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Immunoglobulin transgenes as targets for somatic hypermutation

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ABSTRACT This review describes studies on somatic hypermutation of immunoglobulin genes that were started in the mid-80s in collaboration with Ralph Brinster. Almost all of the experiments were carried out using Ig transgenes as targets for the somatic mutation mechanism. Ig transgenes can be very good targets of somatic mutation, despite many different transgene integration sites. Thus, the required *cis*-acting elements must be present within the approximately 10 kb of the transgene. Only the Ig variable region and its proximate flanks are mutated, not the constant region in unmanipulated sequences. Several Ig gene enhancers are permissive for somatic mutation and they do not have to be associated with the Ig promoter they normally interact with. However, the mutation process does seem to be specific for Ig genes. No mutations were found in several housekeeping genes isolated from cells that had very high levels of somatic hypermutation of their Ig genes. This suggests that the Ig enhancers provide the Ig gene specificity. An exception is the *Bcl-6* gene, encoding a transcription factor, which was found to be mutated in normal human memory B cells. When the transcriptional promoter that is located upstream of the variable region is duplicated upstream of the constant region, this region is mutated as well. This suggests a transcription coupled model in which a mutator factor associates with the RNA polymerase at the initiation of transcription, travels with the polymerase during elongation, and causes mutations during polymerase pausing. Our recent data with an artificial substrate for somatic mutation suggest that the mutations are increased by increased stability of the secondary structures in the nascent RNA, and the specific nucleotides that are mutated are due to preferences of a mutator factor.

KEY WORDS: *immunoglobulin genes, somatic hypermutation, transgenic mice*

Introduction

In the early 80s I called Ralph Brinster to find out if he might have an interest in questions of immunoglobulin (Ig) gene expression. This first contact I had with Ralph revealed him as a very kind and interested man. It was the beginning of a wonderful collaboration that lasted almost ten years. At that time, the transgenic method developed by Ralph was practised in very few labs and most of the mice were produced by his group. The transgenes previously used, that showed expression, all contained viral sequences, but Ralph was very agreeable to try for the first time a mouse gene that had no extraneous additions. The first transgene we experimented with was a functional, rearranged Ig κ light chain gene. It turned out that its expression was completely tissue specific, occurring only in B lymphocytes of several independent transgenic lines (Brinster *et al.,* 1983; Storb *et al.,* 1984,1985). Not only was the transgene expressed normally, it also answered the question we had posed: do B lymphocytes recognize that they contain a functional, rearranged light chain gene? Indeed, the rearrangement of endo-

Abbreviations used in this paper: EPS, EcoRV-PvuII sequence; H and L, heavy and light chains; Ig, immunoglobulin; MMR, mismatch repair; MuF, mutator factor; NT, nucleotide; PC, phosphorylcholine; PNA, peanut agglutinin; V (C) region, variable (constant) region of Ig.

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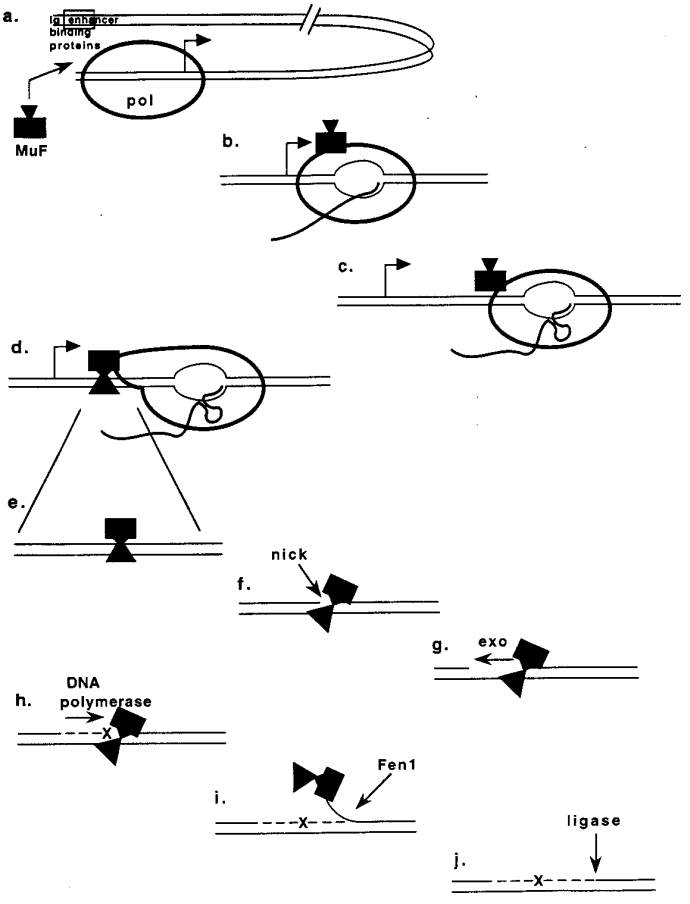

Fig. 1. Model of somatic hypermutation of *Ig* genes. *MuF, mutator factor; pol, RNA polymerase II; exo, DNA exonuclease; x, point mutation; Fen1, flap endonuclease 1. From Storb et al., 1998a.*

genous light chain genes was greatly inhibited in these transgenic mice. Most of the B cells expressed only the transgenic, and not the endogenous light chains (Ritchie *et al.,* 1984).

The collaboration with Ralph produced several other very interesting findings (reviewed in Storb, 1987,1995), but this paper will concentrate on our joint attack on the problem of somatic hypermutation and the more recent outgrowth from those experiments.

Ig transgenes can undergo somatic hypermutation

As mentioned, *Ig* genes are expressed only in B lymphocytes. Their precursors, preB cells, have the ability to create a functional heavy (H) and light (L) chain gene by V(D)J recombination of gene segments. This process occurs in the bone marrow and is rather wasteful, leading to out-of-frame, nonfunctional rearrangements about 90% of the time. Cells that have successfully rearranged both a heavy and a light chain gene leave the bone marrow as B lymphocytes. The encounter of a B cell with an immunizing antigen that has a reasonable binding affinity with the Ig expressed by the cell, can, upon interaction with T cells, lead to somatic hypermutation of the expressed *Ig* genes. Somatic hypermutation occurs in germinal centers of lymphoid tissues. The mutating B cells undergo several rounds of rapid proliferation. In general, only the variable (V) regions and their flanks, not the constant (C) regions of the H and L genes, become mutated. In this way, the antigen binding properties of the encoded Ig proteins can change. Cells with mutations that confer higher affinity to the immunizing antigen are selected to live, cells that have mutated to lower affinity, or that have introduced a stop codon, die by apoptosis (Berek, 1992).

In the mid-eighties, it had become evident that somatic hypermutation can create large numbers of point mutations, up to several percent of the nucleotides (NT) in the V(D)J regions and their proximate flanks can be altered (Kim *et al.,* 1981; Selsing and Storb 1981; Gearhart and Bogenhagen 1983). The sources for the study of mutated *Ig* genes were malignant B cells, such as myelomas and lymphomas, or hybridomas produced from immunized mice. The mechanism generating the somatic mutations was completely obscure.

Rebecca O'Brien, a graduate student at the time, decided to investigate if Ig transgenes can be somatically mutated (O'Brien *et al.,* 1987). She studied mice that carried a Vκ167 transgene that encodes the light chain of anti-phosphorylcholine (PC) antibodies (Storb *et al.,* 1986). To be certain that the cells she chose had undergone somatic hypermutation she selected anti-PC hybridomas whose endogenous heavy chain genes were mutated (O'Brien *et al.,* 1987). She found that most had also mutated the light chain transgene. This was true for two different transgenic lines, suggesting that many integration sites may permit somatic mutation. The data clearly showed that the *cis*-acting elements required for somatic mutation were present in the ~12 kb long transgenes. Furthermore, only the V regions, but not the C regions appeared to be mutated.

Limitation of the mutations to the VJ region and about 1 kb beyond, but sparing of the C region was further supported by findings of John Hackett and Brian Rogerson in more extensive sequencing analyses of these transgenes (Hackett *et al.,* 1990; Rogerson *et al.,* 1991). The mutations were found to extend to the first half of the JC intron and ceased well before the matrix attachment region and the kappa intron enhancer.

Somatic hypermutation is linked to transcription initiation

Findings from the Claflin laboratory showed that the 3' extent of somatic hypermutation is related to the position of the VJ joint in κ genes (Weber *et al.,* 1991a,b). When VJ rearrangement is to *Jκ1*, the mutations peak over the VJ region and then steadily decrease so that no mutations are present in *Jκ5* or the JC intron. However, when V is rearranged to the most 3' J gene, *Jκ5*, the mutations extend into the middle of the JC intron. This is the same distribution as was found by P. Gearhart's laboratory in endogenous *Vκ167/Jκ5* genes and in our lab in transgenes derived from *Vκ167/Jκ5* (Gearhart and Bogenhagen 1983; Hackett *et al.,* 1990; Rogerson *et al.,* 1991). These differences in 3' boundary of hypermutability suggested that something in the 5' end of the gene or the VJ joint determined the mutation tract.

To assess if the transcriptional promoter was responsible for this distribution, A. Peters produced a kappa transgene in which the normal V gene promoter was duplicated, together with the leader segment, just 5' of the constant region gene (Peters and Storb, 1996). Surprisingly, this transgene, P5'C, was hypermutated both in the VJ region and the C region, but not in the region between them. The mutation frequency was similar in the VJ region and in

the C region. Transcripts were found that initiated from the upstream promoter and ended 3' of the constant region at the normal polyA site. Transcripts were also found in at least equal quantities that initiated at the downstream promoter and ended at the same polyA site. These results suggested that susceptibility to mutation is induced at the transcription initiation site and persists for approximately 1 kb into the transcription unit. The findings led to the formulation of a new model of somatic hypermutation.

The model postulated that, directed by the Ig enhancer, a mutator factor (MuF) binds to the RNA polymerase at the promoter, travels with the polymerase during the elongation phase of transcription, and induces pausing of the polymerase (Peters and Storb, 1996; Storb, 1996; Storb *et al.,* 1996,1998b). This pausing was proposed to lead to a stable polymerase/MuF complex which was recognized as a DNA lesion by a DNA repair system, such as transcription coupled nucleotide excision repair (NER). During NER the single stranded DNA with the bound complex was proposed to be excised and resynthesized with the occasional introduction of errors. These errors were postulated to represent the somatic point mutations.

Nucleotide excision repair and DNA mismatch repair appear not to be required for somatic hypermutation

To test the NER aspect of the new somatic mutation model, we have investigated B lymphocytes from patients and mice with severe defects in NER (Kim *et al.,* 1997; Shen *et al.,* 1997). Epstein Bar Virus transformed B lymphocytes from patients suffering from various forms of Xeroderma Pigmentosum (XP-B, XP-D, and XP-V) or of Cockayne syndrome (CS-A) showed high levels of somatic mutation in the V and J regions of their expressed heavy and light chain genes (Kim *et al.,* 1997). The C regions were not mutated, suggesting that the V and J region mutations were indeed due to somatic hypermutation. In further support of somatic hypermutation, V genes associated with switched CH genes were more highly mutated than those associated with Cμ genes, and the ratios of replacement to silent mutations were greater in the complementarity determining regions than in the framework regions of the V genes. The four patients were chosen because they had signs of transcriptional defects, suggesting special deficiencies in transcription coupled DNA repair. It appeared therefore, that transcription coupled NER was not required for somatic hypermutation. Also, an XP-D defect without transcription defects, does not interfere with somatic mutation (Wagner *et al.,* 1996). Finally, we investigated mice with a knockout of yet another NER gene, XP-C, that has been implicated in the repair of the non-transcribed DNA strand (Shen *et al.,* 1997). These mice as well showed normal levels of somatic hypermutation.

Recently, DNA mismatch repair (MMR) deficiency was shown to eliminate the preferential repair of the transcribed DNA strand (Mellon *et al.,* 1996). It was therefore possible, that MMR was required for somatic hypermutation. Mice with defects in the mammalian homologs of the *E.coli MMR* genes Mut L (MLH1, PMS2) have been investigated for their ability to somatically mutate *Ig* genes (Gearhart *et al.,* personal communication; N. Kim, J. Lo, and U. Storb, unpublished). All appeared capable of high levels of somatic hypermutation. There was some suggestion, however, that the distribution and fine patterns of the mutations may be altered (P. Gearhart, personal communication). This question is under further investigation.

These data combined suggested that these general mechanisms of DNA repair are not recruited for the process of somatic hypermutation of *Ig* genes. We will return to a modified transcription-linked model of somatic mutation after an excursion into the question of the *Ig* gene specificity of the mutation process.

Specificity of somatic hypermutation for immunoglobulin genes

In natural *Ig* genes, only the V regions and their proximate flanks are targets for somatic hypermutation. However, in transgenic experiments quite a variety of other sequences have now been shown to be hypermutable when in the context of an *Ig* gene. These include the bacterial genes encoding chloramphenicol acetyl transferase, guanosine phosphoribosyl transferase, and neomycin phosphotransferase, the human β-globin gene, the constant region of Ig light chain genes, and a completely artificial substrate, EPS (Azuma *et al.,* 1993; Yelamos *et al.,* 1995; Peters and Storb, 1996; Klotz *et al.,* 1997). Furthermore, in experiments of nature, the *c-myc* and *Bcl-6* genes translocated into the Ig heavy chain locus in certain naturally occurring lymphomas are also hypermutated (Rabbits *et al.,* 1983; Taub *et al.,* 1984; Migliazza *et al.,* 1995). Thus, V-gene sequences are not unique targets. Likewise, the V-gene promoter is also not specifically required, but can be replaced by the β-globin promoter or the B29 promoter (Betz *et al.,* 1994; Tumas-Brundage and Manser, 1997), or, in the *c-myc* and *Bcl-6* translocations, by these genes' respective promoters. Elimination of the Ig enhancer decreases or eliminates somatic hypermutation (Betz *et al.,* 1994). Replacement of an Ig enhancer with some other enhancer has not been tested, but any of the Ig enhancers appears to be sufficient for the mutation process (Klotz and Storb, 1996). Since different Ig enhancers possess rather different transcriptional motifs, all of which can also be present in enhancers of non-Ig genes, these findings raised the question of whether somatic mutation is really specific to *Ig* genes.

An alternative possibility is that the somatic hypermutation process may be able to target many genes expressed during the short specific period in the life of the B cell when the postulated mutator factor is expressed. To investigate this question in as natural a setting as possible, we have searched for somatic mutations in a number of non-Ig genes that are expressed in B cells while the somatic mutation process is ongoing (A. Peters, N. Kim, B. Rogerson and U. Storb, unpublished). The expressed genes we chose, all have a long 5' untranslated region with the initiator AUG far 3' of where mutations occur in *Ig* genes; so, if mutations occur, they would not change the coding sequences of the gene. Germinal center B cells were isolated from hyperimmunized mice by use of the markers PNA^{hi} and $B220^{hi}$. The VH genes sequenced from rearranged *Ig* genes were found to be mutated at a frequency of about 1% indicating that the cells had undergone an average level of somatic hypermutation. We then sequenced the housekeeping genes *c-myc* and *β-actin*, but found no significant numbers of somatic mutations.

Since these mouse B cells had only average levels of somatic point mutations in their Ig genes, we then turned to memory B cells from a normal human donor. The *Ig* genes in several samples of memory B cells from this donor were mutated at an extremely high frequency. Over 6% on average of V region nucleotides in total expressed *Ig* genes, and 12% on average in *IgG* genes were mutated. In this person, the housekeeping genes *c-myc* and *β-*

actin were again not mutated, nor were the highly expressed ribosomal protein gene S14 or the unexpressed α-fetoprotein gene. However, the transcription factor *Bcl-6* gene was mutated at a significant level, 3x10-4 mutations/nucleotide. This was about 100x lower than the mutation frequency in the *Ig* genes, but at least three orders of magnitude higher than spontaneous mutations. This appeared to be somatic hypermutation, since in non-immune B cells of this donor, the *Bcl-6* gene was not mutated. As a control, the *Bcl-6* gene was also sequenced in the mouse B cells investigated earlier. However, like the *β-actin* and *c-myc* genes, the *Bcl-6* gene was not mutated in the mouse B cells. Given the 100x lower mutations of the *Bcl-6* gene compared with the *Ig* gene in the human cells, mutations in the mouse *Bcl-6* gene would have had to be relatively higher to be detectable. Two other human donors, but not a fourth, also showed relatively high mutation frequency in Bcl-6, but not other genes (Shen *et al*., 1998).

Taken together we interpret the findings with human and mouse non-Ig genes to indicate that the somatic hypermutation process is mostly specific for *Ig* genes. We presume that this specificity is determined mainly by the Ig enhancers. They all share one motif, the E2A site, which is a possible candidate for part of this specificity. The detailed unraveling of the basis of the Ig gene specificity of somatic hypermutation remains a fascinating challenge for further investigation.

The sites of somatic hypermutation appear to be determined by the combination of the secondary structure of the nascent *Ig* gene transcript and the nucleotide preferences of a mutator factor

As described earlier, somatic hypermutation is apparently induced by a mutator factor traveling with the RNA polymerase during transcription. Recent experiments have given some clues as to how the mutations may be introduced. We have created an Ig κ transgene that contains, inserted into the V region, an artificial mutation substrate (Klotz *et al.,* 1997). The substrate consists of alternating EcoRV and PvuII restriction enzyme sites (EPS). Mutations in the EPS are easily assayed by DNA amplification and restriction enzyme analysis. The products can be electrophoretically separated, resulting in a ladder of small fragments when no mutations have occurred. When a mutation exists in the EPS, two small fragments disappear and a larger one is created. The EPS transgene is thus a useful substrate for a quick mutation assay.

When transgene copies with EPS mutations were sequenced, including the flanking V and J regions, we were surprised to find that the EPS is on average mutated 7x more frequently than the flanks (Storb *et al.,* 1998a). Moreover, within the EPS a hierarchy of mutability exists. Certain nucleotide combinations are mutated much more frequently than others in every restriction site that contains the respective nucleotides. For example, GC and TA appear hypermutable, whereas TG and GA are disfavored. The preferred targets turned out to be components of hotspots observed in a number of studies of somatic hypermutations (Rogozin and Kolchanov, 1992; Betz *et al.,* 1993; Smith *et al.,* 1996; Doerner *et al.,* 1997). In fact, comparing the EPS sequence with the sequences analyzed in a large survey of somatically mutated Ig genes (Smith *et al.,* 1996), showed a rather striking correlation of hyper- and hypo-mutability within the EPS with the di- and trinucleotides that were favored or disfavored for somatic mutation of normal *Ig* genes.

This alone, however, did not explain why the EPS was hypermutable compared with the V and J flanks. These flanks also contained similar representation of the susceptible di- and trinucleotides. Different to the flanks, though, the EPS sequence, because of its repeated restriction sites, can form a series of stem-loop structures with high stability of the stems. Such secondary structures could form either on the DNA or the RNA level. At present we favor the view that they play a role in somatic mutation as RNA structures (see the model, below).

A model of somatic hypermutation of *Ig* genes

We propose two major rules for somatic hypermutation from the results with the EPS transgene. First, the overall susceptibility of the region where point mutations occur is determined by the secondary structure of the nascent RNA. Second, the exact nucleotides mutated depend on the nucleotide preferences of a mutator factor that is specific for the somatic hypermutation of *Ig* genes. The findings with the EPS transgene have led us to modify the original transcription based model (Peters and Storb, 1996) by postulating that the polymerase pausing occurs by the natural mechanisms that function during normal transcription (Fig. 1) (Storb *et al.,* 1998a). Furthermore, the role of a mutator factor (MuF) is to interact directly with DNA near the pause site to cause the mutations. For example, MuF could be a nuclease that nicks DNA, which is followed by excision of a short patch of single stranded DNA by general exonucleases. Repolymerization of the patch could be error prone due to the influence of the MuF.

The proposed model is summarized in Figure 1:

(a) MuF associates with RNA polymerase II (pol). This can only happen if the polymerase (or MuF) has interacted with Ig enhancer binding proteins during initiation of transcription at an *Ig* gene promoter. **(b)** MuF travels with the polymerase during transcript elongation. **(c)** If the RNA polymerase encounters an impediment to transcription (e.g., a hairpin in the nascent transcript), it pauses. **(d)** Perhaps under the influence of elongation factors, the conformation of the pausing polymerase changes, resulting in the transfer of MuF to the DNA. **(e)** MuF binds to double-stranded DNA upstream of the polymerase.

How the MuF then causes mutations is rather speculative, but one testable possibility is that:

(f) MuF nicks the non-transcribed strand, remaining associated with the newly created 5' end and also with one or several nucleotides on the transcribed strand. **(g)** Because of the bound MuF, the single-stranded ends cannot be ligated. Exonuclease trims back the single-stranded 3' end. **(h)** A DNA polymerase fills in the gap creating mutation(s) (x) opposite the MuF associated base(s). **(i)** The DNA polymerase continues past the MuF bound residues, creating a 5' flap of the nontranscribed strand. The flap is cut by endonuclease Fen1 (DNase IV). **(j)** The free DNA ends are ligated. MuF has been removed with the flap.

The postulated MuF would only be produced during the short window in the life of the B cell when somatic hypermutation is ongoing. It can only bind to an initiating RNA polymerase II (pol) after interaction with transcription factors bound to the Ig enhancer. It cannot randomly bind to pol or DNA. This property of MuF coupled with a high chance for polymerase pausing within the first

1 kb or so of transcribed DNA explains the extent of somatic hypermutations over only 1-2 kb of the 5' region of the *Ig* gene with sparing of the 3' region (reviewed in Storb, 1996).

This novel model of somatic hypermutation of *Ig* genes has several aspects that can be further tested by studying the proteins associated with the EPS transgene during somatic mutation and creating modifications of the EPS transgene. Some of these studies can be carried out in transgenic mice, others may be feasible in recently developed cell lines that appear to somatically mutate *Ig* genes in culture (Denepoux *et al.,* 1997).

Conclusions

Clearly, the transgenic mouse technology has been invaluable. Due to the lack of a mutating cell line until very recently it has been the only tool to study specifically designed *Ig* genes for the *cis* acting elements in somatic hypermutation. We are extremely grateful to Ralph Brinster. It has been a great pleasure to know Ralph and benefit from his enormous expertise, generosity, and scientific insights during our collaboration. The fruit of our collaborative period continues to enhance our current projects and Ralph is still a wise consultant when we have problems with the transgenic technology.

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References

AZUMA, T., MOTOYAMA, N., FIELDS, L. and LOH, D. (1993). Mutations of the chloramphenicol acetyl transferase transgene driven by the immunoglobulin promoter and intron enhancer. *Int. Immunol. 5:* 121-130.

BEREK, C. (1992). The development of B-cells and the B-cell repertoire in the microenvironment of the germinal center. *Immunol. Rev. 126:* 5-19.

BETZ, A.G., NEUBERGER, M.S. and MILSTEIN, C. (1993). Discriminating intrinsic and antigen-selected mutational hotspots in immunoglobulin V genes. Immunol. Today *14:* 405-411.

BETZ, A., MILSTEIN, C., GONZALEZ-FERNANDES, R., PANNELL, R., LARSON, T. and NEUBERGER, M. (1994). Elements regulating somatic hypermutation of an immunoglobulin K gene: critical role for the intron enhancer/matrix attachment region. *Cell 77:* 239-248.

BRINSTER, R., RITCHIE, K., HAMMER, R., O'BRIEN, R., ARP, B. and STORB, U. (1983). Expression of a microinjected immunoglobulin gene in the spleen of transgenic mice. *Nature 306:* 332-336.

DENEPOUX, S., RAZANAJAONA, D., BLANCHARD, D., MEFFRE, G., CAPRA, D., BANCHEREAU, J. and LEBEQUE, S. (1997). Induction of somatic mutation in a human B cell line *in vitro*. *Immunity 6:* 35-46.

DOERNER, T., BREZINSCHEK, H.-P., BREZINSCHEK, R., FOSTER, S., DOMIATI-SAAD, R. and LIPSKY, P. (1997). Analysis of the frequency and pattern of somatic mutations ithin nonproductively rearranged human variable heavy chain genes. *J. Immunol. 158:* 2779-2789.

GEARHART, P.J. and BOGENHAGEN, D.F. (1983). Clusters of point mutations are found exclusively around rearranged antibody variable genes. *Proc. Natl. Acad. Sci. USA 80:* 3439-3443.

HACKETT, J., ROGERSON, B., O'BRIEN, R. and STORB, U. (1990). Analysis of somatic mutations in k transgenes. *J. Exp. Med. 172:* 131-137.

KIM, N., KAGE, K., MATSUDA, F., LEFRANC, M.-P. and STORB, U. (1997). B lymphocytes of xeroderma pigmentosum or Cockayne syndrome patients with inherited defects in nucleotide excision repair are fully capable of somatic hypermutation of immunoglobulin genes. *J. Exp. Med. 186:* 413-419.

KIM, S., DAVIS, M., SINN, E., PATTEN, P. and HOOD, L. (1981). Antibody diversity: somatic hypermutation of rearranged VH genes. *Cell 27:* 573-581.

KLOTZ, E. and STORB, U. (1996). Somatic hypermutation of a lambda-2 transgene under the control of the lambda enhancer or the heavy chain intron enhancer. *J. Immunol. 157:* 4458-4463.

KLOTZ, E., HACKETT, J.J. and STORB, U. (1998). Somatic hypermutation of an artificial test substrate within an Ig kappa transgene. *J. Immunol. 161:* 782-790.

MELLON, I., RAJPAL, D., KOI, M., BOLAND, C. and CHAMPE, G. (1996). Transcription-coupled repair deficiency and mutations in human mismatch repair genes. *Science 272:* 557-560.

MIGLIAZZA, A., MARTINOTTI, S., CHEN, W., FUSCO, C., YE, B., KNOWLES, D., OFFIT, K., CHAGANTI, R. and DALLA-FAVERA, R. (1995). Frequent somatic hypermutation of the 5' noncoding region of the BCL6 gene in B-cell lymphoma. *Proc. Natl. Acad. Sci. USA 92:* 12520-12524.

O'BRIEN, R., BRINSTER, R. and STORB, U. (1987). Somatic hypermutation of an immunoglobulin transgene in k transgenic mice. *Nature 326:* 405-409.

PETERS, A. and STORB, U. (1996). Somatic hypermutation of immunoglobulin genes is linked to transcription initiation. *Immunity 4:* 57-65.

RABBITS, T., HAMLYN, P. and BAER, R. (1983). Altered nucleotide sequences of a translocated c-myc gene in Burkitt lymphoma. *Nature 306:* 760-765.

RITCHIE, K.A., BRINSTER, R.L. and STORB, U. (1984). Allelic exclusion and control of endogenous immunoglobulin gene rearrangement in k transgenic mice. *Nature 312:* 517-520.

ROGERSON, B., HACKETT, J. and STORB, U. (1991). Somatic hypermutation in transgenic mice. In *Somatic Hypermutation in V-Regions*. (E.J. Steele). CRC Press, Boca Raton, pp. 115-127.

ROGOZIN, I. and KOLCHANOV, N. (1992). Somatic hypermutagenesis in immunoglobulin genes. II. Influence of neighboring base sequences on mutagenesis. *Biochim. Biophys. Acta 1171:* 11-18.

SELSING, E. and STORB, U. (1981). Somatic mutation of immunoglobulin light-chain variable-region genes. *Cell 25:* 47-58.

SHEN, H.M., CHEO, D.L., FRIEDBERG, E. and STORB, U. (1997). The inactivation of the XP-C gene does not affect somatic hypermutation or class switch recombination of immunoglobulin genes. *Mol. Immunol. 34:* 527-533.

SHEN, H.M., PETERS, A., BARON, B., ZHU, X. and STORB, U. (1998). Mutation of BCL-6 gene in normal B cells by the process of somatic hypermutation of Ig genes. *Science 280:* 1750-1752.

SMITH, D., CREADON, G., JENA, P., PORTANOVA, J., KOTZIN, B. and WYSOCKI, L. (1996). Di- and trinucleotide target preferences of somatic mutagenesis in normal and autoreactive B cells. *J. Immunol. 156:* 2642-2652.

STORB, U. (1987). Transgenic mice with immunoglobulin genes. *Annu. Rev. Immunol. 5:* 151-174.

STORB, U. (1995). Ig gene expression and regulation in Ig transgenic mice. In *Immunoglobulin Genes*. (T. Honjo and F. Alt, eds.): Academic Press.

STORB, U. (1996). The molecular basis of somatic hypermutation of immunoglobulin genes. *Curr. Opin. Immunol. 8:* 206-214.

STORB, U., KLOTZ, E., HACKETT, J., KAGE, K., BOZEK, G. and MARTIN, T. (1998a). A hyérmutable insert in an immunoglobulin transgene contains hotspots of somatic mutation and sequences predicting highly stable structures in the RNA transcript. *J. Exp. Med. 188:* 689-698.

STORB, U., O'BRIEN, R.L., MCMULLEN, M.D., GOLLAHON, K.A. and BRINSTER, R.L. (1984). High expression of cloned immunoglobulin k gene in transgenic mice is restricted to B lymphocytes. *Nature 310:* 238-241.

STORB, U., PETERS, A., KLOTZ, E., KIM, N., SHEN, H.M., KAGE, K. and ROGERSON, B. (1998b). Somatic hypermutation of immunoglobulin genes is linked to transcription. *Curr. Top. Microbiol. Immunol. 229:* 11-19.

STORB, U., PETERS, A., KLOTZ, E., ROGERSON, B. and HACKETT, J. (1996). The mechanism of somatic hypermutation studied with transgenic and transfected target genes. *Semin. Immunol. 8:* 131-140.

STORB, U., PINKERT, C., ARP, P., ENGLER, P., GOLLAHON, K., MANZ, J., BRADY, W. and BRINSTER, R.L. (1986). Transgenic mice with m and k genes encoding antiphosphocholine antibodies. *J. Exp. Med. 164:* 627-641.

STORB, U., RITCHIE, K., HAMMER, R., O'BRIEN, R., MANZ, J., ARP, B. and BRINSTER, R. (1985). Expression of a microinjected immunoglobulin κ gene in

transgenic mice. *Bambury Report 20: Genetic manipulation of the Early Mammalian Embryo.* Costantini and Jaenish, eds. Cold Spring Harbor Lab. pp. 197-209.

TAUB, R., MOULDING, C., BATTEY, J., MURPHY, W., VASICEK, T., LENOIR, G. and LEDER, P. (1984). Activation and somatic mutation of the translocated c-myc gene in Burkitt lymphoma cells. *Cell 36:* 339-348.

TUMAS-BRUNDAGE, K. and MANSER, T. (1997). The transcriptional promoter regulates hypermutation of the antibody heavy chain locus. *J. Exp. Med. 185:* 239-250.

WAGNER, S., ELVIN, J., NORRIS, P., MCGREGOR, J. and NEUBERGER, M. (1996). Somatic hypermutation of Ig genes in patients with xeroderma pigmentosum (XP-D). *Int. Immunol. 8:* 701-705.

WEBER, J.S., BERRY, J., LITWIN, S. and CLAFLIN, J.L. (1991a). Somatic hypermutation of the JC intron is markedly reduced in unrearranged k and H alleles and is unevenly distributed in rearranged alleles. *J. Immunol. 146:* 3218-3226.

WEBER, J.S., BERRY, J., MANSER, T. and CLAFLIN, J.L. (1991b). Position of the rearranged Vk and its 5' flanking sequences determines the location of somatic mutations in the Jk locus. *J. Immunol. 146*: 3652-3655.

Yelamos, J., Klix, N., GoyenECHEA, B., LOZANO, F., CHUI, Y.L., GONZALEZ FERNANDEZ, A., PANNELL, R., NEUBERGER, M.S. and MILSTEIN, C. (1995). Targeting of non-Ig sequences in place of the V segment by somatic hypermutation. *Nature 376:* 225-229.