Nucleotides in the panhandle structure of the influenza B virus virion RNA are involved in the specificity between influenza A and B viruses

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Influenza A and B viruses share common sequences and potentially similar panhandle structures in the terminal noncoding regions of virion RNA (vRNA). Interesting differences exist, however, in the number of conserved nucleotides at the 5′ and 3′ ends of the vRNAs, in base pairs constituting the panhandle duplex, and the length of uridine stretch (U stretch) juxtaposed to the RNA duplex. To analyse the contribution of these signals to the specificity between the two viruses, a transient ribonucleoprotein transfection method was used for the expression of the chloramphenicol acetyltransferase (CAT) reporter gene flanked by the noncoding nucleotides derived from influenza B vRNA. While the base pairing in the RNA duplex was primarily important for template activity, mismatch mutations G11¬G12 3′ and C12¬A13 3′ in the terminal RNA duplex region were utilized by influenza B virus, whereas these mutations were detrimental for influenza A virus. Different activity profiles were observed in the length preference of the RNA duplexes: maximum template activity was observed with 11 base pairs for influenza B virus, and 8 base pairs for influenza A virus. When the mutants with various lengths of U stretch were tested, highest CAT activities were observed with 5 to 7 uridine residues in influenza A virus, whereas in influenza B virus the activity was drastically decreased with 7 uridine residues. We suggest that the specific interaction of influenza virus RNA polymerase with these noncoding cis-acting signals in transcription of the RNA genome, along with unique coding strategies adopted by influenza B virus, has contributed to the divergence of these two closely related viruses.

Introduction

Influenza A, B and C viruses are a group of enveloped negative-sense RNA viruses characterized by their segmented genome. Among these, A and B types are important human pathogens (Murphy & Webster, 1990). Although serious infections by influenza B virus are less well documented than are influenza A virus infections, influenza B virus remains the major cause of illness associated with influenza infections among school children (Hall et al., 1979; Kim et al., 1979; Nolan et al., 1980). Influenza B virus exhibits unique diversity in coding strategies (Horvath et al., 1990; Lamb & Choppin, 1983; Lamb & Horvath, 1991; Shaw et al., 1983, 1992), biological properties and host ranges (Kingsbury, 1990).

Moreover, differences between the evolutionary pattern of A and B type viruses are also documented (Smith & Palese, 1989; Yamashita et al., 1988).

Like influenza A viruses, influenza B virus contains eight segments of single-stranded RNA of negative polarity, and each RNA segment is flanked by conserved sequence elements (Stoeckle et al., 1987). The noncoding sequences at the 3′ and the 5′ ends of virion RNA (vRNA) are partially complementary to each other and can form an extended panhandle structure. Despite apparent similarity in genomic structure, there are some interesting differences between influenza A and influenza B virus genomic RNA. First, the base-paired duplex region of influenza B virus is usually longer than influenza A virus (8–10 base pairs in influenza B virus and 4–8 base pairs in influenza A virus). Second, the uridine (U) stretch, a potential polyadenylation signal, usually consists of 5–7 uridine residues in influenza A virus, whereas in influenza B virus the number of uridine residues is 5–6, and no 7 uridine stretches are found. Third, the conserved nucleotides at the 3′ and 5′ ends of vRNA are 9 and 10 nucleotides, respectively, in influenza B virus, and

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12 and 13 nucleotides, respectively, in influenza A viruses. Besides, the position containing natural variations within the conserved region is also different between the two viruses.

Due to recent breakthroughs in reverse genetics technology, much progress has been made in the understanding of RNA signals involved in transcription and replication of the influenza A virus RNA genome (Bergmann & Muster, 1996; Flick et al., 1996; Fodor et al., 1995, 1996; Hagen et al., 1994; Kim et al., 1997; Li & Palese, 1994; Luo et al., 1991; Neumann & Hobom, 1995; Piccone et al., 1993; Yamanaka et al., 1991). Various methods and their modifications have been described for a reverse genetics system for influenza A virus. They include transfection of in vitro-reconstituted ribonucleoprotein (RNP) complex into helper influenza virus-infected cells (Enami & Palese, 1991; Luytjes et al., 1989; Martín et al., 1992; Seong & Brownlee, 1992a) and expression of virus-like RNAs and viral proteins in cultured cells using plasmids or recombinant viruses (Mena et al., 1994; Neumann et al., 1994; Zhang & Air, 1994). Using influenza proteins isolated from purified virions, promoter analysis in vitro has sometimes been complicated due to the presence of influenza-derived short RNA fragments in the polymerase preparations (Seong & Brownlee, 1992b; Fodor et al., 1995; Hagen et al., 1994; Tiley et al., 1994). However, the same enzyme preparations have been successfully used for transient transfection of RNP for analysis of promoters/cis-acting signals involved in transcription of influenza A RNA in cultured cells (Kim et al., 1997; Li & Palese, 1994; Luo et al., 1991; Piccone et al., 1993; Yamanaka et al., 1991). Molecular aspects of influenza B virus RNA replication are still not well understood. Moreover, though accumulated gene sequence data suggest a common structure of vRNA shared by both viruses, very little is known about the cis-acting elements which determine the specificity between the two viruses. In our previous work in vitro, influenza B virus polymerase was shown to have a broader allowance for sequence variations in the panhandle region than influenza A virus polymerase (Lee & Seong, 1996). Extensive analysis, in vivo and in vitro, of the ‘RNA-fork’ model of transcription of influenza A vRNA showed that base pairing in duplex domain II (see Fig. 1) plays an important role in RNA template activity (Fodor et al., 1995; Kim et al., 1997). Since sequence divergence between influenza A and B viruses is more apparent in domain II rather than in domain I, we hoped to find signals influencing the specificity of these two viruses by detailed analysis of this part of the RNA duplex. To investigate the specificity and divergence between these two related viruses in vitro, we used the transient RNP transfection method in influenza virus-infected cells with the CAT gene mimicking an influenza B virus reading frame.

Methods

**Viruses and cells.** Influenza strain B/Lee/40 was grown in 11-day-old embryonated eggs at 37 °C for 48 h and used as helper virus in a transfection assay. B/HK/HG (a gift from Dr Laver, John Curtin Medical School, Canberra, Australia) and B/Panama viruses (a gift from Evans Medical Ltd, Liverpool, UK) were used in core preparation. A batch of A/X-31 was purchased from Evans Medical Ltd. MDBK cells were grown in DMEM enriched with 10% foetal calf serum.

**Preparation of RNP cores.** Preparation of RNP from the A/X-31 virus was as previously described (Seong & Brownlee, 1992a). RNP preparation from B/HK/HG reassortant virus was according to Almond et al. (1979) with slight modification. Briefly, concentrated egg-grown virus was dissolved in disruption buffer containing 10 mM Tris–HCl (pH 7.4), 100 mM NaCl, 100 mM KCl, 50 mM MgCl₂, 4 mM DTT, 1% NP40 and 0.5% lysozyme. After incubation for 30 min at 30 °C, the lysate was fractionated on a glycerol step gradient of 70%, 50% and 30% in 10 mM Tris–HCl (pH 7.4), 100 mM NaCl, 100 mM KCl, 50 mM MgCl₂ and 4 mM DTT. Fractions enriched with nucleoprotein and polymerase proteins were identified by SDS–PAGE and Coomassie blue staining. RNP fractions were pooled and treated with micrococcal nuclease (Seong & Brownlee, 1992a) and subsequently used for reconstitution of RNP.

**Construction of plasmids.** pBCAT-NS carries the CAT gene flanked by the noncoding region of the nonstructural (NS) gene of the B/Lee/40 virus. This plasmid was constructed by PCR using pIVACAT1 (Luytjes et al., 1989) as template with two primers: primer 1, 5’ AGGGATCCGGTTATACGACTCTATAATAGTAAACAAGGAGATTTCATTACATTTACGCCCCGCCC 3’; primer 2, 5’ AGGGATCCGGTTATACGACTCTATAATAGTAAACAAGGAGATTTCATTACATTTACGCCCCGCCC 3’. The noncoding sequence of the NS gene is underlined. Mutagenesis of the influenza B virus noncoding sequences was achieved by PCR using pBCAT-NS as template and derivatives of the above primers carrying the desired mutations. The mutations in all CAT constructs were verified by dideoxy sequencing of plasmids.

**RNP transfection to MDBK cells.** The BCAT-NS RNA was co-transfected with the influenza proteins derived from influenza B virus and transfected into the B virus-infected MDBK cells. For comparison, the same BCAT-NS RNA was reconstituted with influenza A virus-derived proteins and transfected into the A virus-infected cells. RNP complexes were made according to Enami & Palese (1991) using the micrococcal nuclease-digested influenza virus cores as a source of nucleoprotein and polymerase proteins (Seong & Brownlee, 1992b). RNP complexes were identified by SDS–PAGE and Coomassie blue staining. RNP fractions were pooled and treated with micrococcal nuclease (Seong & Brownlee, 1992a) and subsequently used for reconstitution of RNP.

**CAT assay and quantification.** After 12–20 h of incubation post-transfection, cells were harvested and crude cell extracts were used for the CAT assay (Gorman et al., 1982). Acetylated products were separated on a silica thin-layer chromatography plate and exposed to X-ray film. The expression levels were estimated by density scanning of the spots on the X-ray film with LKB Ultrascan XL enhanced laser densitometer.
Results

Effects of base pair mutations in the panhandle structure

In both influenza A and B virus RNA genomes, partially complementary sequences at their termini allow the vRNA to form a panhandle structure. However, the conserved sequences in influenza B virus are confined within 9 and 10 nucleotides at the 3' and 5' ends, respectively, whereas in influenza A virus, the conserved sequences are 12 and 13 nucleotides. To test the potential role of the difference in panhandle nucleotides, the nucleotides and base pairs were systematically changed and their effects on CAT activity were analysed using influenza A or B virus as helper.

3'-11/5'-12 position. Nine different mutants either with base mismatch or with base pair substitutions at the 11:12' position were tested (Fig. 1). When influenza B virus was used as helper and the source of polymerase proteins in RNP reconstitution, most of the mismatch mutants were inactive (C11 → A11 and U11, lanes 1 and 7; G12 → U12', C12', A12', lanes 3 and 9, respectively). However, strong CAT activity was observed with the C11 → G11 mismatch mutant (lane 4, 32% of wild-type). Reconstitution of the same BCAT-NS G11 mutant template with influenza A proteins and transfection into influenza B virus-infected cells did not produce any CAT activity (data not shown). The detrimental effects of mismatch mutations were compensated, at a low level, by restoring the base pair C11:G12 → A11:U12' (lane 2), G11:C12' (lane 5), and U11:A12' (lane 8); 5%, 21% and 10% of wild-type, respectively. However, the CAT activity of the G11:C12' base-paired double mutant was still lower than that of the G11 mismatch mutant (compare lanes 4 and 5). High activity towards the G11 mismatch mutant probably means that, in influenza B virus, the base pairing at the 11:12' position is not crucial for transcriptase activity. The sequence data of B/HK/73 showed that the natural variation at position 11 sometimes did not involve base pairing with the corresponding 12' nucleotide (Desselberger et al., 1980). It should also be remembered that the G11 mismatch was well tolerated by influenza B virus polymerase in previous analysis in vitro (Lee & Seong, 1996).

3'-12/5'-13 position. Fig. 2(A) shows the effects of mutations in the 12:13' base pair on CAT activity. Most single base mismatch mutants, either at the 3'-12 or at the 5'-13 position, were detrimental for CAT activity (U12 → A12 and G12, lanes 1 and 4; A13' → U13', C13' and G13', lanes 3, 6 and 9, respectively). Exceptionally, the U12 → C12 mutant with its C12 × A13' mismatch exhibited strong CAT activity (lane 7, 41% of wild-type). All base-paired double mutants were active in CAT expression (U12:A13' → A12:U13', G12:C13' and C12:G13' in lanes 2, 5 and 8; 88%, 103% and 29% of wild-type, respectively). The level of CAT activity by base pair restoration was higher than the level observed with the adjacent 11:12' pair (Fig. 1). When the same BCAT-NS mutants were rescued with influenza A virus, base pairing ability was again crucial for CAT expression. Moreover, the C12 mutant with the C12 × A13' mismatch was slightly active in the CAT assay (6% of wild-type; data not shown). The results are consistent with similar analyses with influenza A virus using the IVACAT1 RNA containing the noncoding sequence derived from A virus (Kim et al., 1997). The results suggest that the C12:13' position, with the potential exception of the C12 × A13' pair, the base pairing ability, rather than the nature of the nucleotide, is the important determinant for template activity.

During the course of our studies on template specificity of influenza A and B viruses, we observed that the IVACAT1 was nearly inactive with influenza B virus (Fig. 2B, lane 2). Since the U12:A13' base pair is present in the majority of influenza B vRNA segments, we replaced the C12:G13' base pair conserved in the IVACAT1 RNA template with the U12: A13' base pair. This mutant IVACAT1 exhibited low but clearly detectable activity using influenza B virus as helper (9% of wild-type BCAT-NS; Fig. 2B, lane 3). The results showed that influenza B virus polymerase had a preference for the U12:A13' pair over the C12:G13' pair. Previous analyses with influenza A virus with the homologous IVACAT1 template showed that the C12:G13' base pair was threefold more active than the G12: C13' base pair (Kim et al., 1997). The opposite preference of A and B viruses suggests that the nature of nucleotide at the 12:13' position may be involved in the specificity of the influenza B virus polymerase for the A and B virus RNA templates.

13:14' and 14:15' positions. None of the mismatch mutants in the 13:14' base pair, either by the 3' or the 5' mutations, were
active as templates (Fig. 3A, lanes 1, 3, 4, 6, 7 and 9). In all cases, however, the CAT activity was rescued by base pair formation (C13:G14' → A13:U14' (lane 2), G13:C14' (lane 5), U13:A14' (lanes 8); 10%, 87% and 43% of wild-type, respectively). Limited numbers of mutants were also generated at the 14:15 base pair. As shown in Fig. 3(B), base pairing was also critically important for CAT activity (C14:G15' → G14:C15' (lane 2); 109% of wild-type), whereas the single base mismatch mutations were detrimental (C14G in lane 1 and G15C in lane 3). All mutant BCAT-NS templates at positions 13:14 and 14:15 were also reconstituted with influenza A proteins and transfected into influenza A virus-infected cells. The activity profile was similar to Fig. 3(A, B) (data not shown). Therefore, no crucial differences in template specificity were observed between influenza A and B viruses.

From analyses of mutants of each base pair, 11:12, 12:13, 13:14 and 14:15 of the BCAT-NS, our results show that (1) a stable base pair, rather than the nature of nucleotide, is important in the central region of the duplex (e.g. 12:13, 13:14 and 14:15), (2) the nature of the nucleotide as well as the base pair itself becomes important in the distal part of the duplex (e.g. the 11:12 base pair), (3) some mismatch mutations at the 11:12 and 12:13 positions are tolerated with B virus but not with A virus, and (4) the 12:13 base pair may play a
role in the difference in specificity of the A and B virus RNA templates.

**Effect of panhandle length**

Generally, the length of the base-paired RNA duplex in influenza B virus is 8–10, slightly longer than that of influenza A virus, which ranges from 4 to 8. To investigate the potential effect of its length on template efficiency, we generated various mutants with different length RNA duplex regions, either by direct deletion of base pairs within the duplex or by introducing mutation(s) at the 3’ side of the distal end of the panhandle, effectively increasing the number of base pairs.

Direct deletion of the U15:A16 base pair resulted in dramatic decrease in CAT activity (about 8% of wild-type). Deletions of 2 base pairs (14:15 or 15:16) or 3 base pairs (13:14, 14:15 and 15:16) further enhanced the deleterious effect, resulting in no detectable activity (data not shown). The sharp decrease in CAT activity could be due either to the shortening of the duplex length or to the change in distance between the 5’ termini and the U stretch, which has been shown to be an important factor for polyadenylation in influenza A virus (Li & Palese, 1994). To ascertain this, we compared mutants carrying various lengths of the duplex ranging from 4 to 14 base pairs which were generated by cumulative mutations from the distal end of the RNA duplex of the BCAT-NS RNA (Fig. 4A). As shown in Fig. 4(B), the highest activities were observed within the range of 8–13 base pairs (85–150% of wild-type) with influenza B virus, with the peak activity with 11 base pairs. When influenza A virus was used as helper, however, the activity profile was different. The activity was almost saturated within 6–9 base pairs (83–128% of wild-type) with the peak activity with 8 base pairs. It should be noted that, with influenza A virus as helper, good CAT activities were observed with the 8 and 7 base pair mutants, which are 1 and 2 base pairs shorter than the 9 base-paired wild-type, BCAT-NS, respectively.

This suggests that the detrimental effect caused by direct deletion of 1 or 2 base pairs within the duplex is due to the change in distance between the 5’ termini and the U stretch rather than to the shortening of the duplex length, which emphasizes the requirement of the minimal distance between the two (Li & Palese, 1994). Our new finding on the apparent preference of influenza B virus for a longer RNA duplex in the panhandle is consistent with the observation that the base-paired duplex region of influenza B virus RNA is usually longer than influenza A virus RNA.

**Effect of the length of U stretch**

The length of uridine sequence adjacent to the RNA duplex is usually 5–7 nucleotides in A virus, whereas only 5 or 6 residues are present in influenza B virus (Akoto et al., 1987; DeBorde et al., 1988; Desselberger et al., 1980; Kendiririm et al., 1986; Robertson, 1979; Stoedde et al., 1987). To test its potential effect, a series of mutant BCAT-NS RNAs with 3–8 uridine residues were constructed (Fig. 5A). When infected with influenza A virus, the highest CAT activities were observed within the range of 5–7 uridine residues (Fig. 5B).
Fig. 5. Effect of the U stretch on CAT expression by influenza A and B viruses. (A) The U stretch of the 5' noncoding region of the BCAT-NS RNA was changed by addition (shown in bold) or deletion (shown as Δ) of uridine nucleotide(s). (B) While influenza A virus exhibited optimal activity with 5, 6 or 7 uridine residues as previously reported (Li & Palese, 1994), influenza B virus did not show comparable activity with the 7 uridine mutant. The averages of three independent experiments are shown as relative to the BCAT-NS wild-type carrying 5 uridines.

The 7U mutant was almost 90% as active as the 6U mutant. The results are consistent with similar work done with the IVACAT1 template with influenza A virus (Li & Palese, 1994). With influenza B virus as helper, however, the prime activities were observed only with 5 and 6 uridine residues (100 and 130%, respectively) and the activity critically declined with 7 uridine residues (8% of wild-type). Therefore, influenza B virus showed a narrower requirement than A virus for the number of uridine residues. The 3U mutant, generated by deletion of 2 uridine residues from the wild-type template, concomitantly extended the number of base pairs at the RNA duplex (13 base pairs). The mutant template with the same number of base pairs, without deletion of the U stretch, exhibited as a strong CAT activity as wild-type (Fig. 4A, B). Therefore, the low activity with the 3 U mutant could be directly ascribed to the shortening of the poly U stretch.

Other mutations

In influenza A virus natural variation, U or C is observed at position 4 at the 3' end, whereas, in influenza B virus, a similar U or C variation is observed at position 10. To test potential nucleotide specific effects, all three base substitution mutants were generated at both position 4 and 10 (Fig. 6). Only U4 → C4 and U10 → C10 mutants exhibited significant, but lower CAT activity (42% and 10% of wild-type, respectively). Base-paired double mutants (C10:G11 or G10:C11) did not rescue the CAT activity (data not shown). In influenza B virus RNA segments 1, 2 and 3, which encode the polymerase proteins, the C10 nucleotide is present within the C10:A11 mismatch. The apparent activity with the C10 transition mutant without a base pair (Fig. 6) and the lack of activity of base-paired double mutants suggests that while the 10:11 base pair is not essential for template activity, the C10 nucleotide may be involved in down-regulation of polymerase proteins in influenza B virus.

Discussion

The present study on the role of the nucleotides in the panhandle region of the BCAT-NS gene suggests that, primarily, base pairing within the RNA duplex is an important factor for influenza B virus transcriptase activity. The comparative transient transfection analysis using A or B viruses, however, revealed interesting differences between these two
related viruses. We observed that the length of RNA duplex, the nature of base pairs in the RNA duplex and the length of the U stretch all contributed towards differences in specificity between the two viruses.

The greater tolerance of influenza B virus towards mismatch mutations within the RNA duplex is consistent with earlier observations in vitro (Lee & Seong, 1996). Moreover, mismatches are actually observed at the 11:12’ and 12:13’ positions in some natural influenza B isolates (Desselberger et al., 1980). The interpretation, however, is based on the assumption that these nucleotides are part of the elongated double-stranded domain (see Fig. 1). Whether this structure is the only relevant conformation throughout the infectious cycle remains to be determined. Since transfected CAT RNA is expected to undergo multiple rounds of replication, the proposed mismatches in the vRNA template would result in mismatches in the cRNA at the corresponding positions and affect vRNA synthesis as well. Therefore, the observed differences between A and B viruses would be the composite of effects on transcription, replication and potentially on the vRNA/cRNA pool size ratios in infected cells.

The preference of B virus for the U12:A13’ pair over the C12:G13’ pair was the opposite to that for influenza A virus (Kim et al., 1997). Since the U12:A13’ pair is observed in natural influenza B isolates and not in influenza A isolates, the presence of this particular base pair in the influenza B virus RNA template may have a detrimental effect on the interaction of the influenza A virus polymerase with influenza B virus RNA template. Moreover, heterogeneity of sequences in this position observed among different influenza B virus RNA segments may contribute towards the segment-specific regulation of influenza B virus gene expression. The role of discriminator nucleotides in the specificity among pools of similar RNAs and RNA binding proteins has been well documented in tRNA–synthetase interactions (Saks et al., 1994). The proposed role of this base pair was further supported by the inactivity of IVACAT1 RNA template with B virus and partial rescue of CAT activity by replacement of the C12:G13’ pair in the IVACAT1 template by the U12:A13’ base pair. However, this base pair alone was not sufficient; other structural feature such as the number of base pairs in the RNA duplex and the length of the U stretch (see below) were also involved in the discrimination between the A and B virus RNA templates.

The sequence variation observed naturally within the first 12 conserved nucleotides at the 3’ end of the influenza A virus RNA is the U or C variation at position 4. The C4 nucleotide has been thought to be involved in the segment-specific down-regulation of the polymerase genes, since the nucleotide has been invariably documented within the polymerase gene segments (Desselberger et al., 1980; Robertson, 1979). In contrast, sequence conservation at the 3’ end of the influenza B virus RNA’s is confined within the first 9 nucleotides and the U or C variation is observed at position 10 (Stoeckle et al., 1987). According to our results, CAT activities were observed with the U and C nucleotides but not with G or A. The three polymerase genes in influenza B virus carry the C10 nucleotide, and therefore, the lower activity observed with the C10 mutant template than that with the U10 template may contribute, in part, towards the segment-specific down-regulation of polymerase genes in some influenza B viruses. Considering the role of some other nucleotides in the molecular ends of the vRNA, as recently advanced in the ‘cork-screw model’ of transcription of influenza A virus (Neumann & Hobom, 1995; Flick et al., 1996), more extensive analysis of these parts of the promoter element is required for better understanding of transcriptional control of influenza B virus as well.

In our analysis of base pair length mutations, A18:U19’ and A17:U18’ base pairs did not contribute significantly towards template activity. Therefore, four consecutive base pairs, 13:14’, 14:15’, 15:16’ and 16:17’, were essential, although not sufficient, for transcriptase recognition of influenza B virus RNA template. Influenza A and B viruses exhibited slightly different profiles in their preference for the length of the RNA duplex: in B virus, maximal template activity was observed with 11 base pairs, whereas, in A virus, the peak activity was with 8 base pairs. The preference for a longer RNA duplex in B virus was consistent with accumulated sequence data that natural influenza B virus isolates usually carry a larger number of base pairs than influenza A virus (Stoeckle et al., 1987). We observed that BCAT-NS mutants with reduced numbers of base pairs (8–7 base pairs), but keeping the same distance between the 5’ end and the U stretch, still showed strong CAT activities. However, mutant templates with the same reduced number of base pairs which concomitantly shortened the distance between the 5’ end and the U stretch exhibited greatly reduced CAT activity (< 10% of wild-type). This suggests that the distance between the 5’ end of the RNA template and the U stretch of the B virus RNA template should be at least 16 nucleotides, as in A virus. The effect of distance on template activity could, perhaps, be best explained by the polyadenylation model recently proposed in influenza A virus (Tiley et al., 1994; Fodor et al., 1996). The steric hindrance proposed in this model would depend mainly on the distance between the 5’ end and the position of U stretch of the RNA template.

Differential sensitivity between A and B viruses towards the number of uridine residues overlapping to the RNA duplex suggests that the polyadenylation signal (Li & Palese, 1994; Luo et al., 1991) provides further specificity. When tested with influenza A virus, the optimal number of uridine residues was 5–7, consistent with a previous report (Li & Palese, 1994). However, 7 uridine residues critically reduced the template activity in influenza B virus. The results agree with RNA sequence data showing that the number of uridine residues in influenza B virus RNA is usually 5 or 6, and 7 uridine residues are not observed adjacent to the RNA duplex. We believe that,
although influenza A and B viruses share a common mechanism in polyadenylation of mRNA, subtle differences in the polyadenylation signal, both in the length of the RNA duplex and the U stretch, may play a role in divergence of the two viruses.

The base pairing in the RNA duplex is required for initiation of RNA synthesis as proposed in the RNA-fork model (Fodor et al., 1995; Kim et al., 1997). However, to minimize abortive initiation, the duplex should melt to allow passage of the RNA polymerase. Likewise, although the same base pairing is also required for polyadenylation of mRNA (Luo et al., 1991), melting of the duplex is needed for cRNA synthesis. These melting processes may depend, in part, on the processivity of the polymerase. If so, since the duplex region of the B virus RNA is longer and includes a higher number of base pairs, compared to that of A virus RNA, the melting process may require greater processivity of the influenza virus polymerase. The rate of evolutionary change is reported to be higher in A virus than in B virus (Smith & Palese, 1989; Yamashita et al., 1988). It merits further investigation whether greater processivity of influenza B virus polymerase as proposed in this study would be related to a lower rate of nucleotide misincorporation and a lower rate of influenza B virus evolution.

In summary, the sequence, the number of base pairs and the number of residues constituting the U stretch in the noncoding region of the RNA template all affect the influenza A and B virus transcriptases differentially. It is possible that these characteristics, in concert, endow differences in specificity between the two types of influenza virus. While both types of virus share common structural features, the observed differential sensitivities towards these cis-acting signals in transcription of the RNA genome are associated with unique coding strategies in influenza B virus (Barclay & Palese, 1995). Influenza A virus, the endogenous RNA genome and be rescued as transfectant viruses. With dramatic changes in virus-specific RNA synthesis, Bergman & Muster, 1996) or attenuation of virulence (Muster et al., 1991). A good reverse genetics system for influenza B virus would be required to dissect the effects of the specificity signals on virus replication and their potential effect on attenuation. Judicious choice and combination of attenuating mutations would result in genetically engineered influenza B virus suitable for vaccination.

References


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