Volume 330, number 2, 111–113 FEBS 12930 September 1993


# Production of recombinant antibodies in lymphoid and non-lymphoid cells

Sergey M. Deyev[a], Andre Lieber[b], Boris V. Radko[a], Oleg L. Polanovsky[a,*]

[a]*Laboratory of Molecular Genetic Immunology, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov str. 32, 117984 Moscow, Russian Federation*
[b]*Max-Delbruck-Centrum, Robert-Rossie-Strasse 10, O-1115 Berlin-Buch, Germany*

Received 5 July 1993

A recombinant tandem of 'chimeric' mouse/human immunoglobulin (Ig) genes was constructed and inserted into plasmid pGEM1 under the control of the T7 bacteriophage RNA polymerase promoter. Lymphoid (Sp2/0) and non-lymphoid (CHO) cell lines used for transfection contained in their genomes a semisynthetic gene of T7 RNA polymerase and steadily expressed this enzyme. It was shown for the first time that a stable polycystronic transcription of the Ig gene tandem occurs under the control of a single T7 phage promoter, both in lymphoid and non-lymphoid cells. Synthesis of κ-light and ε-heavy Ig chains and functionally active antibodies was observed in the above-mentioned transfected cell lines.

Antibody engineering; Gene tandem expression; Phage T7 promoter; Sp2/0 cell; CHO cell

## 1. INTRODUCTION

Many variants of recombinant antibodies, including the 'humanized' chimeric [1–3], reshaped [4–6], single-chain [7,8], bifunctional [2,9], and others, have been created during the past ten years (for review see [3,10]).

Antibodies are known to be synthesized only by lymphoid B cells. On the one hand, this is due to the structure of promoters and enhancers which regulate the expression of immunoglobulin (Ig) genes [11,12], and on the other to specific transcription factors preferentially synthesized in B cells [13–15]. The recombinant Ig genes may be expressed in lymphoid plasmacytoma cells and in alien systems [16,17]. Earlier [18–20] a high level of the marker gene, chloramphenicol acetyltransferase (CAT), expression was shown in eukaryotic cells under the control of the 'nuclear' T7 RNA polymerase. We were interested in the possibility of expressing the Ig gene tandem in conditions similar to those described in [18]. In the present work a complete tandem of immunoglobulin genes was created consisting of variable (V) genes of light (L) and heavy (H) chains obtained from the mouse hybridoma, and of genes of human constant (C) chains. The whole tandem was put under the control of the T7 RNA polymerase promoter. The eukaryotic cells steadily producing this polymerase were used as targets. Thus, conditions were created that allowed us to investigate the expression of the obtained construction in both lymphoid and non-lymphoid cells.

*Corresponding author. Fax: (7) (095) 135 1405. E-mail: pol@imb.msk.su.

## 2. MATERIALS AND METHODS

### 2.1. *Immunoglobulin genes*

Clones containing the variable gene segments were obtained from the hybridoma PTF-02 genome (obtained courtesy of Dr. F. Franek, Institute of Molecular Genetics, Prague). Both V genes had been cloned and sequenced by us earlier [21,22]. Clones containing C genes of light (κ-type) and heavy (ε-type) chains were obtained from the human genome [23].

Human C ε- and κ-genes, labelled with $^{32}P$, were used as DNA probes.

### 2.2. *Cell lines*

The myeloma cell line, Sp2/0, and the non-lymphoid CHO cells (Chinese hamster ovary) were used for transfection. Both cell lines contain in their genome a semisynthetic gene of T7 RNA polymerase [18] and steadily express this enzyme. An earlier modification of the polymerase [18] consisted of replacing the enzyme's N-terminal part with a synthetic oligonucleotide sequence coding for amino acids 124–133 of SV-40 viral large T antigen.

### 2.3. *Cell transfection*

Co-transfection by plasmid pSV2-neo was carried out simultaneously during the calcium phosphate transfection with plasmid pIG.6ek, and clone selection was done on selective medium containing geneticin G-418 (400 μg/ml for selection of the CHO cells and 1.5 mg/ml for the Sp2/0 cell line.

### 2.4. *Radioimmune analysis*

Rabbit anti-human IgE and anti-ε-chain antibodies, goat antibodies against rabbit antibodies, and protein A labelled with $^{125}I$, were used throughout the work. The antigen (pig transferrin) was obtained courtesy of Dr. F. Franek. To create the gene tandem and insert it into plasmids, recombinant DNA methods were applied.

### 2.5. *Enzymes and reagents*

Restriction endonucleases and other enzymes were obtained from the Institute of Applied Enzymology (Vilnius, Lithuania), agarose from Bio-Rad (USA), and other reagents from Sigma (USA).

## 3. RESULTS AND DISCUSSION

The mouse $V_H$ gene and human $C_H$ gene were inserted into the pGEM1 plasmid under the control of T7 RNA polymerase promoter (Fig. 1). Sticky ends of an *Hin*dIII fragment of the $V_H$ gene and a *Bam*HI fragment of the $C_H$ gene were blunt-ended and intermediates formed by ligating their blunt ends.

The mouse $V_\kappa$ gene and human $C_\kappa$ gene were first inserted into the pUC19 vector (Fig. 1). The *Hin*dIII site within the recombinant gene was removed by blunt-ending using Klenow fragments of DNA polymerase, and the blunt ends of the $V_\kappa$ and $C_\kappa$ fragments were ligated. A linker containing the *Hin*dIII site was attached to both ends of the recombinant $V_\kappa C_\kappa$ gene. The final procedure consisted of binding the $V_\kappa C_\kappa$ gene to the 3′ end of the $V_H C_\varepsilon$ gene.

The lymphoid (Sp2/0) and non-lymphoid (CHO) cells which steadily produced the modified T7 RNA polymerase (see section 2), were transfected with plasmid pIG.6ek, shown in Fig. 1, and geneticin-resistant clones were selected and analyzed. Northern analysis of mRNA synthesized in the transfected cells (not shown) revealed only an extended chain of mRNA (8.5–9.0 kb). This mRNA was able to hybridize with the $^{32}$P-labelled human $C_\varepsilon$ and $C_\kappa$ gene probes. No discrete RNA bands with the mobilities of individual mRNAs of L- and H-immunoglobulin chains were found in transfected cells (unlike the original hybridoma cell line).

The cell lines which steadily synthesized these long mRNAs were used to reveal the expression of the L- and

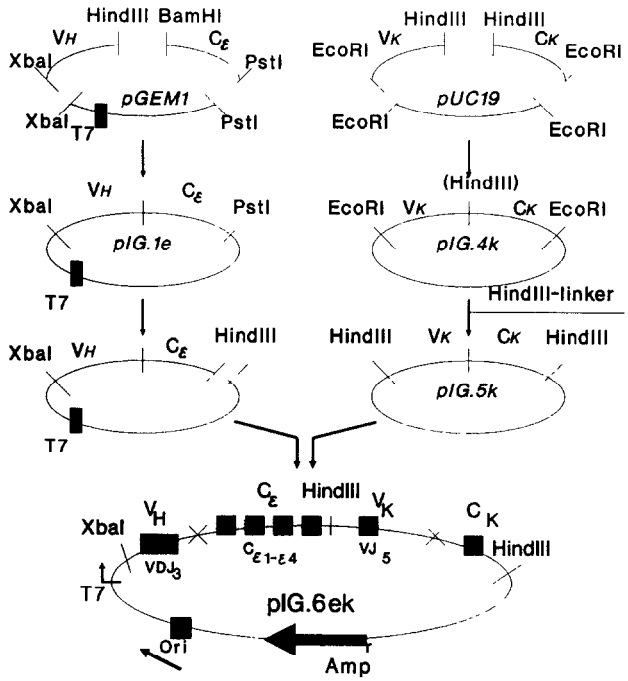


Fig. 1. Construction of plasmid pIG.6ek containing the mouse/human immunoglobulin gene tandem under the control of the T7 RNA polymerase promoter (for details see text).

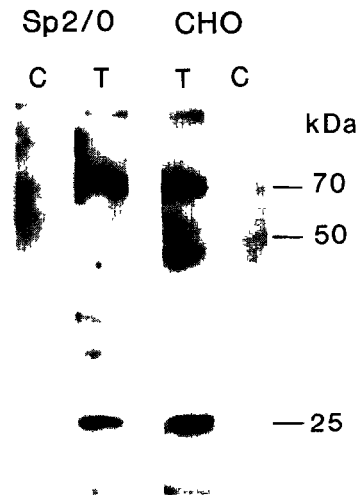


Fig. 2. Western blot analysis of heavy ($\varepsilon$) and light ($\kappa$) immunoglobulin chains synthesized by lymphoid (Sp2/0) and non-lymphoid (CHO) cell lines transfected with plasmid pIG.6ek. Lane C, untransfected; T, transfected cell lines. Numbers to the right of the blots indicate approximate molecular weights in kDa.

H-immunoglobulin chains. These polypeptide chains were identified by radioimmune analysis on electrophoregrams of cellular extracts (rabbit antibodies and $^{125}$I-labelled protein A). The transfected clones of Sp2/0 and CHO cells synthesized both H ($\varepsilon$-type) and light ($\kappa$-type) immunoglobulin chains (Fig. 2). The molecular weights of 70 kDa and 25 kDa, respectively, for the heavy ($\varepsilon$-type) and light ($\kappa$-type) chains coincide in both cell lines; the heavy chain of the original hybridoma, PTF-02, has a much lower molecular weight (50 kDa), which was as expected since it belongs to the G1 class. The level of chimeric antibody production by the myeloma cells, Sp2/0, exceeded that of the CHO cells. Individual clones synthesized up to 150 ng of chimeric antibodies in 1 ml.

T7 RNA polymerase was shown earlier to be effective in gene transcription in eukaryotic cells [18–20]. The T7 transcripts are largely uncapped [19] and therefore their translation must proceed via a cap-independent mechanism. A scanning model has been proposed [24] to explain the mechanism by which most eukaryotic mRNAs initiate translation. Nevertheless, the translation of uncapped poliovirus RNA is not satisfactorily accommodated within the scanning model [25]. In this case the initiation of translation occurs by ribosome binding to an internal sequence within the 5′ non-coding region of viral RNA [25]. Some eukaryotic mRNAs also contain putative internal ribosome entry sites in the 5′ untranslated region [26,27]. Which translation model is realized in our case: the scanning one or the internal initiation of both H- and L-chain reading frames? In this paper we present indirect evidence showing that the second mechanism is more likely (at least in non-lymphoid cells). Rather effective expression at the level of both

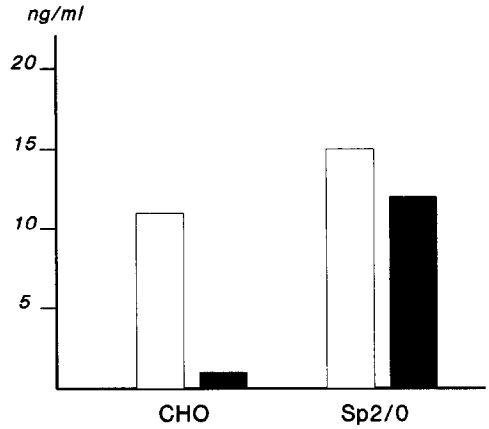


Fig. 3. Accumulation of chimeric antibodies in culture medium and cell extracts of CHO and Sp2/0 cell lines transfected with plasmid pIG.6ek. Open bars, cell extract; filled bars, culture medium.

transcription and translation makes the system described here interesting for further analysis.

The functional activity of chimeric antibodies was detected by their binding to antigen (pig transferrin). The antigen was immobilized on nitrocellulose filters and the antigen-bound antibodies isolated from the cell extracts were identified by radioimmune analysis. It was found that the transfected cells of both types (Sp2/0 and CHO) synthesized functional antibodies of the IgE isotype. In addition, the chimeric antibodies were identified by radioimmune analysis according to their interaction with the rabbit antibodies against human IgE. The effectiveness of chimeric IgE antibody secretion by the transfected cells was also studied. Fig. 3 shows that only Sp2/0 cells are capable of effective antibody secretion. It follows from the obtained results that non-lymphoid CHO cells synthesize functionally active chimeric IgE antibodies but, unlike the lymphoid cells, they are not capable of active secretion of these antibodies. This fact can explain the decreased production of antibodies by the CHO cells compared to the Sp2/0 cells.

*Acknowledgements:* This research was supported by Russian Fund for Fundamental Research, Grant 93-04-6891.

## REFERENCES

[1] Morrison, S.L., Johnson, M.J., Herzenberg, L.A. and Oi, V. (1984) Proc. Natl. Acad. Sci. USA 81, 6851–6855.
[2] Neuberger, M.S., Williams, G.T. and Fox, R.O. (1984) Nature 312, 604–608.
[3] Houghton, A.N. (1988) Immunol. Today 9, 265–268.
[4] Riechman, L. (1988) Nature 332, 323–327.
[5] Verhoeyen, M., Milstein, C. and Winter, G. (1988) Science 239, 1534–1536.
[6] Queen, C. (1989) Proc. Natl. Acad. Sci. USA 86, 10029–10033.
[7] Bird, R.E., Hardman, K.D., Jacobson, Y.W., Johnson, S., Kaufman, B.M., Lee, S.-M., Lee, T., Pope, S.H., Riordan, G.S. and Whitlow, M. (1988) Science 242, 423–426.
[8] Bespalov, I.A., Shiyanov, P.A., Lukashevich, L.V., Lunev, V.E., Tribush, S.S., Gaponova, G.I. and Deyev, S.M. (1993) Molekular. biologia 27, 451–460.
[9] Chaudhary, V.K. (1989) Nature 339, 394–397.
[10] Winter, G. and Milstein, C. (1991) Nature 349, 293–299.
[11] Falkner, F.G. and Zachau, H.G. (1983) Nature 298, 717–720.
[12] Picard, D. and Schaffner, W. (1984) Nature 307, 80–82.
[13] Weinberger, J., Baltimore, D. and Scharp, P.A. (1986) Nature 322, 846–848.
[14] Scheidereit, C., Heguy, A. and Roeder, R.G. (1987) Cell 51, 783–793.
[15] Stepchenko, A.G., Urakov, D.N., Luchina, N.N., Deyev, S.M. and Polanovsky, O.L. (1990) Molekular. biologia 24, 487–494.
[16] Weidle, U.H., Borgya, A., Mattes, R., Lenz, H. and Buckel, P. (1987) Gene 51, 21–29.
[17] Muller, M., Ruppert, S., Schaffner, W. and Mathias, P. (1988) Nature 336, 544–551.
[18] Lieber, A., Kiessling, U. and Strauss, M. (1989) Nucleic Acids Res. 17, 8485–8493.
[19] Elroy-Stein, O., Fuerst, T.R. and Moss, B. (1989) Proc. Natl. Acad. Sci. USA 86, 6126–6130.
[20] Elroy-Stein, O. and Moss, B. (1990) Proc. Natl. Acad. Sci. USA 87, 6743–6747.
[21] Ajalov, V.A., Stepchenko, A.G., Deyev, S.M. and Polanovsky, O.L. (1987) Molekular. Biologia 21, 1137–1141.
[22] Urackov, D.N., Deyev, S.M. and Polanovsky, O.L. (1989) Nucleic Acids Res. 17, 945.
[23] Deyev, S.M., Radko, B.V., Lieber, A. and Polanovsky, O.L. (1991) Doklady Acad. Nauk SSSR 318, 1500–1503.
[24] Kozak, M. (1983) Microbiol. Rev. 47, 1–45.
[25] Jang, S.K., Davis, M.V., Kaufman, R.J. and Wimmer, E. (1989) J. Virology 63, 1651–1660.
[26] Reynolds, G.A., Goldstein, J.L. and Brown, M.S. (1985) J. Biol. Chem. 260, 10369–10377.
[27] Futterer, J. and Hohn, T. (1992) Nucleic Acids Res. 20, 3851–3857.