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The intron requirement for immunoglobulin gene expression is dependent upon the promoter

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ABSTRACT

Transfection assays were used to assess the need for an intron in order to obtain expression of cytoplasmic immunoglobulin μ mRNA. An intron is required when transcription is driven by an immunoglobulin promoter/enhancer combination, although this requirement is not specific for a particular intron. However, this need for an intron is dependent upon the promoter used. Whilst an intron is required in the case of immunoglobulin or β-globin promoters, it is not in the case of cytomegalovirus or heat-shock promoters. The data point to a connection between the promoter and RNA processing or export.

INTRODUCTION

Immunoglobulin genes contain multiple introns. Whilst transcription enhancer elements have been located in the major introns of rearranged heavy and light chain genes, no specific functions have yet been ascribed to the other introns. This lack of any known specific function is, of course, true for the large majority of individual introns in eukaryotic genes.

We have been interested in the construction of plasmid vectors containing immunoglobulin genes lacking introns; this would allow a substantial reduction in the size of the plasmids used for expressing transfected antibody genes. However, initial experiments revealed that driving the expression of an immunoglobulin μ cDNA yielded very little secreted antibody (1). We have therefore investigated the intron requirement for IgH gene expression and conclude that there is a non-specific intron requirement when the expression is driven by the V_H promoter/IgH enhancer combination. This intron requirement can, however, be obviated by the use of different promoters. These results have implications for the design of antibody gene expression vectors. The data also suggest possible explanations for the fact that an intron is required for the expression of some genes but not of others.

MATERIALS AND METHODS

Plasmids

Plasmid pSV-Vμ[ΔIn] (Fig. 1A) is derived from pSV-$V_{NP}H\epsilon$ (2). Plasmid pSV-$V_{NP}H\epsilon$ was digested with BamHI and SacI linkers inserted. The segment extending from the NcoI site spanning the V_H translation start to the introduced SacI linker was replaced by the μ cDNA fragment from plasmid pABμ11 (3) which extended from the NcoI site at the translation start to a SacI site which had been inserted by use of linkers into a HaeII site in the μ 3'-untranslated region. The various intron-containing derivatives of pSV-Vμ[ΔIn] were constructed by substituting appropriate fragments from pSV-Vμ1 (4) for the relevant part of pSV-Vμ[ΔIn]. Thus the regions originating from pSV-Vμ1 are: pSV-Vμ[In1], the NcoI site in the leader exon to the PstI site in the V_H exon; pSV-Vμ[In3,4,5], the BglII site in Cμ1 to the ApaI site in Cμ4; pSV-Vμ[In3,4,5,6,7], the BglII site in Cμ1 to the XhoI site beyond the CμM2 membrane exon; pSV-Vμ[A_n-Ig], from the ApaI site in Cμ4 to the KpnI site in the intron between Cμ4 and the CμM1 membrane exon; pSV-Vμ[In1,3,4,5], the NcoI-PstI fragment spanning the leader-V_H intron as well as DNA extending from the BglII site in Cμ1 to the HaeII site in the 3'-untranslated region of the Cμ4 exon. Intron 1 (82 nucleotides long) separates the leader and V_H exons; intron 3 (110 nucleotides) separates Cμ1 from Cμ2; intron 4 (279 nucleotides) separates Cμ2 from Cμ3; intron 5 (107 nucleotides) separates Cμ3 from Cμ4; intron 6 (1814 nucleotides) separates Cμ4 from CμM1 and includes the μ_s polyadenylation signal which is located 102 nucleotides downstream of the splice donor site; intron 7 (118 nucleotides) separates CμM1 and CμM2.

The heat-shock promoter constructs are based upon pSV-HSVμ2 described previously (5). The ρ-globin promoter fusion was assembled using a ρ-globin gene with a BglII linker inserted at position -24 (gift of R. Treisman); the HCMV fusion was assembled from a HindIII-SacI fragment (gift from G. Yarranton) which spans the start-site (6). The sequences of the fusion points were obtained using the dideoxy method. The plasmids containing the transcription units driven by the β-globin and HCMV promoters are analogous to pSV-Vμ[ΔIn] (Fig. 1A) except (i) that neo is used as the selective marker (32) rather than gpt and (ii) in the plasmid containing the HCMV promoter, the IgH enhancer has been removed by EcoRI digestion. In the case of plasmids containing the β-globin promoter, versions were constructed which contained the IgH enhancer, the SV40 enhancer or no enhancer at position -128 upstream of the promoter.

Transfection and analysis of RNA

The J558L plasmacytoma (7) was grown in DMEM supplemented to 10% in foetal calf serum, transfected by electroporation (8) and pools of stable transfectants selected as previously described (5). Cytoplasmic RNA was prepared by phenol extraction from cells lysed in the presence of 1% Nonidet P40. Nuclear RNA was prepared as described elsewhere (9). For heat-induction, cells were incubated at 42.5°C for 1 hour, diluted into eight volumes of medium that had been preincubated at 32°C and then further incubated at 37°C for 3 hours prior to harvesting for RNA extraction. RNA was quantitated by ribonuclease protection assays (10). Probes for immunoglobulin heavy-chain mRNA spanned the V_H transcription start and extended from the StuI site in V_H back across the relevant promoter. A specific probe was therefore used for each promoter fusion. The lengths of full length probes and of the protected fragments for correctly initiated transcripts are: pV_H (probe 520 and protected fragments 239 and 219 nucleotides); pHS (probe 490 and protected fragment 273 nucleotides); pβG (probe 380 and protected fragment 219 nucleotides) and pHCMV (probe 880 and protected fragment 219 nucleotides). The probe for gpt transcripts (250 nucleotides with 123 nucleotide protected fragment) has been described previously (33). Immunoglobulin λ light chain transcripts were measured using a probe covering the region between the SacI and ScaI sites in the V_λ exon (probe of 120 and protected fragment of 99 nucleotides); neo transcripts were measured using a probe containing the BssHII-RsaI fragment (probe of 127 and major protected fragment of 94 nucleotides). Northern blot analysis of total cytoplasmic RNA was performed as described by Lehrach et al (11).

RESULTS

Intron requirement for μ expression from an IgH promoter

In previous work, we have shown that plasmacytoma cells transfected with a complete V_H-Cμ gene contain abundant amounts of μ mRNA (4). In order to reduce the size of the transfected IgH gene, we constructed a plasmid in which all the introns within the μ gene had been removed and the IgH enhancer placed upstream of the promoter (pSV-Vμ[ΔIn]; Fig.1A) The polyadenylation site for this transcription unit is provided by SV40 sequences. Plasmacytoma cells transfected with pSV-Vμ[ΔIn] contain very little cytoplasmic μ mRNA (Fig. 2). Much higher levels were obtained when the transcription unit was altered so as to include the intron located

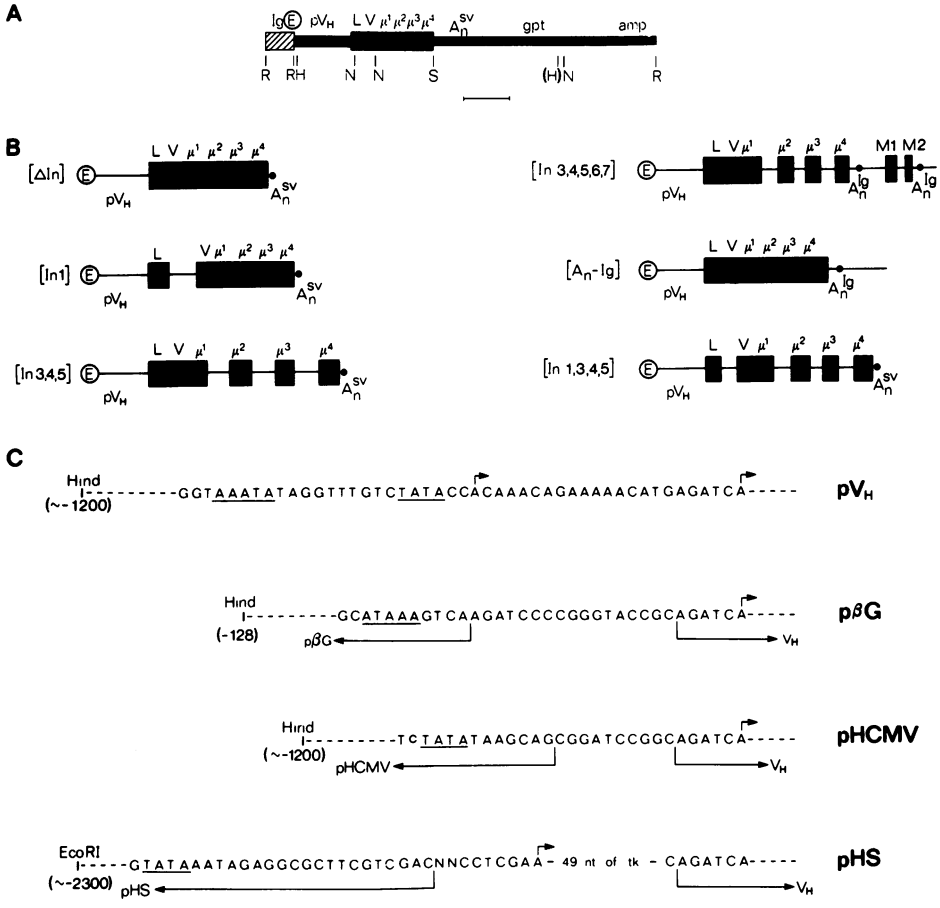

Figure 1. Structures of plasmids. (A) Plasmid pSV-Vμ[ΔIn]. The V_H promoter (pV_H) and the IgH enhancer (Ig E ; hatched) drive transcription of a μ heavy-chain cDNA (thick filled box). Polyadenylation sites (A_n) are given a superscript 'sv' or 'Ig' to indicate that they derive from SV40 or from the μ_m or μ_s polyadenylation sites of the mouse heavy-chain locus. The transcription unit is cloned in the vector pSV2gpt (31). Restriction sites are abbreviated: R, EcoRI; H, HindIII; N, NcoI; S, SacI. The HindIII site in parentheses was destroyed by filling in. A scale bar (1 kb) is provided. (B) Transcription units derived from pSV-Vμ[ΔIn]; these are not drawn to scale. The μ membrane exons are indicated as M1 and M2. (C) Sequence around the V_H transcription start sites in the different promoter fusions. Transcription start sites are indicated by an arrow and TATA elements are underlined.

between the leader and V_H exons (plasmid pSV-Vμ[In1]; Figs. 1B and 2). As was previously demonstrated using the complete genomic μ gene (4), transcripts from the V_H promoter in pSV-Vμ[In1] use two RNA start sites

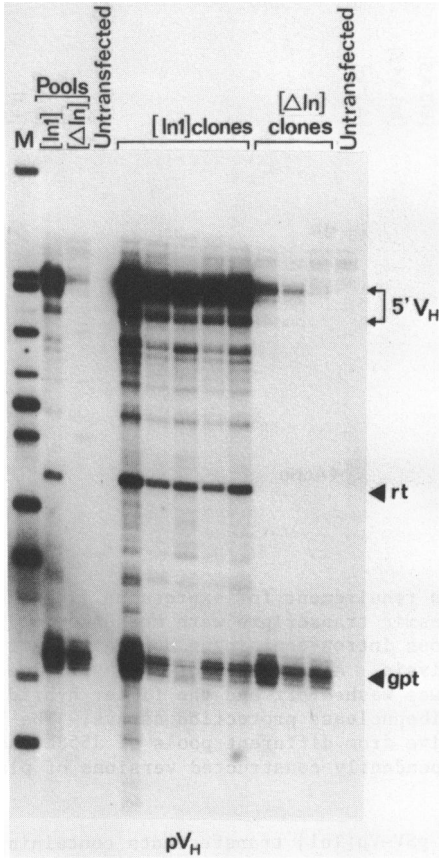

Figure 2. An intron is required for μ expression from the V_H promoter. The cytoplasmic μ transcripts in pSV-Vμ[ΔIn] and pSV-Vμ[In1] transfectants of J558L were measured by ribonuclease protection assays. Transcripts initiating at the two V_H start sites (5'V_H) are indicated; readthrough transcripts (rt) that splice into the acceptor site of the leader-V_H intron are obviously only observed in the case of plasmids containing intron 1. An internal control is provided by measurement of gpt transcripts. The markers (M) are an HpaII digest of pBR322.

located 20 bases apart (Fig. 2) although the majority of transcripts initiate at the 5' cap site.

The initial comparison of plasmids pSV-Vμ[ΔIn] and pSV-Vμ[In1] was carried out using pools of plasmacytoma tranfectants. Measurement of the μ mRNA levels in randomly picked clones (Fig. 2) demonstrates that the difference between the two pools is not a "jackpot" effect created by only

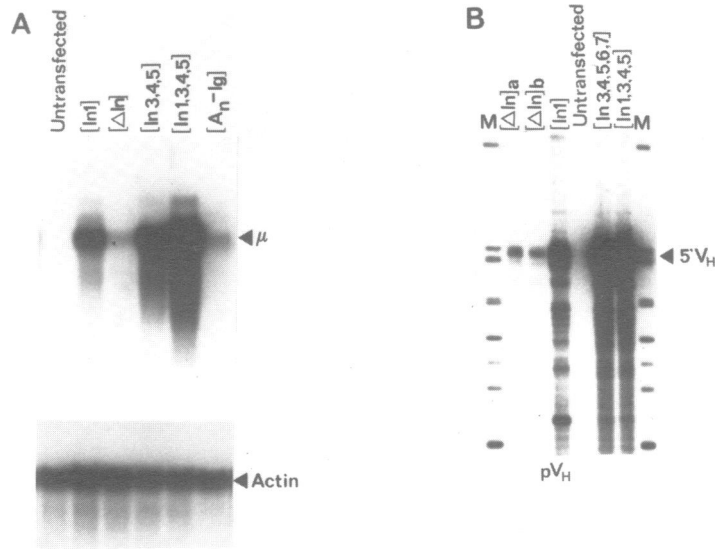

Figure 3. The intron requirement for expression from the V_H promoter is non-specific. Cytoplasmic transcripts were measured in J558L cells transfected with various intron-containing derivatives of pSVp-Vμ[ΔIn]. (A) Northern blot analysis. After the blot was hybridized with a mouse Cμ probe, radioactivity was washed off and the filter hybridized with a β-actin probe. (B) Ribonuclease protection assays. The lanes captioned [ΔIn]a and [ΔIn]b derive from different pools of J558L that had been transfected with independently constructed versions of plasmid pSV-Vμ[ΔIn].

a small proportion of pSV-Vμ[In1] transfectants containing a large amount of μ mRNA.

The intron requirement is not specific for a particular intron

The need for the leader-V_H intron could either reflect a requirement for RNA splicing or it could be an indication that the intron contains essential transcription signals [analogously to the location of the IgH enhancer in the major V_H-C_H intron]. It is interesting in this context that the leader-V_H intron in a human V_H gene has been shown to contain sequences homologous to several enhancer elements (12). Furthermore, there is a sequence in the leader-V_H intron used in this work (TGAGGTCTG) to which an eight out of nine match is found at position -180 in the promoter (4).

In order to test whether the intron requirement simply reflected the fact that RNA splicing was necessary to achieve good levels of cytoplasmic μ mRNA, plasmids were constructed which included various different introns

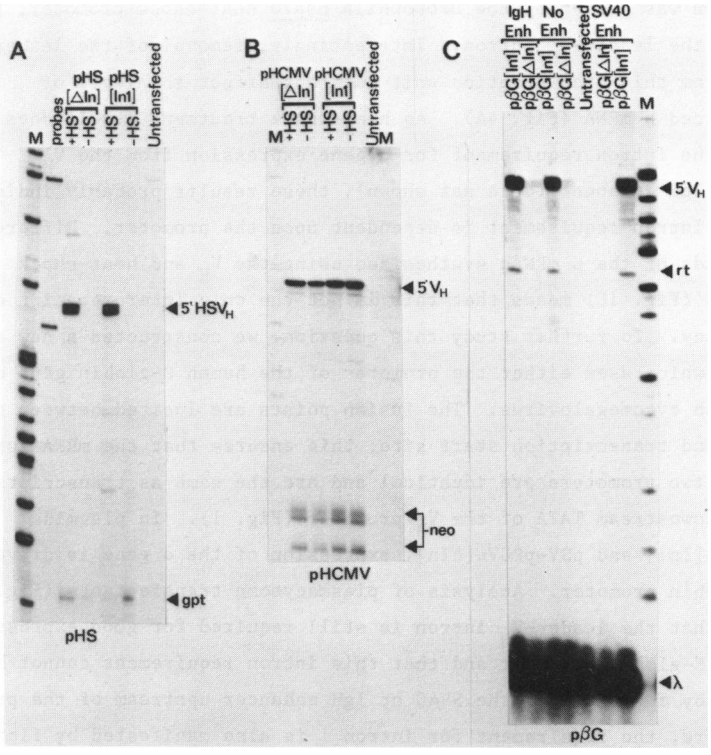

Figure 4. The intron requirement is dependent upon the promoter. Ribonuclease protection assays were carried using cytoplasmic RNA from pools of J558L transfectants and probes spanning the 5' end of the heavy-chain transcription units. (A and B) Expression from the heat-shock and cytomegalovirus promoters was measured before and after heat-shock induction. The gpt and neo transcripts were measured as a reference. (C) Expression from the β-globin promoter was measured from plasmids containing the IgH enhancer, the SV40 enhancer or no enhancer at position -128 upstream of the promoter. Transcripts from the endogenous J558L λ light chain provided a reference.

of the μ gene. As is apparent from the Northern blot and ribonuclease protection assays in Fig. 3, provision of any of the introns tested allowed the production of cytoplasmic μ mRNA. Those constructs with multiple introns directed the synthesis of higher steady-state levels of μ mRNA. As the various introns used show no obvious homology with each other, it is probable that the results obtained here reflect a requirement for splicing rather the presence of promoter elements in the leader-V_H intron.

Promoter dependence of the intron requirement

In previous work (5), we have studied the expression of a μ gene whose

expression was driven by the Drosophila hsp70 heat-shock promoter; the gene included the leader-V_H intron. Interestingly, removal of the leader-V_H intron from this transcription unit does not affect the level of heat-induced μ mRNA (Fig. 4A). As heat-shock treatment itself does not abolish the intron requirement for μ gene expression from the V_H promoter/IgH enhancer (data not shown), these results probably indicate that the intron requirement is dependent upon the promoter. Differences in the 5' ends of the μ mRNAs synthesized using the V_H and heat-shock promoters (Fig. 1C) means that this is not the only interpretation of the discrepancy. To further study this question, we constructed a new set of plasmids which used either the promoter of the human β-globin gene or one from human cytomegalovirus. The fusion points are located between the TATA element and transcription start site; this ensures that the mRNAs produced from the two promoters are identical and are the same as transcripts that use the downstream TATA of the V_H promoter (Fig. 1). In plasmids pSV-pβGVμ[In1] and pSV-pβGVμ[ΔIn], expression of the μ gene is driven by the β-globin promoter. Analysis of plasmacytoma transfectants (Fig. 4C) reveals that the leader-V_H intron is still required for good expression from the β-globin promoter and that this intron requirement cannot be overcome by inclusion of the SV40 or IgH enhancer upstream of the promoter. Furthermore, the requirement for intron 1 is also manifested by fibroblast transfectants and its activity does not therefore appear to be cell-type specific (not shown). However, with the HCMV promoter, an intron is no longer required (Fig. 4B). Thus, the intron requirement for μ gene expression does indeed appear to depend upon the promoter employed.

Intron requirement is post-transcriptional

It was clearly of interest to ascertain whether an intron was required for transcription or for post-transcriptional processes. Attempts to use nuclear run-off assays were unsuccessful as we found that transfected cells contained abundant transcription from both DNA strands as well as there being high RNA polymerase loading in the promoter region upstream of the canonical RNA start sites. We therefore compared steady-state nuclear RNA levels in plasmacytoma cells transfected with pSV-pβGVμ[ΔIn] and pSV-pβGVμ[In1]. The results (Fig. 5) indicate that the two sets of transfectants contain a much larger difference in cytoplasmic than in nuclear μ mRNA levels. This suggests that at least much of the requirement for the intron is associated with nuclear export or RNA processing. However, it should be noted that we cannot be sure that the signals seen in

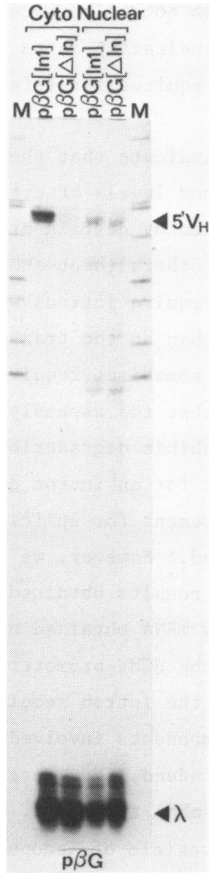

Figure 5. Comparison of steady-state nuclear and cytoplasmic transcripts in pSV-pβG[In1] and pSV-pβG[ΔIn] transfectants of J558L.

the ribonuclease protection assays are due to fully processed mRNA molecules.

DISCUSSION

It has been known for several years that introns are necessary in order to achieve good expression from several eukaryotic genes - for example, SV40 late transcripts (13,14), β-globin (14), dihydrofolate reductase (15) and maize alcohol dehydrogenase (16). However, there are many cellular and viral genes that do not contain introns and, furthermore, the introns in several other genes [adenovirus E1A (17,18), polyoma T antigen (19), Rous sarcoma virus env (20), bean phaseolin (21), yeast actin (22) and chicken

thymidine kinase (23)] are known not to be necessary for good expression. At present, there is no clear indication as to what features of a gene determine whether splicing is required for effective production of cytoplasmic transcripts.

The results presented here indicate that there is a requirement for RNA splicing in order to achieve good levels of cytoplasmic immunoglobulin μ mRNA when transcription is driven by certain promoters (immunoglobulin or β-globin promoters) but not by others (heat-shock or cytomegalovirus). Thus the fact that some genes require introns whereas others do not may depend on the promoter rather than on the transcribed portion of the gene.

The reason why an intron is sometimes required remains wholly unidentified. It is possible that the assembly of splicing complexes may facilitate mRNA transport or inhibit degradation of the primary transcript. Why then should the requirement for an intron depend upon the promoter? One can imagine that the requirement for splicing could be overcome if the gene is very heavily transcribed. However, we do not believe that this is the correct explanation of the results obtained here in view of the fact that the level of cytoplasmic μ mRNA obtained using the β-globin promoter was higher than obtained with the HCMV promoter (not shown). Instead, we propose that the dependence of the intron requirement upon the promoter could reflect the fact that components involved in RNA processing or export may load on at the promoter. Indeed, there are already precedents for the involvement of the promoter in mRNA transport. The export of thymidine kinase mRNA from the germinal vesicle of Xenopus oocytes has been shown by microinjection experiments to be activated by an element in the promoter (24). Another indication comes from the study of the effect of adenovirus infection on the nuclear export of viral and cellular mRNAs. Viral infection blocks the export of many (but not all) host-cell transcripts, but this block can be obviated if the cellular gene is incorporated into the viral chromosome. These experiments have been interpreted (25) in terms of a model which proposes that certain transcripts evade the viral block on export as they are synthesized near a nuclear pore. Thus, following the proposal of Blobel (26) and the experiments of Hutchison and Weintraub (27), some active transcription units could be located near a nuclear pore. When an immunoglobulin μ gene is transcribed under the control of certain (for example, viral or heat-shock) promoters, the transcripts would be synthesized near a nuclear pore and could be readily exported. When other promoters are used, however, the transcripts could be

synthesized further from a pore and might require stabilisation by spliceosome assembly in order to be successfully transported. In view of the results obtained here, it is interesting that transcripts which evade the adenoviral block on export include mRNAs synthesized from heat-shock or viral promoters (25). This model, although highly speculative, provides a framework in which to pursue further investigations. In particular, it will be of interest to identify the element within the promoter that determines whether or not an intron is required for the expression of cytoplasmic μ mRNA.

Finally, it is worth noting the implications of this work for the design of immunoglobulin gene expression vectors. Much of the work carried out so far on the production of chimaeric and recombinant antibodies has made use of vectors containing genomic DNA although expression has been obtained by introducing cDNA inserts into vectors that provide a heterologous intron (28,29). The results presented here indicate that introns should certainly be provided if immunoglobulin transcription is to be driven by a V gene promoter/IgH enhancer; however, no intron is needed if promoters such as that from human cytomegalovirus are used [for example, see (30)].

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