bed a cross-reaction between sheep IgM and erythrocytes was inhibited by hog blood-group substance, suggesting the shared determinant. IgM carbohydrate. This determinant was recognised by antisera to sheep or other mam-
mal species. It has also been observed that in sera of natural antibodies to human tumour cells of several cell types can be absorbed by human IgM (refs. 9, 10). It seems likely that the earlier observations of fluorescent staining of frog thymocytes by rabbit anti- 
Ig antibodies could have been the result of a reaction with the cross-reactive determinant that we have described, and does not necessarily mean that this determinant is present on the surfaces of these cells. Reports of the presence of other pili and Ig on thymocytes of other pili have also may give rise to confusion. Moreover, the fluorescent staining of mouse thymocytes by mouse IgM (ref. 17) may be related to such a cross-reaction. Although rabbit (or other mammalian) antisera to mouse IgM do not usually stain the T lymphocytes, low levels of antibodies to a cross-reactive determinant might be present in such antisera and interfere with T-cell functions such as antigen binding.

V and C parts of immunoglobulin k-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, this has not been directly shown that this is really the case. One way to study this problem which we report here is to hybridise DNA from a plasmacytoma with a purified light chain mRNA in conditions which would not permit remodelling of the gene segments, digest all single-stranded nucleic acid with single-strand-specific nuclease S 
(ref. 4), and determine the size of the DNA segment which was protected by the mRNA from digestion with S 
, 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), we have been able to determine that V and C genes are not contiguous.

We first confirmed that S 
, nuclease does not degrade DNA-
RNA hybrids in this condition by carrying out the following model experiment. 32P-labelled, complementary DNA was synthesised on a purified k-chain mRNA (MOPC 321) template and the full transcript was isolated. The DNA was annealed with the excess k-mRNA and the hybrids, after treatment with the S 
, nuclease, were fractionated by acryl-

amide gel electrophoresis in 9% formamide. Figure 1 shows that the overwhelming majority of the cDNA remains intact when the eDNA is preannealed with excess k-mRNA and subsequently treated with S 
, 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 S 
and S 
 untranscribed 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 k-mRNA. We digested the MOPC 321 DNA with a restriction enzyme and fractionated the digest on 0.9% agarose slab gels, as previously described. Figure 3 shows the pattern of hybridisation of an EcoRI digest of MOPC 321 DNA with homologous, iodinated k-mRNA. A hybridisation pattern of 18S rRNA is also shown to illustrate complete digestion.

Gel fractions with an average molecular weight of 11 megadaltons were pooled as indicated. The pooled DNA should contain the V and C genes, as it is hybridised with both whole and 1 half mRNA, and it was tested 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 S₁ 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 S₁ digestion by hybridised mRNA. This is about half of the estimated length of the κ light chain mRNA minus the poly A tract, 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 EcoRI site near or at the V-C joint of a continuous κ-chain gene. This, however, is incompatible with the amino acid sequence.

Absence of such an EcoRI 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 BamHI was fractionated on 0.9% agarose gel and the 2.4-megadalton component which hybridised to both whole and 3'-half mRNA was isolated. Our more recent studies on a DNA clone which contains the nearly full sequence of the MOPC 321 κ-mRNA demonstrated that BamHI leaves 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 95 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, it should at least contain the C gene sequence. Figure 4 shows that a 590-base-long sequence of the 2.4-megadalton DNA.

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**Fig. 1** Protection against S₁ nuclease of cDNA by preannealing with complementary κ-mRNA. 32P-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 (pH 8.3), 10 mM MgCl₂, 10 mM dithiothreitol, 4 mM tetra-natrium-pyrophosphate, 1 mM each of dATP, dGTP, dUTP, 220 μCi dATP (220 Ci mmol⁻¹), 5 μg mRNA, 2 μg oligo d(T)₁₁⁻₁₈ (P-L, Bochumucals), and 10 units AMV reverse transcriptase. Incubation was at 46°C for 10 min. The reaction product was incubated for 1 hr in 0.3 M NaOH at 37°C, neutralised, extracted with phenol and passed through a 3-ml Sephadex G-100 column in H₂O. The excluded material (cDNA) was fractionated by electrophoresis in 5% acrylamide gel in 9% formamide? The cDNA in the major band (~11,000 bases long) was extracted and incubated with and without excess purified κ-mRNA (0.2 μg) at 70°C for 3 hr. Beside the mRNA, the annealing mixture contained in 20 μl: 225 mM NaPO₄, pH 7.5, and 3,000 c.p.m. 32P-labelled cDNA (specific activity 10⁶ c.p.m. μg⁻¹). After annealing, the sample was diluted 10-fold with the following mixture: 50 mM Na-acetate, pH 4.2, 250 mM NaCl, 1 mM ZnSO₄, and 20 μg ml⁻¹ denatured, sonicated calf thymus DNA. The final pH 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 S₁ nuclease (prepared by the method of Vogt⁸), phenolised, concentrated by alcohol precipitation and electrophoresed in 5% acrylamide gel (1.5 mm thick, 20 cm long) in 9% formamide. The gel was exposed to Kodak X-Omat R film. HindIII or HaeIII digested, end-labelled SV40 DNA fragments were electrophoresed in parallel as size markers. Channel a, cDNA incubated with mRNA followed by S₁ treatment; channel b, cDNA incubated without mRNA followed by S₁ treatment; channel c, cDNA without any treatment; channel d, HaeIII-digested SV40; channel e, HindIII-digested SV40.
Our results indicate that the k-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 lambda chain mRNA, based on evidence obtained by a more direct procedure, namely, by analysis of the DNA fragment cloned from the myeloma cells. In addition, our recent studies on the MOPC 321 k-genes directly confirmed that the V region and the C region 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.

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.

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Gaston Matthessem*  
Susumu Tonegawa

Basel Institute for Immunology,  
Postfach, CH-4005 Basel 5, Switzerland

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* Present address: MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK.


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**Figure 4** MOPC 321 DNA (2 mg) digested with EcoRI was fractionated on 0.9% agarose as described in Fig. 3. The 11-megadalton fraction (1.05 mg) recovered from 2 mg total DNA was denatured in 0.1 M NaOH at 37°C for 15 min, neutralized and incubated with an excess of MOPC 321 k-mRNA (1 µg in 0.2 ml final volume) in 6 X SSC/50% formamide at 45°C for 45 min. The control experiment consisted of the 11-megadalton MOPC 321 DNA fraction (150 µg) also recovered from 2 mg total DNA digested with EcoRI, and incubated in the absence of mRNA. After treating with an excess of S, 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, resistant DNA was fractionated on a 5% acrylamide gel in 9% formamide. The gel was cut into 2-mm slices, DNA was eluted by homogenizing, freezing and thawing, and each fraction was hybridized with 800 c.p.m. of k-mRNA (7 X 10^6 c.p.m. µg^-1) in a 20-µi mixture consisting of 2 X SSC, 200 mM Tris-HCl, pH 7.2, 0.2 mM EDTA at 70°C for 44 h. The bands were separated as described in the legend to Fig. 4. The molecular weight scale was obtained from SV40 DNA digested with HindIII.

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**Figure 1** Quantitation of the release of a prostaglandin (PGI2)-like substance from the cat lung. One collagen strip was superfused with mixed venous blood (lower trace) and another with arterial blood (upper trace) (blood on) of an anaesthetized and heparinized cat. The deposition of platelet clumps resulted in an increase in weight (b) of the strip. This process was faster in mixed venous blood than in arterial blood. An intravenous infusion of PGI2 (1 µg per kg over 3 min) (c) caused platelet disaggregation in arterial blood, but not in mixed venous blood. However, the same intravenous dose of PGI2 was effective on both sides of the lungs when a subthreshold disaggregating concentration of PGI2 (100 µg ml^-1) (d) was continuously infused into mixed venous blood. Therefore, it was assumed that the difference in concentration of endogenous PGI2 between mixed venous blood and arterial blood was approximately 200 µg ml^-1.

The disaggregating response in arterial blood was observed.