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High *RAD51* mRNA levels in young rabbit appendix. A role in B-cell gene conversion?

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Abstract The rabbit has a limited number of V_H genes that rearrange. As in the chicken, the 3′-most V_H1 gene is rearranged in most B lymphocytes. This laboratory reported that by 6 weeks after birth, diversification of rearranged V_H genes occurs, at least in part, by gene conversion-like events in the appendix, suggesting that this organ is a homologue of the avian bursa of Fabricius. Rad51 contributes to the repair of double-strand breaks in DNA during somatic and meiotic recombination. The gene was first identified in lower eukaryotes, and later in vertebrates including chicken, as encoding an *Escherichia coli* RecA-like protein. We report the cloning and sequencing of *RAD51* from the rabbit. Because the chicken bursa was shown to express high levels of *RAD51* message, we investigated the expression of *RAD51* in the rabbit appendix and other tissues. Using a quantitative polymerase chain reaction mimic assay and conventional northern analyses, we found high *RAD51* expression in young rabbit appendix comparable to levels in testis where there is an abundance of meiotic recombination. *RAD51* levels were three times higher in appendix B lymphocytes compared with T lymphocytes and were lower in adult appendix, as well as in spleen and Peyer's patches of young rabbits. We measured the levels of message in several appendix cell sub-populations obtained by fluorescence-activated cell sorting and found that sub-populations of B lymphocytes corresponding to different stages of B-cell development as well as B cells undergoing isotype switch did not have significantly different mRNA levels.

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Introduction

The *RAD51* gene, like many other genes involved in DNA repair activities, has been extensively studied in yeast (Aboussekhra et al. 1992; Muris et al. 1993; Ogawa et al. 1993; Shinohara et al. 1992, 1993). It belongs to the RAD52 epistasis group and appears to be a eukaryotic counterpart of *Escherichia coli* RecA (Shinohara et al. 1992). Its functions in repair of radiation-induced DNA damage as well as its double-strand break repair activity during meiosis and mitosis have been reviewed (Ogawa et al. 1995; Shinohara and Ogawa 1995). Homologues of this gene have been found in various species including human, mouse, and chicken (Bezzubova et al. 1993; Morita et al. 1993; Shinohara et al. 1993). Rad51 and RecA proteins appear to be similar functionally, in that they bind and polymerize on single-stranded DNA and form a similar filamentous structure (Benson et al. 1994; Ogawa et al. 1993). The function of Rad51 has not been completely elucidated. However, human Rad51 protein has been shown to promote ATP-dependent homologous pairing and strand transfer in vitro (Baumann et al. 1996). It binds to tumor-suppressor gene products such as p53 (Lim and Hasty 1996; Sturzbecher et al. 1996), BRCA-1 (Scully et al. 1997), and BRCA-2 (Mizuta et al. 1997; Sharan et al. 1997). It has also been shown to bind to Rad52 protein (reviewed in Shinohara and Ogawa 1995). Human Rad51 was also found in a large RNA polymerase II complex along with transcriptional coactivators (Maldonado et al. 1996). There have been two reports that the targeted disruption of *RAD51* in mouse leads to an embryonic lethal phenotype in $RAD51^{-/-}$ progeny (Lim and Hasty 1996; Tsuzuki et al. 1996). These and other observations suggest that the functions of Rad51 are manifold. Among the many functions pos-

tulated for this gene was its participation in *V*-gene diversification by gene conversion, because *RAD51* mRNA was found to be abundantly expressed in the chicken bursa of Fabricius (Bezzubova et al. 1993). In the chicken, a donor gene contributes part of its sequence information to the downstream rearranged V_H1 gene (reviewed in McCormack et al. 1993). As in chickens, rabbits diversify their antibody repertoire by a gene conversion-like process in gut-associated lymphoid tissue (Weinstein et al. 1994a, 1994b). Combinatorial diversification during *V(D)J* recombination is relatively limited in the rabbit because of preferential usage of the V_H1 gene (Allegrucci et al. 1991; Becker and Knight 1990; Friedman et al. 1994; Knight et al. 1990). In young developing rabbits, the appendix is a bursa-like organ (Weinstein et al. 1994a, 1994b). Immature B cells, with their V_HI gene already rearranged, seed follicles, expand, and undergo diversification by gene conversion (Weinstein et al. 1994a). We found high levels of *RAD51* mRNA in the appendix of young rabbits comparable to levels found in the rabbit testis and thymus, and reported in the chicken bursa of Fabricius (Bezzubova et al. 1993). In order to further examine whether there was a relationship between *RAD51* expression and the B-cell diversification processes that were occurring in this active lymphoid organ, we studied different appendix cell sub-populations obtained by flow cytometric sorting. Recent studies from this laboratory suggest that some de novo B-cell development may also occur in the appendix, with some B lymphocytes undergoing heavy and light chain gene rearrangements (Fuschiotti et al. 1997). RAG1 transcripts and heavy and light chain recombination excision products were detectable in both IgM^-CD43^+ and IgM^-CD43^- cell populations. In addition, based on fluorescence intensity, RAG2 protein appeared to be at the highest levels in the IgM^-CD43^+ followed by the IgM^+CD43^+ population in both three-color analyses and sorting experiments (Fuschiotti et al. 1997). These findings suggested that in addition to B cells, some B-cell precursors may seed the young rabbit appendix. Populations of B cells in the appendix may be dynamically maturing, differentiating, dividing, and also undergoing apoptotic death (Pospisil and Mage 1997; Pospisil et al. 1995).

In order to obtain a more complete picture of the different phases of B-cell development, we measured and compared *RAD51* mRNA levels in appendix T and B cells as well as in different appendix cell populations based on IgM and CD43 staining. In addition, because we found that there is a B-cell sub-population in the appendix with both IgA and IgM at the surface, indicating that a recent class switching event occurred, and because there was a recent report that Rad51 could also be involved in immunoglobulin isotype switching (Li et al. 1996), we used flow cytometry to investigate whether there was a difference in *RAD51* mRNA expression between single-positive IgA^+, IgM^+ and double-positive IgA^+IgM^+ cell sub-populations.

Materials and methods

Cloning and sequencing of rabbit RAD51 cDNA

The mRNA was obtained from the thymus of a 3-month-old rabbit (haplotype *F-I*) using the Micro Fast Track Kit (Invitrogen, Carlsbad, Calif.) and following the procedure indicated by the manufacturer. Synthesis of cDNA and amplification was carried out using the RNA reverse transcriptase polymerase chain reaction (PCR) Kit (Perkin Elmer, Foster City, Calif.) according to the manufacturer's instructions. Primers were from the human RAD51 sequence (Shinohara et al. 1993) at the ends of the open reading frame. The downstream primer (RAD3) used for reverse transcription and the upstream primer (RAD2) that was added to the reverse transcription product for PCR are shown in lowercase letters in Fig. 1. PCR conditions were: melting at 94 °C for 1 min 20 s, annealing at 60 °C for 2 min, and extension at 72 °C for 2 min for a total of 37 cycles followed by an elongation step at 72 °C for 7 min. The PCR product was ligated (pGEM-T Vector, Promega, Madison, Wis.) and transformed into *Epicurean coli* XL1-Blue competent cells (Stratagene). Plasmid DNA inserts from the positive colonies were sequenced with the Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif.) in an automated sequencer (Model 373) (Applied Biosystems). The first sequencing reactions used M13 forward and reverse primers. Subsequent reactions used customized internal primers in order to obtain the complete sequence. To rule out base changes introduced during PCR, we conducted three additional independent PCR cloning and sequencing experiments.

Northern analyses

The mRNA was obtained from different organs using the Invitrogen Fast Track Kit according to the manufacturer's directions. The thymus, spleen, appendix (young), Peyer's patches, and kidneys were taken from 5- to 9-week-old normal rabbits; testis and appendix (adult) were from a 1-year-old-rabbit. After extraction, the mRNA was treated with 20 units of RNase free-DNase I (Boehringer Mannheim, Indianapolis, Ind.) at 37 °C for 30 min, followed by phenol-chloroform-isoamyl alcohol extraction and ethanol precipitation. $Poly(A)^+$ RNA samples (3 μg) were run in a 1% agarose gel under denaturing conditions and then transferred to a nylon membrane (Schleicher & Schuell, Keene, N.H.) using a Turboblotter Rapid Downward Transfer system (Schleicher & Schuell). All the hybridization procedures were carried out using the non-isotopic DIG/Genius System from Boehringer Mannheim. The *RAD51* probe was obtained from the cloned *RAD51* cDNA described above using the PCR DIG Probe Synthesis Kit (Boehringer Mannheim), where digoxigenin-11-dUTP (DIG-dUTP) was incorporated during PCR. The upstream primer was GGTGGAATTGAGACTGGA; the downstream primer was TGATGACCACTGTTACACC. The amplification reaction gave a 452 base pair (bp) DNA segment. A 338 bp probe to be used as a control was obtained similarly from glyceraldehyde-3-phosphate-dehydrogenase (G3PDH) (Applequist et al. 1995). The upstream primer was AATCCACTGGCGTCTTCA and the downstream primer was AGGCAGGGATGATGTTCT. The membrane was pre-hybridized in a 50% formamide prehybridization solution at 42 °C and the *RAD51* probe was added directly to the solution after 2 h. The hybridization reaction was allowed to proceed for 18 h. The membrane was then washed twice at room temperature with 2× standard sodium citrate (SSC)/0.1% sodium dodecyl sulfate (SDS) and twice at 60 °C with 0.5×SSC/0.1% SDS. The membrane was then stripped to remove the bound *RAD51* probe and the blot was rehybridized with the G3PDH probe under the same conditions.

Mimic DNA construction and quantitative PCR

RAD51 quantitation was accomplished by performing a competitive PCR mimic assay employing a heterologous DNA fragment

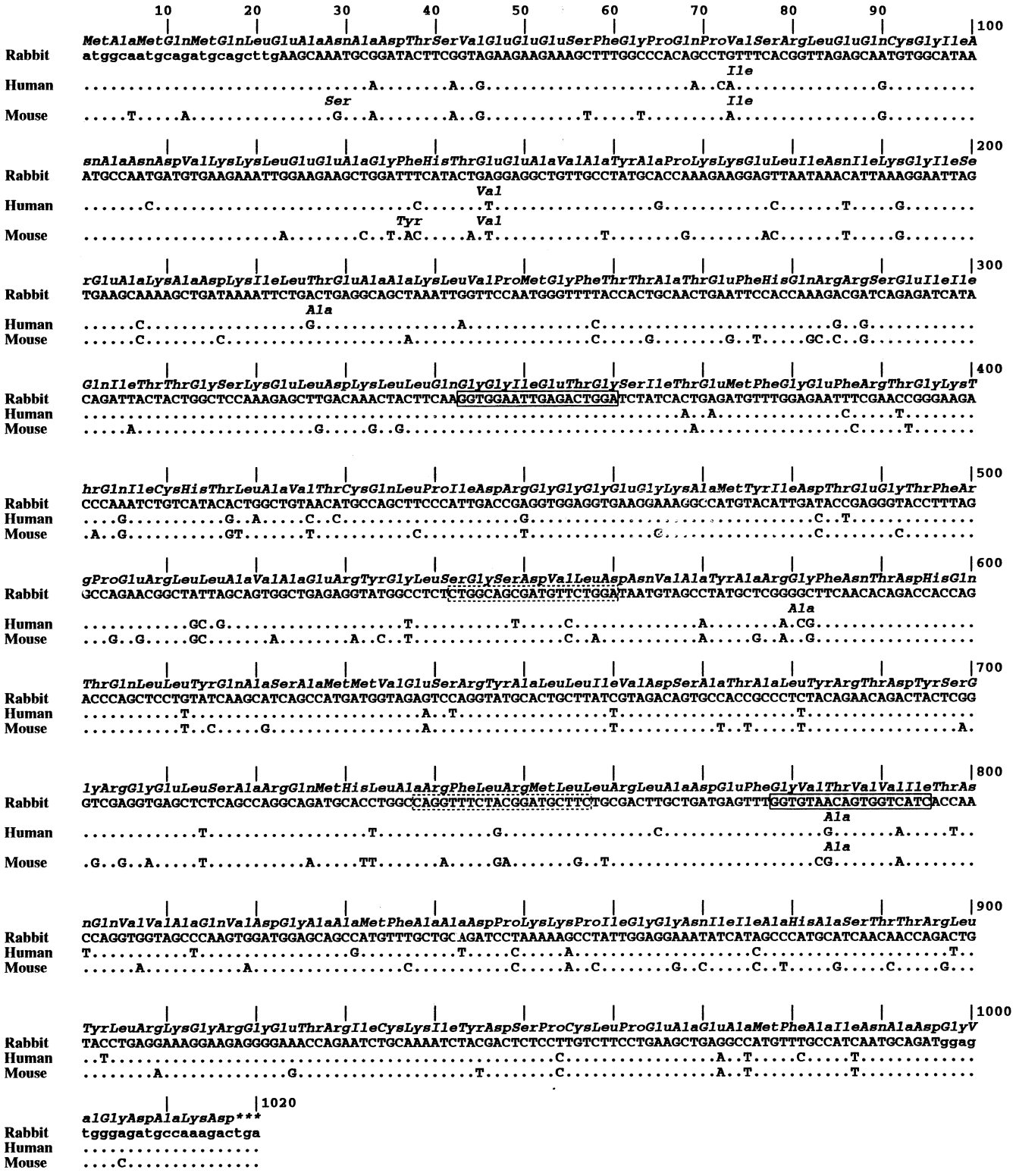

Fig. 1 Rabbit RAD51 open reading frame and deduced amino acid sequence compared with human and mouse *RAD51* sequences. Amino acid replacements in the human and mouse Rad51 sequence compared with the rabbit sequence are indicated. The primers (RAD2 and RAD3) used for amplification are shown in *lowercase* letters. The primers used to synthesize the probe for northern blotting analysis are enclosed in *boxes* with *solid lines*. In the *boxes* with *dashed lines* are shown the primers used for the quantitative PCR mimic assay. The sequence has been submitted to GenBank and given accession number AF017729

supplied by Clontech (Palo Alto, Calif.). Construction of the competitor DNAs was according to the manufacturer's instructions. Briefly, the competitor DNA mimic for *RAD51* was amplified for a first round of 16 cycles using two composite primers that contained RAD51 sequence at the 5' ends of the mimic sequences. The first round of PCR was followed by another round of 16 cycles where only the *RAD51*-specific primers were used in place of the composite primers. The primers used for *RAD51* are shown in Fig. 1 enclosed by dashed lines. The construction of the G3PDH mimic followed the same procedure, with the exception that the primers were specific for the G3PDH sequence. The upstream primer for G3PDH was TATGATTCCACCCACGGCAAGTTC; the downstream primer was CTGCTTCACCACCTTCTTGATGTC (Applequist et al. 1995). The construction of the β-actin mimic took advantage of the mimic DNA specific for human β-actin available from Clontech because the rabbit sequence differed by two bases in the 5' primer and was identical to human β-actin in the 3' primer region. We did not use composite primers but used primers specific for rabbit β-actin. The upstream primer for β-actin was ATCTGGCACCACACCTTCTACAACGAGCTTCG; the downstream primer was CGTCATACTCCTGCTTGCTGATCCACATCTGC (Harris et al. 1992). After reverse transcription of the sample RNA into cDNA, equal 2 ml aliquots (1/10 of the total volume) were added to PCR reactions containing tenfold dilutions of the competitor DNA and amplified. The first set of tenfold dilutions was prepared for each mimic, starting from 100 attomol/μl. Another set of 2-fold dilutions was prepared based on the results of the tenfold series of dilutions. The PCR conditions for RAD51 were as follows: 94 °C for 1 min; 55 °C for 1 min; 72 °C for 1 min for 37 cycles, followed by a final cycle at 72 °C for 7 min. The conditions for G3PDH were: 94 °C for 1 min; 60 °C for 1 min; 72 °C for 1 min 30 s for 30 cycles, and a final extension cycle at 72 °C for 7 min. The values determined for *RAD51* were compared with those found for G3PDH or, in some experiments β-actin was used as the control. PCR conditions for β-actin were as follows: 94 °C for 1 min; 60 °C for 1 min; 72 °C for 1 min 30 s for 37 cycles, and a last extension cycle at 72 °C for 7 min. The PCR products were resolved on an agarose gel, stained with ethidium bromide, and the intensities of the bands corresponding to the amplified competitor and sample fragments were measured using a FluorImager SI and ImageQuaNT Software (Molecular Dynamics, Sunnyvale, Calif.). The amount of target cDNA was calculated by determining the concentration of competitor DNA required to produce bands of equal intensity. The procedure was performed for both RAD51 and the internal control. Once the concentration of the target DNA was estimated, we calculated the ratio of RAD51/internal control and the relative *RAD51* expression was normalized in order to represent it on a log scale from 1 to 100. The differences in relative *RAD51* mRNA expression between cell populations were then calculated. Comparisons of cell sub-populations should be made within the same experiment and not of cell populations from different experiments because we used different enzyme lots and different mimics throughout the 2-year period during which the data were collected.

Sorting of appendix cell sub-populations

In order to detect the level of *RAD51* expression in different appendix cell sub-populations, we sorted lymphocytes from 5- to 9-week-old rabbit appendix using a goat anti-rabbit IgM-PE (Southern Biotechnology Associates, Birmingham, Ala.) and a mouse anti-rabbit CD43-FITC (Spring Valley Laboratories, Woodbine, Md.) as previously described (Fuschiotti et al. 1997). We separated appendix T cells from B cells using goat anti-rabbit IgM-PE and mouse anti-rabbit CD4/8-FITC (Spring Valley Laboratories). The negative controls for both of the above sorts were goat IgG-PE (Southern Biotechnology) and mouse IgG-FITC (Zymed Laboratories, San Francisco, Calif.). In order to obtain IgA^+, IgM^+, and IgA^+IgM^+ cells, we sorted appendix lymphocytes from 5- to 6-month-old rabbits using mouse γ1 anti-rabbit μ-chain-specific hybridoma 3C1-E8 supernatant (Gilman-Sachs and Dray 1985) and α chain-specific goat anti-rabbit IgA (a gift from K. Knight, Maywood, Ill.) as primary antibodies and donkey anti-mouse IgG-PE (Jackson ImmunoResearch, West Grove, Penn.) and donkey anti-goat IgG-FITC (Accurate Chemical and Scientific Corp., Westbury, N.Y.) as secondary antibodies. The negative controls were goat IgG (Pierce, Rockford, Ill.) and mouse IgG (Southern Biotechnology). The mRNA was extracted from the recovered cell populations (3×10^5–10^6 depending on the abundance of the particular population) using the Micro Fast-Track Kit (Invitrogen) and quantitative PCR assays were performed as described above.

Results

RAD51 cloning and sequence comparison

Shown in Fig. 1 is the rabbit *RAD51* cDNA sequence obtained from thymus mRNA of a young rabbit. Reverse transcription and PCR were accomplished by using primers corresponding to the most 5' and 3' ends of the human *RAD51* coding sequence (Fig. 1 in lowercase letters). To rule out base changes introduced during PCR, the sequencing was repeated and confirmed in three additional independent PCR cloning and sequencing experiments. The DNA sequence showed 93.3% and 89.5% identity to human and mouse *RAD51* sequence, respectively; at the protein level only five amino acid differences were detected compared with both human and mouse Rad51.

Detection of RAD51 expression by northern analysis

The *RAD51* probe used for northern analysis was obtained by PCR amplification, where a mixture of deoxyribonucleotides containing DIG-dUTP was used. The primers chosen (shown in Fig. 1, solid boxes) give a 452 bp probe. We observed two bands of ~1.8 and 2.4 kilobases (kb) in appendix from a young animal (6 weeks old) (Fig. 2). In the thymus, the higher molecular mass band was almost exclusively present, while in testis, only the lower molecular mass band was detected. Other tissues such as kidney, spleen, Peyer's patches from young rabbits, and appendix from an adult rabbit (1 year) showed only trace amounts of *RAD51*.

Relative expression of mRNA in sorted appendix cell populations

In order to test whether we could amplify the different size bands visible on the northern experiment (Fig. 2), we carried out a control experiment by quantitating relative *RAD51* message levels present in thymus and testis, which have nearly exclusive expression of only one of the bands. Figure 3A shows that in keeping with the results of northern analysis, the levels of *RAD51* message relative to G3PDH are very similar in thymus and testis, indicating that we were able to amplify the mRNA of different sizes. Figure 3B also demonstrates

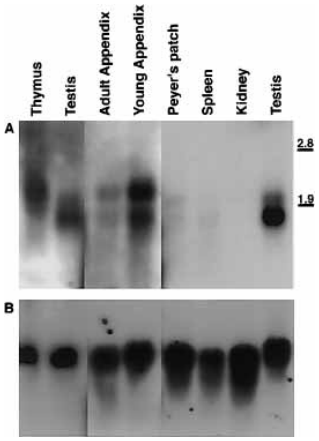

Fig. 2 **A** Profile of *RAD51* expression in several rabbit tissues by northern analysis. Each well was loaded with 3 μg of mRNA. Samples of testis mRNA from two different northern blots are shown. Standard molecular mass markers are indicated on the *right* in kb. **B** Membranes stripped and re-probed with G3PDH as described in the Materials and methods section. The G3PDH band is ~1.9 kb

that there was approximately eight times more *RAD51* in young appendix compared with adult appendix. There was ~150 to 300 times more *RAD51* mRNA relative to G3PDH recovered from young appendix, testis, or thymus compared with kidney (Figs. 3A, B). In order to determine the reproducibility of our quantitative PCR experiments we carried out control experiments by quantitating *RAD51* expression on replicate samples of the same cell populations. Briefly, a single-cell suspension from the appendix was prepared and

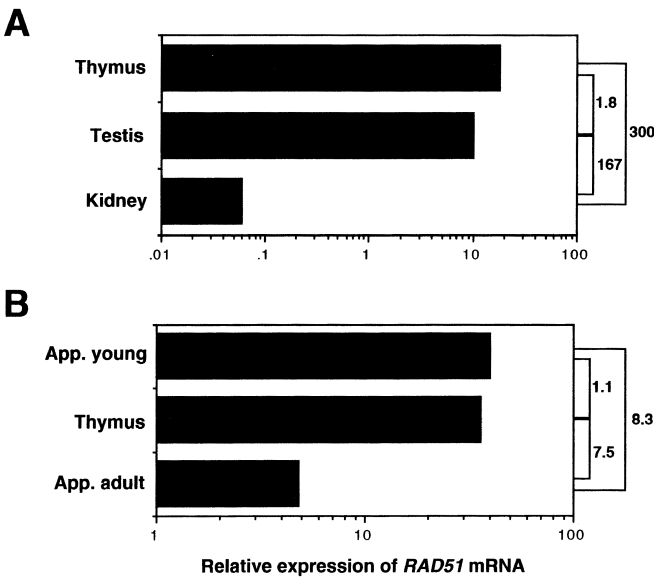

Fig. 3 Relative *RAD51* mRNA expression in **A** rabbit thymus, testis, kidney, **B** young appendix, thymus and adult appendix measured by a quantitative competitive PCR mimic assay. Numbers on the *right* show the differences in relative *RAD51* mRNA expression levels found between the tissue samples connected by the *square brackets*

5×10^6 cells or 1×10^6 cells were deposited in separate tubes. The two aliquots were treated as though they were different cell populations and compared with each other for the relative expression of *RAD51*. Because there should have been no difference, any difference obtained was indicative of experimental error in performing the measurements. The results are shown in Fig. 4A. In two experiments independent measurements of *RAD51* levels were found to be less than twofold different (1.4 and 1.7). Therefore, differences in expression were considered significant only if greater than twofold and reproducible.

The results of typical cell separations obtained by fluoresence activated cell sorting are shown in Fig. 5. Figure 4B shows that in experiments to test the relative *RAD51* mRNA levels in sorted appendix T and B lymphocytes (Fig. 5A), B cells had about three times the amount of relative *RAD51* mRNA expression found in T cells. The slightly higher levels in the B-cell compartment were a consistent finding in duplicate experiments where we obtained similar results. Rabbit appendix is a site where rearranged $V(D)J$ genes are diversified by gene conversion and a primary antibody repertoire is generated (Weinstein et al. 1994a,b). Not only is gene conversion occurring in this organ, but some cells committed to the B-cell lineage may also be undergoing de novo V_HDJ_H and V_LJ_L gene rearrangements (Fuschiotti et al. 1997). In order to test the hypothesis that Rad51 could play a role in somatic gene conversion, we quantitated the level of message in appendix cell populations separated on the basis of markers differentially expressed during B-cell development. For this purpose we used the IgM and CD43 markers of differentiating B cells described in the studies of Fuschiotti and coworkers (1997) to isolate cells of the B lineage at different stages of development. We studied the following cell populations: IgM^+CD43^+, IgM^-CD43^-, and IgM^+CD43^- (Fig. 5B). Presumably gene conversion occurs only during later stages of B-cell development when the immunoglobulin (*Ig*) genes have fully rearranged. Results obtained from these analyses are reported in Fig. 4C. *RAD51* levels were similar in the three cell populations analyzed. The young rabbit appendix is also a site where B cells switch their isotype from IgM to IgA (Weinstein et al. 1994b) and some B cells possess at their surface both IgA and IgM markers indicating that Ig-class switching has recently occurred. In order to investigate the possibility that Rad51 could play a role in Ig-class switching, we conducted a series of quantitative PCR experiments on appendix cell populations sorted for the surface expression of IgA and IgM, to determine whether an increase in *RAD51* expression would be found in cells that recently underwent isotype switching. As shown in Fig. 4D, we detected similar levels of *RAD51* mRNA expression in all three cell populations sorted from the appendix. This suggests that if Rad51 has a role in isotype switching it is played against a background of high steady-state *RAD51* mRNA levels in developing B cells.

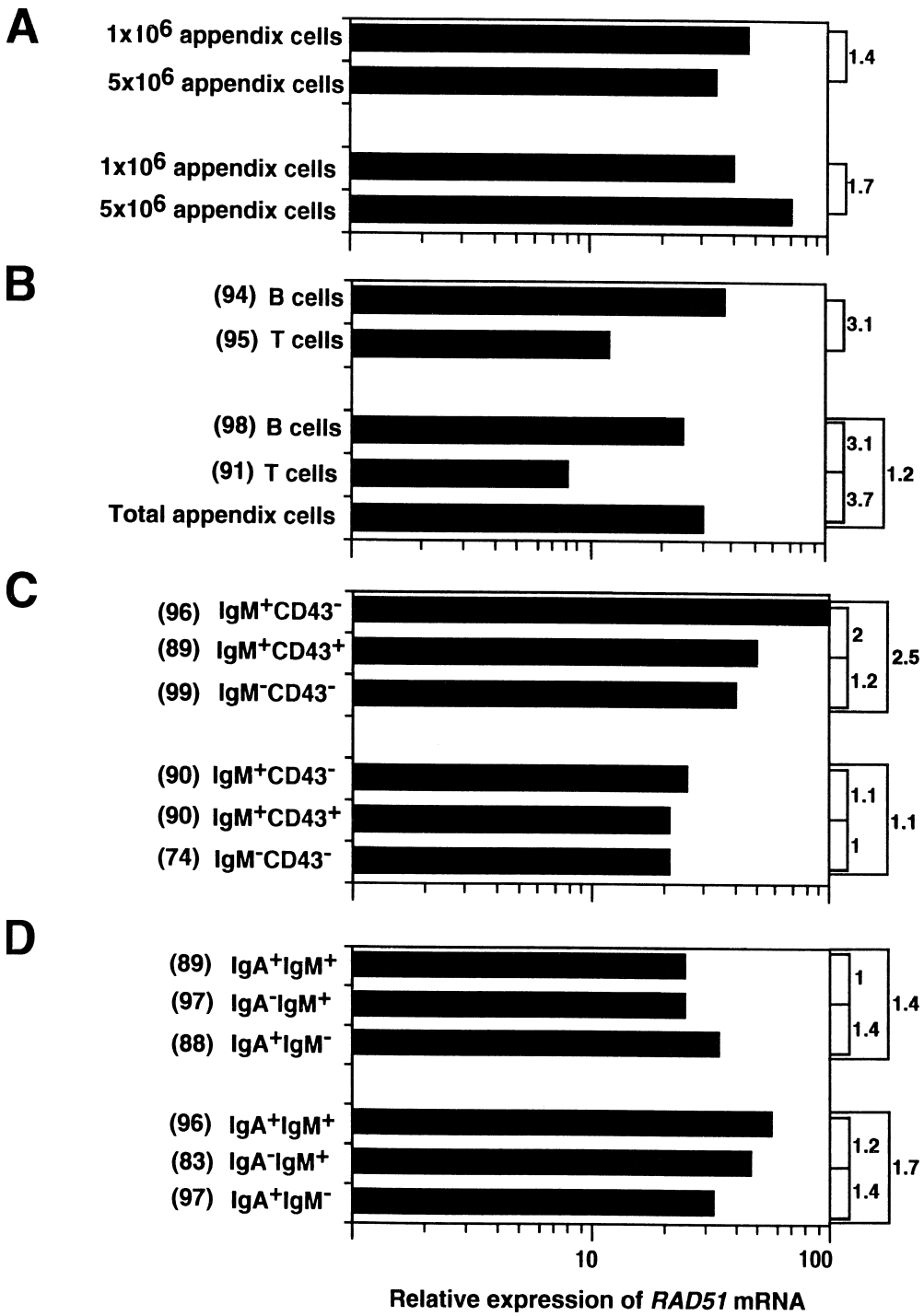

Fig. 4 Results of duplicate experiments quantitating relative *RAD51* mRNA expression by competitive PCR in several lymphocyte populations from rabbit appendix. The numbers in *parentheses* indicate the purity of the sub-population after sorting and reanalysis. Numbers on the *right* show the differences in relative *RAD51* mRNA expression levels found between the cell populations connected by the *square brackets*. In all experiments the internal control was G3PDH except in panel C where β-actin was used. Comparisons of cell sub-populations should only be made within the same experiment

Discussion

Rabbit Rad51 protein appears to be 339 amino acids long with high sequence similarity to mouse and human Rad51. Many of the substitutions detected at the DNA level are silent so that at the protein level rabbit Rad51 differs by only five amino acids from Rad51 of human or mouse. The replacements do not appear in regions of the molecule with known functional relevance. The pattern of expression of *RAD51* in different rabbit tissues is similar to that reported for other species where messages of more than one size have also been observed (Bezzubova et al. 1993; Shinohara et al. 1993). The testis probably exhibits a high level of *RAD51* expression because of its involvement in DNA break repair during meiosis. The ~1.8 kb band seen in testis is slightly smaller than that detected in other tissues such as appendix. The difference in size is almost undetectable and was noticed only after very careful comparisons of the different samples. We did not investigate this further.

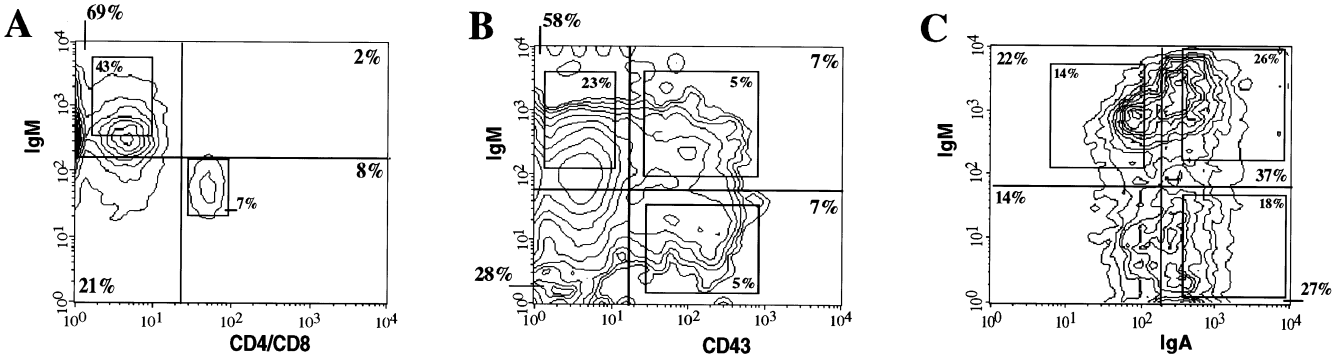

Fig. 5A–C Fluorescence-activated cell-sorter profiles after gating on lymphocytes using forward and side scatter and setting gates based on negative controls (see Materials and methods). **A** Sorting of B and T cells from rabbit appendix, using surface IgM for B cells and CD4 + CD8 for T cells. **B** Sorting of appendix B cells based on surface expression of CD43 and IgM. **C** Sorting of appendix B cells according to surface expression of IgA and IgM. *Boxes* show regions used to collect cells representing each cell population

The high level of *RAD51* mRNA found in the appendix of a young rabbit could be accounted for by the fact that B-lymphocyte development, expansion, as well as V_H gene conversion is occurring in this organ. After the appendix switches from a primary to a secondary lymphoid organ (Weinstein et al. 1994b), the levels of RAD51 message are markedly lower (Figs. 2, 3). High expression of RAD51 was also observed in the chicken bursa of Fabricius, an organ known to be the site where B cells diversify their *Ig* genes by gene conversion (Bezzubova et al. 1993). The increase of Rad51 protein coincident with the establishment of Ig-class switching in splenic B cells stimulated in vitro with LPS was reported to not simply be due to cell proliferation that accompanies isotype switching after stimulation with LPS, but rather to be associated with isotype switching (Li et al. 1996). On the basis of this experiment we tried to detect any possible increase of *RAD51* message in appendix cell populations undergoing class switching from IgM to IgA. However, in our quantitative assays of relative mRNA levels we did not detect significant differences (Fig. 4D). The reason that in our attempts to relate *RAD51* mRNA levels to gene conversion and/or isotype switching we found similar message levels may be that the basal *RAD51* expression in appendix B cells is already so high that an increase was not necessary to allow the sub-populations we analyzed to carry out additional functions. Several studies have reported that Rad51 levels become elevated in proliferating lymphocytes (Flygare et al. 1996; Tashiro et al. 1996; Yamamoto et al. 1996). However, more important may be that the pattern of Rad51 protein distribution within the cell may change. In lymphocytes stimulated with phytohemagglutinin, Rad51 was detected in foci during the S phase of the cell cycle, whereas in other stages a diffuse nuclear distribution was observed (Tashiro et al. 1996). Furthermore, the number of Rad51 foci in fibroblasts subjected to damaging agents (methyl-methanesulfonate, gamma rays and u.v. radiation) increased, suggesting a role of Rad51 in DNA damage repair (Haaf et al. 1995). The amount of protein remained constant, suggesting a change in the distribution rather than in the quantity. In the location where DNA is undergoing rearrangement or repair there could be aggregation of protein to form a focus. We did observe at least threefold higher levels of *RAD51* message in the B cells compared with the T cells of rabbit appendix. This observation could be linked to the additional DNA rearrangements or gene conversions taking place in the B-cell compartment, but we cannot rule out that the difference observed is merely because of the different proliferation status of T and B lymphocytes.

Studies from our laboratory have shown that the appendix of adult rabbits resembles secondary lymphoid tissues such as jejeunal Peyer's patches (Weinstein et al. 1994b). We showed that the level of *RAD51* mRNA is significantly reduced in such appendix tissue from older rabbits as well as in Peyer's patches from a young rabbit. We believe that our finding of high but similar levels of *RAD51* message in several studied cell populations from the young rabbit appendix should enhance interest in this gene whose product is likely to be associated with more than one process in developing B-lymphocytes of rabbit appendix.

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