43: Antibody Engineering

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Introduction

Antibodies can be regarded as natural therapeutic agents which bind to a foreign antigen and mark it for clearance by a variety of mechanisms, ranging from phagocytosis and antibody-dependent cell-mediated cytotoxicity (ADCC) to complement-dependent lysis. In the clinic, horse antiserum has been used since the turn of the century, proving effective, for example, in the treatment of diphtheria and pneumococcal pneumonia, but with complications of immune complex disease and anaphylaxis. Although serotherapy was superseded by antibiotics in the 1940s for treatment of bacterial infections, the discovery of monoclonal antibodies (Köhler and Milstein 1975) may herald its renaissance. For example, monoclonal antibodies directed against cell surface antigens have been used to target and to kill T and B lymphocytes (Hale *et al.* 1983): these antibodies have potential in the treatment of bone marrow transplantation, lymphoma and autoimmune disease.

Recombinant deoxyribonucleic acid (DNA) technology adds a further dimension (for reviews see Morrison 1985; Neuberger 1985; Verhoeyen and Riechmann 1988; Williams 1988). It allows us to build better antibodies, and to dissect antibody binding and effector functions. For example, antibody–enzyme conjugates have been constructed (Neuberger *et al.* 1984), antigen-binding sites have been transplanted from mouse to human antibodies (Jones *et al.* 1986; Riechmann *et al.* 1988a; Verhoeyen *et al.* 1988) and the binding sites for complement 1q (C1q) (Duncan and Winter 1988) and the human high-affinity receptor, FcRI, (Duncan *et al.* 1988) have been localized in the constant domains of the antibody. Such protein engineering studies imply a detailed knowledge of the three-dimensional structure of the antibody and the underlying gene structure.

The antibody molecule consists of four chains: two identical heavy chains and two identical light chains (Fig. 43.1) (Porter 1973). Each chain consists of a string of domains and each domain consists of a β-sheet sandwich, held together by an internal buried disulphide bond. In the assembled molecule, the chains pack together through non-covalent interactions between domains. The two heavy chains are also linked by one or more disulphide bonds in the hinge region, and the heavy and light chains are also held together by a

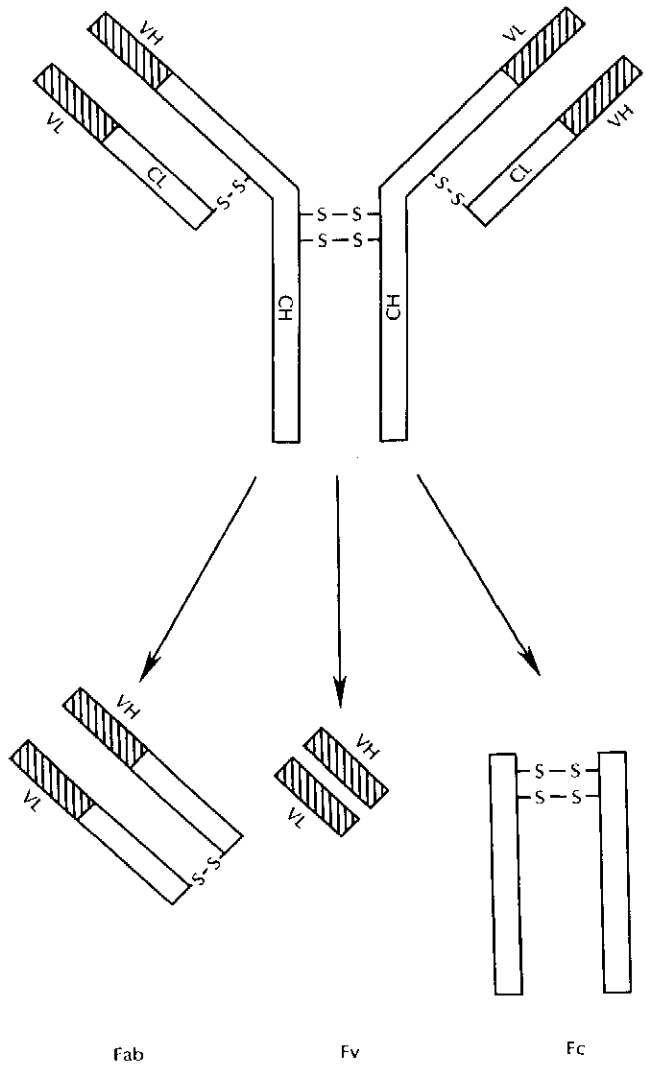

Fig. 43.1. Schematic diagram of the structure of an antibody molecule, showing two heavy and light chains joined by sulphhydryl bonds. The Fab, Fv and Fc fragments are also shown.

disulphide bond in the same region. The antigen-binding site and effector functions are localized in different regions of the antibody molecule, and historically were separated by cleavage of the antibody hinge with papain, to generate the Fab and the Fc fragments (Porter 1973).

The Fab fragment carries the antigen-binding site and is composed of the heavy chain variable domain, first constant domain and part of the hinge, and the entire light chain. The chains are covalently joined by a disulphide bond between the constant domains. Rarely, the antibody has been cleaved into an Fv fragment, which carries the antigen-binding site and consists only of non-covalently associated heavy and light chain variable domains (Inbar *et al.* 1972; Kakimoto and Onoue 1974; Sharon and Givol 1976). The fc fragment carries binding sites for C1q and Fc receptors (Davies and Metzger 1983; Duncan and Winter 1988; Duncan *et al.* 1988) and consists of part of the hinge, and the second and third constant domains of the heavy chain. The chains are covalently joined by one or more disulphides in the hinge.

The domain structure of an antibody is mirrored at the gene level, as the protein domains are arranged as a series of individual exons. The genes encoding an antibody are assembled by DNA rearrangements during the differentiation of antibody-secreting cells (Fig. 43.2). The variable region itself is assembled from the union of germline V elements with separate genetic elements (D segment, and J_H elements for the heavy chain, and Jκ or Jλ elements for the light chain). In the heavy chain assembly, first the D and J_H are joined, followed by the V and DJ_H (Alt *et al.* 1984). A large component of antibody variable region diversity is combinatorial: for example in mouse there are about 500–1000 germline V_H genes (Livant *et al.* 1986), at least 15 D segments and 4 J_H elements. The rearranged V_H genes are brought together with constant region genes: for example in mouse there are several classes of immunoglobulin (Ig) genes, IgG (isotypes γ1, γ2a, γ2b, γ3), IgA (α), IgM (μ), IgD (δ) and IgE (ε). During early differentiation of a B lymphocyte, a given V_H region is expressed as a μ chain. Recombination by class switching (Shimuzu and Honjo 1984) allows immunoglobulins with different isotypes, and therefore effector functions with distinct biological roles, to be produced during differentiation to a plasma cell.

A toolkit for antibody engineering

The domains are the building-blocks for antibody engineering, and their arrangement in individual exons facilitates cutting and pasting by recombinant DNA techniques, allowing assembly of complete antibodies or of groups of domains (for example Fab, Fv or Fc) or fusions with domains from other proteins. A toolkit for antibody engineering requires sets of domains and vectors for expression. The genes encoding the rearranged

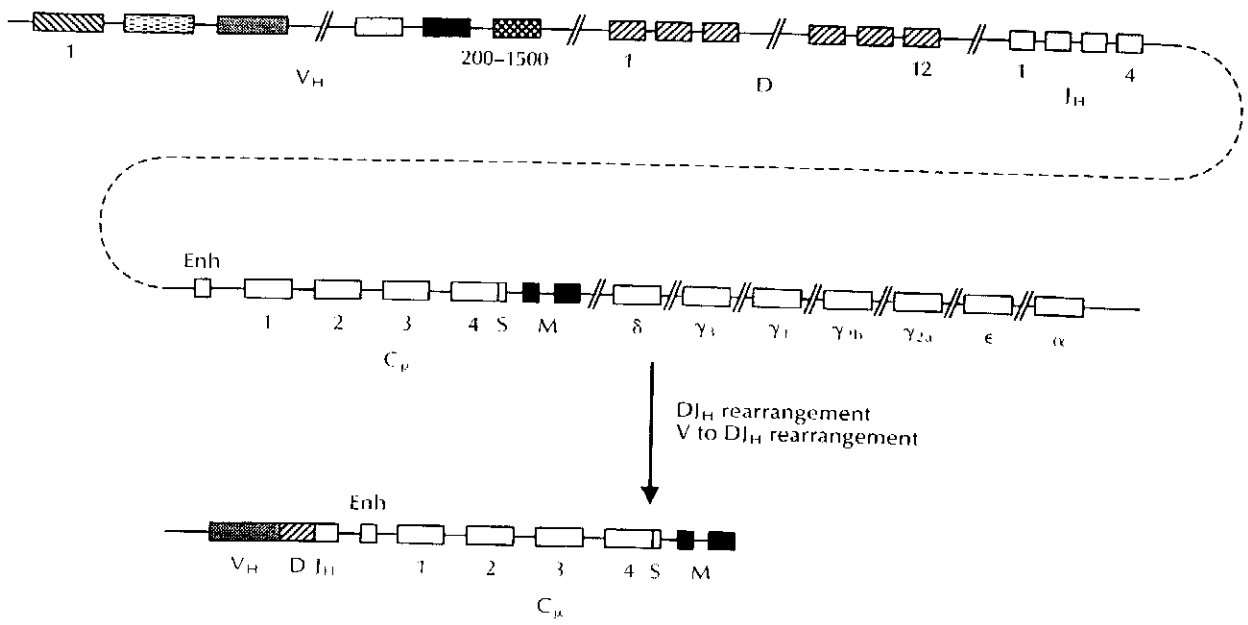

Fig. 43.2. Recombination of mouse Ig germline segments to produce functionally rearranged antibody gene.

variable domains are usually derived from a hybridoma of the required antigenic specificity, and the constant region genes from genomic or complementary DNA (cDNA) libraries. At its simplest, for Ig genes cloned from genomic DNA, the expression of antibody can be driven in cells of lymphoid origin by the Ig promoter and enhancer. Synthetic oligonucleotides are also required, as probes for Ig genes, for gene synthesis, as primers for cDNA synthesis, for sequencing, for mutagenesis and for the polymerase chain reaction (Saiki *et al.* 1985, 1988).

Cloning and sequencing

The sequence of the Ig variable regions is usually derived by direct sequencing of the hybridoma messenger ribonucleic acid (mRNA), or by sequencing cloned DNA. The mRNA can be sequenced directly using primers based at the 5′ end of the constant region if the class or isotype of constant region is known (for example κ or λ light chain). Alternatively the mRNA can be sequenced with reverse transcriptase using primers based in the conserved J regions of either heavy or light chains. Two methods are available for the sequencing reactions: (i) chain termination with dideoxynucleotide triphosphates and α-^{32}P or α-^{35}S nucleotide triphosphates (Sanger *et al.* 1977), or (ii) chemical cleavage of a cDNA transcript derived from primer labelled at its 5′ end with γ-^{32}P adenosine triphosphate (ATP) (Maxam and Gilbert 1977). It is usually desirable, however, to clone the gene.

Rearranged variable regions have been cloned from the cDNA by tailing with homopolymeric tails, attaching linkers or blunt-end cloning, and from genomic DNA by screening λ-phage libraries with probes based at the 3′ end of the J regions. The polymerase chain reaction (PCR) offers new avenues for cloning variable regions from cDNA or genomic DNA. Polymerase chain reaction amplification involves repeated rounds of extension from two primers specific for regions at each end of the gene. Since conserved necleotide sequences are present at both ends of most mouse variable region genes, they are readily amplified and cloned (Chiang *et al.* 1989; Larrick *et al.* 1989a, b; Orlandi *et al.* 1989). However, the primers need not match the gene sequence exactly, and restriction sites can be incorporated within the primers to allow the forced cloning of the amplified DNA (Orlandi *et al.* 1989). By this means the gene can be cloned directly for expression (Fig. 43.3). Immunoglobulin constant region genes have been cloned from the cDNA and from the genomic

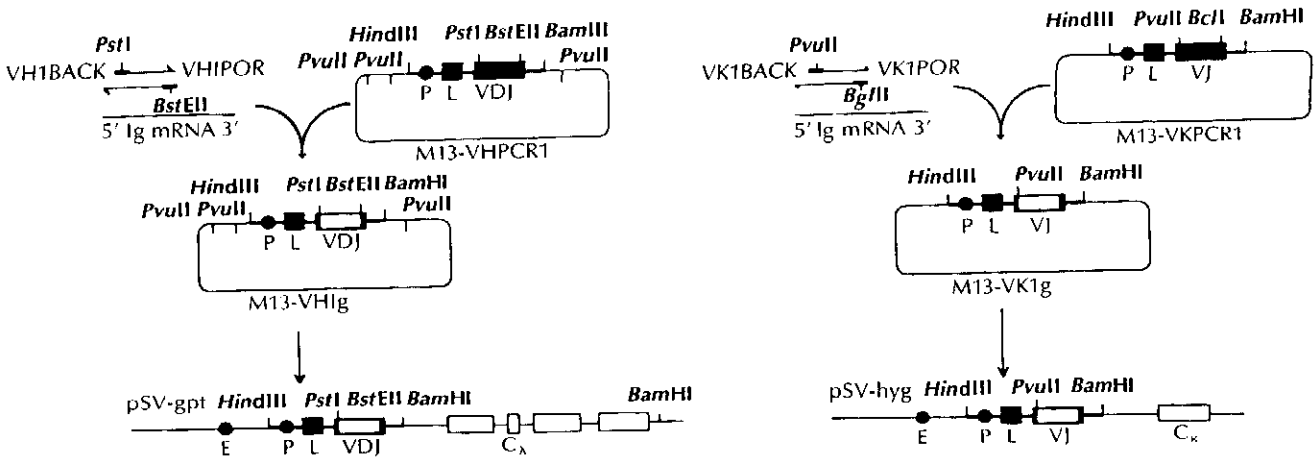

Fig. 43.3. Scheme for the amplification of cDNA and cloning into phage M13 vectors to link up V region genes for expression (Orlandi *et al.* 1989). The vectors M13-VHPCR1 and M13-VKPCR1, for cloning the amplified DNA, contain introns: transcription is driven from the Ig heavy chain promoter (P), and the signal sequence (L) and leader intron are taken from the mouse V47 unrearranged V_H gene (Neuberger 1983). The non-coding sequences to the 3' ends of the V_H and Vκ genes have been previously described (Neuberger 1983; Riechmann *et al.* 1988a).

DNA by screening λ-phage libraries with Ig cDNA probes (same species or even cross species).

Vectors for expression in eukaryotic cells

Vectors for stable expression and secretion of antibodies from eukaryotic cells have several elements: a promoter and enhancer to drive Ig gene expression, a dominant marker for selection of transfected eukaryotic cells, and a colE1 origin of replication and antibiotic resistance gene for a selection in *Escherichia coli*.

The Ig promoter and enhancer have been used to drive transcription of Ig genes in lymphoid cells (for review see Neuberger and Cook 1988). The promoter and enhancer elements are functional in these cells only (see, for example, Neuberger 1983), and are known to bind to a lymphoid-specific transcription factor (Müller *et al.* 1988; Scheidereit *et al.* 1988). Furthermore for the Ig enhancer/promoter there is an intron requirement for expression of the cloned genes (Neuberger and Williams 1988), and therefore genomic constructs are used. Other promoters and enhancers, for example human cytomegalovirus (HCMV) (Boshart *et al.* 1985), heat-shock protein (HSP)-70 promoter (Pelham 1982) or simian virus 40 (SV40) (Okayama and Berg 1983), have also been used to drive expression of genomic or cDNA constructs in lymphoid cells (Liu *et al.* 1987), COS cells (Whittle *et al.* 1987), Chinese hamster ovary (Weidle *et al.* 1987) and neuroglioma cells (Cattaneo and Neuberger 1987) (Fig. 43.4).

For selection of transfected myeloma cells, three dominant markers under the control of the viral SV40 early promoter are currently available. These markers, the *E. coli* xanthine-guanine phosphoribosyltransferase gene, the transposon Tn5 phosphotransferase gene and the hygromycin resistance gene, are derived from the pSV*gpt* (Mulligan and Berg 1981), pSV*neo* (Southern and Berg 1981) and pSV*hygro* (Smith, Miyajima and Strehlow, pers. comm.) vectors respectively. As these markers function in different pathways (Fig. 43.3), they can be used, for example, for independent selection of heavy and light chain expression from two different plasmids. Amplification of the transfected genes in myeloma cells is possible by including a methotrexate-resistant dihydrofolate reductase on the plasmid (Schmike 1984; Dorai and Moore 1987). Vectors devised recently for cloning antibody variable domains by the PCR and the expression of mouse–human chimeric antibodies in myeloma cells are described in Figs 43.3 and 43.4.

The cloned Ig genes are now usually introduced into myeloma cells by electroporation (Potter *et al.* 1984), which has proved more effective than spheroplast fusion (Oi *et al.* 1983) or calcium phosphate co-precipitation (Graham and Van der Eb 1973).

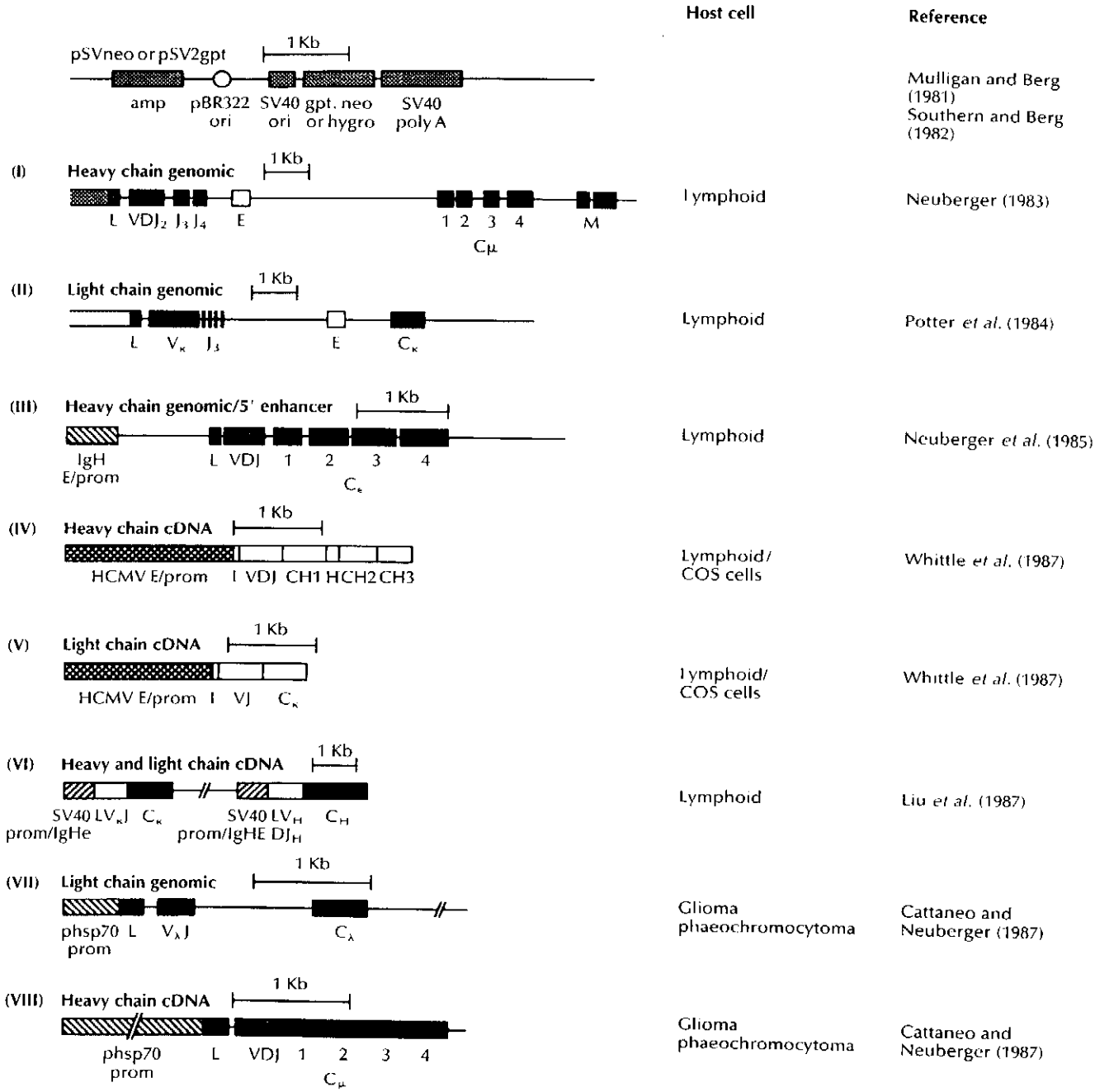

Fig. 43.4. Plasmid constructions for the expression of recombinant antibodies in mammalian cells. promp=promoter, IgH E=immunoglobulin heavy chain enhancer; HCMV E=human cytomegalovirus enhancer, phsp70=heat-shock protein 70. For further details, see references cited in the illustration.

Vectors for expression in *Escherichia coli*

At first sight, the expression of antibody in *E. coli* seems unpromising. Expressed antibody (Boss *et al.* 1984; Cabilly *et al.* 1984) or antibody fragments (Kurokawa *et al.* 1983; Liu *et al.* 1984; Ishizaka *et al.* 1986) accumulate in the cytoplasm as inclusion bodies. It is possible to refold the isolated protein from denaturing buffers but yields of functional antibody are poor (Boss *et al.* 1984; Cabilly *et al.*

1984). Furthermore, *E. coli* is not suitable for the production of glycosylated constant domains, which is necessary for function of IgG isotypes. In contrast, IgE constant domains can be produced in functional form in *E. coli* (Liu *et al.* 1984; Coleman *et al.* 1985; Ishizaka *et al.* 1986) as glycosylation is not necessary for function. Single-chain Fv fragments, however, in which the heavy and light chain variable domains are connected by a peptide linker, show improved yields on refolding from inclusion bodies (Bird *et al.* 1988; Huston *et al.* 1988) (Fig. 43.5). In addition, by attaching N-signal sequences (ompA, phoA or pelB) for export into the periplasm to antibody genes, Fv or Fab fragments have been secreted in an active form, in yields ranging from 0.3 to 10 mg/l (Better *et al.* 1988; Skerra and Plückthun 1988; Ward *et al.* 1989a) (Fig. 43.5). Single (V_H)-domain antibodies have also been expressed and secreted in *E. coli* in yields of 0.2–0.5 mg/ml (Ward *et al.* 1989a). The use of Fv fragments and V_H domains from *E. coli* should permit the rapid alteration and screening of new antigen-binding specifities.

Applications of antibody engineering

Antibody fragments: V_H, Fv, Fab, $(Fab)_2'$, Fc

Perhaps the simplest form of antibody engineering is the expression of individual domains or sets of domains. Antigen-binding fragments based on the Fv fragment (V_H and V_L domains) have been expressed and secreted in *E. coli* (Skerra and Plückthun 1988) and in myeloma cells (Riechmann *et al.* 1988), and have been refolded from *E. coli* inclusion bodies (Boss *et al.* 1984; Cabilly *et al.* 1984). For the secreted Fv fragments, the signal sequence was cleaved on secretion to reveal the N-terminal amino acid of the 'mature' variable domains. The V_H and V_L domains of the Fv fragments can associate non-covalently (Riechmann *et al.* 1988b; Skerra and Plückthun 1988; Ward *et al.* 1989a), and the packing between V_H and V_L domains is highly conserved (Chothia *et al.* 1985). Some interchain contacts are made between the antigen-binding loops of both chains (Chothia *et al.* 1985; Saul *et al.* 1987) and it is therefore difficult to predict whether Fv fragments in general will prove stable. The V_H and V_K domains of an Fv fragment with antilysozyme specificity are in dynamic equilibrium (Riechmann *et al.* 1988b), and it is possible that a chemical cross-link or disulphide bond introduced between the two domains could be advantageous. Recently the generation of single-chain antibodies, in which a V_H is linked by a peptide linker to a V_K, has been reported (Bird *et al.* 1988; Huston *et al.* 1989). Repertoires of V_H domains have recently been expressed and secreted in *E. coli* (Ward *et al.* 1989a). These repertoires of V_H domains were generated using the PCR to amplify and then clone the rearranged V_H genes directly from mouse spleen DNA. Such single domains can have reasonable affinities for antigens (approximately 20 nM). These single V_H domains have been termed 'single-domain antibodies' and they represent a

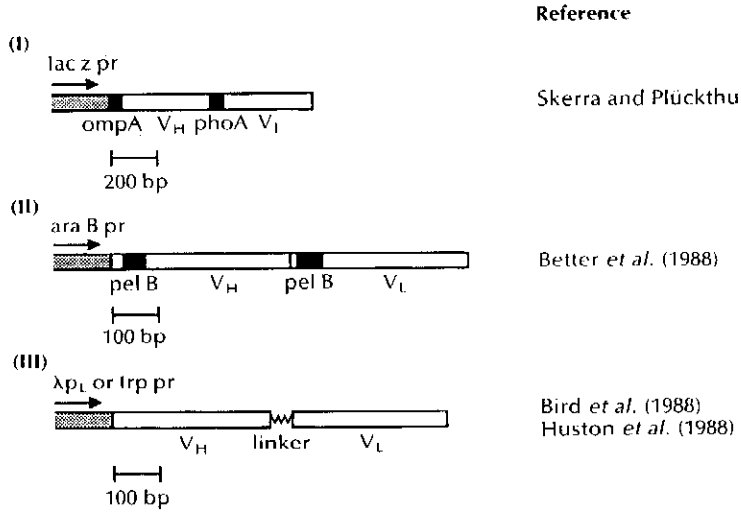

Fig. 43.5. Plasmid constructions for the expression of antibody Fv (I), Fab (II) or single chain Fvs (III) in *E. coli*. Plasmids (I) and (II) have prokaryotic sequences (ompA, phoA and pelB) for secretion of the expressed protein into the periplasm. pr=promoter. For further details, see references cited in the illustration.

new class of recombinant antibody fragments and may be of use in therapy and diagnostics. In addition, it is expected that they could be used as building-blocks for Fv and Fab fragments or complete antibodies. The small size of the Fv fragment and single-domain antibody should facilitate the determination of the three-dimensional structure of the antigen-binding site by both X-ray crystallography and nuclear magnetic resonance (NMR) analyses.

Fab fragments have been secreted from *E. coli* (Better *et al.* 1988). $(Fab)_2'$ fragments (Neuberger *et al.* 1984) and a Fab-like fragment, with V_H and V_L domains each joined to Cκ domains (Sharon *et al.* 1984), have been secreted from myeloma cells. Also a range of fragments containing parts of the Fc region of IgE have been expressed in *E. coli* as inclusion bodies and refolded. This expression system has been used to localize the binding site for the IgE receptor, $Fc_\varepsilon RI$, on the Cε2 and Cε3 domains (Helm *et al.* 1988).

Antibody fragments joined to other proteins: enzymes, toxins and receptors

At its simplest, making a gene fusion with antibody may provide a handle for its detection and purification (Neuberger *et al.* 1984). Furthermore, enzyme activities, such as alkaline phosphatase or horse-radish peroxidase linked directly to Fab fragments could be invaluable for enzyme-linked immunosorbent assay (ELISA) and other diagnostic tests. Genes encoding staphylococcal nuclease and Klenow polymerase (Neuberger *et al.* 1984) were linked to antibody Fab genes as a model system for such antibody fusions. The enzymes, which are monomeric, were attached to the N-terminal portion of the IgG C_H2 domain. This region of the antibody, which leads from the hinge, is disordered in the crystallographic structure, and may provide a flexible link. Both Fab–enzyme and $(Fab)_2'$–enzyme constructs were expressed (the hinge sulph-hydryl groups are present), and the constructs shown to possess both antigen-binding activity and enzyme activity.

Fab–enzyme fusions may also have therapeutic potential, although the enzyme moiety is likely to be immunogenic. For example, tissue plasminogen activator (TPA) has been linked to an anti-fibrin Fab, which should direct the TPA to the vicinity of blood clots, where it can locally activate plasminogen (Schnee *et al.* 1987). Likewise for pro-drug therapy, an enzyme capable of cleaving the pro-drug could be linked to a suitable Fab or $(Fab)_2'$ fragment. Yet another possibility lies in linking a toxin, such as ricin, diphtheria toxin or *Pseudomonas* exotoxin, to the Fab or Fv fragment, to create an immunotoxin (Chaudhary *et al.* 1989; for review, see Ahmad and Law 1988). Here the strategy is different: such toxins must be delivered inside the target cell, where, for example, they catalyse the ribosylation of EFTu. A single molecule of toxin can kill the cell. However the entry of the Fab/Fv–toxin into the target cells is likely to prove difficult, as well as its expression and secretion from myeloma cells.

Other kinds of antibody fusions have been made, for example in which the antibody variable domains have been replaced by a T cell receptor Vα domain, on either the Ig heavy chain (Gascoigne *et al.* 1987) or the Ig light chain (Mariuzza and Winter 1989) constant domains. Likewise CD4, which binds to the acquired immune deficiency syndrome (AIDS) human immunodeficiency virus (HIV)-1 gp(glycoprotein)120 coat protein, has been assembled with Ig heavy chain constant regions to create an 'immunoadhesin'. The fusion enhances the serum half-life of CD4 and the antibody effector functions may prove effective in killing AIDS virus-infected cells (Capon *et al.* 1989).

Simple chimeric antibodies

During the maturation of the immune response, the class of Ig heavy chain is switched from IgM to IgG (for review see Shimizu and Honjo 1984). Likewise the isotype of IgG can be switched and involves bringing the rearranged V_H gene into the proximity of a new set of constant region genes, with deletion of the intervening DNA (Davis *et al.* 1980; Maki *et al.* 1980; Kataoka *et al.* 1981; Obata *et al.* 1981). As expected from the Ig domain structure, class switching does not seem to alter the affinity of the antibody for antigen (Neuberger and Rajewsky 1981). Class switching underlies the concept of simple chimeric antibodies: the variable regions from one source are attached to constant regions from another. This allows the species, class and isotype of constant regions to be selected for the antibody, which in turn dictates its immunogenicity and effector functions.

A variety of simple chimeric antibodies have been made (Boulianne *et al.* 1987; Morrison *et al.* 1984; Neuberger *et al.* 1985; Sun *et al.* 1987). The most extensive collection is based on the mouse B1-8 heavy chain with mouse λ light chain (Brüggeman *et al.* 1987). The heavy chain variable region has been attached to a variety of mouse, rat and human constant regions. These antibodies have a specificity for the haptens NP (and NIP (Neuberger and Rajewsky 1981)), and are readily purified on columns of hapten–Sepharose. Cell surfaces are readily derivatized using NIP-succinimide ester (which reacts with protein cell surface markers) or NIP-cephalin (which inserts into the membrane lipid), and this facilitates the assay of effector functions such as complement lysis and cell-mediated killing (for reviews see Winkelhake *et al.* 1978; Burton 1987). Such 'matched sets' of chimeric antibodies have proved invaluable for comparing effector functions (Brüggeman *et al.* 1987; Stepelewski *et al.* 1988) and segmental flexibility (Oi *et al.* 1984; Dangl *et al.* 1988) of different antibody isotypes. For example, it emerges that the human γ1 isotype is the most active in complement lysis and ADCC, indicating that it is the most suitable for therapy (Brüggeman *et al.* 1987; Riechmann *et al.* 1988a).

Reshaped antibodies

The antigen-binding site is localized to the loops at the tips of the variable region, and correlates with the regions of hypervariable sequence (or complementarity-determining regions, CDRs) in each of the V_H and V_L domains (Kabat *et al.* 1987). Reshaped antibodies, in which the antigen-binding site only is derived from another antibody, have been constructed by transplanting the CDRs (Jones *et al.* 1986; Riechmann *et al.* 1988a; Verhoeyen *et al.* 1988). This not only allows the choice of constant region, as with the simple chimeric antibodies, but also the choice of variable region β-sheet 'framework' and loops adjacent to the constant domains. Such human antibodies, in which only the antigen-binding site is derived from a mouse antibody, may prove less immunogenic in humans than a simple chimeric antibody, in which the entire variable region is taken from a mouse antibody. However, there are several assumptions underlying this approach. In particular, certain structural features of both antibodies must be matched for the graft to 'take' on its new framework: the packing of the heavy and light chain variable domains; the packing of the two β-sheets within a domain; the packing of the CDRs on to the β-sheet framework; and the contact of the antigen only with CDR residues (or conserved residues in the β-sheet). Inspection of the available crystallographic structures suggests that the packing of domains, sheets and loops are relatively conserved (Lesk and Chothia 1982; Chothia and Lesk 1987) and that the vast majority of contacts between antibody and antigen are made via CDR residues (Amit *et al.* 1986; Sheriff *et al.* 1987; Padlan *et al.* 1989). Nevertheless, in reshaping the anti-lymphocyte antibody, CAMPATH-1, to avoid loss of binding activity the framework region of the human antibody had to be adapted to accommodate the CDRs of the rat antibody (Riechmann *et al.* 1988a).

Dissecting antibody affinity and effector functions

The diversity of antibody-binding sites is not only based on the recombinatorial diversity of the genetic elements (V_H, D, J_H; $V\kappa$, $J\kappa$; $V\lambda$, $J\lambda$) and diversity at the junctions, but also on somatic mutation during the muturation of the immune response (for review see Tonegawa 1983). Point mutations in antibody variable regions are sufficient to alter antibody affinity and specificity (Rudikoff *et al.* 1982; Roberts *et al.* 1987). For example, higher-affinity antibodies derived from a V_H gene family which expresses antibodies directed against the hapten NP appear to be determined by a replacement of tryptophan 33 by leucine (Allen *et al.* 1988). It should also be possible to construct higher-affinity antibodies by design: mutations constructed in the hypervariable regions of an anti-lysozyme antibody were shown to increase its affinity for antigen (Roberts *et al.* 1987). Enhancing the affinity and specificity of antibodies has important practical applications, for example in increasing the sensitivity of ELISA or of *in vivo* imaging and therapy.

The binding of antibody to a cell receptor has been illuminated by construction of a single mutant. Binding of antigen–antibody complexes to specialized cells via three receptors, FcRI, FcRII and FcRIII (Anderson and Looney 1986), can trigger ADCC. The high-affinity receptor FcRI appears

to interact via the C_H2 domain of the antibody (Burton 1987) and studies with human myeloma proteins in which the hinge was deleted had indicated that the top of the C_H2 domain must be accessible (Klein *et al.* 1981). Sequence comparisons of several antibodies and their properties led to the proposal that the monocyte-binding site was located mainly in the hinge link, and possibly associated with Leu 235 (Woof *et al.* 1986). This proposal was confirmed by site-directed mutagenesis. The mouse IgG-2b isotype has a glutamic acid residue at position 235 and does not bind to the human FcRI receptor and by mutagenesis binding activity was conferred on this isotype by replacing glutamic acid 235 by leucine (Duncan *et al.* 1988).

The interaction of antibody with C1q has also been dissected by making a range of mutants. The site of interaction of C1q for antibody had been localized to the C_H2 domain. Based on chemical modification studies, inhibition of complement lysis by synthetic peptides and oligosaccharides, and sequence comparisons of antibody isotypes, a wide variety of proposals had been made. Taken together, these residues cover most of the solvent accessible surface of the C_H2 domain. An engineering approach, 'surface scanning' (Bedouelle and Winter 1986) was used, in which side-chains of the antibody were removed systematically and scored for C1q binding. This approach identified three polar side chains, Glu 318, Lys 320 and Lys 322, as essential for C1q binding (Duncan and Winter 1988), confirming aspects of an earlier proposal by Dwek and colleagues (Burton *et al.* 1980). Since these features are conserved in isotypes which do not bind C1q or are non-lytic, there must also be other factors involved, for example steric blocking of the Fc by the Fab arms (Isenman *et al.* 1975) or segmental flexibility (Oi *et al.* 1984).

Future directions

At present, the raw material of antibody engineering is the hybridoma. Although it has proved difficult to derive human monoclonal antibodies by hybridoma technology (Carson and Freimark 1986; Borrebaeck *et al.* 1988; Thompson 1988), antibody engineering offers an indirect route of 'humanizing' rodent monoclonal antibodies, by making simple chimeric antibodies or by reshaping human antibodies. Preliminary clinical results are promising: simple chimeric antibodies have an extended serum half-life compared with the mouse antibodies (LoBuglio *et al.* 1989) and a reshaped human antibody directed against lymphocytes was effective in clearing a large mass of tumour in two patients (Hale *et al.* 1988). Whether such chimeric antibodies or reshaped antibodies will prove much more immunogenic than genuine human monoclonal antibodies and whether this will curtail therapy are open questions. In a mouse model, it seems that the immune response is directed mainly against constant regions, although a significant part of the response is mounted against the framework regions of the variable region.

The ability to tailor effector functions may help define the routes of clearance of pathogen in clinical situations. For example, the construction of a human γ1 antibody which does not activate complement lysis but does bind to receptors could help identify the role of complement-dependent lysis and ADCC in cell killing.

In future, antigen-binding activities may be derived by approaches other than hybridoma technology (Ward *et al.* 1989a), for example: using the PCR to clone variable regions for expression directly from mRNA (Orlandi *et al.* 1989; Sastry *et al.* 1989) or from genomic DNA of spleen (Ward *et al.* 1989a) or of peripheral blood lymphocytes; the mutation of antigen-binding sites with mutagenic primers (Ward *et al.* 1989b); the building of antigen-binding sites by structure prediction (Chothia and Lesk 1987). Furthermore, by tinkering with the antigen-binding site, it may prove possible to enhance antibody affinity (Roberts *et al.* 1987). Finally, the ability to derive catalytic antibodies by immunization with transition state analogues (Pollack *et al.* 1986; Tramontano *et al.* 1986; Napper *et al.* 1987) opens a new avenue for antibody engineering, for example by building in side-chains to selectively stabilize the transition state or by introducing attachment sites for co-factors (Pollack *et al.* 1988; Baldwin and Schulz 1989).

References

Ahmad, A. and Law, K. (1988). Strategies for designing antibody-toxin conjugates. *Trends Biotechnol.* 6, 246–8.

Allen, D., Simon, T., Sablitzky, F., Rajewsky, K. and Cumano, A. (1988). Antibody engineering for the analysis of affinity maturation of an anti-hapten response. *EMBO J.* 7, 1995–2001.

Alt, F.W., Yancopoulos, G.D., Blackwell, T.K. *et al.* (1984). Ordered rearrangement of immunoglobulin heavy chain variable region segments. *EMBO J.* **3**, 1209–19.

Amit, A.G., Mariuzza, R.A., Phillips, S.E.V. and Poljak, R.J. (1986). Three-dimensional structure of an antigen–antibody complex at 2.8 Å resolution. *Science* **233**, 747–54.

Anderson, C.L. and Looney, R.J. (1986). Human leukocyte IgG Fc receptors. *Immunol. Today* **7**, 264–6.

Baldwin, E. and Schulz, P.G. (1989). Generation of a catalytic antibody by site-directed mutagenesis. *Science* **245**, 1104–7.

Bedouelle, H. and Winter, G. (1986). A model of synthetase/transfer RNA interaction as deduced by protein engineering. *Nature* **320**, 371–3.

Better, M., Chang, C.P., Robinson, R.R. and Horwitz, A.H. (1988). *Escherichia coli* secretion of an active chimeric antibody fragment. *Science* **240**, 1041–3.

Bird, R.E., Hardman, K.D., Jacobson, J.W. *et al.* (1988). Single-chain antigen-binding proteins. *Science* **423**, 423–6.

Borrebaeck, C.A.K., Danielsson, L. and Möller, S.A. (1988). Human monoclonal antibodies produced by primary *in vitro* immunization of peripheral blood lymphocytes. *Proc. Nat. Acad. Sci. (USA)* **85**, 3995–9.

Boshart, M., Weber F., Jahn, G., Dorsch-Häsler, K., Fleckenstein, B. and Schaffner, W. (1985). A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* **41**, 521–30.

Boss, M.A., Kenten, J.H., Wood, C.R. and Emtage, J.S. (1984). Assembly of functional antibodies from immunoglobulin heavy and light chains synthesised in *E. coli*. *Nucleic Acids Res.* **12**, 3791–806.

Boulianne, G.L., Isenman, D.E., Hozumi, N. and Shulman, M.J. (1984). Biological properties of chimeric antibodies: interaction with complement. *Mol. Biol. Med.* **4**, 37–49.

Brüggemann, M., Williams, G.T., Bindon, C.I. *et al.* (1987). Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies. *J. Exp. Med.* **166**, 1351–61.

Burton, D.R. (1987). Structure and function of antibodies. In *Molecular Genetics of Immunoglobulins*, ed. F. Calabi and M.S. Neuberger, pp. 1–50, Elsevier, Amsterdam.

Burton, D.R., Boyd, J., Brampton, A.D. *et al.* (1980). The C1q receptor site on immunoglobulin G. *Nature* **288**, 338–44.

Cabilly, S., Riggs, A.D., Pande, H. *et al.* (1984). Generation of antibody activity from immunoglobulin polypeptide chains produced in *Escherichia coli*. *Proc. Nat. Acad. Sci. (USA)* **81**, 3273–7.

Capon, D.J., Chamow, S.M., Mordenti, J. *et al.* (1989). Designing CD4 immunoadhesions for AIDS therapy. *Nature* **337**, 525–9.

Carson, D.A. and Freimark, B.D. (1986). Human lymphocyte hybridomas and monoclonal antibodies. *Adv. Immunol.* **38**, 275–311.

Cattaneo, A. and Neuberger, M.S. (1987). Polymeric immunoglobulin M is secreted by transfectants of non-lymphoid cells in the absence of immunoglobulin J chain. *EMBO J.* **6**, 2753–8.

Chaudhary, V.K., Queen, C., Junghans, R.P., Waldmann, T.A., Fitzgerald, D.J. and Pastan, I. (1989). A recombinant immunotoxin consisting of two antibody variable domains fused to *Pseudomonas* exotoxin. *Nature* **339**, 394.

Chiang, Y.L., Sheng-Dong, R., Brow, M.A. and Larrick, J.W. (1989). Direct cDNA cloning of the rearranged immunoglobulin variable regions. *BioTechniques* **7**, 360–6.

Chothia, C. and Lesk, A.M. (1987). Canonical structures for the hypervariable regions of immunoglobulins. *J. Mol. Biol.* **196**, 901–17.

Chothia, C., Novotny, J. and Bruccoleri, R. (1985). Domain association in immunoglobulin molecules: the packing of variable domains. *J. Mol. Biol.* **186**, 651–63.

Coleman, J.W., Helm, B.A., Stanworth, D.R. and Gould, H.J. (1985). Inhibition of mast cell sensitization *in vitro* by a human immunoglobulin ε-fragment synthesised in *Escherichia coli*. *Eur. J. Immunol.* **15**, 966–9.

Dangl, J.L., Wensel, T.G., Morrison, S.L., Stryer, L., Herzenberg, L.A. and Oi, V.T. (1988). Segmental flexibility and complement fixation of genetically engineered chimeric human, rabbit and mouse antibody. *EMBO J.* **7**, 1989–94.

Davies, D.R. and Metzger, H. (1983). Structural basis of antibody function. *Ann. Rev. Immunol.* **1**, 87–117.

Davis, M.M., Calame, K., Early, P.W. *et al.* (1980). An immunoglobulin heavy-chain gene is formed by at least two recombinational events. *Nature* **283**, 733–9.

Dorai, H. and Moore, G.P. (1987). The effect of dihydrofolate reductase-mediated gene amplification on the expression of transfected immunoglobulin genes. *J. Immunol.* **139**, 4232–41.

Duncan, A.R. and Winter, G. (1988). The binding site for C1q on IgG. *Nature* **332**, 738–40.

Duncan, A.R., Woof, J.M., Partridge, L.J., Burton, D.R and Winter, G. (1988). Localisation of the binding site for the human high-affinity Fc receptor on IgG. *Nature* **332**, 563–4.

Gascoigne, N.R.J., Goodnow, C.C., Dudzik, K.I., Oi, V.T. and Davis, M.M. (1987). Secretion of a chimeric T-cell receptor-immunoglobulin protein. *Proc. Nat. Acad. Sci. (USA)* **84**, 2936–40.

Graham, F.L. and van der Eb, A.J. (1973). A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**, 456–67.

Hale, G., Swirsky, D.M., Hayhoe, F.G.J. and Waldman H. (1983). Effects of monoclonal anti-lymphocyte antibodies *in vivo* in monkeys and humans. *Mol. Biol. Med.* **1**, 321–34.

Hale, G., Dyer, M.J.S., Clark, M.R. *et al.* (1988). Remission induction in non-Hodgkin lymphoma with reshaped human monoclonal antibody CAMPATH-1H. *Lancet* 17 December, 1394–9.

Helm, B., Marsh, P., Vercelli, D., Padlan, E., Gould, H and Geha, R.S. (1988). The mast cell binding site on human immunoglobulin E. *Nature* **331**, 180–3.

Huston, J.S., Levinson, D., Mudgett-Hunter, M. *et al.* (1988). Protein engineering of antibody binding sites: recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in *Escherichia coli*. *Proc. Nat. Acad. Sci. (USA)* **85**, 5879–83.

Inbar, D., Hochman, J. and Givol, D. (1972). Localisation of antibody-combining sites within the variable portions of heavy and light chains. *Proc. Nat. Acad. Sci. (USA)* **69**, 2659–62.

Isenman, D.E., Dorrington, K.J. and Painter, R.H. (1975). The structure and function of immunoglobulin domains. *J. Immunol.* 1726–9.

Ishizaka, T., Helm, B., Hakimi, J., Niebyl, J., Ishizaka, K. and Gould, H. (1986). Biological properties of a recombinant human immunoglobulin ε-chain fragment *Proc. Nat. Acad. Sci. (USA)* **83**, 8323–7.

Jones, P.T., Dear, P.H., Foote, J., Neuberger, M.S. and Winter, G. (1986). Replacing the complementarity-determining regions in a human antibody with those from a mouse. *Nature* **321**, 522–4.

Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M. and Gottesmann, K.S. (1987). *Sequences of Proteins of Immunological Interest*. US Department of Health and Human Services, US Government Printing Office.

Kakimoto, K and Onoue, K. (1974). Characterisation of the Fv fragment isolated from a human immunoglobulin M. *J. Immunol.* **112**, 1373–82.

Kataoka, T., Miyata, T. and Honjo, T. (1981). Repetitive sequences in class-switch recombination regions of immunoglobulin heavy chain genes. *Cell* **23**, 357–68.

Klein, M., Haeffner-Cavallon, N., Isenman, D.E. *et al.* (1981). Expression of biological effector functions by immunoglobulin G molecules lacking the hinge region. *Proc. Nat. Acad. Sci (USA)* **78**, 524–8.

Köhler, G. and Milstein, C. (1975). Continuous culture of fused cells secreting antibody of predefined specificity. *Nature* **256**, 495–7.

Kurokawa, T., Seno, M., Sasada, R. *et al.* (1983). Expression of human immunoglobulin E ε chain cDNA in *E. coli*. *Nucleus Acids Res.* **11**, 3077–84.

Larrick, J.W., Danielsson, L., Brenner, C.A. *et al.* (1989a). Polymerase chain reaction using mixed primers: cloning of human monoclonal antibody variable region genes from single hybridoma cells. *Biotechnology* **7**, 934–8.

Larrick, J.W., Danielsson, L., Brenner, C.A., Abrahamson, M., Fry, K.E. and Borrebaeck, C.A.K. (1989b). Rapid cloning of rearranged immunoglobulin genes from human hybridoma cells using mixed primers and the polymerase chain reaction. *Biochem. Biophys. Res. Comm.* **160**, 1250–5.

Lesk, A.M. and Chothia, C. (1982). Evolution of proteins formed by beta sheets. II. The core of the immunoglobulin domains. *J. Mol Biol.* **160**, 325–42.

Liu, A Y., Mack, P.W., Champion, C.I. and Robinson, R.R. (1987). Expression of mouse:: human immunoglobulin heavy-chain cDNA in lymphoid cells. *Gene* **54**, 33–40.

Liu, F., Albrandt, K.A., Bry, C.G. and Ishizaka, T. (1984). Expression of a biologically active fragment of human IgE ε chain in *Escherichia coli*. *Proc. Nat. Acad. Sci. (USA)* **81**, 5369–73.

Livant, D., Blatt, C. and Hood, L. (1986). One heavy chain variable region gene segment subfamily in the BALB/c mouse contains 500–1000 or more members. *Cell* **47**, 461–70.

LoBuglio, A.F., Wheeler, R.H., Trang, J. *et al.* (1989). Mouse/human chimeric monoclonal antibody in man; kinetics and immune response. *Proc. Nat. Acad. Sci. (USA)* **86**, 4220–4.

Maki, R., Traunecker, A., Sakano, H., Roeder, W. and Tonegawa, S. (1980). Exon shuffling generates an immunoglobulin heavy chain gene. *Proc. Nat. Acad. Sci. (USA)* **77**, 2138–42.

Mariuzza, R.A. and Winter, G. (1989). Secretion of a homodimeric VαCκ T-cell receptor-immunoglobulin chimeric protein. *J. Biol. Chem.* **264**, 7310–16.

Maxam, A.M. and Gilbert, W. (1977). A new method for sequencing DNA. *Proc. Nat. Acad. Sci. (USA)* **74**, 560–4.

Morrison, S.L. (1985). Transfectomas provide novel chimeric antibodies. *Science* **229**, 1202–7.

Morrison, S.L., Johnson, M.J., Herzenberg, L.A. and Oi, V.T. (1984). Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc. Nat. Acad. Sci. (USA)* **81**, 6851–5.

Müller, M.M., Ruppert, S., Schaffner, W. and Matthias, P. (1988). A cloned octamer transcription factor stimulates transcription from lymphoid-specific promoters in non-B cells. *Nature* **336**, 544–51.

Mulligan, R.C. and Berg, P. (1981). Selection for animal cells that express the *Escherichia coli* gene coding for xanthine-guanine phosphoribosyl transferase. *Proc. Nat. Acad. Sci. (USA)* **78**, 2072–6.

Napper, A.D., Benkovic, S.J., Tramontano, A. and Lerner, R.A. (1987). A stereospecific cyclisation catalysed by an antibody. *Science* **237**, 1041–3.

Neuberger, M.S. (1983). Expression and regulation of immunoglobulin heavy chain gene transfected into lymphoid cells. *EMBO J.* **2**, 1373–8.

Neuberger, M.S. (1985). Making novel antibodies by expressing transfected immunoglobulin genes. *Trends. Biochem.* **10**, 347–9.

Neuberger, M.S. and Cook, G.P. (1988). The expression of immunoglobulin genes. *Immunol. Today* **9**, 278–81.

Neuberger, M.S. and Rajewsky, K. (1981). Switch from hapten-specific immunoglobulin M to immunoglobulin D secretion in a hybrid mouse cell line. *Proc. Nat. Acad. Sci. (USA)* **78**, 1138–42.

Neuberger, M.S. and Williams, G.T. (1988). The intron requirement for immunoglobulin gene expression is dependent upon the promoter. *Nucleic Acids Res.* **16**, 6713–24.

Neuberger, M.S., Williams, G.T. and Fox, R.O. (1984). Recombinant antibodies possessing novel effector functions. *Nature* **13**, 602–8.

Neuberger, M.S., Williams, G.T., Mitchell, E.B., Jouhal, S.S., Flanagan, J.G. and Rabbitts, T.H. (1985). A hapten-specific chimaeric IgE antibody with human physiological effector function. *Nature* **314**, 268–70.

Obata, M., Kataoka, T., Nakai, S. *et al.* (1981). Structure of a rearranged γ1 chain gene and its implication to immunoglobulin class-switch mechanism. *Proc. Nat. Acad. Sci. (USA)* **78**, 2437–41.

Oi, V.T., Morrison, S.L., Herzenberg, L.A. and Berg, P. (1983). Immunoglobulin gene expression in transformed lymphoid cells. *Proc. Nat. Acad. Sci. (USA)* **80**, 825–9.

Oi, V.T., Vuong, T.M., Hardy, R. *et al.* (1984). Correlation between segmental flexibility and effector function of antibodies. *Nature* **307**, 136–40.

Okayama, H. and Berg, P. (1983). A cDNA cloning vector that permits expression of cDNA inserts in mammalian cells. *Mol. Cell. Biol.* **3**, 280–9.

Orlandi, R., Gussow, D.H., Jones, P.T. and Winter, G. (1989). Cloning immunoglobulin variable domains for expression by the polymerase chain reaction. *Proc. Nat. Acad. Sci. (USA)* **86**, 3833–7.

Padlan, E.A., Silverton, E.W., Sheriff, S., Cohen, G.H., Smith-Gill, S.J. and Davies, D.R. (1989). Structure of an antibody-antigen complex: crystal structure of the HyHEL-10 Fab-lysozyme complex. *Proc. Nat. Acad. Sci. (USA)* **86**, 5938–42.

Pelham, H.R.B. (1982). A regulatory upstream promoter element in the *Drosophila* Hsp 70 heat-shock gene. *Cell* **30**, 517–28.

Pollack, S.J., Jacobs, J.W. and Schultz, P.G. (1986). Selective

chemical catalysis by an antibody. *Science* **234**, 1570–3.

Pollack, S.J., Nakayama, G.R. and Schultz, P.G. (1988). Introduction of nucleophiles and spectroscopic probes into antibody combining sites. *Science* **242**, 1038–40.

Porter, R.R. (1973). Structural studies of immunoglobulins. *Science* **180**, 713–16.

Potter, H., Weir, L. and Leder, P. (1984). Enhancer-dependent expression of human κ immunoglobulin genes introduced into mouse pre-B lymphocytes by electroporation. *Proc. Nat. Acad. Sci. (USA)* **81**, 7161–5.

Riechmann, L., Clark, M., Waldmann, H. and Winter, G. (1988a). Reshaping human antibodies for therapy. *Nature* **332**, 323–7.

Riechmann, L., Foote, J. and Winter, G. (1988b). Expression of an antibody Fv fragment in myeloma cells. *J. Mol. Biol.* **203**, 825–8.

Roberts, S., Cheetman, J.C. and Rees, A.R. (1987). Generation of an antibody with enhanced affinity and specificity for its antigen by protein engineering. *Nature* **328**, 731–4.

Rudikoff, S., Giusti, A.M., Cook, W.D. and Scharff, M.D. (1982). Single amino acid substitution altering antigen-binding specificity. *Proc. Nat. Acad. Sci. (USA)* **79**, 1979–83.

Saiki, R.K., Scharf, S., Faloona, F. *et al.* (1985). Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230**, 1350–4.

Saiki, R.K., Gelfand, D.H., Stoffel, S. *et al.* (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–91.

Sanger, F., Nicklen, S. and Coulson, H.R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Nat. Acad. Sci. (USA)* **74**, 5463.

Sastry, L., Alting-Mees, M., Huse, W.D. *et al.* (1989). Cloning of the immunological repertoire in *Escherichia coli* for generation of monoclonal catalytic antibodies: construction of a heavy chain variable region-specific cDNA library. *Proc. Nat. Acad. Sci. (USA)* **86**, 5728–32.

Saul, F.A., Amzel, L.M. and Poljak, R.J. (1987). Preliminary refinement and structural analysis of the Fab fragment from human immunoglobulin New at 2.0 Å resolution. *J. Biol. Chem.* **253**, 585–97.

Scheidereit, C., Cromlish, J.A., Gerster, T. *et al.* (1988). A human lymphoid-specific transcription factor that activates immunoglobulin genes is a homoeobox protein. *Nature* **336**, 551–7.

Schmike, R.T. (1984). Gene amplification in cultured animal cells. *Cell* **37**, 705–13.

Schnee, J.M., Runge, M.S., Matsueda, G.R. *et al.* (1987). Construction and expression of a recombinant antibody-targeted plasminogen activator. *Proc. Nat. Acad. Sci. (USA)* **84**, 6904–8.

Sharon, J. and Givol, D. (1976). Preparation of Fv fragment from the mouse myeloma XRPC-25 immunoglobulin possessing anti-dinitrophenyl activity. *Biochemistry* **15**, 1591–4.

Sharon, J., Gefter, M.L. Manser, T., Morrison, S.L., Oi, V.T. and Ptashne, M. (1984). Expression of a VH Cκ chimaeric protein in mouse myeloma cells. *Nature* **309**, 364–7.

Sheriff, S., Silverton, E.W., Padlan, E.A. *et al.* (1987). Three-dimensional structure of an antibody–antigen complex. *Proc. Nat. Acad. Sci. (USA)* **84**, 8075–9.

Shimizu, A. and Honjo, T. (1984). Immunoglobulin class switching. *Cell* **36**, 801–3.

Skerra, A. and Plückthun, A. (1988). Assembly of a functional immunoglobulin Fv fragment in *Escherichia coli*. *Science* **240**, 1038–40.

Southern, P.J. and Berg, P. (1981). Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. *J. Mol. Appl. Genet.* **1**, 327–41.

Stepelewski, Z., Sun, L.K., Shearman, C.W., Ghrayeb, J., Daddona, P. and Koprowski, H. (1988). Biological activity of human-mouse IgG1, IgG2, IgG3, and IgG chimeric monoclonal antibodies with antitumour specificity. *Proc. Nat. Acad. Sci. (USA)* **85**, 4852–6.

Sun, L.K., Curtis, P., Rakowicz-Szulczynska, E. *et al.* (1987). Chimeric antibody with human constant regions and mouse variable regions directed against carcinoma-associated antigen 17-1A. *Proc. Nat. Acad. Sci. (USA)* **84**, 214–18

Thompson, K.M. (1988). Human monoclonal antibodies. *Immunol. Today* **9**, 113–16.

Tonegawa, S. (1983). Somatic generation of antibody diversity. *Nature* **302**, 575–81.

Tramontano, A., Janda, K.D. and Lerner, R.A. (1986). Catalytic antibodies. *Science* **234**, 1566–9.

Verhoeyen, M. and Riechmann, L. (1988). Engineering of antibodies. *Bioessays* **8**, 74–8.

Verhoeyen, M., Milstein, C. and Winter, G. (1988). Reshaping human antibodies: grafting an antilysozyme activity. *Science* **239**, 1534–6.

Ward, E.S., Gussow, D.H., Griffiths, A.D., Jones, P.T. and Winter, G. (1989a). Binding activities of a repertoire of single immunoglobulin variable domains secreted from *Escherichia coli*. *Nature* **341**, 544–6.

Ward, E.S., Gussow, D.H., Griffiths, A.D., Jones, P.T. and Winter, G.G. (1989b). Expression and secretion of repertoires of VH domains in *Escherichia coli*: isolation of antigen binding activities. In *Progress in Immunology*, ed. F. Melcher *et al.* pp. 1144–51. Springer-Verlag, Berlin.

Weidle, H., Borgya, A., Mattes, R., Lenz, H. and Buckel, P. (1987) Reconstitution of functionally active antibody directed against creatine kinase from separately expressed heavy and light chains in non-lymphoid cells. *Gene* **51**, 21–9.

Whittle, N., Adair, J., Lloyd, C. *et al.* (1987). Expression in COS cell of a mouse–human chimaeric B72.3 antibody. *Protein Eng.* **1**, 499–505.

Williams, G. (1988). Novel antibody reagents: production and potential. *Trends Biotechnol.* **6**, 36–42.

Winkelhake, J.L. (1978). Immunoglobulin structure and effector functions. *Immunochemistry* **15**, 695–714.

Woof, J.M., Partridge, L.J., Jefferis, R. and Burton, D.R. (1986). Localisation of the monocyte-binding region on human immunoglobulin G. *Mol. Immunol.* **23**, 319–30.