bed a cross-reaction between sheep IgM and erythrocytes vas inhibited by hog blood-group substance, suggesting he shared determinant is a carbohydrate. This deter- it was recognised by antisera to sheep or other mam- rocytes raised in chickens but not in mammals, and h... . or partially shared by erythrocytes and sera of al mammalian species. It has also been observed that in sera natural antibodies to human tumour cells of several ogical types can be absorbed by human IgM (refs. 9, 10). seems likely that the earlier observation of fluorescent ng of frog thymocytes by rabbit anti-Ig antibodies[11] could iave been the result of a reaction with the cross-reactive minant we have described, and does not necessarily mean fgs are present on the surfaces of these cells. Reports of ce Ig on thymocytes of other poikilotherms[12–16] may have ilar basis. Moreover, the fluorescent staining of mouse ocytes by chicken antisera to mouse IgM (ref. 17) may be related to such a cross-reaction. Although rabbit (or mammalian) antisera to mouse IgM do not usually stain e T lymphocytes, low levels of antibodies to a cross- ive determinant might be present in such antisera and l interfere with T-cell functions such as antigen binding[18]. ever, as the cross-reactive determinant is not found on LMW Ig, our results are probably not relevant to evidence ght chain determinants on T lymphocytes[18,19].

speculate that in all vertebrates an oligosaccharide is d by IgM and a membrane component of thymocytes and l cells, and perhaps of all cells. A strong antibody response is oligosaccharide is usually obtained only by immunising genetically distant species. Carbohydrate antigens, ver, may be sporadically distributed among species and es[20–23], and it is possible that in some species, the postu- immunogenic oligosaccharide of IgM is found only on in cell types. The presence of the same carbohydrate minant on different molecules has been shown for human I ewis blood-group antigens. These oligosaccharides onjugated either to polypeptides or to lipids and are found in secretions and on cell surfaces[22,23].

e constant region of the human $\mu$ chain contains five saccharides; their location on the polypeptide chain is vn[24,25], and they have been partially characterised[26–28]. lts of Hogg and Greaves[29] provide a basis for speculating ie identity of the postulated immunogenic oligosaccharide. found that certain class-specific antisera to mouse IgM ited antigen binding by T lymphocytes. The antibodies onsible for this activity were absorbed by $F(ab')_2$ frag- ts, but not by Fab fragments of IgM. The $F(ab')_2$ fragment, ot the Fab fragment, would be expected to contain the C2 saccharide[24,25,30].

e thank S. Turco for advice on periodate oxidation. This was supported by grants from the NIH.

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# *V* and *C* parts of immunoglobulin κ-chain genes are separate in myeloma

DNA SEGMENTS coding for amino terminal and carboxyl terminal half of immunoglobulin chains are separate in the embryo, and specific rearrangement in these DNA segments occurs during differentiation of lymphocytes[1–3]. Although the simplest model would be that the rearrangement brings a *V* gene in contiguity with a *C* gene, thereby allowing RNA polymerase to transcribe a whole immunoglobulin gene continuously, it has not been directly shown that this is really the case. One way to study this problem and which we report here is to hybridise DNA from a plasmacytoma with a purified light chain mRNA in conditions which would not permit re-naturation of the gene segments, digest all single-stranded nucleic acid with single strand specific nuclease $S_1$ (ref. 4), and determine the size of the DNA segment which was protected by the mRNA from digestion with $S_1$ nuclease. Using the fact that the length of the immunoglobulin mRNA and its two regions corresponding to *V* and *C* genes are known with considerable accuracy[5,6], we have been able to determine that *V* and *C* genes are not contiguous.

We first confirmed that $S_1$ nuclease does not degrade DNA–RNA hybrids in our digestion conditions by carrying out the following model experiment. $^{32}$P-labelled, complementary DNA was synthesised on a purified κ-chain mRNA (MOPC 321) template and the full transcript was isolated. The DNA was annealed with the excess κ-mRNA and the hybrids, after treatment with the $S_1$ nuclease, were fractionated by acrylamide gel electrophoresis in 98% formamide. Figure 1 shows that the overwhelming majority of the $^{32}$P–cDNA remains intact when the cDNA is preannealed with excess κ-mRNA and subsequently treated with $S_1$ nuclease.

*V* and *C* genes can be arranged in three possible ways in the plasmacytoma DNA, depending on whether or not these genes are contiguous with each other and depending on whether or not the 5'and 3' untranslated sequences are contiguous with *V* and *C* translated sequences, respectively. To distinguish between the three possible arrangements shown in Fig. 2, we did a protection experiment similar to that described above using DNA from plasmacytoma MOPC 321 and purified, homologous κ-mRNA. We digested the MOPC 321 DNA with a restriction enzyme and fractionated the digest on 0.9% agarose slab gels, as previously described[1]. Figure 3 shows the pattern of hybridisation of an *Eco*RI digest of MOPC 321 DNA with homologous, iodinated κ-mRNA. A hybridisation pattern of 18S rRNA is also shown to illustrate complete digestion[9].

Gel fractions with an average molecular weight of 11 megadaltons were pooled as indicated. The pooled DNA should contain the $V_\kappa$ and $C_\kappa$ genes, as it hybridised with both whole and 3' half mRNA, and as other regions on the gel hybridised with either of the two RNA probes only to the extent which can be attributed to 'background' counts that are

roughly proportional to the optical density profile. The pooled DNA fragments were annealed to an excess of MOPC 321 κ-mRNA. The single-stranded moiety of the hybrid was digested with $S_1$ nuclease. The nuclease resistant nucleic acids were treated with 0.2 M NaOH at 45°C for 30 min to digest RNA. The remaining DNA was fractionated in acrylamide gel in denaturing conditions. The gel was cut, and DNA from each fraction was hybridised with iodinated MOPC 321 to localise the position of the complementary DNA strand. Figure 4 shows that a DNA fragment of 0.19 megadaltons (590 bases) was protected from $S_1$ digestion by hybridised mRNA. This is about half of the estimated length of the κ light chain mRNA minus the poly A track, suggesting that the *V* and *C* genes in MOPC 321 plasmacytoma are not contiguous. However, the result could also be obtained if there were an *Eco*RI site near or at the *V*–*C* joint of a continuous κ-chain gene. This, however, is incompatible with the amino acid sequence[10].

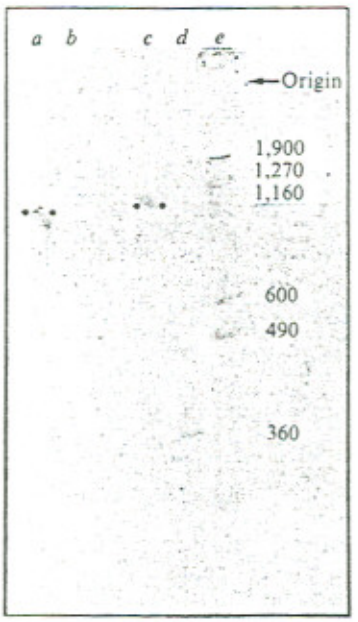



**Fig. 1** Protection against $S_1$ nuclease of cDNA by preannealing with complementary κ-mRNA. $^{32}$P-labelled DNA complementary to purified MOPC 321 κ-mRNA was synthesised by reverse transcriptase. The incubation mixture (in 0.2 ml) consisted of: 50 mM Tris-HCl (*p*H 8.3), 10 mM $MgCl_2$, 10 mM dithiothreitol, 4 mM tetra-natrium-pyrophosphate, 1 mM each of dATP, dCTP, dGTP, dTTP, 200 μCi dATP (250 Ci mmol$^{-1}$), 5 μg mRNA, 2 μg oligo $dT_{12-18}$ (P.-L. Biochemicals) and 10 units AMV reverse transcriptase. Incubation was at 46°C for 10 min. The reaction product was incubated for 12 h in 0.3 M NaOH at 37°C, neutralised, extracted with phenol and passed through a 3-ml Sephadex G-150 column in $H_2O$. The excluded material (cDNA) was fractionated by electrophoresis in 5% acrylamide gel in 98% formamide[7]. The cDNA in the major band (~1,000 bases long) was extracted and incubated with and without excess purified κ-mRNA (0.2 μg) at 70°C for 3 h. Beside the mRNA, the annealing mixture contained in 20 μl: 225 mM $NaPO_4$, *p*H 7.5, and 3,000 c.p.m. $^{32}$P-labelled cDNA (specific activity $10^6$ c.p.m. μg$^{-1}$). After annealing, the sample was diluted 10-fold with the following mixture: 50 mM Na-acetate, *p*H 4.2, 250 mM NaCl, 1 mM $ZnSO_4$, and 20 μg ml$^{-1}$ denatured, sonicated calf thymus DNA. The final *p*H and [$Na^+$] concentration were 4.6 and 300 mM, respectively. The mixtures were incubated at 45°C for 30 min in the presence of 15 units $S_1$ nuclease (prepared by the method of Vogt[8]), phenolised, concentrated by alcohol precipitation and electrophoresed in 5% acrylamide gel (1.5 mm thick, 20 cm long) in 98% formamide. The gel was exposed to Kodak X-Omat R film. *Hin*dIII or *Hae*III digested, end-labelled SV40 DNA fragments were electrophoresed in parallel as size markers. Channel *a*, cDNA incubated with mRNA followed by $S_1$ treatment; channel *b*, cDNA incubated without mRNA followed by $S_1$ treatment; channel *c*, cDNA without any treatment; channel *d*, *Hae*III-digested SV40; channel *e*, *Hin*dIII-digested SV40.

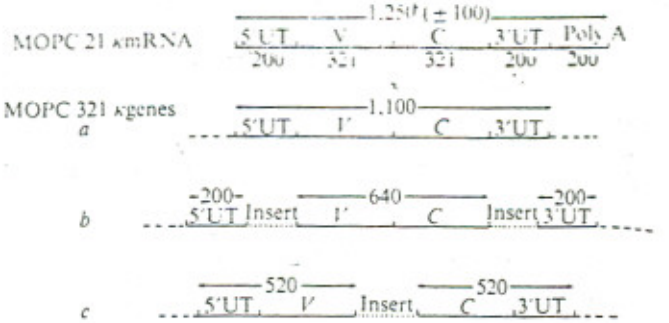
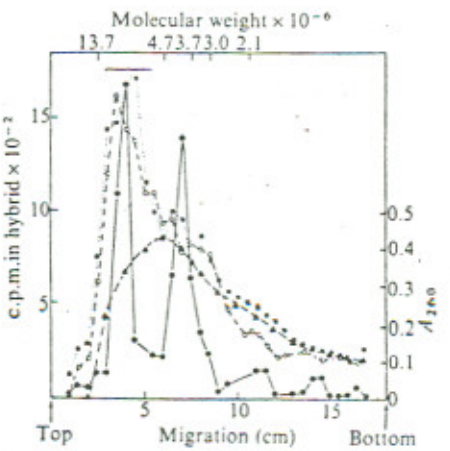



**Fig. 2** Different possible arrangements of *V* and *C* translated and 5' and 3' untranslated sequences of MOPC 321 κ-chain genes. Length in numbers of bases is indicated for each DNA segment and derived from the known numbers for MOPC 21 mRNA (ref. 6).

Absence of such an *Eco*RI site was confirmed by our recent studies in which double-stranded, full-size DNA which was synthesised on MOPC 321 κ-mRNA template using reverse transcriptase and DNA polymerase, was treated with this restriction enzyme (unpublished observation).

The protection experiment data could be compatible with the arrangement shown in model *b* of Fig. 2. In this case, mRNA would protect a sequence of about 640 bases, which is not very different from what was actually observed. To consider this possibility further, we did the following experiment. MOPC 321 DNA digested with restriction enzyme *Bam*HI was fractionated on 0.9% agarose gel and the 2.4-megadalton component which hybridises to both whole and 3' half mRNA was isolated[1]. Our more recent studies on a DNA clone which contains the nearly full sequence of the MOPC 321 κ-mRNA demonstrated that *Bam*HI cleaves the *V* gene DNA at positions corresponding to amino acid residues 64 and/or 66, and 99, and that the same enzyme has no cleavage sites in the *C* gene (unpublished observation). The cleavage site corresponding to amino acid residue 99 is very close to the *V*–*C* junction. Thus, the 2.4-megadalton DNA component should not contain on the same fragment both *V* and *C* gene sequences. On the other hand, as the 3' end half fragment of the κ-mRNA hybridises with this DNA component[1], it should at least contain the $C_\kappa$-gene sequence. Figure 4 shows that a 590-base long sequence of the 2.4-megadalton DNA

**Fig. 3** Gel electrophoresis pattern of MOPC 321 DNA generated by *Eco*RI digestion. $^{125}$I-labelled whole κ-mRNA of MOPC 321 (○) and 3' end half fragment[12] (■) ($5 \times 10^7$ c.p.m. μg$^{-1}$) were annealed with DNA extracted from gel slices as described[1]. The profile obtained with $^{125}$I 18S rRNA is shown for comparison (●). The molecular weight scale was obtained from phage λ DNA, digested by *Eco*RI, which was electrophoresed in parallel. The 11-megadalton fraction of DNA used in the protection experiment is indicated by the bracket. ▲, optical density at 260 Å.



component was protected from $S_1$ digestion by MOPC 321 κ-mRNA. The length of the protected DNA sequence is too large to be accounted for by model *b*. On the other hand, it agrees well with the notion that the translated and untranslated parts of the $C_\kappa$ gene are contiguous. In addition, the fact that the hybridisation extent with this protected DNA is about half that obtained with the *Eco*RI-digested DNA is consistent with model *c*.

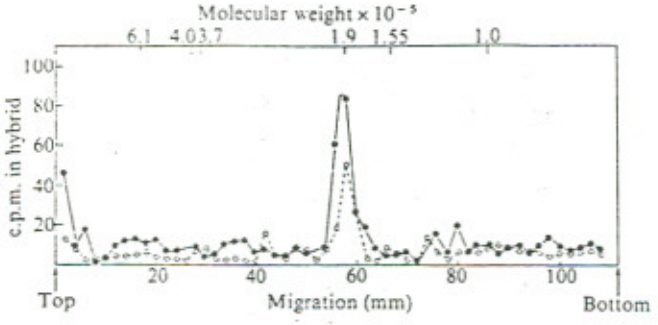



**Fig. 4** MOPC 321 DNA (2 mg) digested with *Eco*RI was fractionated on 0.9% agarose as described in Fig. 3. The 11-megadalton fraction (140 μg) recovered from 2 mg total DNA was denatured in 0.1 M NaOH at 37°C for 15 min, neutralised and incubated with an excess of MOPC 321 κ-mRNA (1 μg in 0.2 ml final volume) in 6×SSC/50% formamide at 45°C for 45 min. The control experiment consisted of the 11-megadalton MOPC 321 DNA fraction (160 μg) also recovered from 2 mg total DNA digested with *Eco*RI, and incubated in the absence of mRNA. After treating with an excess of $S_1$ for 30 min at 45°C (as described in the legend of Fig. 1) and digesting the RNA in the hybrid by alkaline treatment, the $S_1$ resistant DNA was fractionated on a 5% acrylamide gel in 98% formamide. The gel was cut into 2-mm slices, DNA was eluted by homogenising, freezing and thawing, and each fraction was hybridised with 800 c.p.m. $^{125}$I κ-mRNA ($7.5\times10^7$ c.p.m. μg$^{-1}$) in a 20-μl mixture consisting of 2×SSC, 20 mM Tris-HCl, *p*H 7, 2 mM EDTA at 70°C for 44 h. Data are expressed as a difference in counts between the experimental sample and the control sample (●). An analogous experiment was done using the 2.4-megadalton DNA fragment obtained by digesting MOPC 321 DNA with *Bam*HI (ref. 1) (○). The molecular weight scale was obtained from SV40 DNA digested by *Hin*dIII.

Our results indicate that the κ-mRNA from MOPC 321 myeloma contains stretches of polynucleotides which are transcribed from two DNA segments of about equal length that lie separate in the genome. These segments correspond largely to *V* and *C* gene sequences. We recently reached an analogous conclusion for a λ-chain mRNA, based on evidence obtained by a more direct procedure, namely, by analysis of the DNA fragment cloned from the myeloma cells[11]. In addition, our more recent studies on the MOPC 321 κ-gene clones directly confirmed that the $V_\kappa$ and the $C_\kappa$ sequences are separate in the myeloma genome (R. Lenhard-Schuller, C. Brack, B. Hohn and S.T., in preparation). The split nature of coding sequences is not unique to immunoglobulin genes; there are many reported examples[13–18].

After completion of the present studies, we learned that another group had reached the same conclusion based on experiments that are very similar to those described here[19].

We thank Dr J. W. Beard for AMV reverse transcriptase and acknowledge the technical assistance of Ms B. Durrer.

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# Generation of prostacyclin by lungs *in vivo* and its release into the arterial circulation

VASCULAR walls generate from prostaglandin endoperoxides or arachidonic acid an anti-aggregatory and a vasodilator substance, prostacyclin ($PGI_2$)[1,2]. Several prostaglandins are rapidly inactivated as they traverse the pulmonary circulation[3] but prostacyclin passes through unchanged[4,5]. We report here that prostacyclin is continuously generated by the lungs *in vivo* or *in vitro*. Clearly, such an endocrine-like function of the lungs could play an important part in the natural resistance of the organism against intra-arterial thrombosis.

Experiments were carried out in 87 cats anaesthetised with pentobarbitone sodium (40 mg per kg, intraperitoneal); the cats were also heparinised (2,500 IU per kg). A polyethylene cannula was pushed down to the right atrium from the right jugular vein. Through the left carotid artery another cannula was placed in the ascending aorta. Mixed venous blood and aortic blood were withdrawn by a peristaltic pump (3 ml min$^{-1}$) and, after passage (45 s) through a warmed jacket (37°C), each stream of blood superfused a separate collagen strip which was excised from the Achilles tendon of a rabbit[6]. The blood was

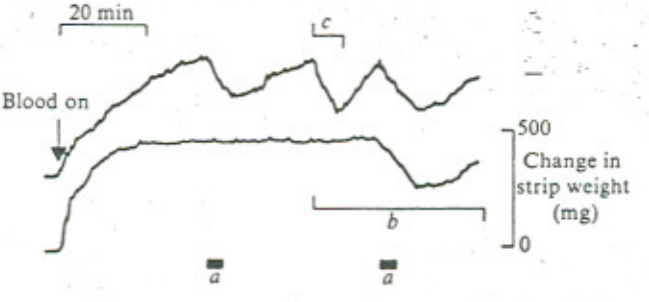


**Fig. 1** Quantitation of the release of a prostacyclin ($PGI_2$)-like substance from the cat lungs. One collagen strip was superfused with mixed venous blood (lower trace) and another with aortic blood (upper trace) (blood on) of an anaesthetised and heparinised cat. The deposition of platelet clumps resulted in an increase in weight (0–500 mg) of the strips. This process was faster in mixed venous blood than in aortic blood. An intravenous infusion of $PGI_2$ (1 μg per kg over 3 min) (*a*) caused platelet disaggregation in aortic blood, but not in mixed venous blood. However, the same intravenous dose of $PGI_2$ was effective on both sides of the lungs when a subthreshold disaggregating concentration of $PGI_2$ (200 pg ml$^{-1}$) (*b*) was continuously infused into mixed venous blood. Therefore, it was assumed that the difference in concentration of endogenous $PGI_2$ between mixed venous blood and aortic blood was approximately 200 pg ml$^{-1}$. The disaggregating response in aortic blood was calibrated