
Unrearranged immunoglobulin variable region genes have a functional promoter

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ABSTRACT

We have tested whether immunoglobulin light chain variable region genes are capable of directing initiation of transcription without undergoing the DNA rearrangement which creates a complete immunoglobulin gene. Two human Vk genes specifically initiated transcription *in vitro* at a site which is approximately 30 bases downstream of a TATA box and 20 bases upstream of the initiation codon. A Vk pseudogene which lacks a TATA box at an homologous position was not transcribed to a detectable extent in the *in vitro* system. One of the Vk genes was injected into *Xenopus* oocytes and it initiated transcription at precisely the same position as in the HeLa cell extract. It is suggested that the promoter which we have identified upstream of unrearranged Vk genes operates in lymphocytes after V-J joining has occurred to initiate transcription of light chain messenger RNA.

INTRODUCTION

Immunoglobulin variable regions are encoded by a family of genes (V genes) that can undergo a DNA rearrangement during lymphocyte differentiation. The rearrangement of light chain V genes joins a particular V gene to a J segment in the proximity of the unique constant region or C gene (1-4). This rearrangement results in a sequence change at the 3' end of the V gene but apparently not at the 5' end of the V gene (5,6).

Rearrangement of a V gene seems to correlate with transcriptional activation of the gene. Evidence for this comes from the observation that the steady state level of nuclear transcripts from a rearranged Vk gene is more than 16,000 times greater than that of an unrearranged Vk gene in the mouse myeloma cell line, MPC 11 (7). In myeloma cells the C gene is transcribed without DNA rearrangement though the transcript is degraded in the nucleus and is not thought to be involved in immunoglobulin production (8). Unrearranged V genes might not be expressed because of factors such as chromatin conformation (9), DNA methylation (10) or specific inhibitors of transcription. Alternatively the actual DNA sequence of a V gene before rearrangement may not be able to support transcription. In this case DNA

rearrangement itself would generate a sequence competent to be transcribed for example by creating a promoter or eliminating an inhibitory sequence. To distinguish between these possibilities, we must first know the location of the Ig gene promoter and in particular whether it is present prior to DNA rearrangement.

In this paper we show by using two transcription systems that V genes in their unrearranged state do possess functional promoters. The two systems employed were a HeLa whole cell extract (11) and injected *Xenopus* oocytes. In both assays unrearranged human kappa light chain variable region genes direct specific initiation of transcription by RNA polymerase II at a site just upstream of the protein coding sequence.

METHODS

Templates for Transcription

The isolation of Vk clones HK100 and HK101 from a phage lambda library of human foetal liver DNA and the construction of subclones HK101/3, HK101/80, and HK100/498 (see Fig. 1) have been described previously (12,13). The sequence of the HK100 Vk pseudogene (13) contains a Bam HI site at the position of codons 66/67. A 2.5 Kb Bam HI fragment of the HK100 phage lambda recombinant was identified as containing the 5' end of the pseudogene by Southern blot hybridisation with nick translated HK101/80 as a probe. The 2.5 kb Bam HI fragment was subcloned in the Bam HI site of pBR 322 with the orientation of the Vk gene identical to that of the tetracycline resistance gene. Clone HK122 was isolated from the same library of foetal liver DNA cloned in Charon 4A (14) by in situ plaque hybridisation using nick translated HK101/80 as a probe. The Vk-containing subclone HK122/80 was isolated from a shotgun cloning of Pst I fragments of HK122 into M13mp2/Pst (13) and its complete sequence was determined (D.L.B. unpublished).

Preparation of plasmid DNAs and M13 replicative form DNAs (RF) was as described (12). Restriction enzymes were purchased from New England Biolabs except for Alu I which was from Boehringer Mannheim. Restriction digests were extracted with phenol, the DNA ethanol precipitated, and then dissolved in 10 mM Tris pH 7.6, 0.1 mM EDTA prior to in vitro transcription.

In vitro Transcription

HeLa (S3) cells were grown in suspension in Joklik's medium containing 5% new born calf serum. Whole cell extracts were prepared essentially as described by Manley et al.(11) and typically had protein concentrations in the range 12-18 mg/ml. Analytical in vitro transcription was performed in

12.5 μ l reactions containing (final concentrations) 60 mM KCl, 7.5 mM MgCl₂, 0.06 mM EDTA, 14 mM Hepes pH 7.9, 1.8 mM Dithiothreitol, 10% glycerol, 8 mM creatine phosphate, 60 μ M each of ATP, GTP, CTP, 60 μ M [α ³²P]UTP (1-5 Ci/mmol), 30-100 μ g/ml DNA, 60% of the reaction volume being cell extract. A range of DNA concentrations was tested for all templates, optimal specific initiation by RNA polymerase II normally being obtained around 60 μ g/ml DNA. After incubation at 30^o for 60 minutes nucleic acids were extracted using phenol/CHCl₃/isoamyl alcohol (20:20:1,v/v) and ethanol precipitated.

Transcription in Oocytes

Oocytes were injected and incubated for 3 days at 18^o in Hepes buffered Barth solution and RNA was then extracted according to ref. 15. Vk transcripts were purified by hybridising total RNA from 30 injected oocytes (150 μ g) to single stranded M13 recombinant DNA immobilised on DBM paper (16). The hybridisation to two 1 cm circles of DBM paper containing about 30 μ g of single stranded DNA was carried out in a 120 μ l final volume containing 50% formamide, 20 mM Pipes pH 6.4, 0.5 M NaCl, 5 mM EDTA, for 9 hrs at 42^o. The filters were washed for five minutes with 20 mM pH Pipes 6.4, 5 mM EDTA. Bound RNA was eluted in 90% formamide, 10 mM Pipes pH 6.4, 5 mM EDTA at 51^o for 10 minutes and ethanol precipitated.

S1 Mapping

A single-stranded end-labelled restriction fragment was used as a probe for S1 mapping the 5' ends of transcripts. Strand separation was carried out according to ref. 17 on the Alu I 148mer from clone HK101/80 by hybridising excess single-stranded M13 recombinant DNA to the isolated restriction fragment. The single-stranded 148mer was treated with calf intestine phosphatase and then labelled with Polynucleotide kinase (P.L. Biochemicals) according to ref.18 and unincorporated nucleotide was removed by gel filtration on Sephadex G100. Specific activities of 1-2 x 10⁷ cpm/ μ g were achieved. This probe was hybridised to RNA in 50% formamide, 20 mM Pipes pH 6.4, 0.5 M NaCl, 1 mM EDTA at 42^o for 60 minutes. To demonstrate the lack of specific initiation upstream of the Alu I 148mer, labelled double-stranded DNA restriction fragments covering the entire 5' flanking region of HK101/3 (see Fig. 1) were used to probe transcripts made in injected oocytes. Hybridisations with RNA were according to ref. 19 in 80% formamide, 50mM Pipes pH 6.4, 0.5M NaCl, 1mM EDTA at 49^o for 2-3 hours. All hybridisation reactions were diluted into 10 volumes of S1 buffer (19) containing 2,000-10,000 units/ml of S1 nuclease (Boehringer) and incubated for 40 minutes at 45^o. Nucleic acids were extracted with phenol:chloroform

(1:1) and ethanol precipitated prior to analysis on sequencing gels.

DNA Sequencing and Gel Electrophoresis

Single stranded recombinant M13 DNA of clones HK122/80 and HK100/498 was sequenced by the dideoxy chain termination method (20).

Denaturing acrylamide gel electrophoresis was performed according to ref. 21. For resolution of larger RNA and DNA species, samples were glyoxylated (22) for 10 minutes at 50⁰ and electrophoresed on 1.4% agarose gels run in 10 mM sodium phosphate pH 6.9. Gels were autoradiographed at -70⁰ with pre-fogged film and intensifying screens (23).

RESULTS AND DISCUSSION

In vitro Transcription of Vk genes

In order to test whether unrearranged Vk genes possess an RNA polymerase II promotor, we used a run-off transcription assay (24) in the HeLa cell extract (11). In this in vitro assay, specific initiation but not termination of RNA synthesis occurs. Restriction fragments are used as templates and the polymerase is allowed to run off the end of the DNA template thus producing an RNA transcript of discrete length. We would expect that a promoter for the Vk gene would be located in the 5' flanking sequence upstream of the coding sequence. We first found evidence for such a promoter by transcribing the Vk clone HK101/80 which contains a 558bp Pst I fragment in pBR 322. This fragment (Fig. 1) which has been completely sequenced (13) contains 130 bases of 5' flanking sequence and extends to codon 79 of the Vk gene including a short intervening sequence. If the 5' flanking sequence in HK101/80 contains a promoter, we would predict that run-off transcription of the 558 bp Pst I fragment would produce an RNA at least 428 bases long. When the Pst I cleaved plasmid was transcribed it in fact gave a major RNA product 440 bases long (Fig. 2, track 4 and Fig. 5, track 1). No transcript of this length was found using pBR 322 DNA cut with Pst I as template (Fig. 5, track 2) and no transcripts were observed when DNA was omitted from the reaction (Fig. 2, track 3). The high molecular weight transcripts seen in Figures 2 and 5 arise from initiation within the vector and aberrant initiation at the ends of the template DNA and are frequently found in this assay system (25,30). When the HK101/80 template was cleaved with Pvu II, the run-off transcript was shortened to 300 bases (Fig. 2, track 6) which is the length expected if transcription initiates 10-20 bases upstream of the first codon and proceeds through the coding sequence to the downstream Pvu II site (see Fig. 1).

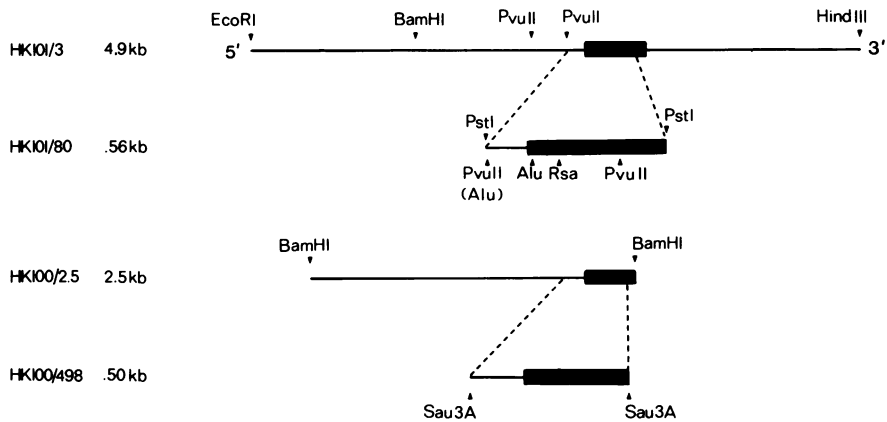


Figure 1. Cloned restriction fragments used as templates for *in vitro* runoff transcription. The names of recombinant clones and the sizes of their inserts are listed on the left. The Vκ sequences (including intervening sequences) are marked as dark boxes and flanking sequences as thin lines. Clone HK122/80 contains a 558 bp Pst I fragment which is homologous to that in HK101/80. All these restriction fragments were cloned in pBR322 except HK100/498 and HK122/80 for which the vectors were M13 derivatives.

To show that the promoter seen in HK101/80 is the principal one for this Vκ gene we also transcribed a clone (HK101/3) containing a much greater length of 5' flanking sequence. Clone HK101/3 is a 4.9kb Eco RI - Hind III fragment inserted into pBR 322 and contains 2.9 kb of 5' flanking sequence and 1.5kb of 3' flanking sequence (Fig. 1). This clone, linearised with Hind III, gave rise to a major 2.0kb transcript (Fig. 2, track 9) consistent with initiation at the same point as that observed in HK101/80. The sequence of pBR 322 contains an adventitious promoter (25) which yields a band of 1.7kb (Fig. 2, track 8) when the plasmid linearised with Hind III is transcribed. With the HK101/3 template the run-off transcript from this pBR 322 promoter is increased in length because the polymerase now traverses the entire insert giving rise to a 6.6 kb RNA which is thought to be the band arrowed in Figure 2, track 9.

To strengthen our conclusions based on the HK101 Vκ gene, we tested a second gene, HK122, for its ability to initiate transcription in the HeLa cell extract. The HK122 clone was isolated from the same library of human foetal liver DNA (14) as HK101 and contains a closely related Vκ gene. The 5' end of the HK122 gene is contained in a 558 b.p. Pst I fragment which has been inserted into the M13mp2/Pst vector in clone HK122/80. The complete sequence (unpublished data) of this fragment shows that it extends

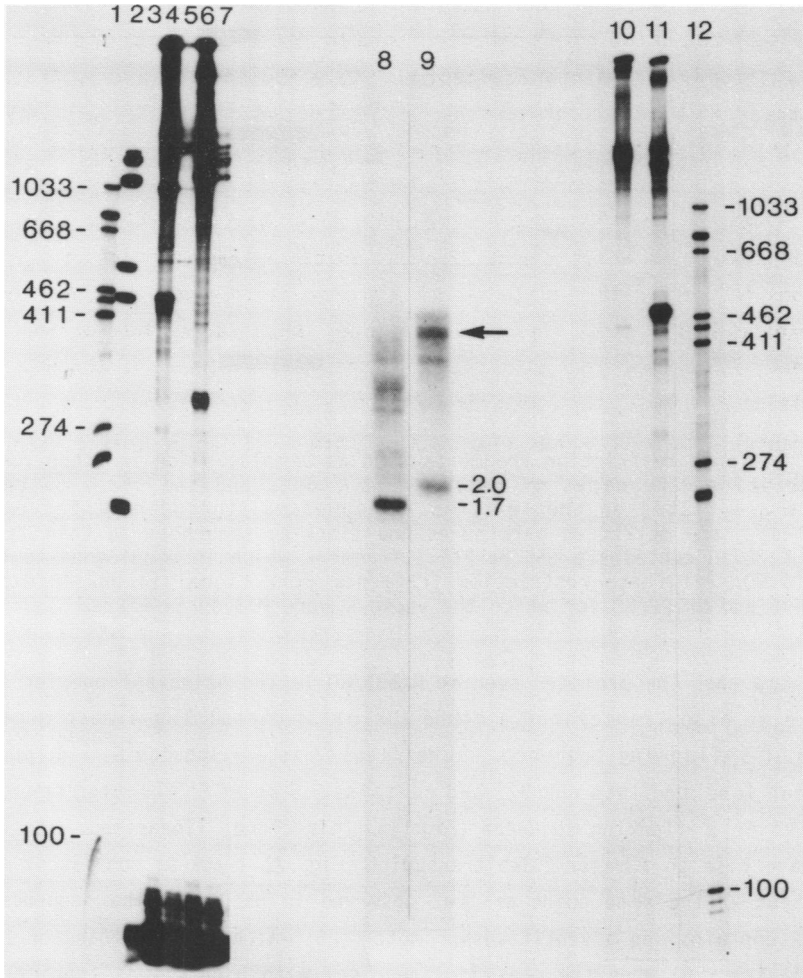


Figure 2. Transcription of the HK101 V κ gene in the HeLa cell extract. Autoradiographs of 5% polyacrylamide (tracks 1-7 and 10-12) and 1.4% agarose gels (tracks 8,9) are shown. HeLa whole cell extract transcription reactions contained 60 μ g/ml of template DNA : track 3; no DNA, tracks 4,5; HK101/80 DNA cut with Pst I, tracks 6,7; HK101/80 DNA cut with Pvu II, track 8; pBR322 DNA cut with Hind III, track 9; HK101/3 DNA cut with Hind III, track 10; HK122/80 DNA cut with Pst I, track 11; human ϵ globin plasmid p ϵ 0.7 (17) cut with Bam HI. Reactions shown in tracks 5 and 7 contained 1 μ g/ml α -amanitin. The band arrowed in track 9 is explained in the text. Markers are end-labelled HaeIII digested bacteriophage G4 RF DNA (tracks 1 and 12) and Hind III digested SV40 DNA (track 2). The sizes of the DNA markers are tracks 1 and 12; 1033, 760, 748, 668, 462, 441, 411, 274, 252, 249, 100, track 2; 1768, 1169, 1101, 526, 447, 215 bases. Selected size markers are indicated in the vertical scale on the left. The vertical scale next to track 9 shows the length in kb of the indicated bands as calculated from the migration of rRNAs on the same gel (not shown).

to codon 79 of the Vk gene. HK122/80 is thus homologous to the Pst I fragment of the HK101 gene contained in HK101/80 (Fig. 1) and its sequence apparently codes for a normal kappa variable region. Figure 2, track 10 shows the runoff transcript from HK122/80 RF digested with Pst I. The size of the transcript, 440 bases, is very similar to that from clone HK101/80 (Fig. 2 track 4) digested with Pst I. No such transcript was obtained from the M13 vector linearised at the same point (Fig. 5, track 4). These results show that both the HK101 and HK122 genes initiate transcription at similar positions indicating that a functional promoter is located just upstream of two unrearranged V genes. It should be noted that, taking into account the different exposures of the autoradiographs in Figures 2 and 5, the HK101 Vk gene yields 2-3 times more transcript than the HK122 gene and about 5 times less transcript than the human ϵ globin plasmid p ϵ 0.7 (17) cleaved with Bam HI (Fig. 2, track 11) which produces a specific runoff RNA about 465 bases long. The significance of the apparently different efficiencies of initiation on these genes is unknown.

The polymerase activity responsible for transcription of these Vk genes can be identified by virtue of its sensitivity to the fungal toxin α -amanitin (26). Addition of α -amanitin at 1.0 μ g/ml abolishes the specific transcription of the Vk gene (Fig. 2, tracks 5 and 7). The α -amanitin sensitivity of the transcription indicates that it is mediated by RNA polymerase II.

Mapping of Transcription Initiation Points

We have mapped the 5' ends of the runoff transcripts from the HK101 Vk gene in order to identify the sites of initiation of transcription. For this we used an S1 protection assay (19). In this assay a 5' end-labelled DNA fragment is hybridised to RNA and the hybrid digested with S1 nuclease. The S1-resistant DNA is then analysed by gel electrophoresis. Its length corresponds to the distance between the labelled nucleotide and the 5' end of the RNA. A single stranded 5' end-labelled Alu I fragment 148 bases long encompassing 123 bases of 5' flanking sequence (Fig. 1) was used as a probe. Figure 3 shows the DNA fragments protected from S1 digestion by hybridisation to transcripts from HK101/3 (tracks 5,6,7), HK101/80 (tracks 3,4) and pBR322 (tracks 1,2). Although there is a complex pattern of S1 protected fragments, it is clear that the 5' ends of the transcripts from clones HK101/3 and HK101/80 are identical. By comparing the length of the protected DNA fragments with Maxam-Gilbert degradations of the labelled probe, we can determine that the majority of transcripts in both cases

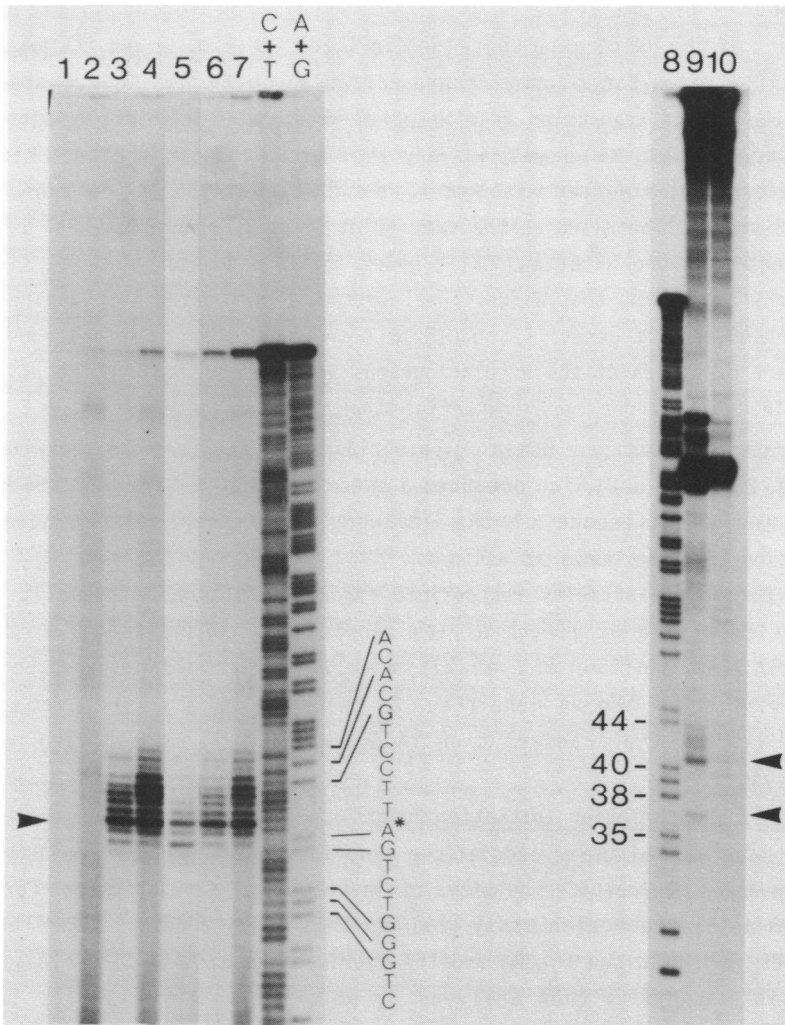


Figure 3. Localisation of the 5' ends of transcripts of the HK101 Vk gene. Left hand panel: S1 mapping of the 5' end of *in vitro* transcripts of the HK101 Vk gene (tracks 1-7). For each track nucleic acids from a 20 μ l transcription reaction were hybridised to 1×10^5 cpm of 5' end-labelled single-stranded 148 base Alu I fragment in a volume of 20 μ l followed by digestion with S1 nuclease. Maxam-Gilbert degradations of the 5' end labelled probe were run in the two righthand tracks of this gel. Tracks 1 and 2: transcripts of Pst I cut pBR322 hybridised to the probe and digested with 5,000 and 2,000 U/ml respectively of S1 nuclease. Tracks 3 and 4: transcripts of Pst I cut HK101/80 hybridised and digested with 5,000 and 2,000 U/ml S1 nuclease. Tracks 5,6 and 7: transcripts of Hind III cut HK101/3, hybridised and digested with 10,000, 5,000, and 2,000 U/ml respectively of S1 nuclease. The major S1-protected band marked with an

arrow corresponds to the A residue marked with a star. Electrophoresis was on a 6% polyacrylamide gel.

Righthand panel: High resolution runoff transcription assay of the HK101 Vk gene. An autoradiograph of a 12% polyacrylamide gel is shown. HeLa cell extract transcription reactions contained: track 9; 80 $\mu\text{g/ml}$ HK101/80 DNA digested with Alu I, track 10; 80 $\mu\text{g/ml}$ pBR322 DNA digested with Alu I. DNA size markers (track 8) were generated by a Maxam-Gilbert degradation of the Alu 1 148mer (A+G reaction) as in the lefthand panel. The two major Vk runoff transcripts in track 9 are arrowed. The dark bands in the region of 70-90 bases are thought to result from labelling of tRNA contaminating the plasmid DNA preparations.

initiate about 20 bases upstream of the first Met codon. The major S1-protected band marked by an arrow corresponds to the starred A residue in the sequencing ladder after taking into account the 1.5 base correction (27). The sequencing ladder is of the non-coding strand and this A residue corresponds to a start at position T₁₀₉ of the sense strand sequence in Figure 6.

The S1 mapping assay frequently yields more protected fragments than there are true start sites (27,28). Also the protected fragments may correspond to initiation sites several bases upstream of the true start site(s) perhaps due to hindrance of the S1 nuclease by the cap structure (29). For these reasons we cannot say precisely how many start sites there are or their exact location, however it is clear that the majority of transcripts initiate in a region about 20 bases upstream of the first Met codon. Only a small fraction of transcripts protect the entire 148 base probe signifying that they are readthrough products from initiation sites further upstream. In the case of Hind III digested HK101/3, these probably correspond to transcripts initiated in the pBR 322 vector. For the Pst I digested clone HK101/80 the readthrough transcripts may correspond to end-to-end transcripts of the 558 b.p. Pst I fragment which are evident in Figure 2, tracks 4 and 5. In summary, the S1 mapping studies confirm that RNA polymerase II initiates transcription in vitro about 20 bases upstream of the HK101 Vk gene. Also, since HK101/80 and HK101/3 yield roughly the same amount of S1 protected fragments (Fig.3), the more extended 5' and 3' flanking sequences in HK101/3 do not seem to affect the efficiency of the promoter in vitro.

As an alternative method of investigating the number of initiation sites, we used a high resolution runoff assay with short blunt-ended restriction fragments as templates. Alu I digested HK101/80 is expected to give runoff transcripts of about 40 bases derived from the 148 b.p. fragment

which spans the 5' end of the gene and ends at codon -15 of the signal peptide (Fig. 1). Figure 3, track 9 shows the transcription products from Alu I cleaved HK101/80 analysed on a sequencing gel. Two major runoff RNA's of the expected size were obtained which apparently differ in length by 4 bases while no comparable transcripts were produced using Alu I digested pBR 322 as a template (Fig.3, track 10). When HK101/80 was cut with Rsa I (see Fig. 1), it also yielded two major runoff products (about 120 bases long) which differ in length by 4 bases (data not shown). The 4 base length difference between the two transcripts probably represents heterogeneity of the 5' end rather than the 3' end since blunt-ended template restriction fragments yield transcripts with a uniform 3' end in this system (30). We are not able to use the length of these short runoff transcripts to determine the precise points of initiation because it is not known whether transcription proceeds to the last base of the template DNA. In conclusion, the in vitro system initiates transcription of the HK101 Vk gene at 2 major sites separated by three bases in the region 105-117. This is the region about 20 bases upstream of the start of the coding sequence of HK101 which is overlined in Figure 6.

Transcription in Injected Oocytes

The in vitro transcription results above identify a promoter upstream of two unrearranged Vk genes. However, it is known that in vivo and in vitro transcription systems recognise somewhat different aspects of a promoter sequence (see for instance Refs. 31 and 32). We have therefore confirmed the results obtained in vitro by injecting the HK101 Vk gene into the nucleus of *Xenopus* oocytes where the template is assembled into chromatin (33). Oocytes were injected with 5-10 ng of supercoiled plasmid DNA of clone HK101/3 and incubated for 3 days. RNA was extracted and the 5' ends of the Vk transcripts were mapped by S1 protection of the same Alu I 148mer as for the in vitro transcripts.

We have compared the S1 protected fragments at the 5' end of transcripts from HeLa cell extract (Fig. 4, track 1) and the injected oocyte (Fig. 4, track 2). The pattern of apparent initiation sites at about 20 bases upstream of the gene is identical in the two transcription systems suggesting that they recognise the same promoter. The yield of protected fragments indicates that it is not a strong promoter in oocytes. In addition the oocyte seems to initiate transcription at a number of sites not found among the in vitro transcripts. The oocyte also produces a much greater fraction of readthrough transcripts initiated further upstream which

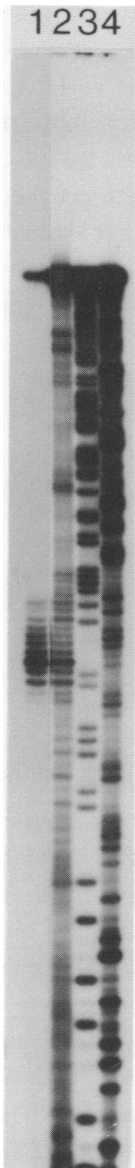


Figure 4. Comparative 5' end analysis of HK101/3 transcripts made *in vitro* and in injected oocytes. Track 1; run off transcripts of Hind III digested HK101/3 analysed as in Fig. 3, track 2; V_k selected RNA from 30 oocytes injected with HK101/3 was hybridized to 5×10^5 cpm of 5' end-labelled single-stranded Alu I 148mer in 35 μ l followed by S1 digestion. S1 nuclease digestions were at 10,000 U/ml. Track 1 was exposed for 20 hours and track 2 for 48 hours. Tracks 3 and 4 show A+G and C+T Maxam-Gilbert degradations respectively of the 5' end labelled Alu 148mer.

protect the entire DNA probe from S1 digestion. However we have eliminated the possibility that these readthrough transcripts initiate at some strong promoter within the 5' flanking sequence of clone HK101/3. This was done by S1 mapping the oocyte transcripts with three restriction fragments covering

the entire 2.8 kb of 5' flanking sequence upstream of the Alu I 148mer probe. The 280 bp. Pvu II fragment, 900 bp. Bam HI-Pvu II fragment, and the 1600 bp. Eco RI-Bam HI fragment (Fig. 1) were each protected over their entire length by RNA from injected oocytes and no discrete initiation sites in the upstream region were detected by this assay. However all the probes did give rise to a uniform smear in addition to the full length protected fragments implying that there is some essentially random initiation or degradation of the RNA. These observations are consistent with others which show that frequent initiations occur in bacterial plasmid sequences injected into oocytes (34,35,36). We can therefore eliminate the possibility that the oocyte uses a strong promoter in the 5' flanking region upstream of the one recognised in vitro. It is still possible, however, that the oocyte recognises other weak promoters in addition to the one which operates in vitro.

A Vk Pseudogene is Not Transcribed in vitro

We recently discovered a pseudogene whose sequence is homologous to a Vk gene (13). This sequence contains several termination codons in phase and therefore cannot code for a normal immunoglobulin. We asked whether the Vk pseudogene HK100 is able to direct transcription in the HeLa cell extract. Two cloned restriction fragments (Fig. 1) were tested as templates. HK100/2.5 contains a 2.5 k.b. Bam HI fragment in pBR322 which extends from 2.1 kb upstream of the gene to codon 67. HK100/498 contains a 498 b.p. Sau 3A fragment in M13mp2/Bam (37). This fragment has been completely sequenced (ref. 13 and unpublished data) and extends from 172 bases upstream of the pseudogene to codon 48. HK100/498 was digested with Eco RI to release the inserted restriction fragment. If this template were transcribed in an analogous fashion to the HK101 and HK122 genes, we would expect a runoff transcript 355 bases long. Figure 5, track 3, shows that there were no detectable runoff transcripts of this Vk pseudogene clone apart from those which derive from the vector DNA (Fig. 5, track 4). We also tested the HK100/2.5 pseudogene template which has 2.1 kb of 5' flanking sequence. This plasmid was linearised with Sal I and would be expected to yield a 680 base transcript if it were transcribed like the other two Vk genes. Again no transcripts of this clone were detected (Fig. 5, track 6) apart from those originating in the vector (Fig. 5, track 7). Controls in which the HK100 templates were mixed with clone HK101/80 showed that the HK100 DNA's did not inhibit transcription of the HK101 gene (Fig. 5, track 5 and data not shown). The HK100 Vk pseudogene therefore is apparently not capable of

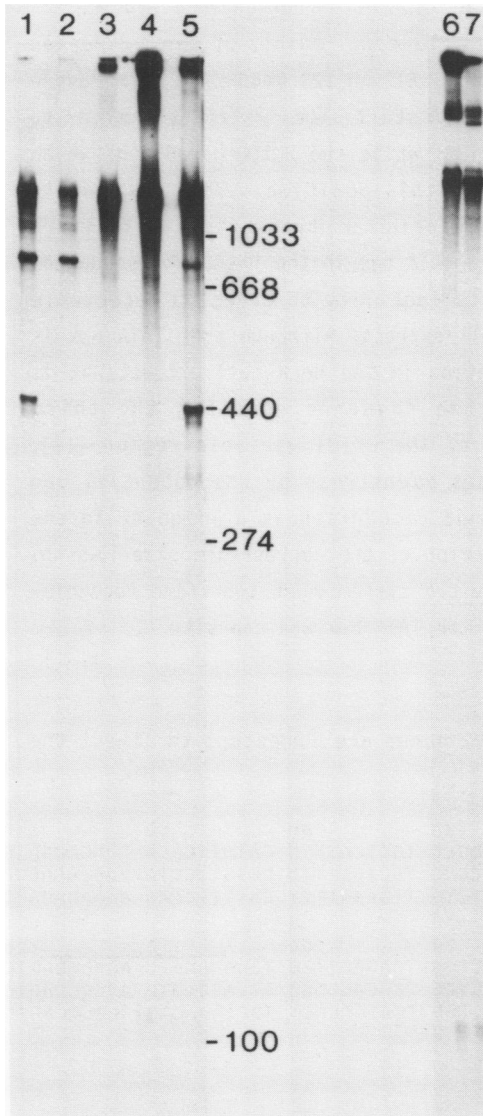


Figure 5. *In vitro* transcription assay of the HK100 Vk pseudogene. Transcription reactions contained 60 μ g/ml of template DNA: track 1; HK101/80 DNA cut with Pst I, track 2; pBR322 DNA cut with Pst I, track 3; HK100/498 DNA cut with EcoRI, track 4; M13mp2/Pst RF DNA cut with Pst I, track 5; HK101/80 DNA cut with Pst I plus HK100/498 DNA cut with EcoRI, track 6; HK100/2.5 DNA cut with Sal I, track 7; pBR322 DNA cut with Sal I. Transcription products were analysed on 5% polyacrylamide gels and detected by autoradiography.

directing transcription in the HeLa cell extract in contrast to the HK101 and HK122 Vk genes. It would be interesting to know whether a promoter mutation caused the initial loss of expression in an evolutionary ancestor of this pseudogene thereby liberating its sequence from the constraints of selection pressure.

Role of the TATA Box in in vitro Transcription of Unrearranged V Genes

We compared the sequences of the 5' flanking regions of the 3 Vk genes which were assayed for transcription (Fig. 6). HK122 and HK101 both have a sequence about 30 b.p. upstream of the RNA start site which is similar to the consensus sequence for a TATA box (38) while the HK100 pseudogene which is not transcribed lacks a TATA box at this position. The TATA box is necessary for promoting specific initiation of transcription *in vitro* (38). It seems likely that the lack of a TATA box in the HK100 Vk pseudogene is at least partly responsible for its inability to direct a detectable amount of transcription in the HeLa cell extract. Although the TATA box is necessary for initiation of transcription in the HeLa cell extract, it is clearly not sufficient. In fact, the sequence encoding the third complementarity determining region of the HK101 variable region (13), includes TATAATA followed thirty bases downstream by the potential cap sequence CACCC (38). This region is not recognised as a promoter in the HK101/3 template since no runoff transcript of the appropriate size (1.5 kb) was produced (Fig. 2 track 9). The *in vitro* system must therefore recognise other promoter elements in addition to the TATA box and cap site.

CONCLUSIONS

We have shown that unrearranged Vk genes are competent to direct the

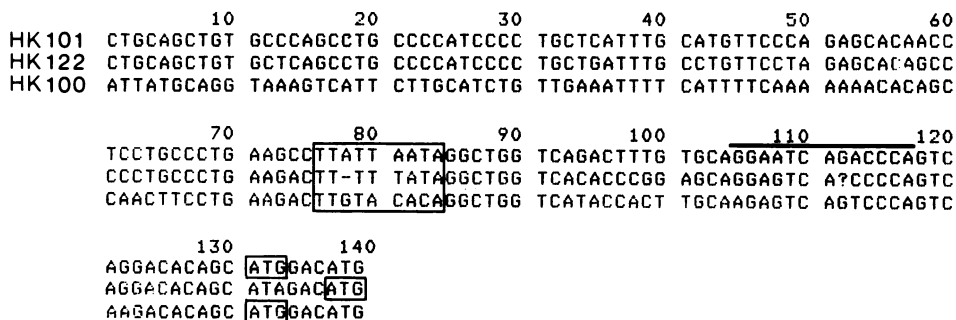


Figure 6. Sequence comparison of 5' flanking regions of the Vk genes HK101 and HK122 and the pseudogene HK100. The sequences have been aligned to maximise homology. The HK101 sequence is from ref.13. The boxed region around base 80 is homologous to the TATA box (37) in HK101 and HK122, but not in HK100. The region containing the 5' end of the HK101 transcripts as defined by S1 mapping is overlined. The first Met codon of each gene is also boxed. The dash at position 78 of the HK122 sequence was inserted to maximise homology. The question mark at position 112 of this sequence refers to a single base whose identity was ambiguous on the sequencing gels.

specific initiation of transcription in vitro and in injected oocytes. The accuracy of initiation of transcription in the HeLa cell extract compared to in vivo transcription has been amply demonstrated for adenovirus major late (11), ovalbumin (39), SV40 (40) and globin genes (41). The fidelity of RNA Polymerase II transcription in injected oocytes has been shown for five sea urchin histone genes (42) and the Herpes Simplex Virus thymidine kinase gene (43). We do not know the location of the 5' end of natural kappa light chain mRNA but it is likely that the promoter which is recognised by these two experimental transcription systems also operates in vivo after V-J joining has occurred. Since we have not tested the transcriptional activity of a rearranged Vk gene, it is still possible that rearrangement supplies DNA sequences which enhance the efficiency of this promoter. Alternatively, rearrangement may activate V gene transcription in other ways for example by conferring a favourable chromatin conformation on the promoter we have identified.

Unrearranged Vk genes are not thought to be transcribed in vivo, at least in myeloma cells (7). Our results show that DNA rearrangement per se is not necessary to make a variable region sequence competent to initiate transcription. Presumably other factors such as chromatin conformation (9) or DNA methylation (10) are responsible for maintaining the transcriptional inactivity of unrearranged V genes in lymphocytes.

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