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Construction of Novel Antibodies by use of DNA Transfection: Design of Plasmid Vectors

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Construction of novel antibodies by use of DNA transfection: design of plasmid vectors

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The DNA that encodes antibodies can be manipulated *in vitro* and reintroduced into lymphoid cell lines. In this way, lymphocyte transfectants can be established which secrete milligram quantities of novel antibody molecules. Here we present data concerning the DNA sequences that are needed for efficient expression of the transfected antibody gene and give examples of the way in which this expression system for immunoglobulin gene DNA can be used for the production of useful antibody-related molecules.

Introduction

The development of monoclonal antibodies by Köhler & Milstein (1975) has given a considerable boost to the extent to which antibodies are used in both biology and medicine. Antibodies are used for affinity chromatography and in a wide range of immunoassays; more recently, monoclonal antibodies and antibody–toxin conjugates (immunotoxins) have been tested in therapy (Ritz & Schlossman 1982; Vitetta & Uhr 1985). The use of antibodies to target toxic agents echoes the proposal of Paul Ehrlich who advocated using 'bodies which possess a particular affinity for a certain organ... as a carrier by which to bring therapeutically active groups to the organ in question' (Ehrlich 1906).

Thus, quite apart from the considerable scientific interest in site-directed mutagenesis experiments to analyse the contacts that antibodies make both with their antigens and with the proteins that mediate their physiological effector functions, an expression system for immunoglobulin gene DNA should be of great value in allowing the construction of novel antibodies or antibody-related molecules that can be used in biology, medicine or industry. Here we present some results concerning expression systems for immunoglobulin gene DNA and then go on to review some of the novel protein molecules that we have constructed with this technology.

Results

Myeloma expression system for immunoglobulin gene DNA

The most effective hosts for the expression of transfected antibody genes have proved to be rodent myeloma cells (Neuberger 1983; Ochi *et al.* 1983; Oi *et al.* 1983). Myelomas are plasma cell tumours, plasma cells being the cells responsible for the synthesis and secretion of immunoglobulin. Myelomas are therefore ideal for expressing transfected antibody genes as they not only recognize immunoglobulin gene transcription signals but are also equipped for antibody secretion at high levels. Other expression systems have been tested. The production of immunoglobulin polypeptides in both *Escherichia coli* and yeast has been demonstrated

(Cabilly *et al.* 1983; Boss *et al.* 1984; Wood *et al.* 1985); however, in these organisms, the immunoglobulin heavy and light chains do not associate efficiently and are not readily secreted. The yields of antibody are therefore poor.

In many of our experiments, we have worked with mouse myeloma cell line J558L, which secretes a λ_1 light chain but expresses no immunoglobulin heavy chain of its own. This cell line has been shown by Oi *et al.* (1983) to be a good host for transfection. The immunoglobulin heavy-chain gene of interest is cloned into the vector pSV2gpt (this vector is described by Mulligan & Berg 1981); the *gpt* gene encodes a xanthine–guanine phosphoribosyl transferase activity which renders the transfected cell resistant to the drug mycophenolic acid and thus provides a selective marker for mammalian cell transfection. Plasmids containing an immunoglobulin heavy-chain gene (pSV-IgH) are introduced into J558L by spheroplast fusion (see figure 1); transfectants are selected in medium containing mycophenolic acid. Thus, if the plasmid encodes an immunoglobulin μ heavy chain, the transfectants secrete an IgM, λ_1 antibody. In our work, the variable region (V_H) of the transfected heavy-chain gene has been chosen such that, on combination with the λ_1 light chain of the J558L plasmacytoma, an antigen binding site for the hapten 4-hydroxy-3-nitrophenacetyl (NP) is created.

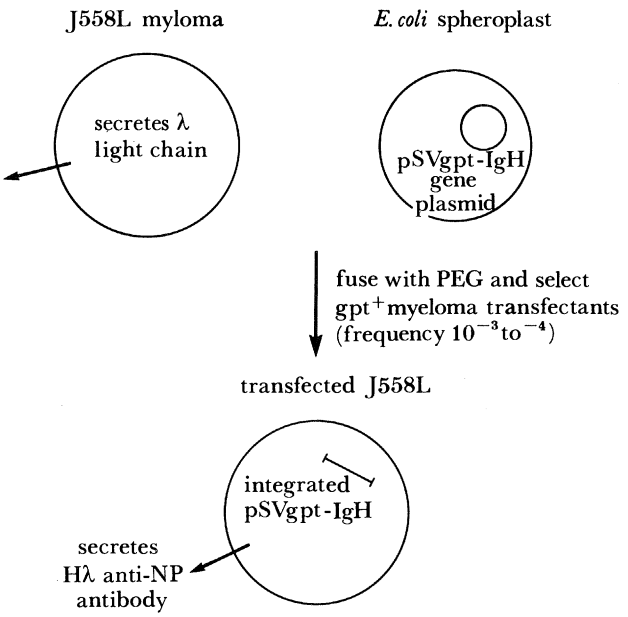

FIGURE 1. Transfection of plasmacytoma cells by spheroplast fusion. Polyethylene glycol (PEG) is used to fuse J558L plasmacytoma cells with spheroplasts of the *E. coli* strain which harbours a pSVgpt-based plasmid that includes an immunoglobulin heavy-chain gene (pSV-IgH). The detailed procedure is described in Neuberger & Williams (1985).

Is the transcription enhancer required for immunoglobulin gene expression?

In our initial experiments (Neuberger 1983; Neuberger *et al.* 1984), the plasmid used for transfection, pSV-Vμ1, contained a complete mouse immunoglobulin μ gene (see figure 2). As most of this 15000 base pair DNA stretch is intron, we were anxious to identify the DNA sequences necessary for high-level immunoglobulin expression so as to be able to simplify the structure of the plasmid vector.

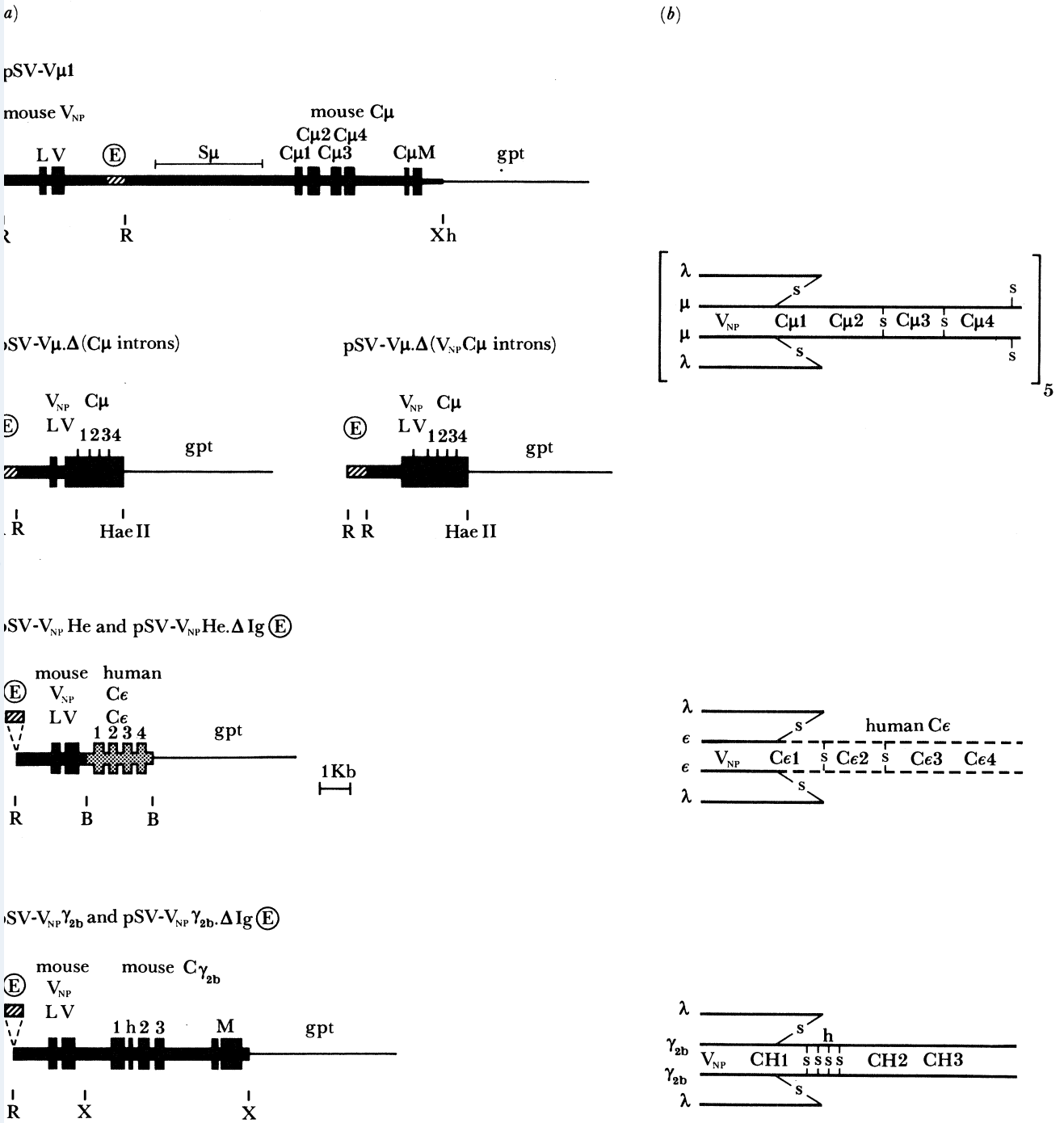

FIGURE 2. (*a*) Structure of pSV-IgH plasmids. Sequences from the mouse immunoglobulin heavy-chain locus are indicated with thick solid lines and exons represented by boxes. The mouse IgH enhancer is hatched, sequences from the human immunoglobulin CH locus are stippled and vector sequences are designated by thin horizontal lines. The locations of the IgH enhancer (E) and the μ switch region (Sμ) are indicated. Restriction endonuclease cleavage sites are abbreviated B, *Bam*HI; R, *Eco*RI; X, *Xba*I; and Xh, *Xho*I. Not all *Hae*II sites are marked. In plasmids pSV-VμΔ(Cμ introns) and pSV-VμΔ(V_{NP}Cμ introns) the polyadenylation signal for the IgH transcripts is provided by SV40 sequences in the pSV2gpt vector. (*b*) Schematic representation of the antibodies produced by pSV-IgH transfectants of plasmacytoma J558L. Mouse polypeptide sequences are shown by solid lines while human sequences are given by a broken line.

The largest intron in the μ heavy-chain gene is that located between V_H and Cμ; this intron is known to contain two biologically significant elements: the switch region (which is involved in heavy-chain class-switching), and the transcription enhancer element – the IgH enhancer (Banerji *et al.* 1983; Gillies *et al.* 1983; Neuberger 1983). While it seemed unlikely that the μ switch region was needed for immunoglobulin heavy-chain gene expression, an enhancer element has been shown to be necessary for transcription initiating at the V_H promoter by using transient gene expression assays (Gillies *et al.* 1983; Neuberger 1983). Therefore, when we constructed a plasmid in which the mouse NP V_H gene was linked to the exons of the human ε constant region (Neuberger *et al.* 1985), all but 300 base pairs of the major intron was removed except that the transcription enhancer element was now placed upstream of the gene. This plasmid, pSV-V_{NP}Hε (figure 2), worked well in directing the synthesis of chimeric ε heavy-chain polypeptide (Neuberger *et al.* 1985). To ascertain whether the immunoglobulin heavy-chain enhancer was in fact required, given that the plasmid already contained the viral SV40 enhancer, a derivative of pSV-V_{NP}Hε was constructed from which the *Eco*RI fragment containing the IgH enhancer had been removed. Both pSV-V_{NP}Hε and the derivative lacking the IgH enhancer (pSV-V_{NP}Hε.ΔIgⒺ (figure 2)) were introduced into J558L by spheroplast fusion. It is clear (table 1) that the presence of the IgH enhancer does not significantly affect the transfection frequency. To ascertain whether the IgH enhancer effected the production of higher antibody levels, radioimmunoassay was used to measure the concentration of anti-NP antibody in the culture supernatants of cloned pSV-V_{NP}Hε and pSV-V_{NP}Hε.ΔIgⒺ transfectants. The results (figure 3) indicate that, whilst there is considerable clone-to-clone variation in antibody yield, the presence of the IgH enhancer in plasmid pSV-V_{NP}Hε does not cause any significant stimulation of antibody production.

TABLE 1. TRANSFECTION FREQUENCY OBTAINED WITH pSV-V_{NP}Hε PLASMIDS

plasmid	frequency of transfection (transfectants/10^4 J558L cells ± s.e.m.)
pSV-V_{NP}Hε	0.64 ± 0.14
pSV-V_{NP}Hε.ΔIgⒺ	0.67 ± 0.15

The transfection frequency was assessed by limiting dilution in the absence of feeder cells and is given relative to the number of J558L cells that entered the fusion.

The pSV-V_{NP}Hε plasmids include the viral SV40 enhancer and it may not therefore seem surprising that the IgH enhancer is dispensable in these constructs. However, these findings are somewhat at variance with the observations of Gillies *et al.* (1983), who worked with pSV2gpt-based plasmids that included a mouse immunoglobulin γ2b gene. The work of these authors showed that deletion of the IgH enhancer from the plasmid resulted in a significant decrease in both transfection frequency and antibody yield. There are several possible explanations for the discrepancy between our results and those of Gillies *et al.* (1983). One possibility is that the human ε gene contains an enhancer element that renders the mouse IgH enhancer dispensable. We therefore repeated the experiment that we had performed with pSV-V_{NP}Hε with a plasmid that contained the mouse immunoglobulin γ2b gene in place of the human ε constant region (figure 2). It will be seen from the data presented in figure 3 that removal of the IgH enhancer from pSV-V_{NP}γ2b has no significant effect on the efficacy

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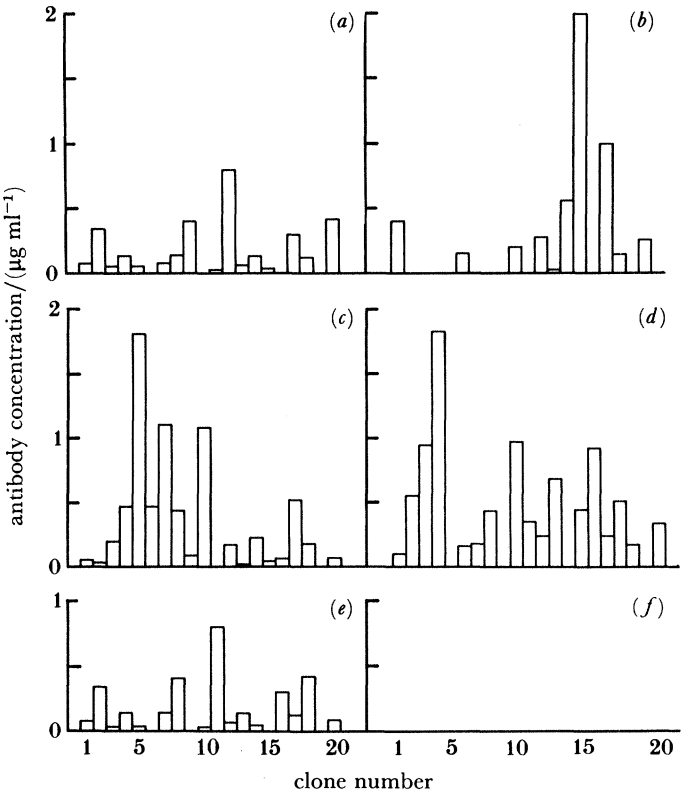

FIGURE 3. Yields of anti-NP antibody from J558L transfectants. The concentration of NP-specific antibody in the supernatants of semi-confluent cultures of cloned transfectants was measured by radioimmunoassay by using affinity-purified monoclonal anti-NP IgG and IgM antibodies as controls. (*a*) pSV-$V_{NP}H\varepsilon$; (*b*) pSV-$V_{NP}H\varepsilon.\Delta Ig\textcircled{E}$; (*c*) pSV-$V_{NP}\gamma_{2b}$; (*d*) pSV-$V_{NP}\gamma_{2b}.\Delta Ig\textcircled{E}$; (*e*) pSV-V$\mu.\Delta$(C$\mu$ introns); (*f*) pSV-V$\mu.\Delta(V_{NP}C\mu$ introns).

of that plasmid in directing immunoglobulin heavy-chain polypeptide synthesis. It is therefore clear that in stable transfection experiments performed by using these pSV2gpt-based plasmids, the presence of the mouse IgH enhancer will often not be necessary to achieve good antibody yields. The explanation for the discrepancy between our findings and those of Gillies *et al.* (1983) is a matter for speculation. Either the results obtained may depend critically on the structure of the plasmid used or the fact that we are using a different V_H gene from the one used by Gillies *et al.* and therefore a different promoter may explain the difference in our findings.

Are the introns required for immunoglobulin gene expression?

A considerable reduction in the size of the expression vectors could be achieved if it were possible to use immunoglobulin complementary DNA (cDNA) rather than genomic DNA. To test this, plasmids were assembled that either lacked all the introns or retained only the intron separating the exons encoding the leader and V_H portions of the antibody heavy chain (figure 2). The results obtained with these plasmids (figure 3) demonstrate that while pSV-V$\mu.\Delta$(Cμ introns) directs the synthesis of slightly reduced levels of antibody, pSV-V$\mu.\Delta(V_{NP}$, Cμ introns) has lost the ability to direct immunoglobulin heavy-chain production. Analysis of the cytoplasmic RNA (data not shown) reveals that the μ transcripts in pSV-V$\mu.\Delta$(Cμ introns) transfected cells are indeed initiated by the V_H gene promoter.

The reason for the requirement for the leader-V_H intron is unknown: it could be required for the stability or processing of the primary RNA transcript. However, one tantalizing possibility is that it contains sequences necessary for transcription initiating from the V_H gene RNA start sites. In fact, when we have made gene fusions linking the V_{NP} promoter to the coding sequence of other genes such as the *hsp70* heat-shock gene or a viral thymidine kinase gene, we have obtained no transcripts initiating from the V_{NP} RNA start sites (unpublished results). The only such fusion that has been effective was that linking the V_{NP} promoter to the human β-globin coding sequence (Mason *et al.* 1985). Transcription of the human β-globin gene is known to be regulated by sequences lying within the body of the gene (Wright *et al.* 1984; Charnay *et al.* 1984), and these observations may therefore be taken as circumstantial evidence supporting the notion that transcription of an immunoglobulin V_H gene requires control sequences located within the body of the V_H gene itself.

Making chimeric antibodies

If monoclonal antibodies are used in therapy, it is clearly desirable to use human rather than rodent antibodies. The production of monoclonal human antibodies of predetermined antigen specificity has therefore been attempted in many laboratories over the past few years. However, quite apart from the difficulties encountered in achieving the efficient *in vitro* immunization necessary for the production of antibodies of predetermined specificity, there have been problems with establishing cell lines which stably secrete large quantities of human antibodies. One of the immediate applications of immunoglobulin gene expression systems has therefore been the preparation of mouse–human chimeric antibodies. We and others (Boulianne *et al.* 1984; Morrison *et al.* 1984; Neuberger *et al.* 1985) have shown that it is possible to produce chimaeric antibodies by using plasmid vectors in which the expressed V gene isolated from a mouse hybridoma cell line is coupled to a human constant region gene. The construction of such hybrid genes is rendered facile owing to the fact that the exons of immunoglobulin genes correspond to domains of the polypeptide chain and that plasmid constructs may therefore be assembled simply by use of exon shuffling. Chimeric antibodies should not only prove useful in therapy but the availability of hapten-specific chimeric antibodies should also allow a detailed and controlled comparison of the effector functions of the different human immunoglobulin classes and sub-classes. In fact this approach has been useful in allowing the construction of a cell line that secretes a hapten-specific chimaeric IgE antibody (Neuberger *et al.* 1985). IgE is present in only low levels in serum but nevertheless plays a central role in allergic reactions; many studies on this class of immunoglobulin have been hampered by the previous lack of a cell line that secretes a human IgE antibody of known antigen specificity.

Making antibodies that exhibit novel effector functions

When antibodies are used for immunoassays, immunotoxins or affinity purification, a novel moiety (e.g. a radionuclide, an enzyme, a toxin, staphylococcal protein A or an insoluble support) is often chemically conjugated to the antibody. If this coupling were performed at the gene level, it might yield a homogeneous conjugate with reproducible properties. Antibodies that lack the Fc portion can be synthesized and secreted by plasmacytoma cells that have been transfected with appropriate plasmid constructs (Sharon *et al.* 1984; Neuberger *et al.* 1984). We have extended this work to show that an antibody–enzyme conjugate could be secreted from cells that had been transfected with a plasmid harbouring a fusion between the DNA

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encoding the Fab portion of an immunoglobulin γ2b heavy chain and the coding sequence of *Staphylococcus aureus* nuclease (Neuberger *et al.* 1984). This recombinant antibody retained both antigen binding and enzymatic activity and could be used for ELISA-type immunoassays. More recently, we have made an analogous hybrid between the DNA encoding the antibody Fab and the sequence that encodes the Klenow fragment of *Escherichia coli* DNA polymerase I (G. T. Williams & M. S. Neuberger, unpublished results). This Fab–Klenow is secreted from the plasmacytoma cells, is easily purified in a single step by affinity chromatography on NP–Sepharose and can then be used for the dideoxy method of DNA sequencing. Thus recombinant antibodies with novel effector functions may find wide application in immunoassays, as immunotoxins and for the tagging of an enzyme moiety for facile expression and purification.

Conclusion

The availability of immunoglobulin gene-expression systems allows site-directed mutagenesis studies on antibodies to be undertaken. Such studies will yield important information on the contacts that antibodies make both with their antigens and with the proteins that mediate their effector functions. The expression system also allows the construction of chimaeric and recombinant antibodies which should prove of use in diagnostic and therapeutic procedures. An improved understanding of the molecular basis of the regulation of immunoglobulin gene expression may allow the use of the plasmacytoma gene-expression system for the large-scale production and secretion of non-immunoglobulin proteins, such as complement components or blood-clotting factors.

References

Banerji, J., Olson, L. & Schaffner, W. 1983 A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. *Cell* **33**, 729–740.

Boss, M. A., Kenten, J. H., Wood, C. R. & Emtage, J. S. 1984 Assembly of functional antibodies from immunoglobulin heavy and light chains synthesised in *Escherichia coli*. *Nucleic Acids Res.* **12**, 3791–3806.

Boulianne, G. L., Hozumi, N. & Shulman, M. J. 1984 Production of functional chimaeric mouse/human antibody. *Nature, Lond.* **312**, 643–646.

Cabilly, S., Riggs, A. D., Pande, H., Shiveley, J. E., Holmes, W. E., Rey, M., Perry, L. J., Wetzel, R. & Heyneker, H. L. 1983 Generation of antibody activity from immunoglobulin polypeptide chains produced in *Escherichia coli*. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3273–3277.

Charnay, P. Treisman, R., Mellon, P., Chao, M., Axel, R. & Maniatis, T. 1984 Differences in human α- and β-globin gene expression in mouse erythroleukemia cells: the role of intragenic sequences. *Cell* **38**, 251–263.

Ehrlich, P. 1906 *Collected studies on immunity*, vol. II, pp. 442–447. New York: John Wiley.

Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. 1983 A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. *Cell* **33**, 717–728.

Köhler, G. & Milstein, C. 1975 Continuous culture of fused cells secreting antibody of pre-defined specificity. *Nature, Lond.* **256**, 495–497.

Mason, J. O., Williams, G. T. & Neuberger, M. S. 1985 Transcription cell-type specificity is conferred by an immunoglobulin V_H gene promoter which includes a functional consensus sequence. *Cell* **41**, 479–487.

Morrison, S. L., Johnson, M. J., Herzenberg, L. A. & Oi, V. T. 1984 Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6851–6855.

Mulligan, R. C. & Berg, P. 1981 Selection for animal cells that express the *Escherichia coli* gene for xanthine–guanine phosphoribosyltransferase. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2072–2076.

Neuberger, M. S. 1983 Expression and regulation of an immunoglobulin heavy chain gene transfected into lymphoid cells. *EMBO J.* **2**, 1373–1378.

Neuberger, M. S., Williams, G. T. & Fox, R. O. 1984 Recombinant antibodies possessing novel effector functions. *Nature, Lond.* **312**, 604–608.

Neuberger, M. S. & Williams, G. T. 1985 Protein engineering of antibody molecules. In *Protein engineering: applications in science, medicine and industry* (ed. M. Inouye & R. Sarma). New York: Academic Press. (In the press.)

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Neuberger, M. S., Williams, G. T., Mitchell, E. B., Jouhal, S. S., Flanagan, J. G. & Rabbitts, T. H. 1985 A hapten-specific chimaeric immunoglobulin E antibody with human physiological effector function. *Nature, Lond.* **314**, 268–270.

Ochi, A., Hawley, R. G., Hawley, T., Shulman, M. J., Traunecker, A., Köhler, G. & Hozumi, N. 1983 Functional immunoglobulin M production after transfection of cloned immunoglobulin heavy and light chain genes into lymphoid cells. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6351–6355.

Oi, V. T., Morrison, S. L., Herzenberg, L. A. & Berg, P. 1983 Immunoglobulin gene expression in transformed lymphoid cells. *Proc. natn. Acad. Sci. U.S.A.* **80**, 825–829.

Ritz, J. & Schlossman, S. F. 1982 Utilization of monoclonal antibodies in the treatment of leukemia and lymphoma. *Blood* **59**, 1–11.

Sharon, J., Gefter, M. L., Manser, T., Morrison, S. L., Oi, V. T. & Ptashne, M. 1984 Expression of V_HC_κ chimaeric protein in mouse myeloma cells. *Nature, Lond.* **309**, 364–367.

Vitetta, E. S. & Uhr, J. W. 1985 Immunotoxins. In *Annual review of immunology* (ed. W. E. Paul, C. G. Fathman & H. Metzger), vol. 3, pp. 197–212.

Wright, S., Rosenthal, A., Flavell, R. & Grosveld, F. 1984 DNA sequences required for regulated expression of β-globin genes in mouse erythroleukemia cells. *Cell* **38**, 265–273.

Wood, C. R., Boss, M. A., Kenten, J. H., Calvert, J. E., Roberts, N. A. & Emtage, J. S. 1985 Synthesis and *in vivo* assembly of functional antibody in yeast. *Nature, Lond.* **314**, 446–449.

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