Review

Structural disorder within paramyxoviral nucleoproteins

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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 (NTAIL) 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 NTAIL–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*) 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...
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. By encapsidating the viral genome the N protein not only protects it from degradation, but it also renders it competent for transcription and replication.

The crystal structure of a monomeric, RNA-free form of the NiV N protein devoid of the NTD-arm and of NTAIL domains could not be resolved in the reconstruction of the nucleocapsid, the fit suggests that NTAIL would point towards the interior of the helical nucleocapsid [40].
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}–PNTD, 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_{-1} and N_{+1} sub-units 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_{STD} is superimposed onto the MeV N_{CORE} structure, the α-helix of the NTD-arm of the MeV N_{-1} protomer perfectly superimposes onto the helix α1 of P_{NTD} (Pα1) from the NiV N_{CORE}–PSTD structure, while the loop of the CTD-arm of the MeV N_{+1} protomer overlaps with helix α2 of P_{NTD} (Pα2) (Fig. 2D). Thus Pα1 competes with the NTD-arm of the N_{-1} protomer, and Pα2 with the CTD-arm of the N_{+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...
In line with this, bioinformatics analyses indicated the presence of short order-prone regions, corresponding to putative Molecular Recognition Elements (MoREs) [28,30,31]. 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 (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 NTAIL and of the mobility of spin labels grafted within Box2 showed that the α-MoRE of MeV NTAIL 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 NTAIL 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 NTAIL 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 NTAIL 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, SDS-PAGE 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 NTAIL 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 NTAIL. 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 NTAIL were observed [32,33,60]. For example, a NiV NTAIL 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 NTAIL proteins have distinct features in spite of their high sequence identity (74%) [33]. In particular, the NiV NTAIL Box2 region experiences a slightly higher extent of α-helical preconfiguration and seemingly plays a role in binding to XD, while HeV NTAIL Box2 does not. A close inspection of the Box2 region reveals a notable difference at position 457, where the Asp of HeV NTAIL is replaced by an Asn in NiV NTAIL. 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 NTAIL 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 NTAIL domains within nucleocapsid-like particles

Beyond being disordered in isolation, the MeV and Henipavirus NTAIL domains are also disordered within full-length N proteins from nucleocapsid-like particles, as judged from NMR studies carried out on 15N-labeled nucleocapsids [19,33,57] (Fig. 3A–C). In those studies, both MeV and Henipavirus NTAIL 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 NTAIL are conformationally restricted. In the case of MeV, by combining those data with previous modelling data [40], a model was proposed where NTAIL escapes from the inner channel to the outside of the nucleocapsid via the interstitial space between successive NCORE helical turns [19,57] (Fig. 3D). Notably, this model provides a plausible explanation for the increased rigidity of nucleocapsids in which the flexible NTAIL 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 NTAIL, 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 NTAIL 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 NTAIL–XD complex formation

The NTAIL domains of MeV, NiV and HeV were shown to bind to XD with an equilibrium dissociation constant (Kd) in the μM range [26,63]. As already mentioned, binding to XD triggers α-helical folding of NTAIL [16,26]. Interestingly, while NMR titration experiments with 15N-labeled NTAIL indicated an α-helical transition within MeV NTAIL upon addition of the homologous X domain, as judged from the appearance of new peaks in the α-helical region of the 15N-TAIL spectra [18,26,51], no such peaks were observed in the HeV and NiV NTAIL 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 NTAIL 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 NTAIL 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 NTAIL (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 Kd observed with an NTAIL variant bearing an Asp at this position [64]. Recently, random mutagenesis studies confirmed the crucial role of NTAIL residue Ser491 in
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 NTAIL–XD complex was confirmed [65].

ITC studies revealed that Henipavirus NTAIL–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 NTAIL at the XD surface, as already observed in the case of MeV [16,22]. Strikingly, while in the case of NiV, NTAIL/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 NTAIL–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 NTAIL was modelled at the hydrophobic surface delimited by helices α2 and α3 of XD using the MeV NTAIL–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 NTAIL–XD complex, the binding interface is dominated by charged residues [67]. Although direct structural data on Henipavirus NTAIL–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 NTAIL [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 NTAIL–XD interaction could be controlled by a combination of...
long-range electrostatic forces that correctly orient NTAIL prior to accommodation in the narrow hydrophobic pocket at the surface of XD. ITC studies carried out on the HeV NTAIL/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 NTAIL 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 NTAIL/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 NTAIL 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 NTAIL 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 NTAIL 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 NTAIL 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 NTAIL 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 NTAIL 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 NTAIL, computational and experimental studies converged to support a folding after binding mechanism. Indeed, the resonance behaviour of NTAIL 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 NTAIL 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 NTAIL, having also been documented in the case of HeV NTAIL [19]. In this latter case, quantitative analysis of peak intensities in the HSQC spectra of NTAIL 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 NTAIL. 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 NTAIL 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 NTAIL–XD complex

A low-resolution model of the MeV NTAIL–XD complex obtained by SAXS showed that most of NTAIL (residues 401–488) remains disordered within the complex [51]. A subsequent study that made use of a combination of SDS-PAGE spectroscopy and modelling showed that a considerable residual flexibility persists also within the NTAIL region encompassing residues 505–525 in the complex [74]. In further support, a recent mass spectrometry study showed that the MeV NTAIL–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 NTAIL 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 NTAIL 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 NTAIL binding to XD could rely on an electrostatic steering mechanism [69], as already proposed in the case of HeV [68].

The MeV NTAIL–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 NTAIL–XD complexes were found to be fuzzy too. Indeed, the experimentally determined hydrodynamic radius of the NiV NTAIL–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 NTAIL–XD complexes, the many observable and relatively sharp NMR resonances that are nearly unaltered upon addition of XD provide evidence that these NTAIL regions remain significantly disordered in the bound state [17–19,26,33]. Strikingly, the Henipavirus NTAIL–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 NTAIL 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 NTAIL 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 (Kd of 10 nM) [78,81]. Since hsp70 competitively inhibits XD binding to NTAIL [76], it has been proposed that hsp70 could enhance transcription and genome replication by reducing the stability of P–NTAIL 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–NTAIL complexes would thus rely on competition between hsp70 and XD for binding to the α-MoRE of NTAIL, 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 NTAIL 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 NTAIL–XD interaction remains however to be elucidated. In the same vein, MeV NTAIL 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 NTAIL–XD interaction and of structural disorder within NTAIL

The NTAIL–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 NTAIL 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 NTAIL–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 NTAIL and/or the inherent flexibility (and hence potential to undergo conformational changes) of the first 50 NTAIL 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 NTAIL 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 NTAIL unexpectedly showed that a reduced binding strength has no impact on the polymerase rate [64]. This tolerance of the polymerase to NTAIL substitutions is probably true only in a certain range of affinities, where in spite of a pronounced drop in the affinity towards XD, the NTAIL–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 carthwheel 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 NTAIL [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 NTAIL–XD interaction. The prevalently disordered nature of NTAIL 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 NTAIL 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 interaction (i.e. promiscuity) [90]. In fact, in addition to P, MeV NTAIL also interacts 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 in proteins of the viral genomes is 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 compensation [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.

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