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Synthesis of stable unspliced mRNA from an intronless simian virus 40–rat preproinsulin gene recombinant

(intron/RNA splicing/mRNA stability)

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ABSTRACT **We constructed a recombinant DNA molecule carrying the coding region and the 3′ flanking sequences of the rat preproinsulin I gene, replacing the late region of a simian virus 40 (SV40) viral vector. The sequences making up the single intron of the preproinsulin I gene, located within the nontranslated leader region, as well as all of the 5′ flanking sequences, have been deleted. The sequences remaining within the late transcriptional unit of SV40 do not retain a functional intron. This "intronless" mutant nevertheless directs the synthesis of substantial quantities of unspliced stable hybrid transcripts that are translated into polypeptides with insulin immunoreactivity. Two novel 5′ termini, not used in wild-type SV40 transcripts, have been detected in the majority of the hybrid RNA molecules. These are located at analogous positions in both of the late SV40 72-base-pair repeats.**

Deciphering the nucleotide signals that regulate the biogenesis of mRNA is crucial to an understanding of gene regulation in eukaryotes. Small DNA tumor viruses, such as simian virus 40 (SV40), have been extensively used as model systems to study the synthesis and processing of mRNA. Knowledge of the entire DNA sequence, a detailed understanding of the structure of transcriptional products, in conjunction with a broad spectrum of genetic data, as well as the ability of this virus to serve as a cloning vector for other eukaryotic genes, has greatly facilitated experimental approaches (see ref. 1 for review). The early and late SV40 coding sequences are situated in opposite halves of the viral genome and transcribed in opposite directions on the antiparallel DNA strands. Recent observations (2, 3) indicate that a remote control element (a 72-base-pair repeated sequence) located approximately 100 nucleotides upstream from the early mRNA capping sites in addition to a Goldberg–Hogness (3, 4) segment forms an essential part of the early viral transcriptional promoter. A number of control elements crucial for posttranscriptional processing have also been identified; in addition to capping and polyadenylylation, it appears that RNA splicing is a requirement at least for the biogenesis of functional SV40 16S mRNA. This observation was based on deletion mutants precisely lacking the intron for 16S mRNA which were unable to generate stable transcripts (ref. 5; B. Howard and P. Berg, personal communication). Such mutants, however, could be "rescued" by the insertion of a heterologous splice site (6). Other investigators, using SV40 recombinant molecules as experimental systems, also found that RNA did not accumulate in the absence of splice sites, and they proposed that splicing might be a prerequisite for stable RNA formation (7, 8). On the other hand, a number of "uninterrupted" genes have been described (e.g., those encoding interferon, histones, herpes virus thymidine kinase, and adenovirus polypeptide IX) for which the primary transcripts after being capped and polyadenylylated appear to be themselves the corresponding mRNAs (9–15).

Also, several unspliced late 19S SV40 mRNAs have been described (15) that appear in greater abundance in cells infected by certain viable deletion mutants in the late region of SV40. More recently, we have generated a recombinant molecule between SV40 and the segment of Harvey murine sarcoma virus that encodes its transforming protein p21 (unpublished). This segment of DNA, which is not known to harbor an intron, nevertheless generates a stable translatable message. In the present study, we have sought to determine whether the removal of an intron from a rat gene that is contained in a SV40 recombinant molecule precludes its ability to be expressed as a stable mRNA. Specifically, the single intron in the rat preproinsulin I gene present in a SV40 recombinant genome has been removed. In spite of the deletion of this intron, substantial quantities of translatable mRNA with novel 5′ ends are synthesized from the reconstructed recombinant molecule. We suggest, therefore, that certain RNA sequences and structures can bypass the requirement for mRNA splicing.

MATERIALS AND METHODS

Virus Strains and DNA and RNA Preparation. The construction of parental SV40–preproinsulin recombinant virus has been described (16). This stock was grown in secondary African green monkey kidney cells (AGMK) in the presence of a helper virus, tsA28 (17), at 40°C. The construction of the deletion mutant of this recombinant virus is described later. Preparation of radiolabeled viral DNA has previously been described (18). Cytoplasmic RNA was harvested from infected AGMK cells 48 hr after infection (19) with 10–20 plaque-forming units per cell of recombinant virus stocks. Recombinant DNA was handled in accordance with National Institutes of Health guidelines.

Analysis of Viral RNAs. Analysis of RNA molecules by nuclease S1 and exonuclease VII was performed as described (16, 20, 21). Labeled DNA from the hybrid molecules was analyzed in alkaline agarose gels (16). RNA blotting was performed as described (22).

Analysis of RNA by Reverse Transcription. The DNA insert of the cloned insulin cDNA plasmid pI19 (23) was removed by cleavage with endonuclease *Pst* I and further digested with *Sau* 96I; this product was then labeled with [γ-^{32}P]ATP (24) and digested with *Hha* I. The two strands of the *Hha* I/*Sau* 96I fragment of preproinsulin cDNA were separated after denaturation on urea/20% polyacrylamide gels (24). The 5′-end-labeled strand complementary to mRNA was purified and used as a primer for reverse transcription. This primer was annealed with oligo(dT)-cellulose-selected cytoplasmic RNA (25) from cells in-

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Abbreviations: SV40, simian virus 40; AGMK cells, African green monkey kidney cells; Exo VII, exonuclease VII.

fected with the SVL_1-rI_1-29 deletion mutant. After ethanol precipitation, reverse transcription was carried out as described (26). The products were purified on urea/10% polyacrylamide gels (24). The two major radiolabeled bands, A (198 nucleotides) and B (126 nucleotides), were purified from the gel and their sequences were determined according to the method of Maxam and Gilbert (24).

RESULTS

Construction of an Intronless SV40-Rat Preproinsulin Gene I Recombinant. Recently, we described the construction of the SV40–rat preproinsulin gene I recombinant molecule (16) that was used as a parental genome in the present study. Most of the late region of the SV40 vector was deleted and replaced by a 1.62-kilobase DNA fragment containing the entire rat preproinsulin I gene (harboring a unique intron in its 5′ noncoding region) and rat chromosomal segments flanking the gene (Fig. 1). Preproinsulin gene transcripts from this parental recombinant were faithfully spliced and translated. A significant amount of proinsulin (about 3×10^6 molecules per host cell) was synthesized and secreted into the culture medium.

The single intron was removed from this parental molecule as follows: The parental recombinant has only two *Hha* I sites, one in the late region of SV40 and one in the preproinsulin DNA sequence (see Fig. 1). The SV40 *Hha* I site corresponds to nucleotide 263 (see ref. 15 for the numbering system) in the late leader sequence, and the cut occurs after the first three codons

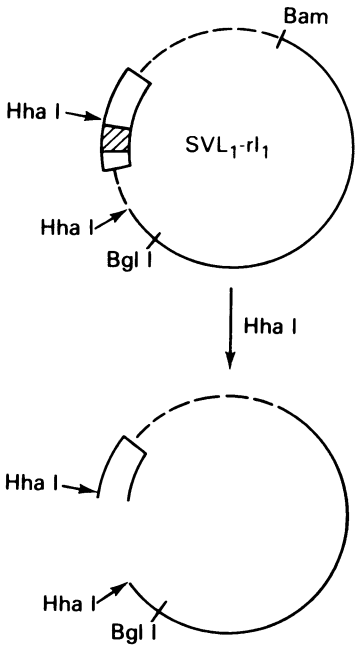

FIG. 1. Construction of mutants lacking the single intron of the rat preproinsulin gene I. The structure of the parental recombinant molecule SVL_1-rI_1, used for this construction, has been described (16). Cloned recombinant DNA molecules were cleaved with *Hha* I; one site is located close to the junction of SV40 (solid line) and the rat preproinsulin (empty bar) DNA sequences, and the second site is positioned just to the distal side of the single intron (hatched bar) in the rI_1 gene (27). The broken line indicates the rat sequences flanking the preproinsulin gene. The large remaining DNA fragment was purified by agarose gel electrophoresis and used for transfection of secondary AGMK cells (28) in an infectious center assay using tsA28 as a complementing helper virus. Individual plaque isolates were analyzed and grown to stocks. The structure of one of these of the deleted recombinants, SVL_1-rI_1-29, was subsequently determined by restriction endonuclease analysis and shown to preserve the *Hha* I ligation site.

of the "agnogene" product (29). The *Hha* I site of the preproinsulin I gene is located after the fifth codon of the preregion, 31 nucleotides behind the acceptor splice junction of the preproinsulin gene. Thus, by eliminating from the parental molecule the DNA fragment bordered by these two *Hha* I sites, we could delete the intron of the preproinsulin I gene and all of the 5′ flanking sequences that include its presumed promoter. Subsequent ligation would substitute the first three codons of the agnogene product for the first five codons of the preproinsulin gene preregion. This deletion mutant retains the SV40 promoters for late transcription.

After digestion of the parental recombinant DNA to completion by *Hha* I, the larger fragment was purified by agarose gel electrophoresis and used to transfect secondary AGMK cells, using the early temperature-sensitive mutant tsA28 as a complementing helper virus (17). Many plaques were isolated and viral stocks were established. Viral DNA was prepared from each plaque individually and the physical structure of the mutants was determined. The cleavage pattern of the deletion mutants precisely missing the small *Hha* I fragment followed the predicted structure. A representative mutant (SVL_1-rI_1-29) was employed for subsequent studies. Preliminary screening showed that this mutant directed the production of an insulin-immunoreactive polypeptide in amounts comparable to those of the parental molecule (data not shown). Thus it was of interest to analyze the structure of the mRNA transcribed from these recombinant molecules.

RNA Analysis by Nuclease Protection Experiments and Blot Hybridization. To evaluate the presence or absence of splice sites, cytoplasmic polyadenylylated RNA was prepared from cells infected with SVL_1-rI_1-29 and analyzed in parallel with polyadenylylated RNA from the parental molecule and a rat insulinoma after hybridization with appropriate DNA probes, uniformly labeled with ^{32}P. Hybrid molecules were treated with either exonuclease VII (Exo VII), which digests protruding single strands, or nuclease S1, which also attacks internal single-strand loops in the DNA. The results are demonstrated in Fig. 2 and an interpretation of the data is shown below the autoradiogram. Initially, we used as a probe a ^{32}P-labeled *Hae* II/*Bam*HI fragment derived from the parental SVL_1-rI_1 recombinant, still carrying the intron. Lanes a to d of Fig. 2 show the analysis using nuclease S1. The recombinant SVL_1-rI_1-29, lacking the single preproinsulin intron, produces stable RNA (lane d) that protects the same segment of the probe as does the RNA from insulinoma cells (lane b) and the RNA from parental SVL_1-rI_1-infected cells (lane c). Moreover, the 240-nucleotide DNA leader fragment representing the spliced parental RNA (lane c) is missing from the analysis of RNA transcribed by the newly generated deletion mutant. This also indicates that the deletion mutant is not contaminated with parental SVL_1-rI_1 molecules. In a parallel experiment, the hybrid molecules were digested with Exo VII (lanes f–i). Because mature mRNA molecules from insulinoma cells and parental SVL_1-rI_1-infected cells have a spliced structure detected by nuclease S1 and not by Exo VII, larger bands are seen in the Exo VII analysis (lanes g and h) than in the nuclease S1 analysis (lanes b and c). The intronless mutant SVL_1-rI_1-29 RNA yields bands of similar size with Exo VII (lane i) or S1 treatment (lane d). This suggests that, within the limits of this probe, the RNA from SVL_1-rI_1-29-infected cells does not contain a novel intron in the coding region. To assess the presence or absence of an intron in SV40 sequences 5′ to the preproinsulin coding region, we used a [^{32}P]DNA probe from the deletion mutant SVL_1-rI_1-29 itself (*Pvu* II to *Bam*). Treatment of the DNA·RNA hybrid molecules with either nuclease S1 or Exo VII revealed a single band of about 440 nucleotides (Fig. 2, right panel). This result suggests that if there is an intron in

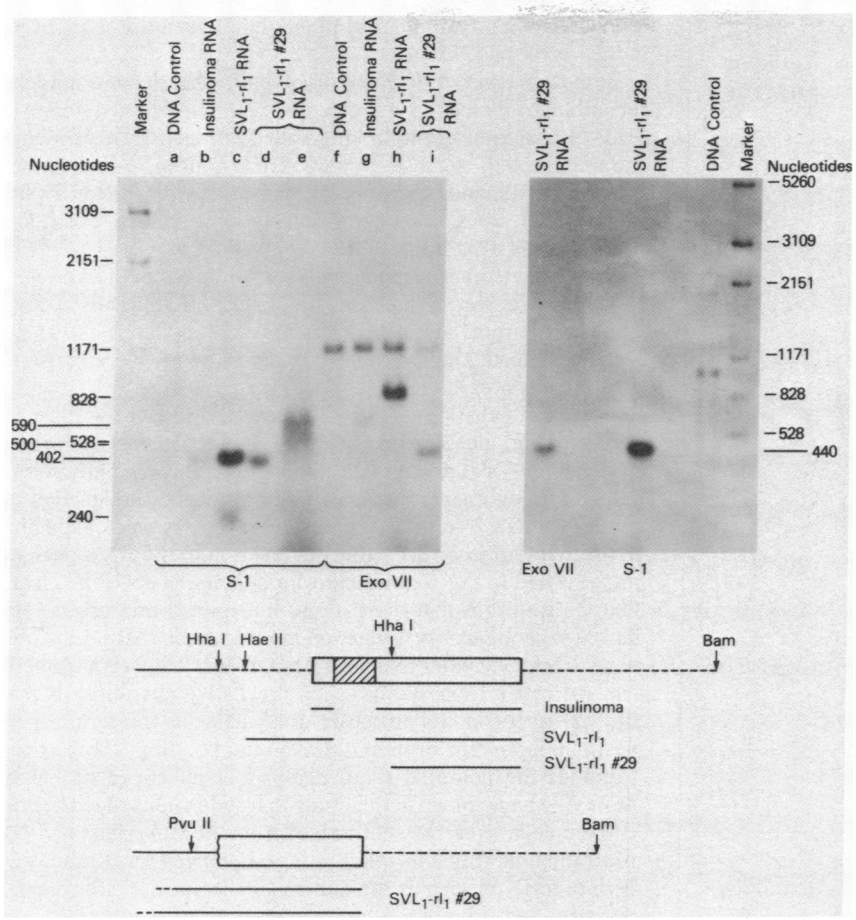

FIG. 2. Nuclease analysis of recombinant RNA molecules. Purified polyadenylylated cytoplasmic RNA from AGMK cells infected by SVL_1-rI_1 (RNA from 1.8×10^6 cells) or SVL_1-rI_1-29 (4.2×10^6 cells) were harvested 48 hr after infection. Polyadenylylated RNA was also obtained from rat insulinoma cells as a control (0.42×10^6 cells). These RNAs were hybridized to 10 ng of a uniformly ^{32}P-labeled probe (*Hae* II/*Bam* fragment) derived from the parental SVL_1-rI_1 recombinant (specific activity 1×10^6 cpm/μg) in lanes c, d, e, and h, and i and treated with nuclease S1 or Exo VII as indicated at the bottom of the figure. A second uniformly labeled probe (10 ng, specific activity 1×10^6 cpm/μg) representing a unit-length *Bam*HI linear fragment of the intron-deficient mutant SVL_1-rI_1-29 was used for hybridization to SVL_1-rI_1-29 RNA (lane f). In the right panel, a uniformly labeled *Pvu* II fragment probe (10 ng) derived from SVL_1-rI_1-29 DNA was annealed with RNA of SVL_1-rI_1-29-infected cells and treated with either nuclease S1 or Exo VII.

the SV40 sequences, it must lie upstream from the *Pvu* II site. When the nuclease S1 protection experiment was repeated using as a probe the entire genome of deletion mutant 29, two bands of approximately 590 and 500 nucleotides were protected (Fig. 2, lane e). Similar results (not shown) were obtained upon digestion of the hybrids with Exo VII. This result suggests that two major 5′ ends exist in the hybrid RNA species, located in the SV40 sequence approximately 60 and 150 nucleotides upstream from the *Pvu* II site.

In a parallel study, direct analysis of the transcripts after RNA blotting (22) and hybridization with an insulin cDNA probe indicated the presence of the same size classes of RNA in both nuclear and cytoplasmic extracts, further supporting the previous results (data not presented). In addition, larger transcripts present at low levels were detected in both the nuclear and cytoplasmic fractions; their size indicates that this class of RNA extends to the SV40 polyadenylylation site (normally used for 16S and 19S mRNA) rather than to the corresponding preproinsulin site.

RNA Analysis by Reverse Transcription and cDNA Sequence Determination. To examine the hybrid RNA transcripts in detail and precisely map their 5′ ends, we reverse transcribed polyadenylylated cytoplasmic RNA derived from SVL_1-rI_1-29-infected cells as described in *Materials and Methods*. The sequences of two predominant transcripts (126 nucleotides and 198 nucleotides in length) were determined by the method of Maxam and Gilbert (24).

The sequencing gel (Fig. 3) shows that the shorter band (B = 126 nucleotides) is separated by 72 nucleotides from the longer band (A = 198 nucleotides), precisely the distance of the set of repeated nucleotides in which they are located. We conclude, therefore, that B is not a partial reverse transcript, but that the two bands correspond to the two predominant species in the mRNA population, as predicted from the nuclease S1 protection experiments (Fig. 2). The last readable nucleotide in A and B is a G. Therefore, the intact DNA molecules should have the next upstream nucleotide T at the end of the sequence (Fig. 4) This corresponds to an A residue in the mRNA strand (Fig. 4, arrow) at the capping site (nucleotides 85 and 157 of the SV40 sequence). It should be noted that an ATA box is located 28 nucleotides upstream from this site, which may function in positioning these new 5′ ends.

The sequence in Fig. 3 is in agreement with the known SV40 sequence in this region. This result demonstrates further the unspliced nature of these hybrid transcripts.

DISCUSSION

The role of RNA splicing in the postranscriptional processing of mRNA precursors is under intense investigation. Previous experiments by several groups have suggested that certain transcripts, generated either from SV40 recombinant molecules or from mutants of the viral genome, require splicing for their stable expression in the cytoplasm (5–8). On the other hand, the recent description of a number of eukaryotic genes that are uninterrupted by intervening sequences (9–14), as well as certain mutants in the late region of SV40 that appear to produce unspliced 19S late mRNAs (15), has suggested that alternative mechanisms must exist for the expression of unspliced transcripts (12). We addressed this question by employing SV40–rat preproinsulin recombinant molecules (16) that retain a single rat preproinsulin intervening sequence in the late region of the genome. All of the late recombinant transcripts appeared to use this intron in the preproinsulin sequence as a splice site, but it was unclear whether this particular splicing event was obligatory to the production of stable mRNA. Mutants of this recom-

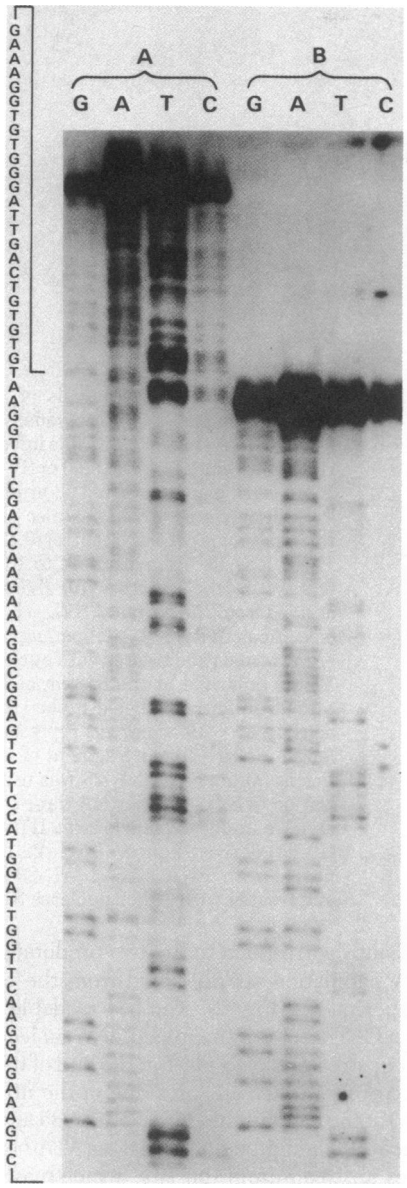

FIG. 3. RNA analysis by reverse transcription and cDNA sequence determination. Cytoplasmic polyadenylylated RNA was purified from cells infected by SVL_1-rI_1-29. A short DNA primer was annealed with the RNA and reverse transcribed into DNA. Two prominent bands, A (198 nucleotides) and B (126 nucleotides), corresponding to the mRNA complementary strand were recovered from a gel and their sequences were determined by the method of Maxam and Gilbert (24). The sequence of the A and the B fragments has been determined in a urea/10% polyacrylamide gel (24). Band B is 72 nucleotides shorter than band A, indicating that the two major RNA species have 5′ ends at corresponding positions in the two SV40 72-base-pair repeated units. A shorter electrophoretic run of the two fragments, beginning at the 5′ end of the primer, is not shown.

binant molecule were constructed in which the unique preproinsulin DNA intron as well as the flanking sequences 5′ to the gene were removed. In the new recombinant molecule, the first five codons of the preproinsulin coding sequence were deleted and replaced "in frame" with the first three codons of the SV40 agnoprotein (29). This resulted in the fusion of the coding region for the rat chromosomal preproinsulin I gene to the 5′ noncoding region of the late SV40 genes. While this construction deleted potential 5′ ends of the rat preproinsulin transcripts, the region of SV40 that contains sequences encoding the 5′ late mRNA ends was preserved and was situated in the immediate vicinity of the new junction between the sequences of the vector and the insert.

Both RNA and protein analyses revealed that the new mutant recombinant molecules were capable of generating stable hybrid colinear transcripts lacking splice junctions. This finding was unexpected, because earlier constructions of SV40 deletion mutants (2, 32) and recombinant molecules with foreign DNA fragments (7, 33, 34) appeared to require a splicing event for production of a stable mRNA.

An associated and perhaps related finding was the absence of RNA molecules containing the 5′ termini generally recognized as the most abundant class of wild-type late SV40 transcripts. Instead, the two most common 5′ ends were situated in analagous sites in the late repeated sequences of SV40 exactly 72 base pairs apart (see Fig. 4). These are in fact identical to certain 5′ ends detected in unspliced RNA molecules generated by a viable SV40 late deletion mutant (15). In view of these results, one might suggest a correlation between the location of the SV40 RNA 5′ end and the presence or absence of splicing in the stable transcripts. How the position of the RNA termini might relate to the requirement for splicing is not at all clear. One possibility is that these altered unspliced transcripts contain new sequences or sequence modifications that permit the novel RNA molecules to bypass a putative splicing requirement and yet retain their stability. An alternative possibility is that the size or secondary structure of a particular transcript or its destination for free or membrane bound polysomes in part determines the potential requirement for splicing in its biogenesis. We have shown in this study that stable nonspliced hybrid RNA molecules can be synthesized from the late region of SV40 recombinants, but the relationship of splicing and stability of certain mRNAs such as the late SV40 16S species still remains obscure.

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1. Tooze, J., ed. (1980) *The Molecular Biology of Tumor Viruses: DNA Tumor Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
2. Gruss, P., Dhar, R. & Khoury, G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 943–947.
3. Benoist, C. & Chambon, P. (1981) *Nature (London)* **290**, 304–310.
4. Ghosh, P. K., Lebowitz, P., Frisque, R. J. & Gluzman, Y. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 100–104.
5. Gruss, P., Lai, C.-J. Dhar, R. & Khoury, G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4317–4321.
6. Gruss, P. & Khoury, G. (1980) *Nature (London)* **286**, 634–639.
7. Hamer, D. H. & Leder, P. (1979) *Cell* **18**, 1299–1302.
8. Buchman, A. R. & Berg, P. (1981) *J. Supramol. Struct. Cell. Biochem. Suppl.* **5**, 443.
9. Schaffner, W., Groos, K., Telford, J. & Birnstiel, M. (1976) *Cell* **8**, 471–478.
10. Nagata, S., Mantei, N. & Weissmann, C. (1980) *Nature (London)* **287**, 401–408.
11. Lawn, R. M., Adelman, J., Franke, A. E., Houck, C. M., Gross, M., Nagarian, R. & Goeddel, D. V. (1981) *Nucleic Acids Res.* **9**, 1045–1052.
12. Alestrom, P., Akusjarvi, G., Perricaudet, M., Mathews, M. B., Klessig, D. G. & Petterson, U. (1980) *Cell* **19**, 671–681.
13. McKnight, S. L. (1980) *Nucleic Acids Res.* **8**, 5949–5966.
14. Wagner, M. J., Sharp, J. A. & Summers, W. C. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1441–1445.

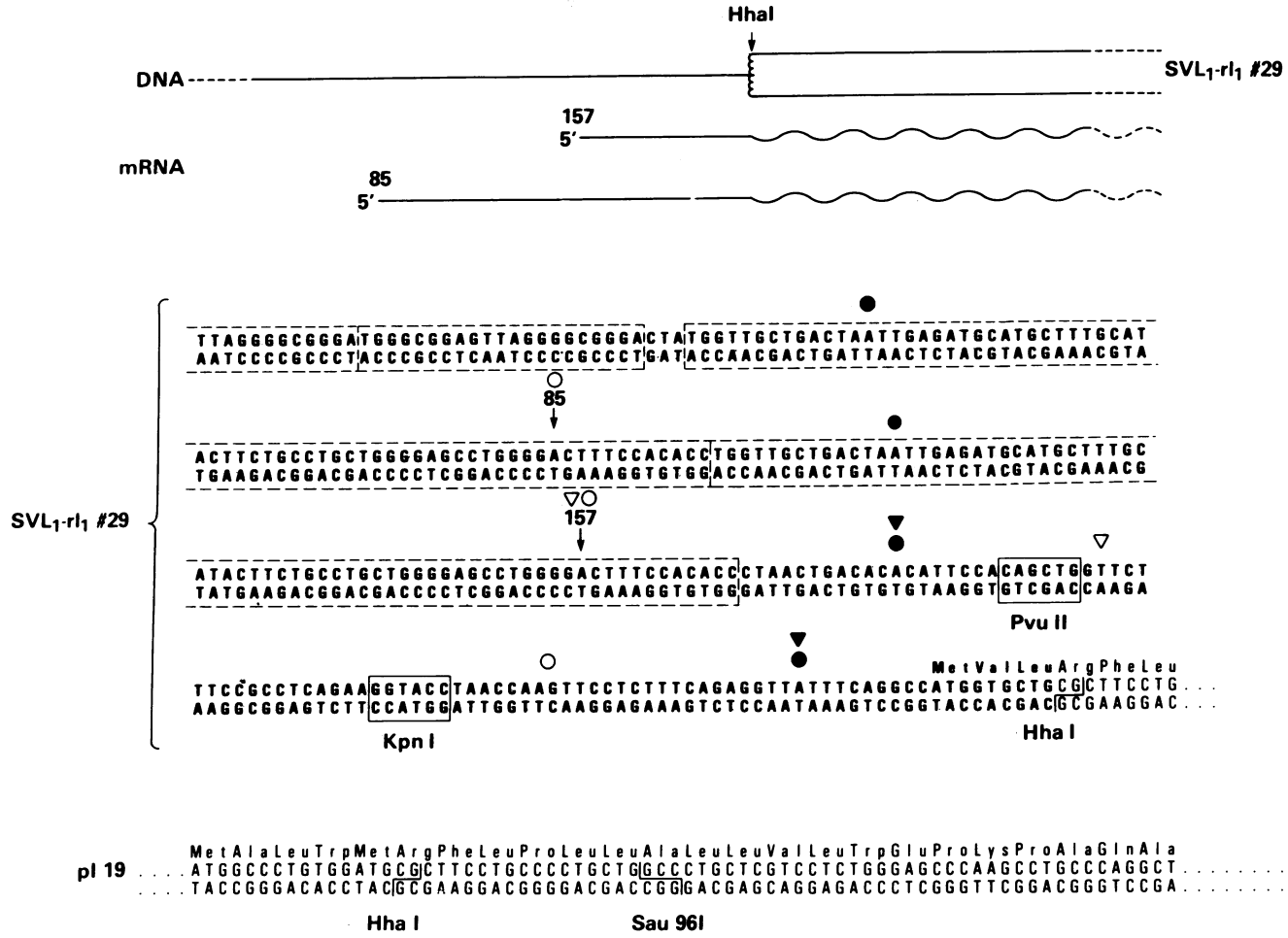

FIG. 4. Schematic representation of transcripts from the intronless SV40–rat preproinsulin recombinant molecules. The late region of SV40 joined at the *Hha* I site to rat preproinsulin sequences in mutant SVL_1-rI_1-29 is shown at the top of the figure. The two major hybrid transcripts synthesized from this recombinant molecule are diagrammed below with their major 5′ ends at nucleotides 85 and 157. In the middle of the figure, these two novel 5′ ends are indicated in the SVL_1-rI_1-29 sequence (↓). The open symbols indicate some of the 5′ ends of the abundant RNAs deriving from a late leader deletion mutant (15), which generates predominantly unspliced 19S mRNAs (○) and spliced 16S mRNA (▽). Also indicated are the positions of the 5′ termini of the predominant wild-type SV40 late 19S (●) and 16S (▼) transcripts (30, 31). Depicted at the bottom of the figure is a segment of the rat preproinsulin DNA sequence. The *Hha* I site of this sequence was used for ligation to the vector (see text for details). The bottom strand between the *Hha* I and *Sau* 96I sites is the primer used for reverse transcription (see text). The amino acids corresponding to the codons of the agnogene and preproinsulin sequences are indicated above the nucleotide sequence.

15. Ghosh, P. K., Roy, P., Barkan, A., Mertz, J. E., Weissman, S. M. & Lebowitz, P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1386–1390.
16. Gruss, P. & Khoury, G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 133–137.
17. Tegtmeyer, P. & Ozer, H. L. (1971) *J. Virol.* **8**, 516–524.
18. Lai, C.-J., Dhar, R. & Khoury, G. (1978) *Cell* **14**, 971–982.
19. Penman, S. (1966) *J. Mol. Biol.* **17**, 117–130.
20. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12**, 721–732.
21. Berk, A. J. & Sharp, P. A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1274–1278.
22. Goldberg, D. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5794–5798.
23. Villa-Komaroff, L., Efstratiadis, A., Broome, S., Lomedico, P., Tizard, R., Naber, S. P., Chick, W. K. & Gilbert, W. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3727–3731.
24. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
25. Casey, J. & Davidson, N. (1977) *Nucleic Acids Res.* **4**, 1539–1552.
26. Efstratiadis, A. & Villa-Komaroff, L. (1979) in *Genetic Engineering: Principles and Methods*, eds. Hollander, A. & Setlow, J. (Plenum, New York), Vol. 1, pp. 15–36.
27. Lomedico, P., Rosenthal, N., Efstratiadis, A., Gilbert, W., Kolodner, R. & Tizard, R. (1979) *Cell* **18**, 545–558.
28. Brockman, W. W. & Nathans, D. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 942–946.
29. Jay, G., Nomura, S., Anderson, C. W. & Khoury, G. (1981) *Nature (London)* **291**, 346–349.
30. Ghosh, P. K., Reddy, V. B., Swinscoe, J., Lebowitz, P. & Weissman, S. M. (1978) *J. Mol. Biol.* **126**, 813–846.
31. Ghosh, P. K., Reddy, V. B., Swinscoe, J., Choudary, P., Lebowitz, P. & Weissman, S. M. (1978) *J. Biol. Chem.* **253**, 3643–3647.
32. Lai, C.-J., Khoury, G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 71–75.
33. Goff, S. P. & Berg, P. (1976) *Cell* **9**, 695–705.
34. Hamer, D. H., Smith, K. D., Boyer, S. H. & Leder, P. (1979) *Cell* **17**, 725–735.