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Immunology

Clonal variants of hybridoma cells that switch isotype at a high frequency

(fluctuation analysis/ELISA spot assay/antibodies)

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ABSTRACT **As B cells differentiate under the influence of antigen and T cells, they frequently switch from the expression of IgM antibody to the expression of other isotypes. This is accomplished by rearranging the expressed variable region gene to downstream constant region genes and deleting the intervening sequences. Some B-cell lines that represent early stages in development switch constitutively in culture at frequencies that approach those of lipopolysaccharide- or lymphokine-stimulated normal B cells. Hybridoma cells represent a later stage of development and rarely switch in culture. In contrast to early B-cell lines, hybridomas produce large amounts of immunoglobulin, and single cells can be assayed easily for the expression of new isotypes. We have used the ELISA spot assay and fluctuation analysis to determine the rate of switching of two hybridoma cell lines. By identifying subclones that switched more frequently, we have progressively enriched for cells that switch spontaneously at higher rates. These cells, like normal cells, switch by rearrangement and deletion, and the frequency of switched cells in some of the clones is comparable to that which has been observed in less differentiated B-cell lines and in normal B cells.**

When antigen and T cell-derived signals trigger the secondary immune response, they stimulate B cells with specific IgM antibodies on their surface to switch to the production of IgG, IgE, and IgA antibodies of the same specificity (1, 2). This is accomplished by rearranging the heavy chain variable region gene from its initial site of assembly 5′ to the μ constant region gene to one of the downstream constant region genes [in mice, γ3, γ1, γ2b, γ2a, ε and α (2, 3)]. The process of immunoglobulin switching allows a particular antigen binding site to be expressed with each of these other isotypes and to mediate a wide variety of effector functions throughout the body. Recombination between variable and constant region genes takes place within or close to repetitive "switch" sequences that are found 5′ to each of the constant region genes, except δ, and results in deletion of the intervening DNA sequences (reviewed in ref. 4). These switch sequences are composed of tandem arrays of reiterated sequences that have some homologies but differ in size and composition for each constant region (2, 3, 5). Studies with both normal B cells and a few B-cell lines that switch in culture show that switching is directed towards particular isotypes by lymphokines such as interleukin 4, γ interferon, and transforming growth factor β that are produced by helper T cells (reviewed in refs. 6 and 7).

Both general and isotype-specific factors are thought to contribute to the switching process. Prior to switching, the constant region of the recipient isotype and its associated switch sequences show increased accessibility to nucleases, and germ-line or sterile transcripts are initiated from lymphokine-responsive intervening region promoters (6, 8–10). Deletion of the γ1 or γ2b intervening region in transgenic mice results in a complete block in switching to the affected isotype (11, 12). Although proteins have been identified that bind to the switch regions and to promotor elements for sterile transcripts (13–22), virtually nothing is known about the enzymes and other factors that mediate switching. Experiments using transfected reporter constructs suggest that the switch recombinase system acts in trans, is regulated during B-cell development, and generally is not expressed in mature B-cell lines such as hybridomas and myelomas (23), which switch in culture at very low frequencies of 10^{-6}–10^{-7} (5, 24–26). Some pre-B-cell and B-cell lines switch more frequently in culture, and in some cases the frequency of switching can be increased by treatment with lipopolysaccharide or lymphokines (27–30) or both, but the rates of switching are probably still too low to allow effective biochemical analysis.

While using the ELISA spot assay to determine the rate at which hybridoma cells switch in culture and to isolate isotype switch variants, we noticed a clonal variation in the rate of switching among subclones obtained from a single parental hybridoma (31). In this report, we illustrate and document that clonal variation and describe how we have used it to sequentially isolate hybridoma subclones that have progressively higher rates of switching. The final products of this process switch constitutively at frequencies that are at best as high as if not higher than those seen in many murine pre-B-cell and B-cell lines and appear to be comparable to those observed in normal B cells.

MATERIALS AND METHODS

Cells. Cell lines were grown in Dulbecco's modified Eagle's medium (DME H-21, GIBCO) supplemented with 10% (vol/vol) fetal calf serum (FCS), 5% National Cancer Tissue Culture (NCTC) 109 (BioWhittaker), 1% nonessential amino acids (GIBCO), 1% penicillin, and 1% streptomycin. PC1.4a and PC1.4.1c are IgG1-producing switch variants isolated from subclones of the PC1 IgM anti-phosphorylcholine-producing hybridoma (32). 36.65 is an IgG1 anti-*p*-azophenylarsonate-producing hybridoma (33).

ELISA Spot Assay. The assay was carried out as described by Greene *et al.* (34). Briefly, flat-bottomed immunoplates were coated with affinity-purified isotype-specific anti-mouse immunoglobulin (Fisher Biotech) for 2 hr and blocked with 2% bovine serum albumin. After five cycles of washing, cells were added and plates were incubated for 6 hr at 37°C in a CO_2 incubator. After extensive washes, plates were treated with the relevant biotin-conjugated antibody (Fisher Biotech, Orangeburg, NY) for 2–4 hr, washed, and treated with avidin-conjugated to alkaline phosphatase for 1–2 hr. Spots were stained by using 1 mg of the phosphate substrate 5-bromo-4-chloro-3-indolyl phosphate (Auresco, Solon, OH) per ml and were counted with a dissecting microscope (31).

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Cloning in Soft Agar. Cells (10^3) were seeded in 0.6% SeaPlaque agarose (35) and allowed to grow for 7–10 days; random clones were picked into 96-well plates and further tested.

Fluctuation Analysis. The rate of isotype switching was determined by fluctuation analysis (36) as described by Lea and Coulson (37). Briefly, for each clone 15–25 subclones were isolated from soft agar and grown to a density of $\approx 5 \times 10^5$ cells; the entire sample was tested for γ2b, γ2a, and α switch variants by using the ELISA spot assay. When the rate of switching was low, 5×10^6 to 10^7 cells were tested.

Southern Analysis. High molecular weight DNA was isolated (38), and 10 μg was digested to completion with *Hin*dIII or *Eco*RI (Boehringer Mannheim). After electrophoresis through 0.8% agarose, DNA was denatured, transferred by capillary action to a nylon membrane (Boehringer Mannheim), and hybridized (38) sequentially to three specific probes: a 10-kb fragment spanning the γ1 switch region (39), a 1.4-kb *Eco*RI fragment from the γ2b switch region (40), and a 600-bp fragment from the C_H3 domain of the γ1 constant region (gift of Barbara Birshtein). Probes were labeled by random priming (Boehringer Mannheim).

RESULTS

Hybridomas secrete large amounts of immunoglobulin. With such cells, the ELISA spot assay (34) can detect a single cell producing a new isotype among 10^5 parental cells and, coupled with fluctuation analysis (36), can provide a rapid and reliable assay for the rate of switching (31).

Using this assay, we have observed a significant clonal variation in the rate of switching of three IgG1-producing hybridomas that switch predominantly to γ2b and γ2a in culture. The two PC-specific clones PC1.4a and PC1.4.1c, which are derived from the same IgM parent, differed in rate by 30-fold, with an intermediate rate for clone 36.65.12.7 PC1.4a switched to both γ2b and γ2a at a very low rate of 4×10^{-7} events per cell per generation, which is typical of hybridomas (Fig. 1). When assayed for each isotype separately, PC1.4.1c switched to γ2b and γ2a at rates of 6.8×10^{-6} and 4.9×10^{-6}, respectively, for a combined rate of 1.2×10^{-5} events per cell per generation. Clone 36.65.12.7 switched to both γ2b and γ2a at a rate of 4×10^{-6} events per cell per generation (Fig. 2*A*).

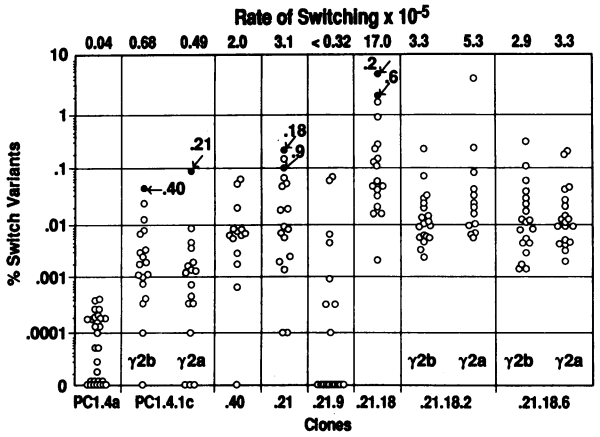

FIG. 1. Fluctuation analysis of clones derived from PC1.4 and isolation of high-switch variants. Unless otherwise noted, cells were assayed by using a mixture of antisera specific for γ2a, γ2b, and α. Each circle represents data from one subclone. The rates of switching, shown above each column, were calculated as described by Lea and Coulson (37). Unswitched cells were recovered from some clones (blackened circles) that contained a large number of switch variants, subcloned and subjected to fluctuation analysis.

This modification of the fluctuation analysis assumes that the underlying rate of the event being measured is the same in each subclone. If true, the number of switch variants scored reflects the timing of the switching event during the clone's propagation; early events will result in the accumulation of more variants than late events (36). We observed significant clonal variation (2–4 orders of magnitude) in the number of switch variants scored (PC1.4a and PC1.4b in Fig. 1; 36.65.12.7 in Fig. 2*A*). This raised the possibility that some clones with large numbers of switch variants were in fact switching at a high rate.

To examine this, we recovered unswitched cells from clones of PC.1.4.1c and 36.65.12.7 that contained a large number of switch variants, subcloned them, and subjected the subclones to fluctuation analysis. Most subclones showed the same or lower rates of switching than the parental clone. However, a few subclones showed a higher rate of switching than the parental cells. For example, PC1.4.1c subclone .21.9 switched at a significantly lower rate than the parent line .21, whereas .21.18 switched at a high rate and gave rise to progeny that also switched at a high rate (Fig. 1). Similarly, three subclones of 36.65.12.7.23 (.1, .10, and .18 in Fig. 2*B*) switched at rates that were lower than or similar to the parental line, whereas one, clone 36.65.12.7.23.20 (20 in Fig. 2*B* and .23.20 in Fig. 2*A*) gave rise to clones with a significantly higher rate of switching. Subsequent progeny of clone .23.20 (Fig. 2*A*) retained this high rate of switching and gave rise to clones that switched at even higher frequencies (Fig. 2*A*).

Using sequential enrichment of subclones that switched at progressively higher rates, we achieved 15-fold and 91-fold

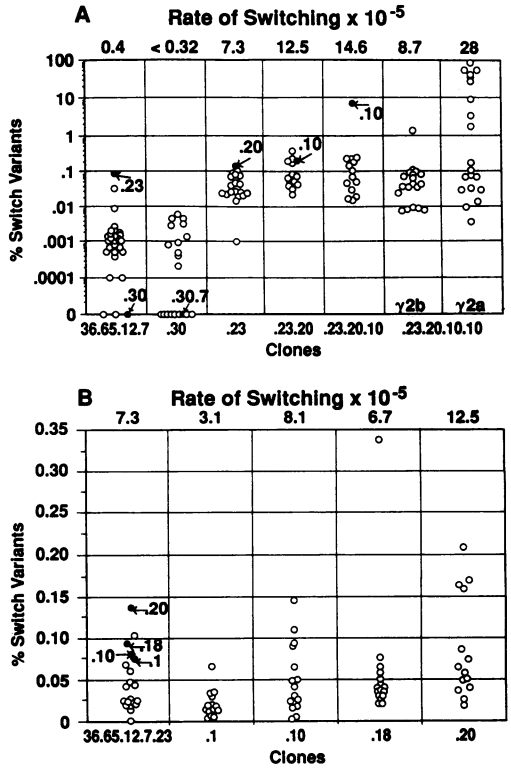

FIG. 2. Fluctuation analysis of clones derived from 36.65.12.7 (*A*) and 36.65.12.7.23 (*B*), showing enrichment for variants that switch at a high rate. Unless otherwise noted, cells were assayed by using a mixture of antisera specific for γ2a, γ2b and α. Each circle represents data from one subclone. The rates of switching, shown above each column, were calculated according to Lea and Coulson (37). The two panels have different ordinates. Unswitched cells were recovered from some clones (blackened circles) that contained a large number of switch variants, subcloned, and subjected to fluctuation analysis.

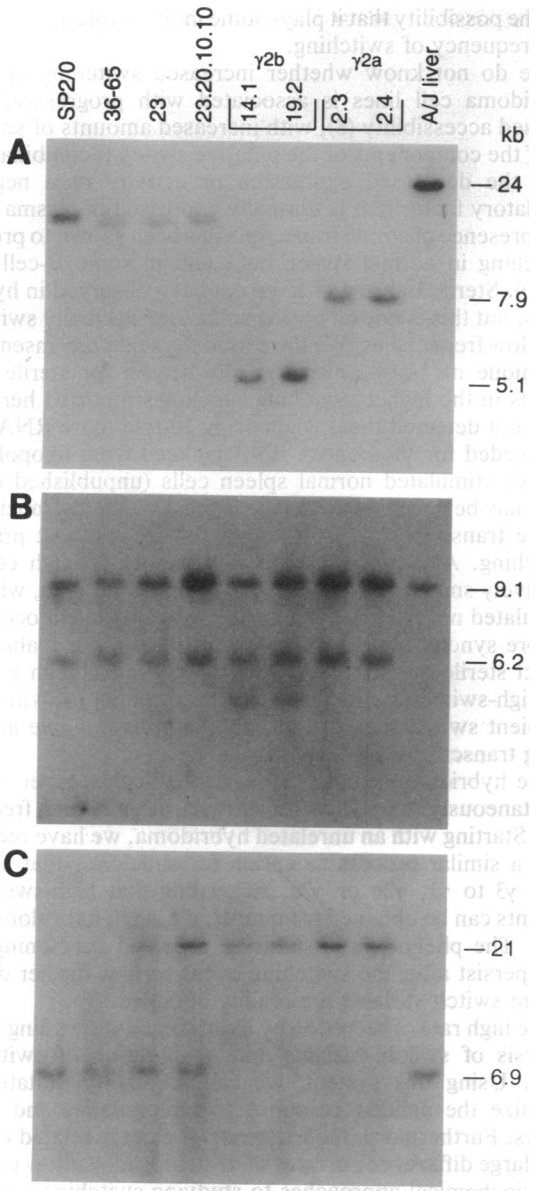

FIG. 3. Genomic Southern analysis of 36.65.12.7 (here 36.65) and subclones. (*A*) Genomic DNA was digested with *Hin*dIII and hybridized with a γ1 switch region probe. The 24-kb germ line fragment is rearranged in 36.65.12.7 to a 16-kb fragment that defines the active γ1 allele. This fragment is replaced by 5.1- and 7.9-kb fragments in the γ2b (14.1, 19.2) and γ2a (2.3, 2.4) switch variants, respectively. (*B*) The blot hybridized to a switch γ2b probe. (*C*) *Eco*RI-digested DNA hybridized to a 600-bp γ1 constant region probe.

increases in rates over the PC1.4.1c and 36.65.12.7 parents, respectively (Figs. 1 and 2). The frequency of switch variants in some PC1.4.1c subclones, such as .18.2, was >1%, or 400 times greater than was seen for PC1.4a, a closely related hybridoma.

As mentioned, some subclones had very low rates of switching (.21.9 in Fig. 1 and .30 in Fig. 2*A*). Analysis of clones like .30.7 required 1×10^7 or more cells to detect switch variants. Like the subclones that switched at a high rate, the variants that switched at a low rate were relatively stable but frequently generated subclones that switched at higher rates. Two 36.65.12.7 subclones with the lowest (.30) and highest (.23.20.10.10) rates of switching differ in their rates by 115-fold (Fig. 2*A*).

Subclones of PC1.4.1c and 36.65.12.7 that switched at the highest frequencies were examined for their ability to switch to either γ2b or γ2a. Whereas PC1.4.1c.21.18 switched at about the same rate to either γ2b or γ2a (Fig. 1), 36.65.23.20.10.10 switched at a somewhat higher rate to γ2a than to γ2b, and the frequency of γ2a switch variants in some subclones often exceeded 10% (Fig. 2*A*). Switching to the further downstream ε and α constant regions occurred at very low frequencies for all clones.

Since our goal is to use these cells to examine the biochemical mechanism of switching, we wanted to determine if switching was associated with gene rearrangement and deletion as occurs *in vivo* (2, 3). 36.65.12.7-derived γ2b and γ2a switch variants were cloned, and antibodies and DNA were examined. The antibodies produced by these switch variants continue to bind the relevant antigen, and their heavy and light chains show the same migration on SDS gels as IgG2b and IgG2a isolated by conventional means (data not shown). DNAs from γ2b and γ2a switch variants were compared by Southern analysis to their progenitors, and the Sp2/0 fusion partner. *Hin*dIII-digested DNAs were hybridized with a γ1 switch region probe (Fig. 3*A*). The 24-kb germ-line fragment was rearranged in 36.65.12.7 to a 16-kb fragment that defines the active γ1 allele. This fragment is replaced by different 5.1- and 7.9-kb fragments in the γ2b (siblings 14.1 and 19.2) and γ2a (siblings 2.3 and 2.4) switch variants respectively, indicating that switching is associated with gene rearrangement. The 5.1-kb fragments also hybridized with a γ2b switch region probe (Fig. 3*B*), confirming that a recombination event had created a hybrid γ1/γ2b switch region in this clone. The active γ1 allele that is represented by the 6.9-kb *Eco*RI fragment was deleted in the γ2b and γ2a switch variants (Fig. 3*C*). Together, these data confirm that rearrangement and deletion occur in association with switching in these clones.

We observed an additional γ1 constant region allele in the high-switching 36.65.12.7 subclones and γ2b and γ2a switch variants. This allele was only detected with a γ1 constant region probe (21-kb R1 fragment in Fig. 3*C*) and did not hybridize to the γ1 switch region probe (Fig. 3*A* and results not shown).

In the course of these experiments, we also observed that PC1.4.1c subclones with a higher rate of switching frequently underwent deletions and/or rearrangements in the donor γ1 switch region (Fig. 4). While PC1.4.1c.21.18 and four of five subclones retain the 9.4-kb germ-line γ1 switch region fragment, additional fragments, some of which are submolar, were seen in the parent and three of five subclones. These altered γ1 switch regions, which dominate some cultures, did not arise from cells that had already switched to γ2b or γ2a

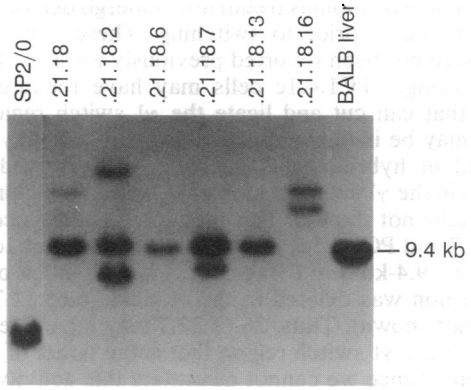

FIG. 4. Genomic Southern analysis of PC1.4.1c subclones. DNA was isolated from γ1-positive cells (minimally 99% γ1 producers), digested with *Sac* I, and hybridized to a γ1 switch region probe. The 9.4-kb germ-line fragment is dominant in the .21.18 parent and three subclones. Three of five subclones have additional bands, and one (.21.18.16) has lost the 9.4-kb germ-line band altogether and gained two new bands.

because the DNA was prepared from cultures that were at least 99% γ1 producers. In addition, when the same blot was hybridized sequentially with γ2b and γ2a switch region probes, no changes from the germ-line configuration of these downstream recipient switch regions were detected, confirming that the cultures contained few switched cells (results not shown). Because Southern analysis is a relatively insensitive technique, we do not know if ongoing deletion of the γ1 switch regions is restricted to the clones switching at a high rate or even correlates with the frequency of switching.

DISCUSSION

Clonal variation has been used to obtain hybridoma cell lines that switch frequently from IgG1 to the production of IgG2b or IgG2a in culture. We have used fluctuation analysis (36) to compare the rate of switching in different cell lines and subclones. This may not be completely valid, since switching is not a typical rare mutational event. In the higher switching clones, the distribution of the frequencies may provide a more valid representation of the behavior (Figs. 1 and 2). However, it is clear that each subclone of the higher switching variants has the potential to switch and that some subclones accumulate switched cells at frequencies comparable to mitogen-stimulated normal spleen cells. A direct comparison of switching rates is not possible, because fluctuation analysis has not been previously applied to switching in spleen cells or most other cultured cell systems.

We have considered whether the size or composition of the donor switch sites influences the rate of switching. Two subclones of PC1 switched at quite different rates; 4×10^{-7} events per cell per generation for PC1.4a and 1.2×10^{-5} events per cell per generation for PC1.4.1c (Fig. 1). After switching from μ to γ1, PC1.4a retained about 300 bp of the μ switch region and none of the γ1 switch region, while PC1.4.1c retained 1.5 kb of the μ switch region and all of the γ1 switch region (41). Clone 36.65.12.7 switches to γ2b and γ2a at a relatively high rate (4×10^{-6} events per cell per generation, Fig. 2), yet retains little or no μ switch region and has a large deletion in its γ1 switch region (unpublished data). There is thus no simple relationship between the donor switch site structure and rates of switching in these clones.

Some have suggested a link between deletions in donor switch regions and commitment to switching. Deletions of the μ switch region occur in IgM-producing spleen cells prior to switching (1, 6), but the role of this event in subsequent switching to downstream isotypes is unknown (1, 6, 42).

As reported here, we have observed that the higher switching PC1.4.1c hybridomas frequently undergo deletions of the γ1 switch region prior to switching. These very frequent events have not been reported previously for hybridomas to our knowledge. PC1.4.1c cells may have reexpressed an activity that can cut and ligate the γ1 switch region. This activity may be involved in switching and normally may be repressed in hybridomas. We have not observed similar deletions in the γ1 switch region of 36.65.12.7 subclones (Fig. 3 and results not shown). This may reflect differences in the 36.65.12.7 and PC1.4.1c γ1 switch regions. PC1.4.1c retains a germ-line 9.4-kb *Sac* I fragment, whereas much of the γ1 switch region was deleted in the original 36.65.12.7 isolate (results not shown). Thus, 36.65.12.7 may have already lost the part of the γ1 switch region that is the target of deletion in PC cells. Since we cannot quantitate this activity, we do not know if it correlates with the frequency of switching, but we have observed a modest increase in the incidence of deletions in the course of isolating high-switching subclones.

We also observed a duplication of the γ1 constant region gene in high-switch variants of 36.65.12.7. Although this duplicated gene does not appear to have a 5′ switch region or to participate directly in the switching events, we cannot rule out the possibility that it plays some indirect role in increasing the frequency of switching.

We do not know whether increased switching in these hybridoma cell lines is associated with progressively increased accessibility (8), with increased amounts of some or all of the components of the putative switch recombinase, or with the decreased expression or activity of a negative regulatory factor that is normally expressed in plasma cells. The presence of sterile transcripts has been shown to precede switching in normal spleen cells and in some B-cell lines (8–10). Sterile transcripts have not been observed in hybridomas, but this is not surprising since they normally switch at very low frequencies. We have used the relatively insensitive technique of Northern analysis to search for sterile transcripts in the higher switching subclones reported here and have not detected them when using 10-fold more RNA than we needed for the control RNA isolated from lipopolysaccharide-stimulated normal spleen cells (unpublished data). This may be because even in the highest switching subclones, sterile transcripts are only expressed in cells just prior to switching. At any given time the number of such cells is relatively small even in the high-switching variants, while in stimulated normal spleen cells the switching event occurs in a more synchronous fashion. Nevertheless, our inability to detect sterile transcripts suggests that even though each of the high-switching subclones has the potential to switch, the recipient switch sites of the population as a whole are not being transcribed.

The hybridoma subclones described in this paper switch spontaneously from γ1 to γ2b and γ2a at quite high frequencies. Starting with an unrelated hybridoma, we have recently used a similar process to enrich for subclones that switch from γ3 to γ1, γ2b or γ2a, suggesting that high-switching variants can be obtained from most, if not all, hybridoma cell lines. The phenotype is stable to repeated subcloning and may persist after the switching event so that further downstream switch variants are readily obtained.

The high rate of secretion by hybridomas allows single-cell analysis of switch variants and quantitation of switching rates. Using this system, we can readily quantitate and optimize the cellular response to lymphokines and other agents. Furthermore, the existence of closely related clones with large differences in rates of switching may allow genetic and biochemical approaches to studying switching and lead ultimately to an understanding of the underlying mechanism and its absence in terminally differentiated plasma cells.

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1. Cebra, J. J., Komisar, J. L. & Schweitzer, P. A. (1984) *Annu. Rev. Immunol.* **2**, 493–548.
2. Honjo, T., Nakai, S., Nishida, Y., Kataoka, T., Yamawaki-Kataoka, Y., Takahashi, N., Obata, M., Shimizu, A., Yaoita, Y., Nikaido, T. & Ishida, N. (1981) *Immunol. Rev.* **59**, 33–68.
3. Marcu, K. B., Lang, R. B., Stanton, W. L. & Harris, L. J. (1982) *Nature (London)* **298**, 87–89.
4. Dunnick, W., Hertz, G. Z., Scappino, L. & Gritzmacher, L. (1993) *Nucleic Acids Res.* **21**, 365–372.
5. Radbruch, A., Bruger, C., Klein, S. & Muller, W. (1986) *Immunol. Rev.* **89**, 69–84.
6. Esser, C. & Radbruch, A. (1990) *Annu. Rev. Immunol.* **8**, 717–735.
7. Snapper, C. M. & Mond, J. J. (1993) *Immunol. Today* **14**, 15–17.

8. Alt, F. W., Blackwell, T. K., DePinho, R. A., Reth, M. G. & Yancopoulos, G. D. (1986) *Immunol. Rev.* **89**, 5–30.
9. Stavnezer, J., Radcliffe, G., Lin, Y. C., Nietupski, J., Berggren, L., Sitia, R. & Severinson, E. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7704–7708.
10. Shockett, P. & Stavnezer, J. (1991) *J. Immunol.* **147**, 4374–4383.
11. Jung, S., Rajewsky, K. & Radbruch, A. (1993) *Science* **259**, 984–987.
12. Zhang, J., Bottaro, A., Li, S., Stewart, V. & Alt, F. W. (1993) *EMBO J.* **12**, 3529–3537.
13. Waters, S. H., Saikh, K. U. & Stavnezer, J. (1989) *Mol. Cell. Biol.* **9**, 5594–5601.
14. Wuerffel, R., Nathan, A. T. & Kenter, A. L. (1990) *Mol. Cell. Biol.* **10**, 1714–1718.
15. Williams, M. & Maizels, N. (1991) *Genes Dev.* **5**, 2353–2361.
16. Schultz, C. L., Elenich, L. A. & Dunnick, W. A. (1991) *Int. Immunol.* **3**, 109–116.
17. Rothman, P., Li, S. C., Gorman, B., Glimcher, L., Alt, F. & Boothby, M. (1991) *Mol. Cell. Biol.* **11**, 5551–5561.
18. Liao, F., Giannini, S. L. & Birshtein, B. K. (1992) *J. Immunol.* **148**, 2909–2917.
19. Lin, Y. A. & Stavnezer, J. (1992) *J. Immunol.* **149**, 2914–2925.
20. Illger, H. & Radbruch, A. (1992) *Mol. Immunol.* **29**, 1265–1272.
21. Xu, L. Z., Kim, M. G. & Marcu, K. B. (1992) *Int. Immunol.* **4**, 875–887.
22. Mizuta, T. R., Fukita, Y., Miyoshi, T., Shimizu, A. & Honjo, T. (1993) *Nucleic Acids Res.* **21**, 1761–1766.
23. Ott, D., Alt, F. W. & Marcu, K. (1987) *EMBO J.* **6**, 577–584.
24. Preud'homme, J.-L., Birshtein, B. K. & Scharff, M. D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1427–1430.
25. Dangl, J. L., Parks, D. R. & Herzenberg, L. A. (1982) *Cytometry* **2**, 395–401.
26. Spira, G., Bargellesi, A., Teillaud, J. L. & Scharff, M. D. (1984) *J. Immunol. Methods* **74**, 307–315.
27. Stavnezer, J., Sirlin, S. & Abbott, J. (1985) *J. Exp. Med.* **161**, 577–601.
28. Burrows, P. D., Beck, G. B. & Wabl, M. R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 564–568.
29. Lutzker, S. & Alt, F. W. (1988) *Mol. Cell. Biol.* **8**, 1849–1852.
30. Whitmore, A. C., Prowse, D. M., Arnold, L. W. & Haughton, G. (1989) *Int. Immunol.* **1**, 532–539.
31. Spira, G. & Scharff, M. D. (1992) *J. Immunol. Methods* **148**, 121–129.
32. Desaymard, D., Giusti, A. M. & Scharff, M. D. (1984) *Mol. Immunol.* **21**, 961–967.
33. Rothstein, T. L. & Getter, M. L. (1983) *Mol. Immunol.* **20**, 161–168.
34. Greene, G., Hodous, J., Dintzis, R. Z. & Dintzis, H. M. (1990) *J. Immunol. Methods* **129**, 187–197.
35. French, D., Kelly, T., Buhl, S. & Scharff, M. D. (1988) *Methods Enzymol.* **151**, 50–66.
36. Luria, S. E. & Delbruck, M. (1943) *Genetics* **28**, 491–511.
37. Lea, D. E. & Coulson, C. A. (1949) *J. Genet.* **119**, 264–275.
38. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
39. Mowatt, M. R., Dery, C. & Dunnick, W. (1985) *Nucleic Acids Res.* **13**, 225–237.
40. Eckhardt, L. A. & Birshtein, B. K. (1985) *Mol. Cell. Biol.* **5**, 856–868.
41. Aguila, H. L. (1988) Ph.D. dissertation (Albert Einstein College of Medicine, Bronx, NY).
42. Mukherjee, J., Casadevall, A. & Scharff, M. D. (1993) *Mol. Immunol.* **30**, 1049–1055.