news and views

Arrangement of immunoglobulin genes

from Alan R. Williamson

THE complete sequences of the first two light chains of human immunoglobulin determined by Hilschmann and Craig (*Proc. natn. Acad. Sci., U.S.A.*, **53**, 1403–1409; 1965) showed that the unique features of each chain lay in the N-terminal half whereas the C-terminal halves of the two chains were identical. Extensive sequence data from both light and heavy chains have since shown that each chain has a unique N-terminal sequence (variable region) of about 110–120 amino acids. The hypothesis that two genes code for each immunoglobulin chain was advanced by Dreyer and Bennett in 1965 to account for these data, and patterns of inheritance have supported the proposal that a single constant (C) region gene is expressed in conjunction with any one of a set of variable (V) region genes.

The question then arises of the stage at which the information from the V and C genes is combined to produce the complete antibody chain. Evidence against joining at the polypeptide or RNA levels has steadily accumulated. Contiguous translation of V and C regions from the same mRNA (Milstein *et al.*, *Nature*, **252**, 354 ; 1974) and the absence of V and C scrambling in hybrid cells (Köhler and Milstein, *Nature*, **256**, 495; 1975) both fit more easily the hypothesis that the V and C genes are rearranged and joined together in the genome.

Evidence that the physical arrangement of V and C genes differs in the genome of embryonic and somatic cells has now been presented by Hozumi and Tonegawa (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 3628; 1976). The experiment is elegantly simple in concept; there are however limitations to the interpretation. DNA (either from BALB/c mouse embryos (12 or 13 d old) or from the BALB/c myeloma tumour MOPC321) is specifically fragmented with the restriction endonuclease *Bam*H1. The fragments are separated electrophoretically on slabs of agarose which are then cut into sections and the DNA extracted from each one. Each fraction of DNA is tested for sequence complementarity to κ-chain mRNA prepared from MOPC321 tumour cells.

Different patterns of complementary DNA fractions were found with embryo and tumour DNA. In a digest of embryonic DNA, two fragments (about 6.6 and 9.2 kb) showed significant hybridisation with the κ 321 mRNA probe whereas a digest of tumour DNA showed significant hybridisation to only one fragment (about 3.5 kb). The peaks of hybridisation are broad and only a fraction of input radioactivity was hybridised (< 20%). Identification of the peaks is made using a second probe specific mainly for the C region of κ chain. This probe was prepared after the fragmentation of mRNA during iodination by selection of the appropriately sized 3′-end fragments containing poly(A). By this test the 9.2 kb fragment of embryonic DNA contains C_K sequences but no V_{321} sequence whereas the 6.6 kb fragment contains V_{321} sequence but no C_K sequence. The 3.5 kb fragment of tumour DNA apparently contains both C_K and V_{321} sequences. These results are interpreted by the authors as showing that V_{321} and C_K genes are located separately in embryonic DNA but occur in the same short stretch of 321 tumour DNA. Clearly this interpretation is consistent with somatic rearrangement of V_{321} and C_K to give a contiguous gene. Alternative explanations involving two short stretches of DNA in 321 (one V_{321} and one C_K) or mutations introducing and removing *Bam*H1 sites are considered less probable than the contiguous gene hypothesis.

It is tempting to accept that somatic rearrangement of V and C genes is now beyond reasonable doubt and to seek for some mechanism. But it is when one comes to consider the possible mechanisms that the present data appear too simple in the light of some of the other evidence available from studies of immunoglobulin genes in myeloma cells.

Immunoglobulin-bearing cells are functionally haploid (that is, only one allele is expressed). The restriction mapping, which results in only one type of fragment homologous to the V or C probes in the tumour cell, suggests therefore that either MOPC321 cells are physically haploid or that some form of homozygosis has occurred yielding homologous chromosomes with the same VC pair on both. There is also the complication that myeloma cells are usually aneuploid but the authors do not comment on this point. The C_K gene seems to be in a linkage group on chromosome 6 in the mouse, so knowledge of the number of copies of this chromosome in 321 tumour cells would clarify the interpretation.

Studies of somatic mutation in myeloma proteins suggest that myeloma cells have only one expressed VC_K gene (Milstein *et al.*, in *Molecular Approaches to Immunology*, edit. by Smith, E. E., and Ribbons, D. S., 131–148, Academic, New York and London, 1975) although some myeloma tumours possibly express an additional C_K gene (Kuehl *et al.*, *Cell*, **5**, 139; 1975) and there is also evidence for more than one C_K gene per haploid genome (Stavnezer *et al.*, **88**, 43; 1974). The absence of C_K restriction fragments in myeloma DNA is therefore puzzling and a single $V_{321}C_K$ restriction fragment may be too simple an interpretation of the data. Alternatively MOPC321 may be an unusual tumour which has lost all but the functional C_K gene.

A different way of asking the rearrangement question would be to map the DNA of a tumour other than 321 (that is, not expressing V_{321}) using the V_{321} mRNA probe. Analysis of 321 tumour DNA with another κ mRNA would also be valuable. In these experiments the relative locations of expressed and non-expressed V region sequences could be determined on the same DNA digest.

Hozumi and Tonegawa comment that their choice of hybridisation technique was limited by the purity of κ mRNA currently achievable. Mach and Rougeon reported at the 9th Harden Conference the successful cloning of κ cDNA in a bacterial plasmid (see *News and Views*, **263**, 726; 1976). The availability of homogeneous nucleic acid probes will make possible the application of hybridisation techniques using excess probe; even 90% purity of probe is insufficient for such techniques.

Conclusive evidence for somatic rearrangement of antibody genes may not be far away. Knowledge of the mechanism is probably a little more remote. □