PU.1 Is Involved in the Regulation of B Lineage-associated and Developmental Stage-dependent Expression of the Germinal Center-associated DNA Primase GANP*

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Germinat center-associated DNA primase (GANP) associated with MCM3 of the DNA replication complex is up-regulated selectively in germinal center B cells. We studied promoter activity of the 5′ region involved in the developmental-stage-dependent expression in B lineage cells by luciferase reporter assay. Selective regulation of ganp expression was observed in the −737-bp promoter region in B and plasma cell lines but was significantly low in pre-B and T cell lines. The deletion constructs displayed a gap decrease after shortening the region from −134 to −108 bp. Further narrowing suggested the involvement of the PU.1 consensus sequence at −126 bp by electrophoretic mobility shift assembly. The protein component PU.1 complex is not inhibited with mutated probes at the consensus site but is inhibited with the known PU.1 probe of CD72 and with anti-PU.1 antibody. Moreover, introduction of PU.1 cDNA enhanced the reporter gene activity in a dose-dependent manner in B cells, whereas the reporter construct with the mutated PU.1 site did not respond. Anti-CD40 stimulation induced the reporter activity with a 100% increase, which is not observed with the PU.1-mutated reporter construct. These results demonstrate that the germinal center-associated DNA primase expression is partly regulated by the transcription factor PU.1 expressed in B lineage cells.

Immunization with T cell-dependent antigen induces proliferation of specific B cell clones in the germinal center (GC) region of secondary lymphoid follicles of the spleen and lymph nodes (1, 2). Antigen-driven B cells develop into large centroblasts that rapidly move through the cell cycle, localize at the dark zone, and further differentiate into small centrocytes whose proliferation is arrested abruptly in the light zone of GCs (3, 4). During the transition and cell cycle regulation, antigen-driven B cells undergo various molecular events for clonal expansion, affinity maturation, class switching from IgM to IgG, and the selection of B cells for further differentiation into long lived memory B cells or terminally differentiated antibody (Ab)-secreting plasma cells. Among these molecular events, the mechanism that controls the proliferation of antigen-specific B cells in the specified area of GCs remains undetermined and is a particularly important issue.

Antigen-driven B cells differentiate with expression of various cell differentiation markers such as Peanut Agglutinin, GL-7, CD23, and CD21 but with reduced expression of the cell surface B cell receptor (BCR) (5–7). GC-B cells proliferate with rapid DNA synthesis and expression of the cell cycle-associated molecules such as Ki-67 and proliferating cell nuclear antigen (7, 8). During the differentiation of GC-B cells, various molecular events appear to be associated with an effective Ab production, some of which are probably closely associated with cell cycle progression that is inevitable for clonal expansion of antigen-reactive B cell clones. Some events also include somatic hypermutation in immunoglobulin (Ig)V region genes (9–11), isotype switching (12, 13), and the arrest of cell proliferation before selection in the follicular dendritic cell network (10, 14). Details of such molecular mechanisms, including clonal expansion, somatic hypermutation, and class switching, remain undetermined.

Recently, a mAb identified a differentiation antigen named GC-associated DNA primase (GANP) that is up-regulated selectively in GC-B cells (15–17). GANP protein is hardly detectable in normal lymphoid tissues and organs but is up-regulated in GCs of antigen-immunized spleen and lymph nodes. GANP protein levels are nearly undetectable in resting primary spleen cells but are induced markedly by stimulation with anti-IgM Ab plus anti-CD40 monoclonal antibody (15). GANP protein binds to MCM3 through a domain identical to an MCM3-binding molecule Map80 that possesses an acetyltransferase activity for MCM3 (16, 18). Recent studies of DNA replication in eukaryotic cells (19–22) have demonstrated a requirement of a complex that recognizes the origin of DNA replication and the associated components designated as minichromosome maintenance proteins (MCM). The MCM complex required for DNA replication is composed of at least six identified components known as MCM2, 3, 4, 5, 6, and 7; some of which are phosphorylated during cell cycling (23–25). GANP also bears a DNA primase activity that is inducible by phosphorylation of Ser502 of GANP (17). The findings regarding the structure and associated components of GANP suggest a role for GANP in the proliferation and DNA replication of lymphoid lineage cells. The up-regulation of GANP appears selectively in B cells stimulated with anti-CD40 mAb in vitro or in vivo.
in GC-B cells stimulated with T cell-dependent antigen in vivo, but it does not appear in the T cell area of GCs. These observations imply the selective up-regulation of GANP expression in B lineage cells undergoing maturation and differentiation into Ab-secreting cells for T cell-dependent antigens. The stage-associated expression of DNA primase GANP might be involved in maturation of antigen-driven B cells in secondary lymphoid follicles.

To understand the molecular mechanism that regulates the selective up-regulation of GANP, we isolated a genomic DNA clone carrying the 5'-flanking region adjacent to the first exon of ganp. Using a luciferase assay with DNA-transfected cells, we studied the promoter element required for the expression of the ganp gene. The results demonstrated that a 737-bp 5'-flanking region of the ganp gene is responsible for selective up-regulation in GC-B cells.

**EXPERIMENTAL PROCEDURES**

**Library Screening, Subcloning, and DNA Sequencing**—To isolate the 5'-flanking region of the ganp gene, a BALB/c mouse genomic library in the EMBL-3 vector was screened according to a standard procedure (26) with the 1.39-kb BamHI-EcoRI fragment of the pUC19/1.38-kb. Briefly, ~108 phage clones were screened with the 5'-end of the ganp cDNA (1.1-kb BamHI-Asp718 fragment) radiolabeled probe. Four independent clones named pG-23, pG-16, pG-23, and pG-25 were selected and analyzed with the restriction endonucleases BamHI, HindIII, EcoRI, NdeI, XbaI, and SacI and were compared with BALB/c liver genomic DNA.

Appropriate genomic DNA fragments recovered from the longest phage clone pG-25 (~17 kb) were subcloned in the vector pBluescript II KS(+) (pBS) and partially sequenced. By comparing genomic and cDNA sequence data, we identified a 6.38-kb flanking region as the putative promoter region, which extends from the XbaI site (in the middle of exon I) to the EcoRI site further upstream. A 1.38-kb EcoRI-XbaI fragment was subcloned and designated as pBSI/3.8-kb and pBSI/3.8-kb (3). The reporter construct was designated pGV-B (Toyo Ink Mfg., Tokyo, Japan) upstream from the luciferase gene. The 737-bp, -367-bp, -397-bp, and -264-bp fragments were introduced. The ganp promoter fragments of −168, −134, −108, and −54 bp were generated by PCR using the 1.38-kb DNA fragment as a template. All inserted genomic DNA fragments representing the 5'-flanking sequence, extending varying distances upstream from the major transcription start site (designated as +1), were confirmed by restriction enzyme digestion and sequencing to verify proper insertion and orientation.

The pGV-P construct in which the luciferase gene is regulated by the SV40 early promoter (PicaGene, Toyo Ink Mfg.) and the pSV-β-galactosidase construct (Promega, Madison, WI) in which the β-galactosidase gene is controlled by the SV40 promoter and enhancer were used in the transient transfection experiment as a positive control (to measure the maximum reporter gene activity) and as an internal control (to normalize the transcription efficiency).

Mutations in the PU.1 site were generated using oligonucleotide primers carrying point mutations. The nucleotides 5'-GGAA-3' were mutated to 5'-GGTG-3'. Oligonucleotides were synthesized by adding the primer-RNA hybrids (10 ng of the primer) containing 500 units/ml streptomycin, 100 units/ml penicillin, 2 mM l-glutamine (BioWhittaker, Walkersville, MD), and 50 mM 2-mercaptoethanol at 37 °C in a 5% CO2 incubator. The mature B cells, A20, were transfected as described below and then stimulated with anti-CD40 mAb (LB429, 10 μg/ml).

**Preparation of Nuclear Extracts**—Nuclear extracts were prepared from splenocytes and as described previously (29), frozen in aliquots and kept at −80 °C after determination of the protein content by Bradford assay (Bio-Rad).

**Transient Transfection and Reporter Gene Assay**—Transfection of all cell lines was conducted by electroporation using the Bio-Rad Gene Pulser II (Bio-Rad). Cells (~1 × 106) were harvested and resuspended in 0.5 ml of complete culture medium. Equivalent amounts of recombinant proteins were added relative to 10 μg of the pGV-B-luciferase reporter construct. One microgram of pSV-β-galactosidase construct was added as an internal control for the transcription efficiency. Cells were incubated with DNA for 5 min at room temperature in a 0.4-cm cuvette and then electroporated at 950 microfarads with 280 V (7023), 300 V (A20), 270 V (X63), or 320 V (BW5147). After 5 min at room temperature, cells were transferred to culture dishes containing 10 ml of the complete culture medium and incubated at 37 °C for 48 h. Luciferase assay was performed using a luciferase assay kit (PicaGene) as described (30). Cells were harvested, and protein extracts were prepared for luciferase and β-galactosidase assays. Luciferase activities were normalized against the β-galactosidase activities for transcription efficiency as described previously (30). Each transfection was repeated at least six times (three times in duplicate) using three preparations of DNA, and representative data are shown. The mouse PU.1 cDNA, introduced into the mammalian expression vector pCMV (Stratagene), was co-transfected with increasing doses as shown in Figs. 5 and 6.

**EMSA**—Electrophoretic mobility shift assay (EMSA) was performed with double strand synthetic oligonucleotides between −255 and −108 bp of the ganp promoter (see Fig. 1A). The double strand probes were radiolabeled with γ-[32P]ATP (Amersham Biosciences) by T4 polynucleotide kinase and purified through a Sephacryl G-50 column.

For the binding reaction, 15 μg of the nuclear extract, 1 μg of poly(dI-dC) as a non specific competitor, and 0.5 ng (−1 × 106 cpm) of the labeled probe were mixed in a buffer (total volume, 20 μl) containing 400 mM KCl, 150 mM NaCl, 0.5 mM MgCl2, 0.2 mM EDTA, and 0.5 mM dithiothreitol. Otherwise stated, binding reaction mixtures were incubated for 10 min at room temperature with a molar excess of the unlabeled nucleotide probe as a specific competitor for the labeled probe. Binding reactions were carried out for an additional 20 min at room temperature and were analyzed on 5% nondenaturing polyacrylamide gels containing 2.5% glycerol for 2 h at 150 V. The bands were visualized by autoradiography with a phosphorimager (Molecular Dynamics).

For Ab inhibition, 4 μl of Ab against the DNA-binding domain of PU.1 (Santa Cruz Biotechnology) was added to the binding mixture for 15 min prior to the addition of the labeled probe.

**Primer Extension Mapping**—A DNA primer (5'-TCCGCCGACATACGGCACCAGTGTC-3') complementary to mouse ganp sequence nucleotides from +42 to +21 was end-labeled with [γ-32P]ATP (2 × 106 cpm) and incubated with 4 μl of poly(A)1 RNA extracted from the myeloma cell line SP2/0. After being denatured at 80 °C for 4 min and then annealed for 2 h at 50 °C, reverse transcription reaction was performed in a total volume of 50 μl by adding the primer-RNA hybrids (10 μl) to 40 μl of 1.25 × reverse transcription buffer containing 1.25 mM each of dNTP, 12.5 mM dithiothreitol, 12.5 mM Tris-HCl (pH 8.4), 7.5 mM MgCl2, 40 units of the avian myeloblastosis virus (AMV) reverse transcriptase, and 40 units of RNasin for 30 min at 42 °C. The extended product was analyzed on a 6% sequencing gel in parallel with a sequencing reaction of the genomic DNA.

**RESULTS**

Cloning and Structure of the 5'-Flanking Region of Mouse ganp Gene—To study the mechanism of GC-associated ganp expression, we isolated DNA clones from the EMBL3 genomic DNA library of C57BL/6 with the mouse ganp cDNA probe (15). A clone (lane +25) carrying the terminal 5' region was analyzed by subcloning (Figs. 1A and 2), and the 1.38-kb region was sequenced (EMBL accession number AJ338868). By analyzing this region, we determined the longest 5' end of the mouse ganp CDNA by the 5'-RACE method (15). To determine the transcription initiation site of the mouse ganp gene, we performed primer extension analysis and identified one major transcription start site (Fig. 1, A and B) and two other minor sites (data not shown). One of two minor sites was identified as the longest 5' site by the
Based on these findings, we tentatively concluded the major initiation site of the mouse ganp gene (as indicated by 1 in Fig. 1, A and B).

In the region from ~400 bp to the major transcription site, significant similarities were found to the consensus sequences of E2F (at ~357), E-box (at ~333), BSAP (at ~235), PU.1 (at ~126), and an initiator-like sequence (at ~61). In addition, an obvious canonical TATAA or CCAAT box is not found in the 5′-flanking region of the ganp gene.

**Functional Analysis of the ganp Promoter** — The function of the putative promoter region was analyzed using the reporter construct containing the DNA fragment from ~737 bp to +1 and the transfection into cell lines representing various stages as pre-B (70Z/3), mature B (A20), plasma (X63), and T lineage.

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**Fig. 1. Mouse ganp 5′-flanking sequence and transcription initiation sites.** A, nucleotide sequence of the 400-bp fragment of the ganp promoter region. The major transcription start site has been numbered +1, and the minor start sites are indicated by solid dots below the sequence. Curved arrows show the 5′-end points of the reporter constructs. Several potential binding sites for transcription factors are boxed. Oligonucleotides used in EMSAs are shown. B, analysis of the transcription initiation by primer extension. An end-labeled oligo primer was annealed to poly(A)+ RNA extracted from myeloma cell line SP2/0 and extended using avian myeloblastosis virus reverse transcriptase as described under “Experimental Procedures.” The extended product (lane #) was analyzed on a sequencing gel together with a size marker. An arrow to the right indicates the major transcription initiation site. The two minor sites detected in this assay are indicated in A by solid dots below the sequence.
Table 1. Deletion analysis of the mouse ganp promoter. A series of deletions from the ganp 5'-flanking sequence was inserted upstream from the promoterless/enhancerless luciferase gene. On the left is a schematic representation of ganp-based reporter constructs. All constructs are designated according to the insert size relative to the major transcription start site (designated as +1). The restriction enzyme sites used for promoter cloning and positions of three regulatory DNA motifs (black boxes) are indicated. On the right, luciferase activity in 70Z/3, A20, X63, and BW5147 cells 48 h after transfection with the indicated promoter constructs is shown. The relative luciferase activity is expressed as fold activity above the background conferred by the promoterless/enhancerless control plasmid (pGV-B). Each histogram represents the value of luciferase activity for three independent experiments. Values shown are normalized by β-galactosidase activity.

Fig. 2. Deletion analysis of the mouse ganp promoter. A series of deletions from the ganp 5'-flanking sequence was inserted upstream from the promoterless/enhancerless luciferase gene. On the left is a schematic representation of ganp-based reporter constructs. All constructs are designated according to the insert size relative to the major transcription start site (designated as +1). The restriction enzyme sites used for promoter cloning and positions of three regulatory DNA motifs (black boxes) are indicated. On the right, luciferase activity in 70Z/3, A20, X63, and BW5147 cells 48 h after transfection with the indicated promoter constructs is shown. The relative luciferase activity is expressed as fold activity above the background conferred by the promoterless/enhancerless control plasmid (pGV-B). Each histogram represents the value of luciferase activity for three independent experiments. Values shown are normalized by β-galactosidase activity.

Further truncation of the promoter region demonstrated that the region from −264 to −168 bp is involved in the stage-dependent regulation of ganp expression in comparison with the reporter construct of −168 bp. The deletion of this element resulted in a decrease in A20 (from 42- to 31-fold), but a slight increase in X63 (from 48- to 48-fold) (Fig. 2). Using all of the constructs, similar differential expression was obtained in another set of representative cell lines as pre-B (18-81), B (BAL17), and T cells (EL-4) (data not shown).

The data presented here clearly suggest that the 5'-flanking region up to −737 bp is partly involved in the B lineage-associated and stage-dependent expression of the ganp gene.

Involvement of PU.1 in DNA-Protein Interactions at the ganp Promoter—To explore lineage-associated regulatory molecules involved in ganp expression, we conducted a series of EMSAs using radiolabeled oligonucleotides and nuclear protein extracts from the cell lines characterized above. Initially, we generated six oligo probes covering the entire region from −264 to −108 bp (Fig. 1A) and analyzed the DNA-protein interaction by EMSA. The GB-1 complex, detected with oligo 8 covering the PU.1 consensus site (5'-GCGGAA-3'), could be inhibited by the cold 15-nucleotide probe (oligo 9) containing the same PU.1 consensus site (Fig. 3A, upper band). The GB-1 complex is not detected by the 15-nucleotide probe with the mutation at the PU.1 site (oligo 9Mut M1 and M2) (Fig. 3B), but the GB-1 complex is not detected by the 15-nucleotide probe with the mutation at the PU.1 site (oligo 9Mut M1 and M2) (Fig. 3B).
complex, which is detected by the oligo 8 probe containing the consensus PU.1 site, could not be inhibited by the cold probe with the mutation at the PU.1 site. The GB-1 complex was clearly inhibited by the cold probe with a known PU.1 consensus site at the promoter region of CD72 (27) as a specific competitor, suggesting that the GB-1 complex contains the PU.1 protein (Fig. 3C). We also examined whether the formation of a GB-1 complex is affected by the addition of anti-PU.1 Ab. Specific Ab may cause a shift of the complex at the lower migration site on EMSA or the disappearance from the gel by the inhibition of complex formation. The anti-PU.1 Ab used here showed rather an inhibition of the formation of the complex, indicating that the complex formed with the oligo 8 sequence contains in part PU.1 protein (Fig. 3C). These results narrowed down the sequence element to the motif of the PU.1 consensus site. The contribution of the region with the PU.1 consensus element to the function of the ganp promoter activity was obviously significant (Fig. 2). The lack of this element in a reporter construct (from −134 to −107 bp) caused decreases of 51% (A20), 64% (X63), 55% (70Z/3), and 18% (BW5147) in the reporter gene activity. Collectively, these data suggest that the sequence element corresponding to the PU.1 consensus site is responsible for positive regulation of ganp promoter activity.

Effect of PU.1 in Regulation of ganp Promoter—To determine the role of PU.1 in the regulation of ganp expression, we transfected the PU.1 expression construct together with a reporter construct containing the PU.1 site (−134/PU.1WT) or a construct harboring a mutation in the PU.1 site (−134/PU.1Mut). PU.1 mRNA is expressed in B lineage cells (70Z/3, A20, and X63) but not in the T cell line BW5147 (32), which was also confirmed before these experiments (data not shown). As shown in Fig. 4, the mutation introduced into the PU.1 binding site resulted in a decrease of reporter activity in A20 and in X63. This finding indicates that the PU.1 binding site is involved in the regulation of ganp promoter activity.

To investigate whether PU.1 protein is involved in the regulation of reporter activity, we introduced the cDNA construct that produces PU.1 protein in the transfectants. Introduction of PU.1 cDNA caused enhancement in reporter activity in a dose-dependent manner from 1 to 8 μg in A20 and X63 cells (Fig. 4).
The mutation at the PU.1 site abolished the potential for enhancement in reporter activity even with the highest concentration of 8 μg.

The longer construct (−264 bp) carrying more motifs (Fig. 1) showed a similar enhancement in reporter activity in response to the exogenous PU.1 introduction, but the activity was much higher than that of the shorter construct (Fig. 5). The negative effect of the mutation was not apparent in B cells presumably because of the much enhanced activity or the cooperative regulation by the other elements in the longer construct; however, the mutant construct at the consensus PU.1 site could not augment the reporter gene activity in response to PU.1 introduction (8 μg). These results indicate that PU.1 is involved in the regulation of ganp gene expression through the PU.1 site (at −126 bp).

Stimulation-dependent Induction of ganp Promoter—GANP protein expression in resting primary spleen cells are markedly increased by treatment with anti-CD40 mAb (15–17). Because the ganp promoter potentially regulates the B cell by stimulation in vitro with anti-CD40 mAb, we examined whether this promoter region with the PU.1 site is a target of anti-CD40 signaling. We compared the reporter activity of a promoter construct (−134 and −264 bp) with or without the PU.1 element in A20 cells after stimulation (Fig. 6). Based on nonstimulated controls, anti-CD40 mAb significantly induced the luciferase activity by about 100%. The mutants of the PU.1 site could not show the up-regulation by anti-CD40 stimulation. Both the longer and shorter constructs showed similar results. These results suggest that the PU.1 site in the promoter region is involved in the anti-CD40-induced up-regulation of the ganp gene.

DISCUSSION

GANP is a new member of RNA/DNA primase that associates with the DNA-helicase complex of MCMs, and interestingly both the expression and the activity of DNA primase are induced in antigen-driven B cells in GCs (17). The role of GANP may be more diverse; GANP may be involved in DNA replica-
tion of not only GC-B cells but also various other cells, especially in rapid proliferation. Analysis of promoter activity in the 5′ putative ganp promoter region of −737 bp demonstrates that the region serves for the B lineage-associated and developmental stage-dependent regulation in cell lines maintained in vitro.

As with many lymphoid-specific genes, the mouse ganp promoter lacks an obvious TATAA box and initiates transcription at multiple sites. The initiator element, which is present in promoters with or without the TATAA box, is believed to be necessary for positioning the transcription initiation complex in promoters lacking the TATAA box (33). Interestingly, the sequence element 5′-CACA(N)5GAGNC-3′ encompassing nucleotides −174 to −161 of the ganp promoter shows a 7 of 8-bp identity among 13 nucleotides at its 5′ and 3′ ends with the TdT initiator sequence 5′-CTCA(N)5GAGNC-3′ (34).

Transient transfection analysis indicates that the ganp promoter is much more active in B cells than in T cells. Importantly, the ganp promoter is increasingly active as the B cell maturation proceeds, and this is consistent with a previous study showing that ganp expression is predominant at a later stage(s) of B cell development (15, 17). The ganp promoter is less active during the pre-B cell stage, but the activity increases with the maturation of B cells to sIgM-positive B cells and is at its highest in terminally differentiated plasma cells. Such an increase in the promoter activity through B cell differentiation is not caused by variations among the individual cell lines analyzed, because transfection of other cell lines representing similar stages and lineages displayed equivalent profiles of ganp promoter activity (data not shown).

We determined the element recognized by PU.1 as one of the transcription factors involved in ganp gene expression. PU.1 is an Ets family transcription factor specifically expressed by erythroid, myeloid, and B cells (35). Gene-targeting experiments of the mouse PU.1 locus demonstrate the necessity of PU.1 for the development of granulocytes, myeloid cells, B cells, and T cells (36). PU.1 has been reported to target many B cell-specific genes including the J chain promoter (37), 3′-κ enhancer (38), heavy chain Eκ enhancer (39), Btk promoter (40), CD18 (41), and IL-1β (42). The PU.1 consensus site at −126 bp of the ganp gene promoter region is recognized by the component including PU.1 in B lineage cells. This was confirmed by cold inhibition (shown on EMSAs), and the mutation at the PU.1 consensus site abolished the binding completely. The anti-PU.1 Ab inhibited the formation of the DNA-protein

**Fig. 5.** Reporter gene activity of ganp promoter PU.1 element with the promoter construct −264 bp. Point mutations in the PU.1 binding site within the promoter construct −264 bp and the co-transfection was carried out as described for Fig. 4. The induction of the promoter activity was measured after introduction of PU.1 as relative luciferase activity in 70Z/3 (A), A20 (B), X63 (C), and BW5147 (D).
complex (with only a low level of the complex remaining), which is presumably recognized by related transcription factors such as Spi-B (43). The reporter assay clearly demonstrates the positive effect of PU.1 on the PU.1 site (−126 bp), whose mutation abolished the up-regulation by exogenous PU.1 cDNA co-transfection. In the shorter construct, the mutation clearly reduced the spontaneous promoter activity in B cell lines; however, the longer PU.1-mutated construct (−264 bp) with other putative consensus sites did not show such a decrease. The longer construct with the mutation did not respond at all to the exogenous PU.1 cDNA.

The mechanism of how the longer PU.1-mutated construct did not show a decrease in B cells remains to be determined. It seems that the transcriptional activity of PU.1 might be suppressed by the distal elements(s) or the molecules expressed at pre-B and B cells. This notion may be consistent with the gap region(s) is involved in the repressive activity of PU.1.

in B cell activation with induction of the nuclear transcription factor NF-κB/Rel (50). There are no apparent binding sites for NF-κB in the ganp promoter region analyzed here, and the inactivation of IκB did not show any effect on reporter gene activity (data not shown). On the contrary, CD40 signaling showed a positive effect on ganp promoter activity that is mediated through the PU.1 site, suggesting that PU.1 might be involved in the up-regulation of endogenous GANP in GC-B cells stimulated with antigen and CD40/CD40L interaction. The effect of anti-CD40 stimulation on A20 cells is not strong in comparison with the exogenous introduction of PU.1 cDNA, but the mutation at the PU.1 site abolished the enhancement induced by stimulation with anti-CD40 mAb. The results presented here are based on the reporter gene assay using cell lines maintained in vitro. Normal naïve B cells in the quiescent cell cycle stage do not express GANP protein at a detectable level, but GANP expression is up-regulated by immunization with T-dependent antigen in vivo and by stimulation with anti-CD40 mAb in vitro. The ganp promoter is regulated throughout B cell development and is the target of cell signaling events via PU.1 cis-acting regulatory element(s). This information would also be helpful to characterize the stage-dependent regulatory mechanism that might regulate the expression of other functional molecules required for the activation of antigen-specific B cells in differentiating into Ab-secreting plasma cells.

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