Structure, Transcription, and Replication of Measles Virus

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1 Virion Structure 35
2 Overview of the Infectious Cycle 37
3 Experimental Systems for Synthesis of Viral RNA In Vitro 38
4 RNA Polymerase 39
5 Transcription 41
6 RNA Replication 44
7 Future Studies 46
References 47

1 Virion Structure

Measles virus, a member of the morbillivirus genus of the paramyxovirus family, is an enveloped virus containing a single-stranded, minus (–) sense 50S RNA genome (Baczko et al. 1983; Dunlap et al. 1983; Udem and Cook 1984). Negatively stained preparations of virus particles appear roughly spherical but pleomorphic by electron microscopy, with the diameters of the particles ranging from 300 um to 1000 um (Lund et al. 1984). The envelope of the virion consists of a lipid bilayer membrane; the integral viral membrane proteins hemagglutinin (H, 80 kDa) (Gerald et al. 1986) and the two subunits of the fusion protein (F1, 40 kDa and F2, 20 kDa) (Buckland et al. 1987; Richardson et al. 1986; Stallcup et al. 1979) can be released by trypsin treatment. The matrix protein (M, 37 kDa) (Greer et al. 1986) appears to lie on the inner surface of the membrane and can be released with detergent and high salt (Bellini et al. 1986; Stallcup et al. 1979). Virion RNA is packaged in a helical ribonucleoprotein particle or nucleocapsid (Nakai et al. 1969; Robbins et al. 1980; and Lund et al. 1984) by the nucleocapsid protein (N, 60 kDa) (Rozenblatt et al. 1985). Measles nucleocapsids can be purified from virus banded on CsCl gradients as ribonucleoprotein particles at a density of 1.32 g/cm^3

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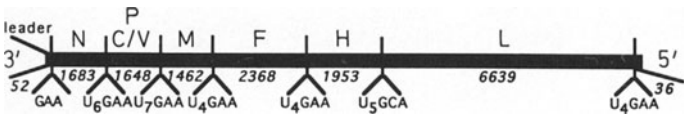

Fig. 1. Gene organization of the measles virus RNA. The measles virion RNA is indicated by the *horizontal line*, with the gene and boundary regions indicated *above* and *below the line*, respectively. The number of nucleotides in each gene is indicated *below* the gene. The data are a summary of the sequencing data contributed by the authors (referenced in the text)

(Stallcup et al. 1979; Robbins et al. 1980; Udem and Cook 1984). The association between RNA and N is very stable, being resistant to dissociation by high salt, and the encapsidated RNA is resistant to nuclease digestion (Andzhaparidze et al. 1987; Moyer et al. 1990). The virus-encoded RNA-dependent RNA polymerase consists of two subunits, the P (70 kDa) and L (~250 kDa) proteins, and is associated with the nucleocapsid in the virion (Bellini et al. 1985; Blumberg et al. 1988; Seifried et al. 1978).

The measles virus gene order, 3' leader-N-P/ C/ V-M-F-H-L-trailer 5' (Fig. 1), was first inferred from northern analyses of the mono- and polycistronic mRNAs isolated from measles-infected cells (Barret and Underwood 1985; Richardson et al. 1985; Dowling et al. 1986; Rima et al. 1986; Yoshikawa et al. 1986) and then directly determined by sequencing (Bellini et al. 1985, 1986; Rozenblatt et al. 1985; Gerald et al. 1986; Alkhatib and Briedis 1986; Richardson et al. 1986;

A. Gene start Sequences

Gene	Intergenic spacer	Genomic sequence
	3'	5'
N	GAA	UCCUAAGUUC.....
P/C/V	GAA	UCCUUGGUCC.....
M	GAA	UCCUCGUUUC.....
F	GAA	UCCCGGUUCC.....
H	GAA	UCCCACGUUC.....
L	GCA	UCCCAGGUUC.....

B. Gene stop Sequences

Gene	Genomic sequence	
	3'	5'
N	.....AGGUAGUAACAAUA	UUUUUU
P/C/V	.....GGAUUUAGGUAAUA	UUUUUUU
M	.....GGCCGUUUGAUUUG	UUUU
F	.....UAGCCAUCAAUUAA	UUUU
H	.....UGUAGUCUUAAUUC	UUUUU
L	.....UUAUAUAUAAUUUC	UUUU

Fig. 2A,B. Sequences at the 3' and 5' ends of the measles virus genes. The data from Crowley et al. (1988) are presented as (–) sense RNA

that the reaction proceeds in a standard manner, through concerted joining of the two ends of an excised linear transposon to the target DNA.

3.3 The Chemistry of DNA Breakage and Joining

The double-strand breaks that excise Tn7 from the donor backbone cleanly expose the 3' ends of the transposon but are staggered, such that 3 nucleotides of flanking donor DNA remain attached to the 5' transposon ends (BAINTON et al. 1991; P. Gary and N.L. Craig, unpublished; Fig. 4B). This contrasts with the excision of the bacterial transposon Tn10, which also transposes via an excised linear transposon where the 5' strands are also cut at the transposon termini (BENJAMIN and KLECKNER 1989, 1992). Tn7 is inserted into the target DNA by the joining of the exposed 3' transposon ends to staggered positions on the top and bottom strands of *attTn7*; these positions are separated by 5 bp (BAINTON et al. 1991). The joining of the 3' transposon ends to 5' positions in the target DNA results in the concomitant generation of exposed 3' ends in the target DNA. Thus the newly inserted Tn7 transposon is covalently linked to the target DNA through its 3' ends and is flanked by short gaps at its 5' ends, reflecting the staggered positions of end joining; the intact strands of the flanking gaps are the top and bottom strand of the target DNA between the positions of end joining. The repair of these gaps by the host DNA repair machinery results in the characteristic 5-bp duplications that flank Tn7 insertions; this repair process is also presumed to remove the few nucleotides of donor DNA attached to the 5' transposon ends in the initial recombination product.

The same chemistry of DNA breakage and joining, i.e., breakage to expose the 3' ends of the transposon and subsequent covalent linkage of these ends to 5' ends of target DNA, has been determined for all other elements that have been investigated at the biochemical level including Mu, retroviruses and retrotransposons, and other bacterial elements such as Tn10 (reviewed in MIZUUCHI 1992).

It should be noted that although it appears as if the excised transposon has joined to the target DNA by joining of the transposon ends of a staggered double-strand break at the insertion site, there is no evidence to support the view that Tn7 transposition proceeds through such a mechanism. No such double-strand

Fig. 4A, B. The pathway of transposition. **A** Transposition substrates, intermediates, and products are shown. On the *left* are the substrates, a donor plasmid containing Tn7 (white box) and a target plasmid containing *attTn7* (*black box*). Recombination initiates with double-strand breaks at either transposon end; pairs of breaks on the same substrate generate an excised linear transposon which inserts into the target DNA. The transposition products are a simple insertion and a gapped donor backbone. **B** The chemistry of breakage and joining during Tn7 transposition is shown. Recombination begins with staggered double-strand breaks at the ends of Tn7 which cleanly expose the 3' terminal-*CA*s and leave several nucleotides of donor DNA (*d*) attached to the 5' ends of the transposon. Tn7 joins to the displaced 5' positions on the target DNA though its terminal 3' *A*s. The simple insertion transposition product has covalent linkages between the 3' ends of the transposon and 5' ends of target DNA. The 5' transposon ends are flanked by short gaps; repair of these gaps by the host repair machinery generates 5-bp duplications of target sequences

the synthesis of the (+) strand nucleocapsid which in turn serves as the template for the synthesis of progeny nucleocapsids containing the (–) strand genome RNA. The newly synthesized (–) strand nucleocapsids are templates for secondary transcription, amplifying the viral mRNAs and proteins in the infected cell, as well as for budding of progeny virus.

The envelope components of the virion, the H and F proteins, are synthesized on membrane-bound ribosomes, transported through the endoplasmic reticulum, glycosylated in the Golgi, and become integral plasma membrane proteins (Morrison and Portner 1991; Ray et al. 1991b). The M protein is synthesized on cytoplasmic ribosomes and binds both progeny nucleocapsids (Stallcup et al. 1979; Hirano et al. 1992) and one or both of the viral glycoproteins at the cytoplasmic surface of the plasma membrane (Peebles 1991). The virions form by budding of the nucleocapsids through the plasma membrane, a process largely defined only by electron microscopy (Nakai et al. 1969). Virus containing both single and multiple nucleocapsids with both the (+) and (–) sense RNAs are formed, indicating a lack of specificity in the maturation process (Lund et al. 1984).

Cellular proteins are thought to be required for measles virus maturation. Cytochalasin B, an inhibitor of actin microfilaments, inhibits virus formation and results in the accumulation of measles nucleocapsids within the cell (Stallcup et al. 1983). Actin binds M protein (Guiffre et al. 1982), is also packaged in virions (Tyrrell and Norrby 1978; Stallcup et al. 1979; Robbins et al. 1980) and has been shown to be associated with measles virus nonreplicating nucleocapsids in vitro (Moyer et al. 1990). From this and electron microscopy data (Bohn et al. 1986), it is proposed that progeny nucleocapsids attach to growing actin filaments, possibly through their association with M protein bound to the nucleocapsid, and the vectoral growth of the actin filament is used as the means of transport of the nucleocapsids from the cytoplasm to the plasma membrane to initiate budding.

3 Experimental Systems for Synthesis of Viral RNA in Vitro

We will concentrate in this review specifically on the mechanisms of measles virus RNA synthesis. While many aspects of the transcription and replication of the measles virus genome appear to be similar to other nonsegmented negative strand RNA viruses, detailed studies of this virus are not as advanced. RNA synthesis of two other viruses, vesicular stomatitis virus (VSV), a rhabdovirus, and Sendai virus, a paramyxovirus, has been extensively studied (for reviews, see Banerjee 1987; Banerjee and Barik 1992; Kingsbury 1991) and these will serve as models for the present discussion when specific data on measles virus are not available. The initial studies of measles virus focused on the characterization of the various RNA products from infected cells, as summarized above, from which models for RNA synthesis were derived. Major advancements came with the

establishment and characterization of a variety of cell-free systems that would support RNA synthesis in vitro. We will first discuss the various systems that have been employed and then describe the mechanisms of both transcription and RNA replication in detail.

Seifried et al. (1978) first described in vitro measles transcription using detergent disrupted virus and an assay measuring the incorporation of radiolabeled GTP into TCA precipitable material. They showed that the polymerase activity copurified with virus in sucrose gradients. More recently we have also utilized purified measles virus to study various parameters of transcription in vitro (Horikami and Moyer 1991). Other in vitro transcription systems utilizing cytoplasmic extracts of, or nucleocapsids from, virus-infected cells were developed and permitted the synthesis of individual virus-specific mRNAs and some genome RNA (Ray and Fujinami 1987; Moyer et al. 1990).

A powerful mammalian expression system utilizing cloned viral genes was developed to study VSV and Sendai virus RNA replication in vivo (Pattnaik and Wertz 1990; Curran et al. 1991). Following this methodology, we extended this system to study in vitro RNA synthesis of Sendai virus (Horikami et al. 1992) and more recently of measles virus (unpublished data). In this procedure cells are infected with a recombinant vaccinia virus containing the T7 RNA polymerase gene. Newly formed T7 RNA polymerase drives expression of the subsequently transfected plasmids containing the essential Sendai virus genes NP, P, and L, which were cloned downstream of the T7 promoter. The viral proteins in extracts of these cells are used for in vitro RNA synthesis reactions. This system is very versatile, since the individual genes can be modified and then expressed in various combinations to monitor different steps in either transcription or RNA replication.

4 RNA Polymerase

The measles RNA polymerase is thought to consist of a complex formed from two different components, the L and P proteins. For VSV and Newcastle disease virus (NDV), each subunit of the RNA polymerase was purified and reconstitution experiments showed that both P and L are required for viral transcription (Hamaguchi et al. 1983; Emerson and Yu 1975). A similar, direct biochemical analysis of the measles virus RNA polymerase has not yet been performed. Although there is no direct experimental evidence as yet for any negative strand virus, it is believed that the L protein contains the majority, if not all, of the catalytic activities. Consistent with this hypothesis is the large size of the L protein (measles virus, 2183 amino acids). In addition, complementation studies with VSV temperature-sensitive mutants defined several intragenic complementation groups in the L gene which are also consistent with multiple functional domains in this protein (Pringle 1987).

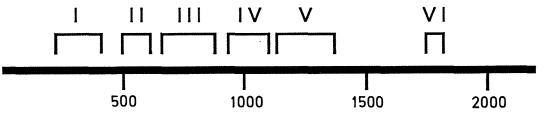

Fig. 3. Conserved regions in the measles virus L protein. The amino acid sequence of the measles L protein is depicted by the *horizontal line* with the positions of the conserved domains, I–VI, indicated *above the line*. The data were adapted from POCH et al. (1990)

The measles virus L amino acid sequence has been compared with that of other negative strand RNA viruses. Six conserved regions, I-VI (Fig. 3), have been described (BLUMBERG et al. 1988; POCH et al. 1990), although their functions have not yet been identified. The only transcription-associated activities assigned so far to the L protein have been described in VSV. The mRNA methyltransferase activities (HAMMOND and LESNAW 1987; HERCYK et al. 1988) and an aberrant polyadenylation activity (HUNT and HUTCHINSON 1993) map to the L protein. In the latter case, sequence analysis of mutants and revertants suggests that the polyadenylation mutation maps between conserved boxes V and VI in the COOH-terminus of the protein. Mutation of the GDN site in conserved region III abolished transcription of the VSV L protein (SLEAT and BANERJEE 1993), suggesting that this motif is important for catalytic activity. In other experiments CANTER et al. (1993) showed that deletion of a possible nucleotide binding motif in box VI in the VSV L protein also abrogated transcription. Future site-directed mutagenesis of the L gene in these systems will be an invaluable approach in mapping the functional domains of the protein.

Among the paramyxoviruses, the P gene is unique because it alone codes for multiple proteins, P, C and V. In measles virus the largest open reading frame in the P mRNA starts at the first AUG at nucleotide 60 and encodes the P protein (507 amino acids) in the O reading frame (BELLINI et al. 1985). In the overlapping +1 reading frame starting at nucleotide 82 is a second AUG used for the translation of the C protein (186 amino acids). The V protein will be discussed later. Both the P and C proteins are expressed from the same mRNA in measles virus-infected cells and during translation in vitro. The measles P protein is phosphorylated at multiple sites, but the C protein is unphosphorylated (BELLINI et al. 1984; ALKHATIB et al. 1988). Phosphorylation of P protein does not depend on other measles proteins, since this modification occurs when the P gene alone is expressed in an adenovirus virus vector (ALKHATIB et al. 1988). The functional role, if any, of phosphorylation is unknown, although in VSV, specific P phosphorylation sites are essential for transcription (BANERJEE and BARIK 1992).

Immunofluorescence microscopy shows that the measles C protein is present in the cytoplasm of infected cells in both diffuse and punctate staining, and it was found in the nucleus as well in one study (BELLINI et al. 1985; ALKHATIB et al. 1988). The C protein is apparently not present in purified measles virions. In Sendai virus, data from in vitro RNA synthesis reactions suggest that the C protein plays a role specifically in the regulation (inhibition) of mRNA synthesis (CURRAN et al. 1992). For measles virus we found that the addition of a rabbit anti-

measles C peptide antibody to in vitro reactions had no effect on RNA synthesis (unpublished data); however, future studies using proteins expressed from cloned genes will be required to more directly address a possible role of C protein in measles virus transcription.

Although not done directly with measles virus, the P protein of the paramyxoviruses NDV and Sendai virus was shown to be required for RNA synthesis by biochemical fractionation as mentioned above and by the inhibition of transcription with monoclonal antibodies to P, respectively (Hamaguchi et al. 1983; Deshpande and Portner 1985). P protein appears to have multiple roles in RNA synthesis, since Sendai virus P protein forms separate complexes with the L and N proteins (Horikami et al. 1992). Ryan and coworkers showed that the Sendai P protein will bind to the N protein of Sendai nucleocapsids in the absence of L protein (Ryan and Portner 1990; Ryan et al. 1991). Specifically, they have identified two separate regions of the P protein, one at the COOH-terminal and one at an upstream region, that are both required for binding to viral nucleocapsids. These regions are separated by a 66 amino acid domain that is not required for N binding. The individual measles virus N and P proteins also form a complex, since both full length P and a polypeptide encompassing the COOH-terminal amino acids 322–507 of P protein bound N protein in an assay in which monoclonal antibodies to either P or N coimmunoprecipitate the other protein (Huber et al. 1991). While the N binding domain of measles P clearly resides in its COOH-terminal half, further mapping of the essential domain(s) is needed to determine if it also has the bipartite organization found in the Sendai P protein. The function of the N-P complex in RNA replication will be discussed later.

We showed for Sendai virus that RNA polymerase activity requires a complex of P and L proteins that forms only when the proteins are coexpressed in mammalian cells (Horikami et al. 1992). This was demonstrated both by coimmunoprecipitation of both proteins by a monoclonal antibody to P protein and by cosedimentation of the proteins on glycerol gradients. Similar analyses of overlapping deletion mutants of the Sendai P protein expressed in mammalian cells have mapped the region between the two nucleocapsid binding regions as the L binding site on the P protein (Smallwood, et al. 1994). We have recently shown for measles virus as well that the P and L proteins expressed from plasmids also form a complex (unpublished data), but it remains to be determined if the L binding site on the measles virus P protein maps to a region similar to that in Sendai virus.

5 Transcription

The measles RNA polymerase initiates transcription on the nucleocapsid RNA at the 3' terminal nucleotide, synthesizing a 55 nucleotide (+) strand leader RNA from purified virus in vitro (Horikami and Moyer 1991). Free leader RNA has also

been detected in measles-infected A549 cells but not in infected Vero cells (Crowley et al. 1988; Castenada and Wong 1989; Horikami and Moyer 1991), suggesting that the stability of the leader RNA in vivo is cell line-dependent. The synthesis of leader RNA demonstrates that the measles RNA polymerase initiates transcription in a manner similar to that of other non-segmented negative strand RNA viruses. Ray et al. (1991a) reported that leader RNA accumulates in the nucleus of measles virus-infected cells; however, since their assay was not specific for free leader RNA this observation requires confirmation.

Transcription then occurs sequentially following the gene order with the synthesis of the individual N, P/C/V, M, F, H, and L mRNAs. The polymerase apparently detaches from the nucleocapsid at the intergenic regions with increasing frequency as a function of the distance of the boundary from the promoter, leading to the polar accumulation of the mRNAs. mRNA abundance, therefore, is thought to be the main determinant of protein abundance. The transcription frequencies of the mRNAs in infected cells relative to N mRNA as 100%, are P (81%), M (67%), F (49%), and H (39%) (Cattaneo et al. 1987b; Schneider–Schaulies et al. 1989). A polar gradient of mRNAs was also synthesized from purified virus in vitro, except with an even steeper slope and little synthesis of the more distal mRNAs (Horikami and Moyer 1991). These results suggest that the in vitro reaction is either intrinsically inefficient or perhaps requires a cellular factor for the continued elongation or processing ability of the enzyme. At the end of each gene boundary, except for the leader RNA which is not processed, the polymerase presumably terminates synthesis following polyadenylation and then reinitiates at the consensus sequence of the next gene without transcription of the inter-genic trinucleotide (Banerjee 1987; Banerjee and Barik 1992).

As in eukaryotic mRNAs, the viral mRNAs are processed at their 5' and 3' ends. The measles virus mRNAs are capped and methylated at the 5' end with the structure 7mGpppAm (Yoshikawa et al. 1986). The mechanisms of these reactions are not known, although the conserved consensus sequence at the beginning of each gene, 3' UCC(U/C)N(G/A/C)(G/U)U(U/C)C 5' (Fig. 2A), may specify the initiation of mRNA synthesis and capping and methylation of the 5' end (Banerjee 1987). At the stop region of each gene (Fig. 2B), there is an A/U-rich sequence followed by four to seven Us, which is thought to be the signal for polyadenylation of the mRNAs (Udem and Cook 1984; Yoshikawa et al. 1986). The poly A tail varies from 70 to 140 bases (Hall and ter Meulen 1977) and appears to be synthesized by a slippage or stuttering mechanism in which the polymerase slips or backs up and copies the multiple Us reiteratively (Kolakofsky et al. 1991). It has not been determined if all these mRNA processing events occur during in vitro transcription employing purified virus, although this has been demonstrated with other negative strand viruses. Since an infectious genomic cDNA is not yet available, testing the function of the putative genomic *cis* sequences in mRNA processing has not been possible.

Measles virus transcription is not precise. Both monocistronic and various polycistronic mRNAs are synthesized in vivo and in vitro (Dowling et al. 1986; Moyer et al. 1990). Cattaneo et al. (1987a) as well as Wong and Hirano (1987)

have shown for measles variant SSPE viruses that only a P-M bicistronic mRNA, and not the individual P and M mRNAs, are synthesized. Sequencing showed that the junction between the P and M regions is the exact copy of the intergenic region and polyadenylation occurs only at the end of the RNA. The synthesis of these mRNAs, therefore, is not due to mutations of the junction sequence, but seems to be a function of altered polymerases. Only the first cistron of this RNA was functional in the infected cell or in in vitro translation yielding synthesis of the P, but not the M, protein.

Transcription of the P gene of most paramyxoviruses has another unique feature, designated mRNA editing (Thomas et al. 1988; Cattaneo et al. 1989; Kolakofsky et al. 1991). Cattaneo et al. (1989) showed in measles virus-infected cells that half of the P mRNA is complementary to genomic RNA and half is altered by the insertion of a single nontemplated G nucleotide at position 751 giving a second mRNA (V) to access a third open reading frame. The putative measles virus editing sequence derived from the conserved regions in other paramyxoviruses is 5' UUAAAAAGGG*CACAGA 3' ((+) sense), in which the G insertion takes place within the three Gs indicated by the asterisk. In Sendai virus a 24 nucleotide sequence spanning the G insertion site and the three G residues were necessary and sufficient for editing in a model system (Park and Krystal 1992). We showed that P mRNA editing also occurs during in vitro transcription from purified measles virus, although the insertion of the nontemplated G occurred at a somewhat lower frequency (20%–30%) than in the infected cell (Horikami and Moyer 1991). These data suggest that RNA editing is an additional function of the RNA polymerase, presumably of the L protein, as was also proposed for Sendai virus (Vidal et al. 1990b). P mRNA editing is thought to occur by a stuttering or slippage mechanism, similar to that proposed for polyadenylation (Vidal et al. 1990a).

The translation product of the edited mRNA, V protein shares with the P protein a common NH_2-terminal 230 amino acids but has a unique, cysteine-rich COOH-terminal polypeptide segment encompassing 68 amino acids from the third open reading frame. The V protein is phosphorylated and by immunofluorescence microscopy of measles virus-infected cells, V can be seen to have a diffuse cytoplasmic distribution distinct from that of viral nucleocapsids (Wardrop and Briedis 1991; Gombart et al. 1992). Studies on Sendai virus show that V protein is not present in virions, but is in infected cells and appears to interfere specifically with viral RNA replication. Thus, it may fulfill a regulatory role in virus reproduction (Curran et al. 1991); however, this remains to be determined for measles virus.

In vitro transcription of measles nucleocapsids from infected cells or of purified virus was markedly stimulated by a cytoplasmic extract of uninfected cells, suggesting the involvement of a cellular protein in viral RNA synthesis (Moyer et al. 1990; Horikami and Moyer 1991; Blumberg et al. 1991). Tubulin appears to be at least one of the required proteins, since transcription is inhibited by an anti-tubulin monoclonal antibody and stimulated by the addition of purified tubulin. Similar results were reported for the effect of tubulin on VSV and Sendai virus RNA synthesis (Moyer et al. 1986; Chattopadhyay and Banerjee 1988; Moyer

and HORIKAMI 1991). The anti-tubulin antibody coimmunoprecipitated the measles L protein with tubulin, suggesting one model in which tubulin might act as a subunit of the viral RNA polymerase (MOYER et al. 1986). Other models propose that tubulin or microtubules may act instead as an acidic activator of the RNA polymerase (CHATTOPADHYAY and BANERJEE 1988) or as an anchoring site for the transcription/replication apparatus (HAMAGUCHI et al. 1985).

6 RNA Replication

The most unusual feature of RNA replication of negative strand viruses, which distinguishes it from transcription, is that the synthesis of both the (+) and (–) genome length RNAs is coupled to their concomitant encapsidation by the nucleocapsid protein, while mRNAs remain unencapsidated. The viral polymerase responsible for genome RNA replication is thought to be the same protein complex, P-L, which catalyzes transcription and it is the availability of the N protein which is thought to regulate the transition from transcription to replication (BANERJEE 1987; BLUMBERG et al. 1981). The overall model is based on extensive data with VSV (summarized in BANERJEE 1987): The N protein, normally an insoluble protein, is complexed with P protein to form the soluble substrate used for encapsidation. To initiate encapsidation the RNA polymerase catalyzes the binding of the N protein from the complex (releasing P) to nascent leader RNA in a sequence-specific manner. The cooperative encapsidation with N coupled with further RNA synthesis masks the putative consensus signals at each gene boundary, preventing termination and mRNA processing, to yield the full-length, assembled nucleocapsid. Based on this model for RNA replication, one would predict at least three functional domains for the N protein: (1) a P binding site for the formation of the N-P complex used as the substrate for encapsidation; (2) an RNA binding site for initiation and elongation during encapsidation; and (3) an N binding (assembly) site. In addition, the RNA polymerase must recognize the N-P encapsidation substrate. None of these sites have been mapped, but some details of replication are beginning to emerge.

The measles N protein (525 amino acids) is a phosphoprotein, although the phosphorylation sites are not known. In Sendai NP, however, the nonconserved COOH-terminal region contains most of the phosphorylation sites (HSU and KINGSBURY 1982). Studies in Sendai and measles viruses suggest that the NH_2-terminal two-thirds of the N protein contain the domain that interacts with the RNA and the adjacent N polypeptides, while the nonconserved COOH-terminus (~ 100 amino acids) protrudes from, or lies on the surface of, the nucleocapsid. Trypsin treatment of the nucleocapsid, for instance, yields a large NH_2-terminal fragment still associated with the RNA and releases a COOH-terminal fragment (12K) (HEGGENESS et al. 1981; GIRAUDON et al. 1988). Deletion constructs of measles virus N have been used to map three antigenic, antibody binding sites on N

protein (amino acids 122–150, 457–476, and 519–525), suggesting that there are surface epitopes at each end of the protein (Buckland et al. 1989). Gombart et al. (1993) described a different NH_2-terminal antibody binding site (amino acids 23–239) in measles N, which is conformation-dependent and recognizes N protein after it is folded and then incorporated into nucleocapsids. This is confirmation that an NH_2-terminal domain is also exposed on the surface of the nucleocapsid.

The N protein expressed from a recombinant vaccinia virus containing just the measles N gene appears to self-assemble into nucleocapsid-like structures, although it is not clear if these assembled structures contain RNA (Spehner et al. 1991). Huber et al. (1991) have shown by immunofluorescence microscopy that, when measles N protein is expressed from a plasmid in transfected cells, the protein migrates predominantly to the nucleus, while the coexpression of P with N protein retains the N protein in the cytoplasm as an N-P complex. Thus, as in VSV, the P protein appears to solubilize N protein via the complex to provide a suitable substrate for replication in the proper cellular location.

It is the folded form of N protein which binds P, and the NH_2-terminus of N protein may contain the P binding site, since a monoclonal antibody to amino acids 23–239 prevents P binding (Gombart et al. 1993). For comparison, studies in Sendai virus show that deletion of amino acids 189–293 in N protein abolished binding to the Sendai P protein, although deletions elsewhere also affected binding (Homann et al. 1991). Additional studies are needed to fine map the P binding site on measles N. For the other component of the complex, the COOH-terminal 322–507 amino acids of the measles P protein are sufficient to bind N protein; however, amino acids 205–507 of P are required to prevent N from migrating to the nucleus (Huber et al. 1991). Since the V and C proteins are not synthesized from these P mutants, they appear not to be involved in N-P complex formation or its cytoplasmic retention.

The precise location of the other putative functional domains of N protein (N protein and RNA binding) have also not been determined. Sequence comparisons of the N proteins of many paramyxoviruses show a conserved amino acid sequence, spanning amino acids 261–359 (Miyahara et al. 1992), which may be important for these latter functions; however, no replication data are available for measles virus N protein deletions. Analyses of in vitro Sendai virus replication assays demonstrate that N deletions anywhere from amino acids 1 to 399 abolish replication, while proteins with deletions in the COOH-terminal 400–525 amino acids still give significant replication (Curran et al. 1993). These data support the importance of the NH_2-terminal domain in RNA binding and N-N protein interaction. The nonconserved COOH-terminus appears malleable and, in fact, can be substituted entirely by the COOH-terminus of the N protein of a bovine parainfluenza virus in vitro.

Udem and Cook (1984) reported a ratio of two to three (–) to (+) strand nucleocapsid RNAs in measles-infected cells, suggesting that the abundance of each strand is regulated. The disproportionate synthesis of (+) and (–) nucleocapsids may simply be due to the relative level of initiation at the (+) and (–) leader templates. Although the 3' sequences of (+) and (–) RNAs are conserved

for 18 nucleotides (with three changes, at positions 5,12 and 17), they diverge thereafter in the leader template (BLUMBERG et al. 1991; CROWLEY et al. 1988). The enhanced synthesis of (–) leader RNA, and thus (–) strand nucleocapsids, may occur as a result of these sequence differences.

A role for host cell proteins in RNA replication has recently been postulated. LEOPARDI et al. (1993) have shown two proteins, 22 and 30 kDa, to UV cross-link to (+) strand leader RNA, while BLUMBERG et al. (1991) found leader RNA bound to a 90 kDa cellular protein in a northwestern blot. It has been suggested that host proteins may somehow facilitate the initial binding of N protein to leader RNA to initiate replication; however, additional work is needed to establish which proteins are involved and their significance for this process.

Just as measles virus transcription seems to be inexact, since there is synthesis of polycistronic RNAs, virus infection also yields unusual replication products. In virus-infected cells, CASTENADA and WONG (1989, 1990) found novel encapsidated, but polyadenylated RNAs which contained the leader RNA fused to N RNA or to a N-P bicistronic RNA. They had a density identical to nucleocapsids, were not found on ribosomes and were apparently not translated. These RNAs seem to start as replication products but may be formed by an uncoupling of encapsidation and RNA synthesis specifically at the ends of these genes, so that the RNAs are terminated by polyadenylation.

7 Future Studies

The mammalian expression system utilizing viral genes expressed from plasmids in conjuction with biochemical assays of the parameters of RNA synthesis is a versatile, powerful approach for studying protein interactions and function. Viral genes with various mutations, deletions, truncations, or site-directed changes can be expressed and tested in vitro for transcription and replication and for binding to other proteins. The construction of an infectious measles virus cDNA is the next major research goal. This cDNA would allow the analysis of both genomic *cis* signals and the study of various gene mutations in the context of their effect on the full virus reproductive cycle. This goal should be feasible in the near future, based on the recent successes in preparing infectious subgenomic defective interfering cDNAs in several paramyxoviruses. With these reagents detailed mechanistic questions about measles virus RNA synthesis could be addressed.

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