Transfection Analysis of Expression of mRNA Isoforms Encoding the Nuclear Autoantigen La/SS-B*

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Transcription of the gene encoding for the nuclear autoantigen La resulted in La mRNA isoforms. A promoter switching combined with an alternative splicing pathway replaced the exon 1 with the exon 1′. The exon 1′ contained GC-rich regions and an oligo(U) tail of 23 uridine residues. Moreover, it encoded for three open reading frames upstream of the La protein reading frame. Despite this unusual structure, when exon 1′ La mRNAs were expressed in transfected cells, both exon 1 and 1′ La mRNAs were translated to La protein, whereas the upstream open reading frames of the exon 1′ were not translated. In addition to full-length exon 1′ La mRNAs 5′-shortened exon 1′ La mRNAs were detected. The exon 1′ 5′-starts varied in dependence on the analyzed tissues. Like the full-length exon 1′ La mRNA a 5′-shortened exon 1′ construct starting downstream of the oligo(U) tail but upstream of the open reading frames 2 and 3 was also well translated when transfected in mouse cells. Thus all La mRNA forms represent functional La mRNAs.

One of the target antigens of sera suffered from autoimmune patients with rheumatoid diseases such as systemic lupus erythematosus or primary Sjögren’s syndrome (pSS) is the nuclear autoantigen La (SS-B) (1). The La protein was described to associate at least transiently with all primary RNA polymerase III transcripts including precursor molecules of ribosomal 5 S RNA, tRNAs, and some 4.5 S RNAs, as well as a portion of the uridine-rich small nuclear RNAs U1 and U6 (2–5). Common to all primary RNA polymerase III transcripts is their 3′-terminal oligo(U) tail, which is transcribed during the transcription termination step. These oligo(U) tails were shown to be a binding site for the La protein (6). In addition to the oligouridylated RNA polymerase III transcripts, an association of the La protein with some nonoligouridylated RNAs has been reported especially for some viral RNAs including the leader RNAs of the vesicular stomatitis virus and rabies virus (7, 8).

The La protein is assumed to be involved in transcription termination of RNA polymerase III and internal initiation of translation of at least the poliovirus mRNA (9–12).

Most recently five La cDNAs were isolated when a cDNA library made from peripheral blood lymphocytes (PBLs) of a patient with pSS was screened with her own anti-La serum (13). In two of these five La cDNAs the exon 1 was replaced with an alternative 5′-end. Genomic analysis revealed that these La cDNAs represented alternatively spliced transcripts of the La gene. An additional promoter site was identified in the intron between exons 1 and 2, which served as initiation site for transcription of the alternative exon 1′.

The exon 1′ La mRNA form had an unusual 5′-terminus. It contained GC-rich regions and an oligo(U) tail of 23 uridine residues and encoded for three upstream open reading frames (ORF1 to 3). The ORF1 encoded for a putative peptide of 5.4 kDa. It was interrupted from the La protein reading frame by two stop codons. The ORF2 and ORF3 were not in the reading frame of the La protein. Qualitative and quantitative analysis of expression of the exon 1 and exon 1′ La mRNAs showed that both La mRNA forms represented finally processed abundant cytoplasmic mRNAs. Exon 1 to exon 1′ La mRNAs were expressed at ratios between 1:1 and 1:5 (15, 16).

Due to the unusual structure of the exon 1′ La mRNA it still remained unclear whether the exon 1′ La mRNA is a translatable mRNA and if so, which of the reading frames is used for translation. This was of special interest because one of the exon 1′ La cDNAs contained a frameshift mutation in a recently detected hot spot region in the exon 7 of the La gene (14). The frameshift mutation caused a premature stop codon in the La protein reading frame. Thus the mutant exon 1′ La mRNA could encode for a C-terminally truncated mutant La peptide of 29 kDa.

Here we present evidence that exon 1′ La mRNAs can be translated to La protein, whereas the upstream ORFs are not used for translation.

**EXPERIMENTAL PROCEDURES**

Materials—The following materials were obtained: BstEII, EcoO109, EcoRI, KpnI, pGEX-2T, and T7-sequencing kit from Pharmacia Biotech Inc. (Freeburg, Germany); SalI, NheI, and NcoI from MBI Fermentas (St. Leon-Rot, Germany); QIAprep-spin kit and QIAEX were from QIAGEN (Hilden, Germany); DMEM, Opti-MEM medium, LipofectAMINE, and Taq polymerase from Life Technologies, Inc. (Eggenstein, Germany); BstI, Pfu polymerase, pBluescript SK(+) from Stratagene GmbH (Heidelberg, Germany); CDP-Star Tropix, pCI-neo, pCI, and pGEM-T vector systems from Promega (Serva, Heidelberg, Germany); shrimp alkaline phosphatase, BstXI, HindIII, Taq buffer (10 ×), DNA molecular weight marker VI, blocking reagent from Boehringer Mannheim (Mannheim, Germany); agarose and NuSieve-agarose from Biozym (Hameln, Germany); anti-mouse IgG conjugated with peroxidase developed in sheep (Fab′)2 fragments adsorbed with human serum proteins, anti-human and anti-rabbit IgG-conjugated with alkaline phosphatase, and isopropyl β-D-thiogalactopyranoside from Sigma; the ECL-Western blotting detection reagents from Amersham-Buchler (Braunschweig, Germany); 4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate from Roth (Karlsruhe, Germany); and polyvinylidene
difluoride membrane (IPVH 000 10; pore size 0.45 μm) obtained from Millipore (Bedford, MA, U.S.A.). As anti-La serum we used the serum of the patient (Ma) found to be directed to the N-terminal domain of La protein (17), was used. For the first strand cDNA synthesis we chose the 5'-rapid amplification of cDNA ends system (5'-RACE) supplied by Life Technologies, Inc. It includes a first strand cDNA synthesis and a PCR amplification step. During reverse transcription 40 units of RNase inhibitor were added to the 5'-Germ plasmid DNA (see also Fig. 2A). The downstream primer P1 located in the exon 4 downstream of the La sequence. The reaction mixtures were prepared using as upstream primer P10 (P10, CGCTTTACTAGTTTTGGAATACTC) and as downstream primer P1 as downstream primer and La23 as substrate. The downstream primer P1 located in the exon 4 downstream of the La sequence. The exon 1 PCR fragments were further amplified using as upstream primer P1 as downstream primer and La23 as substrate. The primer P1 was obtained from the La cDNA La19. For this purpose La23 was linearized with SpeI, and the isolated linearized DNA was partially digested with EcoRI. The exon 1 and 1' La inserts were amplified using the primer P1 as downstream primer and P1 as upstream primer. The PCR fragment was prepared using as upstream primer P10 (P10, CGCTTTACTAGTTTTGGAATACTC) and as downstream primer and La23 as substrate. The fragment was cleaved with BstEII and after isolation of the linearized DNA partially digested with BglIII. Then the BglII-BstEII fragment of La19 was cloned in the appropriate sites of pBluescript II. The final constructs were sequenced.

Transfection, Cell Culture, and Staining Procedures—The human Raji and XPT cells were grown in RPMI 1640 medium containing 10% FCS in a humidified CO2 atmosphere. Mouse LTA and NIH 3T3 cells and human HeLa cells were grown in DMEM containing 10% FCS. Transfection was performed in 6-well tissue culture plates (35 mm) containing coverslips. Cells were grown to confluence of 70–80% in 4 ml of DMEM containing 10% FCS. Prior to transfection the serum/medium was replaced with 2 ml of DMEM lacking FCS and antibiotics were added to the cells, and the DNA mixture followed. After an 5-h incubation 1 ml of DMEM containing 20% FCS and antibiotics was added. 20 h after the beginning of transfection the medium was replaced by 2 ml of DMEM containing 10% FCS and antibiotics. 44 h after transfection the cells were either harvested for preparation of total extracts or fixed with methanol/EGTA for immunofluorescence microscopy. In general we observed that even after optimization of the transfection conditions the expression level of the pC1-neo constructs was only 25% compared with the pC1 constructs. In additio to transiently transfected cells, permanently transfected LTA cell lines were used as probes. These cell lines were obtained from Dr. K. Keech of the group of Prof. J. McCluskey and Prof. T. Gordon (Flinders Medical Center, Bedford, South Australia). The cells were transfected with the La cDNA La19. The permanently transfected LTA cells were grown in the presence of 0.02% genetin.

Immunofluorescence microscopy the cells were fixed with meth-
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Preparation of Cell Extracts—Total extracts of transiently transfected cell lines were prepared by incubation with 350 μl of SDS-PAGE sample solution (95 °C). Total extracts from XPTA cells or permanently transfected LTA cells were harvested by adding 100 μl of hot cell lysis buffer (100 mM NaHPO4, pH 8.3, 200 mM dithiothreitol, 1% SDS, 10% glycerol (v/v)) per cm². Raji cells were harvested by centrifugation (250 × g, 10 min). The lysed cells were heated for 5 min to 95 °C and centrifuged at 14,000 × g for 5 min at 4 °C. 5-μl aliquots were mixed with SDS-PAGE sample solution and used for SDS-PAGE.

Preparation of ORF1 Constructs—To obtain antibodies against the ORF1, a fusion protein consisting of glutathione S-transferase (GST) and a portion of ORF1 was constructed. For this purpose an exon 1 fragment spanning nucleotides 171–260 (6) was amplified by PCR using the upstream primer P11 (P11, CCCGGGATTCTACTCCAGATCTCGG; the artificial BamHI site is underlined; A indicates a point mutation introduced in the native BamHI site) and the downstream primer P12 (P12, AGGATCCCAAACGCAATGGGATGAG; the artificial BamHI site is underlined; subsequently subcloned into pGEX-2T and after restriction with BamHI inserted into the BamHI site of pGEX-2T. To proof the specificity of the anti-ORF1 serum and to look for or to rule out an expression of the ORF1 further constructs were prepared. In a first step the complete exon 1 was cloned in the transfection vector pCI. Because the translation initiation site of the ORF1 in the exon 1 is not optimal according to Kozak (24), a further construct was prepared in which the translation initiation site was optimized according to the Kozak rules. This construct was termed ORF1 Kozak construct. Finally, the ORF1 was cloned downstream of a 3′-terminally truncated exon 1′-La mRNA. This clone was termed as ORF1 La(N)-ORF1 (see also Fig. 6, I).

The ORF1 in the pCI transfection vector was obtained as follows. The pCI vector containing the full-length exon 1′ La cDNA was cut with SpeI. Then the cleaved DNA was partially digested with BalI and religated. The resulting clone contained the 5′ leader sequence of the exon 1′ La mRNA (nucleotides 1–137), the complete ORF1 (starting at nucleotide 138 and ending at nucleotide 276 including the oligo(DT) tail). It ended at nucleotide 293 in the exon 1′.

The ORF1 Kozak construct was prepared as follows. The full-length exon 1′ La cDNA served as substrate for PCR using as upstream primer the primer P13 (P13, TCTAGAGGCGACACATGCTGGGATGAGG; the artificial XhoI site is underlined) and as downstream primer P15 (P15, TGTAAAACGACGGCCAGTG) and as downstream primer P16 (P16, ATGAAATCAGTATGTTGACTACACCGACACC; the SpeI site is underlined). The resulting PCR fragment was cleaved with SpeI and XhoI and cloned in the XhoI site of the N-terminal truncated La deletion construct.

RESULTS

In a recent study Keech et al. (21) describe a mouse LTA cell line transfected with the human La gene. This cell line expressed human La protein in addition to the endogenous mouse La protein. As shown in Fig. 1 a differentiation between the human La protein and the endogenous mouse La protein in this mouse LTA cell line was possible by two techniques, including immunofluorescence microscopy (Fig. 1A) and SDS-PAGE/imunoblotting (Fig. 1B). As shown in Fig. 1A (a) LTA cells transfected with the human La gene were stained with the anti-La mAb SW5, whereas untransfected cells were not stained (Fig. 1A, b and c). After SDS-PAGE/immunoblotting, the anti-La mAb SW5 reacted with a single protein band according to a molecular mass of 50 kDa from the extract of the LTA cell line transfected with the human La gene (Fig. 1B, lane 2). The same band was also recognized by the patient’s anti-La antibody (Fig. 1B, lane 4). The anti-La mAb SW5 did not react with the total extract of the untransfected LTA cells (Fig. 1B, lane 1). In contrast, the patient’s antibody reacted with a further protein band according to a molecular mass of 45 kDa in the extract of both the transfected (Fig. 1B, lane 4) and the untransfected cells (Fig. 1B, lane 3). These data allowed the following conclusions: (i) the human and the mouse La protein can be separated by SDS-PAGE, (ii) the protein band with a molecular mass of about 50 kDa represented the human La protein, (iii) the protein band with a molecular mass of 45 kDa...
allowed the expression of the exon 1 mRNA. Therefore, in a first step we analyzed if the mouse LTA cells permanently transfected with the human exon 1 construct but also the LTA cell line transfected with the human La gene should be useful to represent the endogenous mouse La protein, (iv) the patient’s anti-La antibody reacted with both the human and the mouse La protein, and (v) the anti-La mAb SW5 reacted only with the human but not with the endogenous mouse La protein. Consequently, transfection of mouse cells with a human exon 1 La construct should allow the decision if the exon 1 La mRNA is a translatable mRNA. Moreover, a mouse cell line transfected with an exon 1 La construct but also the LTA cell line transfected with the human La gene should be useful to look for translation products of the upstream ORFs of the exon 1 La mRNA.

One prerequisite to use the LTA cell line transfected with the human La gene for such a purpose was that the exon 1 La mRNA was made and expressed similarly to human cells. Therefore, in a first step we analyzed if the mouse LTA cells allowed the expression of the exon 1 La mRNA type from the human La gene.

Detection of Exon 1 La mRNAs in Mouse LTA Cells Transfected with the Human La Gene—For this purpose 5'-RACE experiments were performed as schematically summarized in Fig. 2 (S). The PCR products obtained after the first amplification step were further amplified using a primer combination consisting either of the common exon 2 downstream primer together with an exon 1 (Fig. 2, A1, PCR 2a) or exon 1 (Fig. 2, A1, PCR 2b) specific upstream primer or of the common UAP upstream primer in combination with an exon 1 specific downstream primer (Fig. 2, B1, and also Fig. 3, S, B).

As shown in Fig. 2 (A2, lanes 1), both the exon 1' and exon 1 human La mRNA forms could be detected in the mouse LTA cells transfected with the human La gene, whereas they were not detectable in the untransfected cell line (Fig. 2, A2, lanes 3). The human exon 1 La mRNAs were also not detectable in a LTA cell line permanently transfected with the human exon 1 La cDNA (Fig. 2, A2, exon 1', lane 2), whereas the human exon 1 La mRNA was detectable in this LTA cell line (Fig. 2, A2, exon 1, lane 2). Finally, when the exon 1 and 1' bands were excised, eluted, subcloned, and sequenced, the 5'-start of the exon 1 (A1) and exon 1' (B1–B4) fragments were schematically summarized. The exon 1' fragments started upstream of the ORF2 (B1), upstream of the ORF2 but downstream of the ORF1 (B2), upstream of the ORF3 but downstream of the ORF2 (B3), or downstream of the ORF3 (B4). The ORF1 was in the La protein reading frame but interrupted by two stop codons (*). ORF2 was in the +1 reading frame, and ORF3 was in the −1 reading frame. Due to the upstream ORFs (gray bars followed by stop codons in B1–B4), it was not clear (indicated by question marks) if the La protein reading frame (black bar in B1–B4) can be translated to La protein. The black box ((U)23) represents the oligo(U) stretch in the exon 1' (gray box). m, marker lanes.

**Fig. 2.** Analysis of expression of exon 1 and 1' La mRNAs in transfected mouse cells. S, schematic summary of the 5'-RACE procedure and the localization of the used primers (P). A1 and A2, PCR of 5'-RACE products using a nested exon 1 (P4) or exon 1' (P5) specific upstream primer in combination with the exon 2 specific downstream primer (P9). Lanes 3, LTA cells untransfected; lanes 1, LTA cells permanently transfected with the human La gene; lanes 2, LTA cells permanently transfected with the exon 1 construct. B1 and B2, PCR of 5'-RACE products from mouse LTA cells transfected with the human La gene. PCR was performed using the UAP as upstream primer and an exon 1' (P6) specific downstream primer. m, marker lanes.

**Fig. 3.** Identification of varying 5'-starts of the exon 1' La mRNA isofrom. S, 5'-RACE products were prepared from different mRNA preparations according to the procedure that is schematically summarized in Fig. 2 (S). Then the PCR products were further amplified using the UAP as upstream primer in combination with either the exon 1 (P7) specific downstream primer (A1) or the exon 1' (P6) specific downstream primer (B1). A and B, the PCR products were prepared from different human tissues including fetal spleen (FS, lanes a), peripheral blood lymphocytes of the patient with pSS (Pp, lanes b) or a healthy donor (P, lanes c), and human liver (L, lanes d). All the exon 1 bands (A) and the four regions of the separated exon 1' products as indicated in B were excised, eluted, subcloned, and sequenced. The 5'-start of the exon 1 (A1) and exon 1' (B1–B4) fragments were schematically summarized. The exon 1' fragments started upstream of the ORF1 (B1), upstream of the ORF2 but downstream of the ORF1 (B2), upstream of the ORF3 but downstream of the ORF2 (B3), or downstream of the ORF3 (B4). The ORF1 was in the La protein reading frame but interrupted by two stop codons (*). ORF2 was in the +1 reading frame, and ORF3 was in the −1 reading frame. Due to the upstream ORFs (gray bars followed by stop codons in B1–B4), it was not clear (indicated by question marks) if the La protein reading frame (black bar in B1–B4) can be translated to La protein. The black box ((U)23) represents the oligo(U) stretch in the exon 1' (gray box). m, marker lanes.
cDNAs were isolated. The decision of the 5′-start of the exon 1′ La mRNA was made on the longest 5′-exon 1′ La cDNA fragment obtained by the 5′-RACE technique. However, when the 5′-RACE products obtained for mRNAs isolated from the LTA cells transfected with the human La gene were separated on a NuSieve-agarose gel (Fig. 2, B2, lane 1), several bands were obtained. Therefore, further 5′-RACE studies were performed, and the PCR products were characterized. For this purpose exon 1 and exon 1′ 5′-RACE products were prepared in parallel from different mRNA preparations according to the procedure that is schematically summarized in Fig. 2 (S). After the first amplification (Fig. 2, S, PCR(1)) the PCR products were further amplified as schematically summarized in Fig. 3 (S). The second amplification was performed using the common UAP primer in combination with either an exon 1 (Fig. 3, S, (A)) or an exon 1′ (Fig. 3, S, (B)) specific downstream primer. The mRNA preparations used for the 5′-RACE experiments were obtained from different human tissues including liver (Fig. 3, A and B, lanes L), PBLs of a patient with pSS (Fig. 3, A and B, lanes Ph) and a healthy donor (Fig. 3, A and B, lanes Pd), and fetal spleen (Fig. 3, A and B, lanes FS).

As shown in Fig. 3 (A, lanes a–d), the exon 1′ 5′-RACE products gave a single band when separated on an agarose gel. The bands obtained from the mRNAs of the different tissues were excised and subcloned, and a representative amount of clones were sequenced. All exon 1 inserts started around the predicted 5′-start of the exon 1 La mRNA as schematically summarized in Fig. 3 (A1). No difference was observed between the different mRNA preparations. In contrast, when the exon 1′ 5′-RACE products were separated on an agarose gel (Fig. 3B, lanes a–d) up to at least five bands were obtained. Moreover, the exon 1′ 5′-RACE patterns differed between the liver tissue (Fig. 3B, lane d) and the patterns for the PBL preparations (Fig. 3B, lanes b and c) and the fetal spleen (Fig. 3B, lane a). Four regions of the agarose gel as indicated by the bars in Fig. 3 (B, 1–4) were excised. The extracted DNAs were subcloned, and representative amounts of clones were sequenced. The results of these experiments were schematically summarized in Fig. 3 (B1, B2, B3, and B4). The characterized exon 1′ La cDNAs started either upstream of the ORF1 ATG at the predicted 5′-start of the exon 1′ (Fig. 3, B1), around the oligo(T) stretch (Fig. 3, B2), downstream of the ORF2 ATG but upstream of the ORF3 ATG (Fig. 3, B3), or downstream of the ORF3 ATG (Fig. 3, B4).

In consequence, these data increased the complexity, and we had to look for translational products not only of the exon 1′ full-length La mRNA but also of the 5′-shortened exon 1′ La mRNAs.

**Translation of Exon 1′ La mRNAs in Mouse LTA Cells**—In a first step, full-length exon 1, full-length exon 1′, and a 5′-shortened exon 1′ construct starting downstream of the oligo(U) tail but upstream of the ORF2 AUG were prepared (see “Experimental Procedures”) and mouse LTA cells were transiently transfected. The transfected cell lines were analyzed for expression of human La protein by cLSM (Fig. 4) and SDS-PAGE/immunoblotting (Fig. 5). As shown in Fig. 4 (a–c) the anti-La mAb SW5 gave the same nuclear staining pattern, which was also obtained for the LTA cell line permanently transfected with the human La gene (see Fig. 1A, a), on the LTA cells being transiently transfected with either the full-length human exon 1, full-length exon 1′, or the 5′-shortened exon 1′ La pCI-neo construct. No staining was found on cells transfected with a pCI-neo control lacking a La specific insert (Fig. 4d).

The results obtained with cLSM were confirmed by SDS-PAGE/immunoblotting. As shown in Fig. 5 (lane 1) the patient anti-La antibody reacted only with the endogenous mouse La protein in LTA cells transiently transfected with the pCI-neo control construct lacking a La insert. The endogenous mouse La protein was also detected in all the other extracts obtained from the transiently transfected LTA cells (Fig. 5, lanes 2–4). In addition to the endogenous mouse La protein, the patient’s anti-La antibody also detected the human La protein in the mouse LTA cells transfected with full-length exon 1 (Fig. 5, lane 2), full-length exon 1′ (Fig. 5, lane 3), and the 5′-shortened exon 1′ (Fig. 5, lane 4) pCI-neo construct. There was no obvious difference between the molecular mass of La protein translated from the exon 1 or the exon 1′ La mRNAs.

From the epifluorescence and the SDS-PAGE/immunoblotting results, we concluded that all La mRNA forms can be translated to the same La protein. The shortening of the 5′-end of the full-length exon 1′ La mRNA might increase the translational efficiency. None of the upstream ORFs should be fused to La protein when translated from an exon 1′ La mRNA.

**Analysis of an Expression of the ORF1 Reading Frame**—Finally, we analyzed whether the ORF1 of the exon 1′ is translated. For this purpose a recombinant GST-ORF1 fusion protein was prepared and used for immunization of a rabbit. After adsorption of the rabbit serum to GST, the anti-serum did no more react with the GST-carrier protein but still reacted with the recombinant GST-ORF1 fusion protein (data not shown). No reactivity was detected in extracts of several human cell lines including HeLa (see below), Raji, or XPTA cells and mouse cells transfected with the human La gene or full-length exon 1′ La mRNA (data not shown).

To proof the specificity of the ORF1 serum and to look for a translational product of the ORF1 further exon 1′ La mRNA, derivatives were constructed as schematically summarized in
Fig. 5. SDS-PAGE/immunoblotting analysis of transiently transfected mouse LTA cells. An extract of mouse LTA cells transfected with either a pCI-neo control construct (lane 1) or a pCI-neo construct of the exon 1 (lane 2), the exon 1' (lane 3), or the shortened exon 1' (lane 4) was analyzed with the monospecific patient’s (La(P)) anti-La antibody. m, marker; lane 1, 97.5 kDa; lane 2, 66 kDa; lane 3, 45 kDa; lane 4, 31 kDa.

Fig. 6 (I). The ORF1 La(N) construct represented a full-length exon 1’ La cDNA in pCI but contained a frameshift mutation in the exon 7. The frameshift mutation caused a premature stop codon. Thus the mutant exon 1’ La mRNA encoded a C-terminally truncated La peptide of 29 kDa (14). Because the epitope recognized by the anti-La mAbs SW5 is located upstream of the frameshift mutation, it still reacted with the truncated La protein (see below). The resulting N-terminal La coding region contained a XbaI site upstream of the premature stop codon. This site was used to fuse a second ORF1 sequence to the C terminus of the N-terminal La peptide. Thus the resulting construct ORF1 La(N)-ORF1 (Fig. 6, I) contained a first ORF1 located upstream of but interrupted by two stop codons from the La coding region and a second ORF1 fused to the C terminus of the truncated N-terminal La peptide. As shown in Fig. 6 (II, A, lane b) only the ORF1 La(N)-ORF1 La extract reacted with the anti-ORF1 serum. No reactivity was found for the ORF1 La(N) construct irrespective of whether the construct was transfected into human HeLa (Fig. 6, II, A, lane d) or mouse 3T3 (Fig. 6, II, A, lane f). Furthermore, no reactivity was found in the control HeLa and 3T3 cell extracts. The anti-ORF1 immune complexes were eluted from the blot. Then the same blot was processed a second time using the anti-ORF1 immune complexes were eluted, and the same blot was processed a second time with the same ORF1 La(P) rabbit serum (Fig. 6, II). After detection the anti-ORF1 immune complexes were eluted, and the same blot was processed a second time with the same ORF1 La(P) rabbit serum (Fig. 6, II). After detection the anti-ORF1 immune complexes were eluted, and the same blot was processed a second time with the same ORF1 La(P) rabbit serum (Fig. 6, II). After detection the anti-ORF1 immune complexes were eluted, and the same blot was processed a second time with the same ORF1 La(P) rabbit serum (Fig. 6, II).

DISCUSSION

Frequently sera of patients with pSS or systemic lupus erythematosus contain self-reacting antibodies directed to nuclear antigens. One of the targets of anti-nuclear antibodies is the nuclear autoantigen La/SS-B (1). The La protein was described as a housekeeping protein (19). It was proposed to be involved in the optimization of the translation initiation site of the ORF1 (see “Experimental Procedures”). However, in both cases no ORF1 translation product was detected when these constructs were transfected into human HeLa or mouse 3T3 cells (data not shown). All the results that were obtained by SDS-PAGE/immunoblotting for all the ORF1 constructs were confirmed by using enzyme-linked immunosorbent assay and indirect immunofluorescence technique. In none of these studies was an ORF1 peptide detected (data not shown).

In summary, these results allow the following conclusions: (i) the ORF1 of an exon 1’ La mRNA is not translated to either a separate ORF1 peptide or a ORF1 La fusion protein and (ii) the La protein reading frame in an exon 1’ La mRNA is efficiently translated.
in transcription/termination of RNA polymerase III and in internal initiation of translation especially of (polio)virus mRNAs (9–12).

In a recent study we identified alternative La mRNAs (13). Both La mRNA forms were characterized as abundantly finally processed cytoplasmic mRNAs (see the Introduction). However, due to the unusual 5′-terminus of the exon 1′ La mRNA isoform its function still remained obscure. The exon 1′ was GC-rich, contained an oligo(U) tail of 23 uridine residues, and encoded for three ORFs locating upstream of the La protein reading frame. mRNAs with GC-rich 5′-termini and/or upstream ORFs had also been described in the case of other housekeeping proteins and also in the case of (proto)oncogenes, growth factor receptors, and proteins involved in immune response and inflammation (24). In most of these cases it was suggested that the alternative mRNA forms may play a regulatory role. It was assumed that the mRNA isoforms are either expressed in parallel to throttle the protein production of critical protooncogene products or represent nonfunctional mRNAs, which are alternatively expressed instead of the functional gene product. Only in few cases were the upstream ORFs used for translation.

Based on this background, we asked which if any of the reading frames encoded by the exon 1′ La mRNA can be translated into protein. Looking to the upstream ORF1 to ORF3 it appeared likely that only the ORF1 could be translated to a 5.4-kDa peptide, whereas the ORF2 and ORF3 reading frames are (i) most likely too short and (ii) not in the La protein frame. Thus, we looked for expression of a peptide from either the ORF1 or the La protein reading frame of the exon 1′ La mRNA. To look for an expression of the ORF1 or the human La protein reading frame a cell system and antibodies had to be established that allowed a specific detection of the human La protein and an ORF1 peptide when made. During these studies we looked to find out whether the selected mouse cell system was able to express the human La gene similarly to a human cell. Thereby it became evident that the 5′-start of the human exon 1′ La mRNAs differed. However, similar results had also been observed when analyzing mRNA preparations that were obtained from a series of other human tissues. The observed 5′-start variations might not be the result of a 5′-terminal nonspecific RNA degradation because the exon 1 La mRNAs analyzed in parallel from the same RNA preparations did not show 5′-terminal variations, and the longest 5′-starts of exon 1′ La mRNAs were found in human liver tissue, which certainly contained the highest RNase concentration during preparation of the mRNAs. It is also unlikely that the distinct 5′-terminal start regions of the exon 1′ La cDNAs are the result of premature stops during reversed transcriptase due to secondary structures in the exon 1′ La mRNAs because the pattern of a certain mRNA preparation could be repeated (data not shown). Because exon 1′ La mRNAs lacking the ORF1 could have different properties if compared with the full-length exon 1′ La mRNAs, we included a 5′-shortened exon 1′ La mRNA derivative in our expression studies. The selected 5′-shortened exon 1′ La mRNA form started like the two exon 1′ La cDNAs isolated from the patient’s cDNA library at the oligo(U) tail in the exon 1′.

The two independent techniques, immunofluorescence microscopy and SDS-PAGE/immunoblotting, showed that the La protein reading frame in full-length exon 1 and exon 1′ and the 5′-shortened exon 1′ La mRNAs can be translated in transiently transfected cells, whereas the ORF1 reading was not used. The translation of the full-length exon 1′ product appeared to be less efficient if compared with the exon 1 mRNA, whereas the 5′-shortened exon 1′ La mRNA appeared to be more efficient despite the still existing two upstream ORFs 2 and 3. However, future studies using reporter gene constructs will be required to determine the translational efficiency of the different La mRNA forms.

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