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COMPLEMENTARY OLIGONUCLEOTIDE PROBE OF VESICULAR STOMATITIS VIRUS MATRIX PROTEIN mRNA TRANSLATION

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We decided to predict the secondary structure of a messenger ribonucleic acid (mRNA), then pick a single-stranded region as a target for hybridization arrest by complementary oligonucleotides (1), and test the predictions in vitro. Translation of Rous sarcoma virus RNA was inhibited in vitro by a tridecanucleotide complementary to a site near its 5′ end, with a 50% inhibition dose of ~100 nM (1). Translation of all five vesicular stomatitis virus (VSV) mRNA was inhibited in mouse L cells by a methylphosphonate octanucleotide complementary to the ribosome binding site of the N protein mRNA, with a 50% inhibition dose of ~150 μM (2).

VSVmatrix protein (M) is a 26,024 d polypeptide coded by an mRNA with 831 nucleotides (nt) (3). The mRNA is translated on free polyribosomes in the cytoplasm (4), so it may be more accessible than in membrane-bound polyribosomes. Its prominence and separation from other VSV proteins on polyacrylamide gels (5), as well the modest size of its mRNA, identified it as a plausible model system for complementary oligonucleotide probes.

MATERIALS AND METHODS

RNA secondary structures were predicted with the program RNAFLD (6). Equilibrium constants for the association of oligonucleotides with predicted single-stranded regions of VSV M mRNA were calculated at 37°C from nearest-neighbor enthalpies and entropies of base pairing (7). No correction of the free energies was made for RNA-DNA hybridization, instead of RNA-RNA hybridization, nor was any correction made for topological constraints on RNA loops or bulges.

Oligonucleotides were synthesized (8) on an Applied Biosystems (Foster City, CA) oligonucleotide synthesizer, purified by gel electrophoresis, and sequenced chemically (9). The full length M gene was isolated as a Pst I fragment of the plasmid pM309 (3).

VSV mRNAs were isolated from infected baby hamster kidney (BHK) cells as described (5). VSV mRNAs were translated in rabbit reticulocyte lysate (Promega Biotec, Madison, WI), in the presence of [^{35}S]Met, 1064 Ci/mol (New England Nuclear, Boston, MA), with or without added oligonucleotides. The oligonucleotides and mRNA were annealed for 3 min at 52°C, then cooled for 10 min at room temperature prior to translation. Labeled translation products were analyzed by hot 5% trichloroacetic acid precipitation and by 10% polyacrylamide gel electrophoresis (10) followed by fluorography.

RESULTS

A potential secondary structure for the 5′ half of the VSV M mRNA was calculated (Fig. 1), and then a structure was calculated for the entire 831 nt message (Fig. 2). In Fig. 1, nucleotides 17–31 appear to form a single-stranded bulge, a likely target for a complementary oligonucleotide; this site is midway between the cap and the initiation codon. In Fig. 2, nucleotides 37–46 form a somewhat smaller single-stranded bulge, which is thus a less favorable target for a complementary oligonucleotide. However, this site includes the initiation codon.

Thermodynamic calculations (7) at 37°C for the association of 5′-TTGGGATAACACTTA-3′ with nucleotides 17–31 of the structure in Fig. 1 yield an association constant of 2.0×10^{15}/M. The calculated association constant drops to 6.2×10^{6}/M if one assumes the secondary structure calculated for the entire sequence (Fig. 2).

When total VSV mRNA was translated in rabbit reticulocyte lysate with added 5′-TTGGGATAACACTTA-3′, we observed inhibition of overall VSV mRNA translation (Table I) with 50% inhibition at ~14 μM. Polyacrylamide gel electrophoresis revealed that production of all VSV proteins was inhibited equally (not shown). Brome mosaic virus mRNA translation was also inhibited nonspecifically, with 50% inhibition at 45μM observed with a pentadecamer complementary to nucleotides 37–46 (unpublished results). Hybridization arrest of M mRNA translation occurred after annealing with the full length M gene, the Pst I fragment of pM309. The M band on a gel fluorograph was reduced by 83%, relative to the N band, compared with a control lane of labeled VSV proteins.

DISCUSSION

Coordinate suppression of all VSV mRNA translation by the complement of M mRNA nucleotides 17–31 correlates with a similar observation for N mRNA (2), and suggests

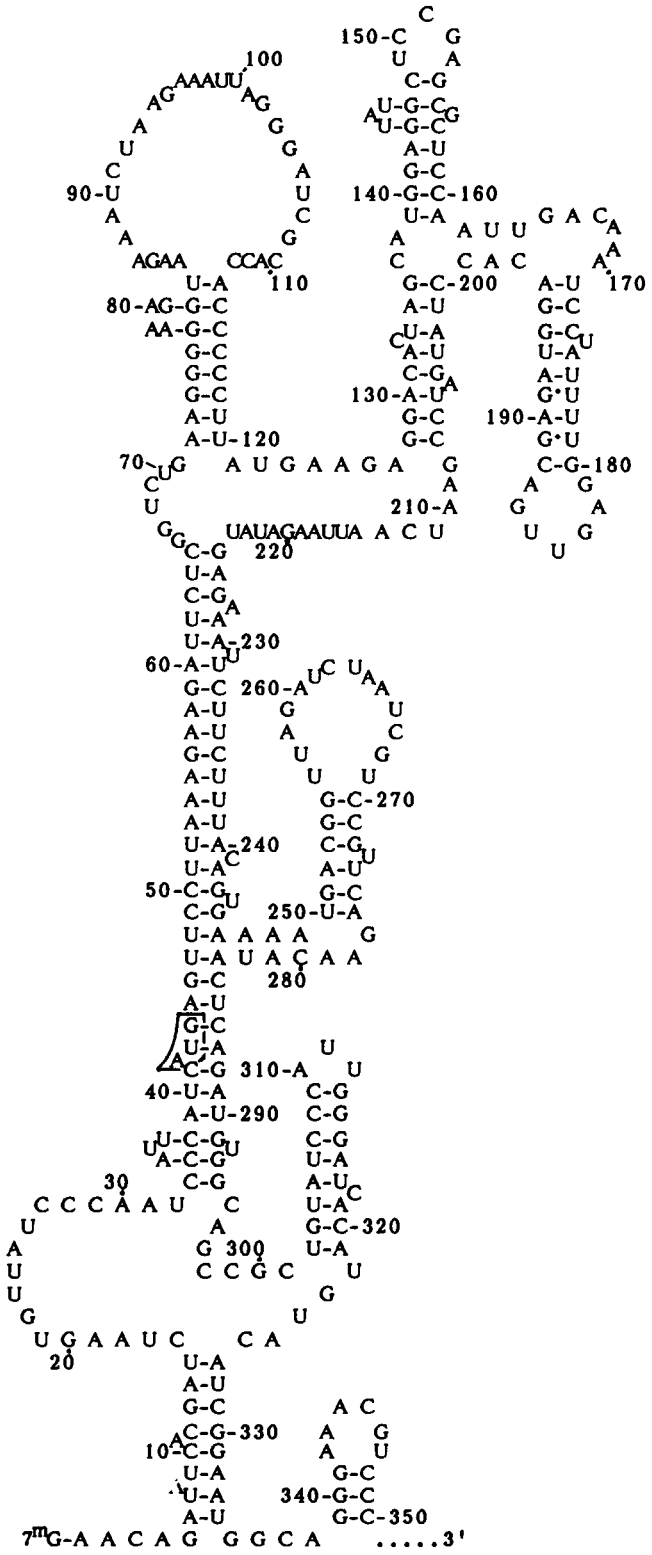

FIGURE 1 Calculated secondary structure of the 5′ 350 nucleotide of the VSV M mRNA, with the initiation codon outlined. Residues 17–31 appear in a single-stranded bulge, a potential target for hybridization arrest.

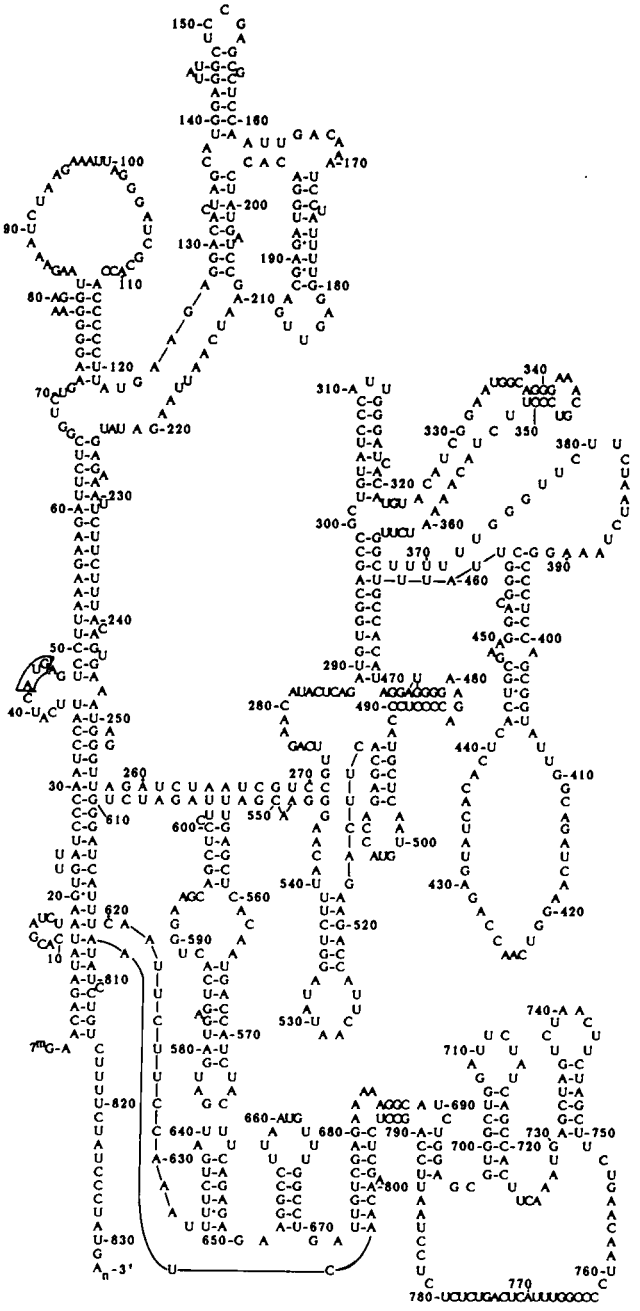

FIGURE 2 Calculated secondary of the entire VSV M mRNA, with the initiation codon outlined. Residues 37–46 appear in a single-stranded bulge, a potential target for hybridization arrest.

the possibility of coordinate regulation of translation by both the M and the N proteins. However, the weaker inhibition of BMV mRNA implies that the pentadecanucleotide used may also have inhibited the rabbit reticulocyte ribosomes nonspecifically, as seen before (1). No sequence homology is immediately apparent between the pentadecamer and BMV mRNA or rabbit 18S rRNA. The concentration required for 50% inhibition is far higher than would be expected from Fig. 1 and the corresponding association constant, but is only two orders of magnitude

TABLE I
PERCENT INHIBITION OF mRNA TRANSLATION BY 5'-TTGGGATAACACTTA-3'

15-mer	mRNA	
concentration, μM	VSV	BMV
5	23	ND (not done)
10	41	2
20	61	11
30	71	26
40	88	43
50	74	ND (not done)

300-500 ng aliquots of VSV mRNA on 1μg of BMV mRNA were translated in rabbit reticulocyte lysate; inhibition was calculated as a percentage of acid precipitable [^{35}S]Met cpm at 30 min. found in the absence of oligonucleotide, less blank without mRNA. Experiments were done at least twice, on different days.

greater than the predictions from the Fig. 2 structure. We presume that the oligonucleotide is competing with initiation factors and ribosomes for access to its target on the M mRNA. Further probing with an oligonucleotide complementary to the ribosome binding site, and with M gene antisense RNA of varying sizes may better identify susceptible and resistant portions of the M mRNA.

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ARCHITECTURE OF PRE-MESSENGER, NUCLEAR RIBONUCLEOPROTEIN MONOPARTICLES

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Nascent pre-messenger RNA transcripts in eukaryotic nuclei are entirely associated with protein and organized into a series of globular particles spaced by thin ribonucleoprotein fibrils; the RNA is folded along these nuclear ribonucleoproteins, or nRNP, to achieve a packing ratio of at least 6:1–10:1 (1). Electron microscopic observations and biochemical studies on nRNP argue against a simple repeating structure similar to the chromatin fiber (1–3). Instead, electron micrographs of nascent transcripts suggest a gene-specific nRNP organization (1).

Brief ribonuclease digestion of purified mammalian nuclei releases individual RNP complexes or monoparticles, sedimenting broadly ~40S, that contain pre-messenger RNA (along with introns and other nRNA species) and a set of nucleus-restricted polypeptides. The six most abundant polypeptides species (constituting up to 90% of the total protein mass) have been termed A1 (molecular weight 32,000 d from SDS gels), A2 (34K), B1 (36K), B2 (37K), C1(42K), and C2 (44K), respectively (2). Our previous analyses suggest that A1, A2, and C1 are equimolar and are present at three times the abundance of B1, B2, and C2, which are also probably equimolar with each other (3).

We have used biochemical methods and the scanning transmission electron microscope (STEM) to begin a structural characterization of human (HeLa cell) monoparticles (isolated from purified nuclei following ribonuclease digestion: see references 2 and 3 for details of the