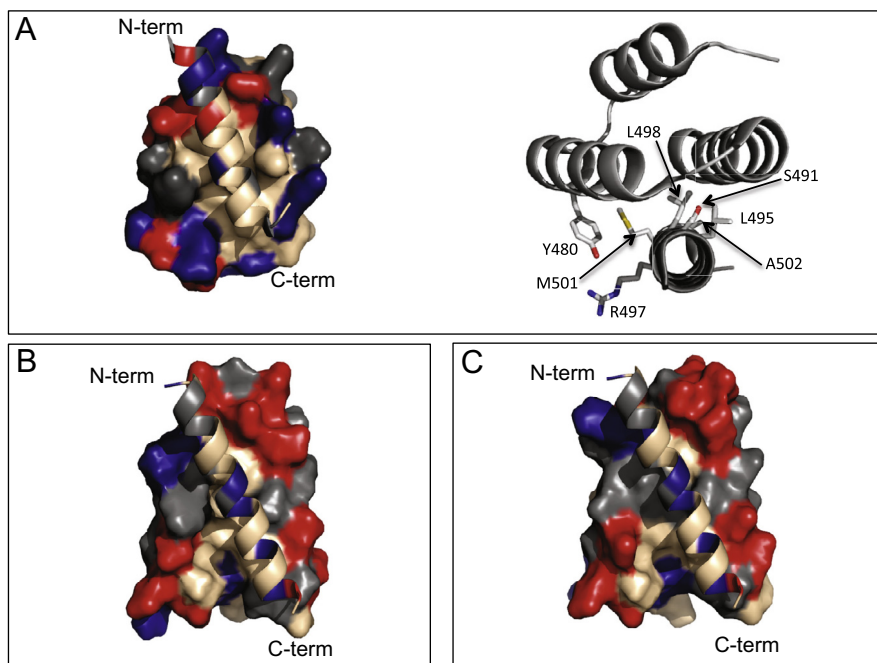
Fig. 4. Structures of XD and of the interacting MoRE of N_{TAIL} in MeV, NiV and HeV. (A) Structure of the MeV Box2/XD complex (PDB code 1T6O [17]). XD is shown either in surface (left) or ribbon representation (right). In the right panel, the side chains of the Box2 residues involved in the interaction are shown in sticks and in atom type colour. (B) and (C). Structural models of the NiV (B) and HeV (C) Box3/XD complexes with XD in surface representation and Box3 in ribbon [19,26]. Panel B shows a model of NiV XD [26], while panel C shows the crystal structure of HeV XD [19]. Box3 is shown in a parallel orientation according to [68]. In panel A left and in panels B and C, hydrophobic residues are represented in beige, while basic (Arg and Lys) and acidic residues (Asp and Glu) are shown in blue and red, respectively. All other residues are shown in grey.

long-range electrostatic forces that correctly orient N_{TAIL} prior to accommodation in the narrow hydrophobic pocket at the surface of XD. ITC studies carried out on the HeV N_{TAIL}/XD pair at different pH values confirmed the role of electrostatics in complex formation, a conclusion further strengthened by mutational studies that targeted charged residues both within N_{TAIL} and XD [68]. Collectively, those studies provided direct evidence that charged residues surrounding the hydrophobic binding interface play a crucial role in complex formation, thus arguing for a multiparametric interaction and emphasizing the role of residues located in the neighbourhood of the binding interface. It is therefore conceivable that the HeV N_{TAIL}/XD complex formation relies on the so-called "electrostatic steering mechanism" [69]. According to this model, long-range electrostatic forces pull an IDP towards the relevant acidic (or basic) patch on the surface of a target protein, thus establishing contact in the vicinity of the binding site. The resulting state, in which the IDP is loosely anchored at the periphery of the binding site, has been described as "electrostatic encounter complex" [70]. This mechanism effectively increases the local concentration of the ligand in the vicinity of the binding site and thus promotes binding. The corollary of this assumption is that HeV N_{TAIL} would fold according to a folding after binding mechanism, a hypothesis supported by quantitative analysis of NMR titration data (see below) [19].

Notably, neither chemical shifts nor electrostatic interactions are able to distinguish rotational symmetry about the axis of the N_{TAIL} helix, although two conformations are most probable, both having the hydrophobic face of the α-MoRE in contact with the hydrophobic interface of XD (Fig. 4). In both conformations, the two arginine residues flanking the hydrophobic face on HeV N_{TAIL} interact with acidic residues on the surface of XD. Through a combination of mutational and SAXS studies, experimental evidence was recently gathered supporting a parallel orientation of the MoRE at the XD surface [68]. The parallel orientation of the MoRE at the XD surface is thus a conserved feature between MeV

and HeV, arguing for a functional relevance. It is tempting to speculate that this could be related to the relative orientation of the whole P protein with respect to the N_{TAIL} region protruding from the nucleocapsid. In its turn, this might be related to optimal positioning of the polymerase onto the nucleocapsid template and might impart directionality to the polymerase movement along the nucleocapsid (see [5,10,11] for reviews).

The finding that the α-MoRE of MeV and *Henipavirus* N_{TAIL} is transiently populated as an α-helix might be taken as a hint suggesting that the molecular mechanism governing the folding coupled to binding of N_{TAIL} could rely on conformational selection [71]. Two different, but not exclusive, binding mechanisms have been described in the literature for IDPs/IDRs (see [14] and references cited therein). In the first one, called "folding after binding", the binding event takes place before folding [72]. In the second mechanism, the partner binds to the pre-folded MoRE, thereby shifting the equilibrium of the conformational ensemble to the folded form. In the case of MeV N_{TAIL}, computational and experimental studies converged to support a folding after binding mechanism. Indeed, the resonance behaviour of N_{TAIL} in titration experiments with XD indicated a very poor fit to a two-state process, suggesting that binding may imply the formation of a binding intermediate in the form of a weak encounter complex [18]. In support of this hypothesis, recent data obtained by molecular dynamics simulations confirmed that binding preferentially occurs *via* an induced folding mechanism in spite of the partial pre-configuration of the α-MoRE [73]. That MeV N_{TAIL} does fold after binding to XD has been definitely proven by kinetic studies [63].

The presence of a preconfigured MoRE with nevertheless a folding-after-binding mechanism is not a unique feature to MeV N_{TAIL}, having also been documented in the case of HeV N_{TAIL} [19]. In this latter case, quantitative analysis of peak intensities in the HSQC spectra of N_{TAIL} at each XD titration point showed that the signal intensity decreases faster for the residues located at the

extremities of the MoRE and for which a smaller amount of residual helical structure is observed in the isolated state of N_{TAIL}. This differential broadening suggests that XD binds to a short, central helix within the α-MoRE, and that this helix is subsequently extended via helical folding of the adjacent residues. Data therefore indicate that HeV N_{TAIL} interacts with XD via a folding-upon-binding mechanism, with the folding event occurring on the micro- to millisecond time scale [19].

6. Residual flexibility within the N_{TAIL}–XD complex

A low-resolution model of the MeV N_{TAIL}–XD complex obtained by SAXS showed that most of N_{TAIL} (residues 401–488) remains disordered within the complex [51]. A subsequent study that made use of a combination of SDSL EPR spectroscopy and modelling showed that a considerable residual flexibility persists also within the N_{TAIL} region encompassing residues 505–525 in the complex [74]. In further support, a recent mass spectrometry study showed that the MeV N_{TAIL}–XD complex is characterized by a high structural heterogeneity. In those studies, distinct conformers of the complex could be detected by electrospray ionization-mass spectrometry (ESI-MS). Beyond documenting structural heterogeneity, those studies enabled to capture a collapsed form of the complex that had escaped detection in previous studies. Indeed, a bimodal charge state distribution was observed with a high-charge component (18+) and a low-charge (11+) component. While the former would correspond to an "open" conformation, in which the disordered arms of N_{TAIL} flanking the α-MoRE fluctuate maintaining high solvent accessibility, the low-charge component likely represents a compact or "closed" conformation of the complex in which the N_{TAIL} arms collapse onto the surface of the folded partner [34]. Computational modelling of the "open" complex in solution, using experimental chemical shifts as restraints, provided atomic-resolution structural models with calculated solvent accessible surface area (SASA) in good agreement with that experimentally determined by ESI-MS. In the resulting models, the intermolecular interactions are predominantly hydrophobic, not only in the ordered core of the complex, but also in the disordered regions. Interestingly, the more compact states were found to involve electrostatic interactions [34], suggesting that MeV N_{TAIL} binding to XD could rely on an electrostatic steering mechanism [69], as already proposed in the case of HeV [68].

The MeV N_{TAIL}–XD complex therefore provides an illustrative example of "fuzziness", where this term has been coined by Tompa and Fuxreiter to designate the persistence of conspicuous regions of disorder within protein complexes implicating IDPs [75]. *Henipavirus* N_{TAIL}–XD complexes were found to be fuzzy too. Indeed, the experimentally determined hydrodynamic radius of the NiV N_{TAIL}–XD complex is 1.6 times higher than expected for a compact complex [26]. In further support of the "fuzziness" within MeV, HeV and NiV N_{TAIL}–XD complexes, the many observable and relatively sharp NMR resonances that are nearly unaltered upon addition of XD provide evidence that these N_{TAIL} regions remain significantly disordered in the bound state [17–19,26,33]. Strikingly, the *Henipavirus* N_{TAIL}–XD complexes are even fuzzier, as judged from the vanishing of resonances of the MoREs at the beginning of titration with no reappearance even at saturation [19,33]. This observation suggests that even when bound to XD, the α-MoRE of both NiV and HeV N_{TAIL} remains highly dynamic, undergoing exchange between different conformers at the XD surface [19,33].

The functional relevance of this fuzziness may reside in the ability of the disordered appendages to serve as a platform to capture other (regulatory) binding partners. In line with this speculation, the fuzzy Box3 region of MeV N_{TAIL} was shown to constitute a

binding site for the major inducible heat shock protein hsp70 [76,77], where the latter is known to stimulate both viral transcription and replication [78–80]. Box3 constitutes however a low-affinity binding site for hsp70, with Box2 providing a high-affinity binding site (K_D of 10 nM) [78,81]. Since hsp70 competitively inhibits XD binding to N_{TAIL} [76], it has been proposed that hsp70 could enhance transcription and genome replication by reducing the stability of P–N_{TAIL} complexes, thereby promoting successive cycles of binding and release that are essential to polymerase movement along the nucleocapsid template [51,76]. The hsp70-dependent reduction of the stability of P–N_{TAIL} complexes would thus rely on competition between hsp70 and XD for binding to the α-MoRE of N_{TAIL}, with recruitment of hsp70 being ensured by both Box2 and Box3 [76] (see also Section 7).

Finally, fuzzy regions flanking MoREs can also serve as natural modulators of the interactions established by IDPs. In fact, a recent descriptive random mutagenesis study of MeV N_{TAIL} led to the identification of five regulatory regions that are located in the upstream fuzzy region and dampen the interaction [82]. This finding is consistent with recent observations based on mini-replicon studies [83] (see Section 7). The molecular mechanism by which these regulatory regions modulate the N_{TAIL}–XD interaction remains however to be elucidated. In the same vein, MeV N_{TAIL} variants devoid of Box3 were found to exhibit enhanced interaction with XD, suggesting that Box3 would naturally serve as a dampener.

7. Functional impact of the N_{TAIL}–XD interaction and of structural disorder within N_{TAIL}

The N_{TAIL}–XD interaction is thought to be critical as it allows the P/L complex to be recruited onto the nucleocapsid thereby allowing transcription and replication to take place (see [9,84,85] for reviews). A recent study by the Plemper group has however challenged the model according to which Box2 is strictly required to recruit the MeV polymerase complex; indeed, Box2 was shown to be dispensable for MeV transcription and replication in the absence of the upstream N_{TAIL} region, which was found to act as a negative modulator (i.e. to prevent binding of the L–P complex to the nucleocapsid) [83].

In *Paramyxovirinae*, the N_{TAIL}–XD interaction is also thought to trigger the opening of the nucleocapsid to provide access of the polymerase to the viral RNA. In agreement, EM studies showed that addition of XD triggers unwinding of MeV nucleocapsids (unpublished data). This dramatic conformational change is accompanied by an increased exposure of viral RNA to the solvent as indicated by its increased sensitivity to RNase. In line with these observations, recent studies documented the ability of the MuV P protein to induce nucleocapsid uncoiling, with both N- and C-terminal P domains being involved [86]. In striking contrast with these findings, NMR studies have shown that addition of XD to HeV nucleocapsids does not trigger any major nucleocapsid rearrangement [19]. We can speculate that the expectedly necessary HeV nucleocapsid unwinding requires either the full-length P protein, or the P–L complex and/or cellular cofactors. One such possible cellular cofactor could be hsp70, by analogy with previous studies that showed that hsp70-nucleocapsid complexes of the closely related canine distemper virus exhibit an expanded helical diameter, an increased fragility, and an enhanced exposure of the genomic RNA to nuclease degradation [87,88].

The P XD-induced folding of N_{TAIL} and/or the inherent flexibility (and hence potential to undergo conformational changes) of the first 50 N_{TAIL} residues stacked between successive nucleocapsid turns, could also affect the structure of the replication promoter. Indeed, the replication promoter, located at the 3′ end of the viral

genome, is composed of two discontinuous elements that form a functional unit when juxtaposed on two successive helical turns [89]. The switch between transcription and replication could be dictated by variations in the helical conformation of the nucleocapsid, which would result in a modification in the number of N monomers (and thus of nucleotides) per turn, thereby disrupting the replication promoter in favour of the transcription promoter (or *vice versa*). Morphological analyses, showing the occurrence of a large conformational flexibility within *Paramyxoviridae* nucleocapsids [27,37,87,88], corroborate this hypothesis.

A tight N–P complex is predicted to hinder the polymerase processivity, according to the cartwheeling mechanism, which posits that contacts between N_{TAIL} and XD have to be dynamically made/broken to allow the polymerase to progress along the nucleocapsid template in order to allow transcription and replication to take place. Mutational studies that targeted the Box2 region of MeV N_{TAIL} unexpectedly showed that a reduced binding strength has no impact on the polymerase rate [64]. This tolerance of the polymerase to N_{TAIL} substitutions is probably true only in a certain range of affinities, where in spite of a pronounced drop in the affinity towards XD, the N_{TAIL}–XD interaction remains strong enough to ensure recruitment of the polymerase. These results suggest that the accepted model whereby the interaction has to be relatively weak to allow the polymerase to cartwheel on the nucleocapsid template needs to be revisited. A relatively labile complex can result from either an inherently low affinity of the binding reaction, or from a tight complex whose strength is modulated by co-factors. Taking into account the ability of hsp70 to compete out XD for binding to N_{TAIL} [76], it is tempting to speculate that the progression of the MeV polymerase complex along the template could be ensured by hsp70. In this model hsp70 would promote successive cycles of binding and release thanks to its destabilizing effect on the N_{TAIL}–XD interaction. The prevalently disordered nature of N_{TAIL} even in the bound form would facilitate recruitment of hsp70, thus providing an easy means to modulate the N–P interaction strength, which would ultimately result in modulation of transcription and replication rates.

The presence of the disordered N_{TAIL} domain protruding at the surface of the viral nucleocapsid confers the ability to establish a complex molecular partnership with a panel of structurally distinct cellular and viral partners, in agreement with previous reports that underscored a relationship between disorder and protein interactivity (i.e. promiscuity) [90]. In fact, in addition to P, MeV N_{TAIL} does also interact with the M protein [91] and many cellular proteins [49,50,77,92–96], which leads to plethora of functional effects including virus assembly, stimulation of transcription and replication, and evasion of the antiviral response. The presence of long disordered regions is not unique to paramyxoviral N proteins, being also a conserved property of P proteins of *Paramyxovirinae* members [28,97,98]. The occurrence of long disordered domains on both N and P proteins would allow for coordinated interactions between the polymerase complex and a large surface area of the nucleocapsid template, including successive turns of the helix. Indeed, the maximal extension of MeV PNT as measured by SAXS is 40 nm (unpublished data). In comparison, one turn of the MeV nucleocapsid is about 6 nm high [37,39,45]. PNT could thus easily stretch over several turns of the nucleocapsid, and since MeV P is multimeric [99,100], N°–P might have a considerable extension. Likewise, the maximal extension of MeV N_{TAIL} in solution is 13 nm [30]. The very long reach of disordered regions could enable them to act as scaffolding engines to tether partners. It is conceivable that during replication, the extended conformation of PNT and N_{TAIL} would allow the establishment of contacts between the assembly substrate (N°–P) and the polymerase complex (L–P), leading to a tripartite N°–P–L complex. The plasticity of IDRs

within N and P would therefore confer a considerable reach to the elements of the replication machinery.

8. Conclusions

The presence of large disordered regions in proteins of the replicative complex of *Paramyxovirinae* members is far from being a unique feature in the virosphere. Several bioinformatics studies have indeed shown that viral proteins, and in particular proteins from RNA viruses, are enriched in short disordered regions [101–104]. Beyond these computational studies, a considerable body of experimental evidence has been collated that points to the abundance of disorder within viral proteins (see [105,106] for reviews). The wide occurrence of disordered regions in viral proteins beyond affording a broad partnership would also represent a strategy for buffering the deleterious effects of mutations, with this being particularly relevant in RNA viruses that have high mutation rates.

Viruses are obligate intracellular parasites. They thus live in a very hostile environment and have to find strategies to survive in their host and to counteract the host immune response. In the course of evolution, viruses have "learned" to hijack and manipulate host proteins for their benefit and to evade host defence mechanisms. A recent study by Davey and co-workers showed that viruses have achieved this ability through broad mimicry of host protein short linear motifs (SLiMs), where the latter are embedded in disordered regions and play a variety of roles [107]. Binding to cell proteins through sites that mimic SLiMs also helps viral proteins to elude the host cell's immune system by rendering viral epitopes poorly recognizable by it (see [106] and references therein cited).

Finally, disorder has also been reported to provide a means to tolerate insertions and/or deletions, and therefore to be abundant in regions with dual coding capacity [108–110]. Taking into account the correlation between overlapping genes and disorder and the typically high compaction of viral genomes that often contain overlapping reading frames, one major advantage of disorder within viral proteins would reside in pleiotropy and genetic compaction [106]. Indeed, disorder provides a solution to reduce both genome size and molecular crowding, where a single gene would (i) encode a single (regulatory) protein product that can establish multiple interactions via its disordered regions and hence exert multiple concomitant biological effects, and/or (ii) encode more than one product by means of overlapping reading frames. In fact, since disordered regions are less sensitive to structural constraints than ordered ones, the occurrence of disorder within one or both protein products encoded by an overlapping reading frame can represent a strategy to alleviate evolutionary constraints imposed by the overlap. As such, disorder would give viruses the ability to "handle" overlaps, thus further expanding the coding potential of viral genomes.

References

[1] Longhi, S. and Canard, B. (1999) Mécanismes de transcription et de réplication des *Paramyxoviridae*. Virologie 3, 227–240.
[2] Lamb, R.A. and Parks, G.D. (2007) Paramyxoviridae: the viruses and their replication in: Fields Virology (Knipe, D.M. and Howley, P.M., Eds.), pp. 1450–1497, Lippincott Williams & Wilkins, Philadelphia, PA.
[3] Albertini, A.A.V., Schoehn, G. and Ruigrok, R.W. (2005) Structures impliquées dans la réplication et la transcription des virus à ARN non segmentés de sens négatif. Virologie 9, 83–92.
[4] Roux, L. (2005) Dans le génome des Paramyxovirinae, les promoteurs et leurs activités sont façonnés par la « règle de six. Virologie 9, 19–34.
[5] Blocquel, D., Bourhis, J.M., Eléouët, J.F., Gerlier, D., Habchi, J., Jamin, M., Longhi, S. and Yabukarski, F. (2012) Transcription et réplication des Mononégavirales: une machine moléculaire originale. Virologie 16, 225–257.
[6] Bourhis, J.M., Canard, B. and Longhi, S. (2006) Structural disorder within the replicative complex of measles virus: functional implications. Virology 344, 94–110.
[7] Bourhis, J.M. and Longhi, S. (2007) Measles virus nucleoprotein: structural organization and functional role of the intrinsically disordered C-terminal domain in: Measles Virus Nucleoprotein (Longhi, S., Ed.), pp. 1–35, Nova Publishers Inc., Hauppage, NY.
[8] Longhi, S. and Oglesbee, M. (2010) Structural disorder within the measles virus nucleoprotein and phosphoprotein. Protein Pept. Lett. 17, 961–978.
[9] Longhi, S. (2011) Structural disorder within the measles virus nucleoprotein and phosphoprotein: functional implications for transcription and replication in: Negative Strand RNA Virus (Luo, M., Ed.), pp. 95–125, World Scientific Publishing, Singapore.
[10] Habchi, J. and Longhi, S. (2012) Structural disorder within paramyxovirus nucleoproteins and phosphoproteins. Mol. BioSyst. 8, 69–81.
[11] Habchi, J., Mamelli, L. and Longhi, S. (2012) Structural disorder within the nucleoprotein and phosphoprotein from measles, Nipah and Hendra viruses in: Flexible viruses: structural disorder in viral proteins (Uversky, V.N. and Longhi, S., Eds.), pp. 47–94, John Wiley and Sons, Hoboken, New Yersey.
[12] Communie, G., Ruigrok, R.W., Jensen, M.R. and Blackledge, M. (2014) Intrinsically disordered proteins implicated in paramyxoviral replication machinery. Curr. Opin. Virol. 5, 72–81.
[13] Dunker, A.K., Babu, M.M., Barbar, E., Blackledge, M., Bondos, S.E., Dosztányi, Z., Dyson, H.J., Forman-Kay, J., Fuxreiter, M., Gsponer, J., Han, K.-H., Jones, D.T., Longhi, S., Metallo, S.J., Nishikawa, K., Nussinov, R., Obradovic, Z., Pappu, R.V., Rost, B., Selenko, P., Subramaniam, V., Sussman, J.L., Tompa, P. and Uversky, V.N. (2013) What's in a name? Why these proteins are intrinsically disordered. Intrinsically Disordered Proteins 1, e24157.
[14] Habchi, J., Tompa, P., Longhi, S. and Uversky, V.N. (2014) Introducing protein intrinsic disorder. Chem. Rev. 114, 6561–6588.
[15] Morin, B., Rahmeh, A.A. and Whelan, S.P. (2012) Mechanism of RNA synthesis initiation by the vesicular stomatitis virus polymerase. EMBO J. 31, 1320–1329.
[16] Johansson, K., Bourhis, J.M., Campanacci, V., Cambillau, C., Canard, B. and Longhi, S. (2003) Crystal structure of the measles virus phosphoprotein domain responsible for the induced folding of the C-terminal domain of the nucleoprotein. J. Biol. Chem. 278, 44567–44573.

[17] Kingston, R.L., Hamel, D.J., Gay, L.S., Dahlquist, F.W. and Matthews, B.W. (2004) Structural basis for the attachment of a paramyxoviral polymerase to its template. Proc. Natl. Acad. Sci. U.S.A. 101, 8301–8306.
[18] Gely, S., Lowry, D.F., Bernard, C., Ringkjobing-Jensen, M., Blackledge, M., Costanzo, S., Darbon, H., Daughdrill, G.W. and Longhi, S. (2010) Solution structure of the C-terminal X domain of the measles virus phosphoprotein and interaction with the intrinsically disordered C-terminal domain of the nucleoprotein. J. Mol. Recognit. 23, 435–447.
[19] Communie, G., Habchi, J., Yabukarski, F., Blocquel, D., Schneider, R., Tarbouriech, N., Papageorgiou, N., Ruigrok, R.W., Jamin, M., Ringkjøbing-Jensen, M., Longhi, S. and Blackledge, M. (2013) Atomic resolution description of the interaction between the nucleoprotein and phosphoprotein of Hendra virus. PLoS Pathog. 9, e1003631.
[20] Kingston, R.L., Gay, L.S., Baase, W.S. and Matthews, B.W. (2008) Structure of the nucleocapsid-binding domain from the mumps virus polymerase; an example of protein folding induced by crystallization. J. Mol. Biol. 379, 719–731.
[21] Blanchard, L., Tarbouriech, N., Blackledge, M., Timmins, P., Burmeister, W.P., Ruigrok, R.W. and Marion, D. (2004) Structure and dynamics of the nucleocapsid-binding domain of the Sendai virus phosphoprotein in solution. Virology 319, 201–211.
[22] Kingston, R.L., Walter, A.B. and Gay, L.S. (2004) Characterization of nucleocapsid binding by the measles and the mumps virus phosphoprotein. J. Virol. 78, 8630–8640.
[23] Tran, T.L., Castagne, N., Bhella, D., Varela, P.F., Bernard, J., Chilmonczyk, S., Berkenkamp, S., Benhamo, V., Grznarova, K., Grosclaude, J., Nespoulos, C., Rey, F.A. and Eleouet, J.F. (2007) The nine C-terminal amino acids of the respiratory syncytial virus protein P are necessary and sufficient for binding to ribonucleoprotein complexes in which six ribonucleotides are contacted per N protein protomer. J. Gen. Virol. 88, 196–206.
[24] Yegambaram, K., Bulloch, E.M. and Kingston, R.L. (2013) Protein domain definition should allow for conditional disorder. Protein Sci. 22, 1502–1518.
[25] Karlin, D., Longhi, S. and Canard, B. (2002) Substitution of two residues in the measles virus nucleoprotein results in an impaired self-association. Virology 302, 420–432.
[26] Habchi, J., Blangy, S., Mamelli, L., Ringkjobing, Jensen M., Blackledge, M., Darbon, H., Oglesbee, M., Shu, Y. and Longhi, S. (2011) Characterization of the interactions between the nucleoprotein and the phosphoprotein of Henipaviruses. J. Biol. Chem. 286, 13583–13602.
[27] Bhella, D., Ralph, A. and Yeo, R.P. (2004) Conformational flexibility in recombinant measles virus nucleocapsids visualised by cryo-negative stain electron microscopy and real-space helical reconstruction. J. Mol. Biol. 340, 319–331.
[28] Habchi, J., Mamelli, L., Darbon, H. and Longhi, S. (2010) Structural disorder within henipavirus nucleoprotein and phosphoprotein: from predictions to experimental assessment. PLoS ONE 5, e11684.
[29] Lieutaud, P., Canard, B. and Longhi, S. (2008) MeDor: a metaserver for predicting protein disorder. BMC Genomics 9, S25.
[30] Longhi, S., Receveur-Brechot, V., Karlin, D., Johansson, K., Darbon, H., Bhella, D., Yeo, R., Finet, S. and Canard, B. (2003) The C-terminal domain of the measles virus nucleoprotein is intrinsically disordered and folds upon binding to the C-terminal moiety of the phosphoprotein. J. Biol. Chem. 278, 18638–18648.
[31] Bourhis, J., Johansson, K., Receveur-Bréchot, V., Oldfield, C.J., Dunker, A.K., Canard, B. and Longhi, S. (2004) The C-terminal domain of measles virus nucleoprotein belongs to the class of intrinsically disordered proteins that fold upon binding to their physiological partner. Virus Res. 99, 157–167.
[32] Martinho, M., Habchi, J., El Habre, Z., Nesme, L., Guigliarelli, B., Belle, V. and Longhi, S. (2013) Assessing induced folding within the intrinsically disordered C-terminal domain of the Henipavirus nucleoproteins by site directed spin labeling EPR spectroscopy. J. Biomol. Struct. Dyn. 31, 453–471.
[33] Baronti, L., Erales, J., Habchi, J., Felli, I.C., Pierattelli, R. and Longhi, S. (2015) Dynamics of the intrinsically disordered C-terminal domain of the Nipah virus nucleoprotein and interaction with the X domain of the phosphoprotein as unveiled by NMR spectroscopy. Chem. BioChem. 16, 268–276.
[34] D'Urzo, A., Konijnenberg, A., Rossetti, G., Habchi, J., Li, J., Carloni, P., Sobott, F., Longhi, S. and Grandori, R. (2015) Molecular basis for structural heterogeneity of an intrinsically disordered protein bound to a partner by combined ESI-IM-MS and modeling. J. Am. Soc. Mass Spectrom. 26, 472–481.
[35] Rudolph, M.G., Kraus, I., Dickmanns, A., Eickmann, M., Garten, W. and Ficner, R. (2003) Crystal structure of the borna disease virus nucleoprotein. Structure (Camb) 11, 1219–1226.
[36] Warnes, A., Fooks, A.R., Dowsett, A.B., Wilkinson, G.W. and Stephenson, J.R. (1995) Expression of the measles virus nucleoprotein gene in *Escherichia coli* and assembly of nucleocapsid-like structures. Gene 160, 173–178.
[37] Bhella, D., Ralph, A., Murphy, L.B. and Yeo, R.P. (2002) Significant differences in nucleocapsid morphology within the Paramyxoviridae. J. Gen. Virol. 83, 1831–1839.
[38] Tan, W.S., Ong, S.T., Eshaghi, M., Foo, S.S. and Yusoff, K. (2004) Solubility, immunogenicity and physical properties of the nucleocapsid protein of Nipah virus produced in *Escherichia coli*. J. Med. Virol. 73, 105–112.
[39] Schoehn, G., Mavrakis, M., Albertini, A., Wade, R., Hoenger, A. and Ruigrok, R.W. (2004) The 12 A structure of trypsin-treated measles virus N-RNA. J. Mol. Biol. 339, 301–312.

[40] Desfosses, A., Goret, G., Farias, Estrozi L., Ruigrok, R.W. and Gutsche, I. (2011) Nucleoprotein-RNA orientation in the measles virus nucleocapsid by three-dimensional electron microscopy. J. Virol. 85, 1391–1395.

[41] Barbet-Massin, E., Felletti, M., Schneider, R., Jehle, S., Communie, G., Martinez, N., Jensen, M.R., Ruigrok, R.W., Emsley, L., Lesage, A., Blackledge, M. and Pintacuda, G. (2014) Insights into the structure and dynamics of measles virus nucleocapsids by 1H-detected solid-state NMR. Biophys. J. 107, 941–946.

[42] Tawar, R.G., Duquerroy, S., Vonrhein, C., Varela, P.F., Damier-Piolle, L., Castagné, N., MacLellan, K., Bedouelle, H., Bricogne, G., Bhella, D., Eleouet, J.F. and Rey, F.A. (2009) 3D structure of a nucleocapsid-like nucleoprotein-RNA complex of respiratory syncytial virus. Science 326, 1279–1283.

[43] Alayyoubi, M., Leser, G.P., Kors, C.A. and Lamb, R.A. (2015) Structure of the paramyxovirus parainfluenza virus 5 nucleoprotein-RNA complex. Proc. Natl. Acad. Sci. U.S.A. 112, E1792–E1799.

[44] Yabukarski, F., Lawrence, P., Tarbouriech, N., Bourhis, J.M., Delaforge, E., Jensen, M.R., Ruigrok, R.W., Blackledge, M., Volchkov, V. and Jamin, M. (2014) Structure of Nipah virus unassembled nucleoprotein in complex with its viral chaperone. Nat. Struct. Mol. Biol. 21, 754–759.

[45] Gutsche, I., Desfosses, A., Effantin, G., Ling, W.L., Haupt, M., Ruigrok, R.W., Sachse, C. and Schoehn, G. (2015) Near-atomic cryo-EM structure of the helical measles virus nucleocapsid. Science.

[46] Oldfield, C.J., Cheng, Y., Cortese, M.S., Romero, P., Uversky, V.N. and Dunker, A.K. (2005) Coupled folding and binding with alpha-helix-forming molecular recognition elements. Biochemistry 44, 12454–12470.

[47] Vacic, V., Oldfield, C.J., Mohan, A., Radivojac, P., Cortese, M.S., Uversky, V.N. and Dunker, A.K. (2007) Characterization of molecular recognition features, MoRFs, and their binding partners. J. Proteome Res. 6, 2351–2366.

[48] Diallo, A., Barrett, T., Barbron, M., Meyer, G. and Lefevre, P.C. (1994) Cloning of the nucleocapsid protein gene of peste-des-petits-ruminants virus: relationship to other morbilliviruses. J. Gen. Virol. 75 (Pt 1), 233–237.

[49] Laine, D., Trescol-Biémont, M., Longhi, S., Libeau, G., Marie, J., Vidalain, P., Azocar, O., Diallo, A., Canard, B., Rabourdin-Combe, C. and Valentin, H. (2003) Measles virus nucleoprotein binds to a novel cell surface receptor distinct from FcgRII via its C-terminal domain: role in MV-induced immunosuppression. J. Virol. 77, 11332–11346.

[50] Laine, D., Bourhis, J., Longhi, S., Flacher, M., Cassard, L., Canard, B., Sautès-Fridman, C., Rabourdin-Combe, C. and Valentin, H. (2005) Measles virus nucleoprotein induces cell proliferation arrest and apoptosis through NTAIL/NR and NCORE/FcgRIIB1 interactions, respectively. J. Gen. Virol. 86, 1771–1784.

[51] Bourhis, J.M., Receveur-Bréchot, V., Oglesbee, M., Zhang, X., Buccellato, M., Darbon, H., Canard, B., Finet, S. and Longhi, S. (2005) The intrinsically disordered C-terminal domain of the measles virus nucleoprotein interacts with the C-terminal domain of the phosphoprotein via two distinct sites and remains predominantly unfolded. Protein Sci. 14, 1975–1992.

[52] Blocquel, D., Habchi, J., Costanzo, S., Doizy, A., Oglesbee, M. and Longhi, S. (2012) Interaction between the C-terminal domains of measles virus nucleoprotein and phosphoprotein: a tight complex implying one binding site. Protein Sci. 21, 1577–1585.

[53] Morin, B., Bourhis, J.M., Belle, V., Woudstra, M., Carrière, F., Guigliarelli, B., Fournel, A. and Longhi, S. (2006) Assessing induced folding of an intrinsically disordered protein by site-directed spin-labeling EPR spectroscopy. J. Phys. Chem. B 110, 20596–20608.

[54] Belle, V., Rouger, S., Costanzo, S., Liquiere, E., Strancar, J., Guigliarelli, B., Fournel, A. and Longhi, S. (2008) Mapping alpha-helical induced folding within the intrinsically disordered C-terminal domain of the measles virus nucleoprotein by site-directed spin-labeling EPR spectroscopy. Proteins: Struct. Funct. Bioinform. 73, 973–988.

[55] Bernado, P., Blanchard, L., Timmins, P., Marion, D., Ruigrok, R.W. and Blackledge, M. (2005) A structural model for unfolded proteins from residual dipolar couplings and small-angle X-ray scattering. Proc. Natl. Acad. Sci. U.S.A. 102, 17002–17007.

[56] Jensen, M.R., Houben, K., Lescop, E., Blanchard, L., Ruigrok, R.W. and Blackledge, M. (2008) Quantitative conformational analysis of partially folded proteins from residual dipolar couplings: application to the molecular recognition element of Sendai virus nucleoprotein. J. Am. Chem. Soc. 130, 8055–8061.

[57] Ringkjøbing Jensen, M., Communie, G., Ribeiro Jr., E.D., Martinez, N., Desfosses, A., Salmon, L., Mollica, L., Gabel, F., Jamin, M., Longhi, S., Ruigrok, R.W. and Blackledge, M. (2011) Intrinsic disorder in measles virus nucleocapsids. Proc. Natl. Acad. Sci. U.S.A. 108, 9839–9844.

[58] Bischak, C.G., Longhi, S., Snead, D.M., Costanzo, S., Terrer, E. and Londergan, C.H. (2010) Probing structural transitions in the intrinsically disordered C-terminal domain of the measles virus nucleoprotein by vibrational spectroscopy of cyanylated cysteines. Biophys. J. 99, 1676–1683.

[59] Jensen, M.R., Bernado, P., Houben, K., Blanchard, L., Marion, D., Ruigrok, R.W. and Blackledge, M. (2010) Structural disorder within sendai virus nucleoprotein and phosphoprotein: insight into the structural basis of molecular recognition. Protein Pept. Lett. 17, 952–960.

[60] Blocquel, D., Habchi, J., Gruet, A., Blangy, S. and Longhi, S. (2012) Compaction and binding properties of the intrinsically disordered C-terminal domain of Henipavirus nucleoprotein as unveiled by deletion studies. Mol. BioSyst. 8, 392–410.

[61] Das, R.K. and Pappu, R.V. (2013) Conformations of intrinsically disordered proteins are influenced by linear sequence distributions of oppositely charged residues. Proc. Natl. Acad. Sci. U.S.A. 110, 13392–13397.

[62] Bhella, D. (2007) Measles virus nucleocapsid structure, conformational flexibility and the rule of six in: Measles Virus Nucleoprotein (Longhi, S., Ed.), Nova Publishers Inc., Hauppage, NY.

[63] Dosnon, M., Bonetti, D., Morrone, A., Erales, J., di Silvio, E., Longhi, S. and Gianni, S. (2015) Demonstration of a folding after binding mechanism in the recognition between the measles virus NTAIL and X domains. ACS ChemBiol. 10, 795–802.

[64] Shu, Y., Habchi, J., Costanzo, S., Padilla, A., Brunel, J., Gerlier, D., Oglesbee, M. and Longhi, S. (2012) Plasticity in structural and functional interactions between the phosphoprotein and nucleoprotein of measles virus. J. Biol. Chem. 287, 11951–11967.

[65] Gruet, A., Dosnon, M., Vassena, A., Lombard, V., Gerlier, D., Bignon, C. and Longhi, S. (2013) Dissecting partner recognition by an intrinsically disordered protein using descriptive random mutagenesis. J. Mol. Biol. 425, 3495–3509.

[66] Meszaros, B., Tompa, P., Simon, I. and Dosztanyi, Z. (2007) Molecular principles of the interactions of disordered proteins. J. Mol. Biol. 372, 549–561.

[67] Houben, K., Marion, D., Tarbouriech, N., Ruigrok, R.W. and Blanchard, L. (2007) Interaction of the C-terminal domains of sendai virus N and P proteins: comparison of polymerase-nucleocapsid interactions within the paramyxovirus family. J. Virol. 81, 6807–6816.

[68] Erales, J., Beltrandi, M., Roche, J., Maté, M. and Longhi, S. (2015) Insights into the Hendra virus NTAIL–XD complex: evidence for a parallel organization of the helical MoRE at the XD surface stabilized by a combination of hydrophobic and polar interactions. BBA-Proteins and Proteomics 1854 (8), 1038–1053.

[69] Schreiber, G., Haran, G. and Zhou, H.X. (2009) Fundamental aspects of protein–protein association kinetics. Chem. Rev. 109, 839–860.

[70] Xue, Y., Yuwen, T., Zhu, F. and Skrynnikov, N.R. (2014) Role of electrostatic interactions in binding of peptides and intrinsically disordered proteins to their folded targets. 1. NMR and MD characterization of the complex between the c-Crk N-SH3 domain and the peptide Sos. Biochemistry 53, 6473–6495.

[71] Tsai, C.J., Ma, B., Sham, Y.Y., Kumar, S. and Nussinov, R. (2001) Structured disorder and conformational selection. Proteins: Struct. Funct. Bioinform. 44, 418–427.

[72] Shoemaker, B.A., Portman, J.J. and Wolynes, P.G. (2000) Speeding molecular recognition by using the folding funnel: the fly-casting mechanism. Proc. Natl. Acad. Sci. U.S.A. 97, 8868–8873.

[73] Wang, Y., Chu, X., Longhi, S., Roche, P., Han, W., Wang, E. and Wang, J. (2013) Multiscaled exploration of coupled folding and binding of an intrinsically disordered molecular recognition element in measles virus nucleoprotein. Proc. Natl. Acad. Sci. U.S.A. 110, E3743–E3752.

[74] Kavalenka, A., Urbancic, I., Belle, V., Rouger, S., Costanzo, S., Kure, S., Fournel, A., Longhi, S., Guigliarelli, B. and Strancar, J. (2010) Conformational analysis of the partially disordered measles virus NTAIL-XD complex by SDSL EPR spectroscopy. Biophys. J. 98, 1055–1064.

[75] Tompa, P. and Fuxreiter, M. (2008) Fuzzy complexes: polymorphism and structural disorder in protein–protein interactions. Trends Biochem. Sci. 33, 2–8.

[76] Zhang, X., Bourhis, J.M., Longhi, S., Carsillo, T., Buccellato, M., Morin, B., Canard, B. and Oglesbee, M. (2005) Hsp72 recognizes a P binding motif in the measles virus N protein C-terminus. Virology 337, 162–174.

[77] Couturier, M., Buccellato, M., Costanzo, S., Bourhis, J.M., Shu, Y., Nicaise, M., Desmadril, M., Flaudrops, C., Longhi, S. and Oglesbee, M. (2010) High Affinity Binding between Hsp70 and the C-Terminal Domain of the Measles Virus Nucleoprotein Requires an Hsp40 Co-Chaperone. J. Mol. Recognit. 23, 301–315.

[78] Zhang, X., Glendening, C., Linke, H., Parks, C.L., Brooks, C., Udem, S.A. and Oglesbee, M. (2002) Identification and characterization of a regulatory domain on the carboxyl terminus of the measles virus nucleocapsid protein. J. Virol. 76, 8737–8746.

[79] Carsillo, T., Zhang, X., Vasconcelos, D., Niewiesk, S. and Oglesbee, M. (2006) A single codon in the nucleocapsid protein C terminus contributes to in vitro and in vivo fitness of Edmonston measles virus. J. Virol. 80, 2904–2912.

[80] Oglesbee, M. (2007) Nucleocapsid protein interactions with the major inducible heat shock protein in: Measles Virus Nucleoprotein (Longhi, S., Ed.), pp. 53–98, Nova Publishers Inc., Hauppage, NY.

[81] Carsillo, T., Traylor, Z., Choi, C., Niewiesk, S. and Oglesbee, M. (2006) Hsp72, a host determinant of measles virus neurovirulence. J. Virol. 80, 11031–11039.

[82] Gruet, A., Longhi, S. and Bignon, C. (2012) One-step generation of error-prone PCR libraries using Gateway(R) technology. Microb. Cell Fact. 11, 14.

[83] Krumm, S.A., Takeda, M. and Plemper, R.K. (2013) The measles virus nucleocapsid protein tail domain is dispensable for viral polymerase recruitment and activity. J. Biol. Chem. 288, 29943–29953.

[84] Longhi, S. (2007) Measles Virus Nucleoprotein, Nova Publishers Inc., Hauppage, NY.

[85] Longhi, S. (2009) Nucleocapsid structure and function. Curr. Top. Microbiol. Immunol. 329, 103–128.

[86] Cox, R., Pickar, A., Qiu, S., Tsao, J., Rodenburg, C., Dokland, T., Elson, A., He, B. and Luo, M. (2014) Structural studies on the authentic mumps virus nucleocapsid showing uncoiling by the phosphoprotein. Proc. Natl. Acad. Sci. U.S.A. 111, 15208–15213.

[87] Oglesbee, M., Ringler, S. and Krakowka, S. (1990) Interaction of canine distemper virus nucleocapsid variants with 70K heat-shock proteins. J. Gen. Virol. 71, 1585–1590.

[88] Oglesbee, M., Tatalick, L., Rice, J. and Krakowka, S. (1989) Isolation and characterization of canine distemper virus nucleocapsid variants. J. Gen. Virol. 70 (Pt 9), 2409–2419.

[89] Tapparel, C., Maurice, D. and Roux, L. (1998) The activity of Sendai virus genomic and antigenomic promoters requires a second element past the leader template regions: a motif (GNNNNN)3 is essential for replication. J. Virol. 72, 3117–3128.

[90] Haynes, C., Oldfield, C.J., Ji, F., Klitgord, N., Cusick, M.E., Radivojac, P., Uversky, V.N., Vidal, M. and Iakoucheva, L.M. (2006) Intrinsic disorder is a common feature of hub proteins from four eukaryotic interactomes. PLoS Comput. Biol. 2, e100.

[91] Iwasaki, M., Takeda, M., Shirogane, Y., Nakatsu, Y., Nakamura, T. and Yanagi, Y. (2009) The matrix protein of measles virus regulates viral RNA synthesis and assembly by interacting with the nucleocapsid protein. J. Virol. 83, 10374–10383.

[92] tenOever, B.R., Servant, M.J., Grandvaux, N., Lin, R. and Hiscott, J. (2002) Recognition of the measles virus nucleocapsid as a mechanism of IRF-3 activation. J. Virol. 76, 3659–3669.

[93] Colombo, M., Bourhis, J.M., Chamontin, C., Soriano, C., Villet, S., Costanzo, S., Couturier, M., Belle, V., Fournel, A., Darbon, H., Gerlier, D. and Longhi, S. (2009) The interaction between the measles virus nucleoprotein and the Interferon Regulator Factor 3 relies on a specific cellular environment. Virol. J. 6, 59.

[94] Sato, H., Masuda, M., Miura, R., Yoneda, M. and Kai, C. (2006) Morbillivirus nucleoprotein possesses a novel nuclear localization signal and a CRM1-independent nuclear export signal. Virology 352, 121–130.

[95] Watanabe, A., Yoneda, M., Ikeda, F., Sugai, A., Sato, H. and Kai, C. (2011) Peroxiredoxin 1 is required for efficient transcription and replication of measles virus. J. Virol. 85, 2247–2253.

[96] Moyer, S.A., Baker, S.C. and Horikami, S.M. (1990) Host cell proteins required for measles virus reproduction. J. Gen. Virol. 71, 775–783.

[97] Karlin, D., Ferron, F., Canard, B. and Longhi, S. (2003) Structural disorder and modular organization in Paramyxovirinae N and P. J. Gen. Virol. 84, 3239–3252.

[98] Karlin, D., Longhi, S., Receveur, V. and Canard, B. (2002) The N-terminal domain of the phosphoprotein of morbilliviruses belongs to the natively unfolded class of proteins. Virology 296, 251–262.

[99] Communie, G., Crepin, T., Maurin, D., Jensen, M.R., Blackledge, M. and Ruigrok, R.W. (2013) Structure of the tetramerization domain of measles virus phosphoprotein. J. Virol. 87, 7166–7169.

[100] Blocquel, D., Habchi, J., Durand, E., Sevajol, M., Ferron, F., Erales, J., Papageorgiou, N. and Longhi, S. (2014) Coiled-coil deformations in crystal structures: the measles virus phosphoprotein multimerization domain as an illustrative example. Acta Crystallogr. D 70, 1589–1603.

[101] Tokuriki, N., Oldfield, C.J., Uversky, V.N., Berezovsky, I.N. and Tawfik, D.S. (2009) Do viral proteins possess unique biophysical features? Trends Biochem. Sci. 34, 53–59.

[102] Xue, B., Williams, R.W., Oldfield, C.J., Goh, G.K., Dunker, A.K. and Uversky, V.N. (2010) Viral disorder or disordered viruses: do viral proteins possess unique features? Protein Pept. Lett. 17, 932–951.

[103] Xue, B., Dunker, A.K. and Uversky, V.N. (2012) Orderly order in protein intrinsic disorder distribution: disorder in 3500 proteomes from viruses and the three domains of life. J. Biomol. Struct. Dyn. 30, 137–149.

[104] Peng, Z., Yan, J., Fan, X., Mizianty, M.J., Xue, B., Wang, K., Hu, G., Uversky, V.N. and Kurgan, L. (2015) Exceptionally abundant exceptions: comprehensive characterization of intrinsic disorder in all domains of life. Cell. Mol. Life Sci. 72, 137–151.

[105] Uversky, V.N. and Longhi, S. (2012) Flexible Viruses: Structural Disorder in Viral Proteins, John Wiley & Sons, Hoboken, New Jersey.

[106] Xue, B., Blocquel, D., Habchi, J., Uversky, A.V., Kurgan, L., Uversky, V.N. and Longhi, S. (2014) Structural disorder in viral proteins. Chem. Rev. 114, 6880–6911.

[107] Davey, N.E., Trave, G. and Gibson, T.J. (2011) How viruses hijack cell regulation. Trends Biochem. Sci. 36, 159–169.

[108] Rancurel, C., Khosravi, M., Dunker, K.A., Romero, P.R. and Karlin, D. (2009) Overlapping genes produce proteins with unusual sequence properties and offer insight into de novo protein creation. J. Virol. 83, 10719–10736.

[109] Romero, P.R., Zaidi, S., Fang, Y.Y., Uversky, V.N., Radivojac, P., Oldfield, C.J., Cortese, M.S., Sickmeier, M., LeGall, T., Obradovic, Z. and Dunker, A.K. (2006) Alternative splicing in concert with protein intrinsic disorder enables increased functional diversity in multicellular organisms. Proc. Natl. Acad. Sci. U.S.A. 103, 8390–8395.

[110] Kovacs, E., Tompa, P., Liliom, K. and Kalmar, L. (2010) Dual coding in alternative reading frames correlates with intrinsic protein disorder. Proc. Natl. Acad. Sci. U.S.A. 107, 5429–5434.