Octamer Binding Factors and Their Coactivator Can Activate the Murine PU.1 (spi-1) Promoter*

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PU.1 (spi-1), a member of the Ets transcription factor family, is predominantly expressed in myeloid and B cells, activates many B cell and myeloid genes, and is critical for development of both of these lineages. Our previous studies (Chen, H. M., Ray-Gallet, D., Zhang, P., Hetherington, C. J., Gonzalez, D. A., Zhang, D.-E., Moreau-Gachelin, F., and Tenen, D. G. (1995) Oncogene 11, 1549–1560) demonstrate that the PU.1 promoter directs cell type-specific reporter gene expression in myeloid cell lines, and that PU.1 activates its own promoter in an autoregulatory loop. Here we show that the murine PU.1 promoter is also specifically and highly functional in B cell lines as well. Oct-1 and Oct-2 can bind specifically to a site at base pair −55 in vitro, and this site is specifically protected in B cells in vivo. We also demonstrate that two other sites contribute to promoter activity in B cells; an Sp1 binding site adjacent to the octamer site, and the PU.1 autoregulatory site. Finally, we show that the B cell coactivator OBF-1/Bob1/OCA-B is only expressed in B cells and not in myeloid cells, and that OBF-1/Bob1/OCA-B can transactivate the PU.1 promoter in HeLa and myeloid cells. This B cell restricted coactivator may be responsible for the B cell specific expression of PU.1 mediated by the octamer site.

PU.1 is the product of the spi-1 oncogene identified as a consequence of its transcriptional activation in Friend virus induced erythroleukemias (1–4). The overexpression of the PU.1 gene from a retroviral construct in long term bone marrow cultures is able to provoke the proliferation of proerythroblast-like cells that differentiate at a low frequency into hemoglobinized cells (5). PU.1 antisense oligonucleotides reduce the capacity of Friend tumor cells to proliferate, and competitor oligonucleotides block myeloid colony formation in human CD34+ cells (6, 7). Moreover, disruption of the PU.1 gene by homologous recombination in murine embryonic stem cells is lethal for homozygous PU.1 mutant progeny (8). Embryos present with multilineage defects in white cell development, including B cells. Taken together, these data demonstrate the importance of proper regulation of PU.1 in the hematopoietic system in general, and particularly in B cells.

PU.1 is a member of the Ets family of transcriptional activators and is involved in the regulation of expression of multiple genes specific for different lineages. PU.1 has been shown to bind to the promoters of many myeloid genes and is critical for their myeloid specific expression (listed in Ref. 9 and reviewed in Ref. 10). In B cells, PU.1 regulates the J chain promoter and immunoglobulin promoters, the immunoglobulin \( \lambda \) 2–4 and \( \kappa \) 3- enhancers, the \( \mu \) heavy chain gene enhancer, the B29 gene promoter, and Epstein-Barr viral promoters (11–18).

Since PU.1 is such an important transcription factor in hematopoietic development, and abnormalities of PU.1 expression can lead to experimental erythroleukemia, we investigated the PU.1 promoter in order to understand its regulation. Our previous investigation of the murine PU.1 promoter in myeloid cells demonstrate that as little as 334 bp\(^2\) of upstream promoter sequence directs cell-type specific transcription. This promoter includes a binding site for PU.1 itself in this region, which may play an important role in positive autoregulation of PU.1 (9).

As discussed above, in addition to myeloid cells, PU.1 is also strongly expressed in B lymphocytes and regulates many B cell-specific genes. The functional region of the PU.1 promoter has octamer and Sp1 sites which are conserved in both the murine and human PU.1 promoters (9). The octamer sequence ATGGAAT was originally found in all immunoglobulin heavy and light chain promoters, as well as the intronic heavy chain enhancer (reviewed in Refs. 19 and 20). Octamer binding proteins belong to the POU homeodomain transcription factor family (21). Two of the best characterized octamer binding proteins are Oct-1 and Oct-2. The Oct-1 protein is ubiquitously expressed, whereas Oct-2 is expressed mainly in B lymphocytes (22–27). Constitutively expressed Oct-2 can preserve expression of B cell-specific genes in cell hybrids (28, 29). Oct-2 gene disruption shows that it plays an important role in B cell maturation at late stages, but not in early B cell development (30). In addition, several reports suggested the existence of octamer coactivators, proteins which augment the ability of octamer factors to stimulate promoters in a factor, promoter, and cell type-specific manner (31, 32). Recently, both Oct-1 and Oct-2 have been shown to interact with a non-DNA binding octamer coactivator, known as Bob1 (33), OBF-1 (34), or OCA-B (35), and that this interaction is critical for octamer function. In these reports, the Oct-1/Oct-2 coactivator was reported to be expressed in B cells and not T cells, but its expression in myeloid cells was not extensively studied.

In this study, we show that the murine PU.1 promoter is highly functional in B lymphocytes and that an octamer site at bp −55 of the PU.1 promoter is an important determinant of its B cell specificity and activity. We also show that the octamer

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1 The abbreviations used are: bp, base pair(s); CMV, cytomegalovirus; hGH, human growth hormone; EMSA, electrophoretic mobility shift assay; PCR, polymerase chain reaction.
coactivator is not expressed in myeloid cells, suggesting why the octamer site is relatively more important for PU 1 expression in B cells than in myeloid cells, in which the PU 1 site itself is most important. Interaction between Oct-1/Oct-2 and the PU 1 site in B cells (38), and murine B-lymphoma cell line A20 cells (ATCC no. TIB 208) are maintained in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% newborn calf serum and 2 mM L-glutamine. Human epithelial HeLa cells (ATCC no. CCL 2) were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% newborn calf serum and 2 mM L-glutamine. The murine myeloma line Sp2/0-Ag14 (ATCC no. CRL 1581) was maintained in Dulbecco’s modified Eagle’s medium supplemented with 4.5 g/liter glucose and 10% FCS. The human monocytic cell line Mono Mac 6 (ATCC no. TIB 192), human myelomonocytic pro-B cell line Ly9 were maintained as described previously (39, 40).

Plasmid Constructions—The murine 0.5-kb BamHI fragment containing the PU 1 promoter region from bp 334 to 147 was subcloned into the promoterless vector pX2 just upstream of the luciferase reporter gene to generate mPU-334/luc (9). Unidirectional deletions of mPU-334/luc were prepared using exonuclease III as described (9) to create 5’ deletions starting between bp 197, 86, 61, 48, 39, 7, 3, and +107, to the end of bp +147, to generate mPU-197/luc, mPU-186/luc, mPU-61/luc, mPU-48/luc, mPU-39/luc, mPU-37/luc, mPU-34/luc, and mPU-107/luc plasmids, respectively (see Fig. 1).

Site-directed mutagenesis (41, 42) was employed to create single mutations in the PU 1 site, the Sp1 site, or in the octamer site as described above; 1 μg of the Sp1 site or in the octamer site as described above) and 1 μg of a plasmid containing the CMV-hGH, and measuring growth hormone level in the supernatant. Cell lines were harvested at 5 h. Data represent the mean and the standard error of the mean for three independent experiments, and all transfections were performed with three independent DNA preparations for each construct.

For cotransfection experiments, 10 μg of PU 1 promoter constructs and 2 μg of either the Oct-1 (pcGOct-1) or Oct-2 (pcGOct-2) expression vectors (in which the octamer cDNAs are expressed by a CMV promoter) were transfected with or without the coactivator expression vector pCATH NLS-BOB1, in which the BOB1 cDNA is expressed by a CMV promoter) (33) into HeLa cells using the CaPO 4 (33) and co-coprecipitation method (42), or into U937 cells by electroporation (43). Luciferase assays were performed 48 h after transfection for HeLa and 24 h for U937 cells. Transfection efficiency was normalized by cotransfecting a 2,2-kb fragment of a plasmid containing the CMV-hGH, and measuring growth hormone levels in the supernatant. Data represent the mean and the standard error of the mean for three independent experiments, and all transfections were performed with three independent DNA preparations for each construct.

In Vivo Footprinting—1 μg of plasmid DNA (Sp1 site or in the octamer site as described above) and 1 μg of a plasmid containing the CMV-hGH, and measuring growth hormone level in the supernatant. Cell lines were harvested at 5 h. Data represent the mean and the standard error of the mean for three independent experiments, and all transfections were performed with three independent DNA preparations for each construct.

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formamide/formaldehyde, followed by electrophoresis in 1% agarose/formaldehyde gels. RNA was then transferred to Biotrans nylon membrane (ICN, Irvine, CA) and hybridized to a human Bob1 probe (corresponding to bp 49–866 of the human Bob1 cDNA) (33) and a 18 S oligonucleotide probe (bp 938 to 921 of the human 18 S ribosomal RNA gene (54), 5'-TCGGGCTGCTTTGAACA-3'). The Bob1 probe was labeled by the random priming as described previously (55). Hybridization was performed at 65°C in 0.5 M sodium phosphate buffer (pH 7.2), 7% SDS, and 1% BSA for 15 h. Membranes were washed twice in 2× SSC and 0.2% SDS at room temperature for 5 min and once in 0.2× SSC and 0.2% SDS at 65°C for 20 min. Autoradiography was performed at −80°C with Kodak XAR-5 film using an intensifying screen for 4 days. Subsequently, the Bob1 probe was removed by shaking in 10 ml sodium phosphate buffer (pH 6.5) and 50% denatured formamide for 1 h at 65°C, followed by washing in 2× SSC and 0.1% SDS for 15 min at room temperature. The blot was then hybridized to the [α-32P]dCTP 3'-end-labeled (56) 18 S oligonucleotide overnight in 6× SSC, 5× Denhardt's solution and 0.5% SDS at 48°C, washed twice in 2× SSC and 0.2% SDS at room temperature for 5 min, and once in 0.2× SSC and 0.2% SDS at 48°C for 30 min. Autoradiography was performed at room temperature for 3 min.

**RESULTS**

The Murine PU.1 −334-bp Promoter Is Functional in B Cell Lines, and an Octamer Site at bp −55 Contributes to This Tissue Specificity—Our previous studies have shown that the murine 334-bp PU.1 promoter, as well as the human 290-bp promoter, demonstrate tissue-specific reporter gene expression in myeloid cell lines but not in T cells and HeLa (nonhematopoietic) cells (9). To determine if this region of the PU.1 promoter was functional in B cells, which also express high levels of PU.1 (3, 50), the −334 PU.1 promoter/luciferase construct was transfected into the B cell line BJ A-B. As shown in Fig. 1B, the murine PU.1 −334-bp promoter luciferase construct (mPU.1−334/luc) is highly active in B J A-B cells, with luciferase activities 47-fold higher than in HeLa cells. The activity in BJ A-B is 5-fold higher than that in the myeloid cell line U937 (Fig. 1B).

To locate the important regulatory region of the PU.1 promoter in B cells, a 5' deletion series of the −334 PU.1 promoter was transfected into BJ A-B cells. As shown in Fig. 1B, in BJ A-B cells, deletion from bp −334 to bp −61 did not significantly change promoter function, but deletion from bp −61 to −48, which removes an octamer binding site, decreased activity to 20% of the bp −334 promoter. Deletion from bp −48 to −39, which contains a Sp1 binding site, did not result in a significant change in luciferase activity. Deletion from bp −7 to −34, which removes a PU.1 binding site, decreased activity to 50% of the bp −7 promoter. Similar results were obtained with the murine B cell line K46 (data not shown).

The Transcription Factors Oct-1 and Oct-2 Bind to the PU.1 Promoter at bp −55—The functional analysis of the PU.1 promoter deletion series suggested that the murine PU.1 promoter −55 octamer site was important for its function in B cell lines. To determine whether the octamer binding proteins bind the PU.1 promoter, we performed gel shift assays. A 25-bp (bp −66 to −42) oligonucleotide containing a central octamer site was used as a probe. As shown in Fig. 2A, nuclear extracts from the Oct-1 or Oct-2 transiently transfected COS cells, which express undetectable levels of Oct-2 were used as sources of Oct-1 and Oct-2 proteins for controls. Nuclear extracts from transfected...
COS cells bound to an oligonucleotide probe from the PU.1 promoter bp 66 to 42 (Fig. 2A, lanes 1 and 4). Binding was competed by self oligonucleotide (Fig. 2A, lanes 2 and 5), demonstrating specific binding activity. The complexes of Oct-1 and Oct-2 could be supershifted by anti-Oct-1 antibody or anti-Oct-2 antibody, respectively (shown as S1 or S2 in Fig. 2A, lanes 3 and 6). An Oct-1 complex was formed using nuclear extracts from U937 cells (Fig. 2A, lane 7). The complex was specifically competed out by self oligonucleotide or by an oligonucleotide which has a wild type octamer site and mutant Sp1 site (Fig. 2A, lanes 8 and 10), but not by a mutated octamer site oligonucleotide from the PU.1 promoter, a wild type Sp1 site oligonucleotide from the PU.1 promoter, or wild type PU.1 site oligonucleotide from the PU.1 promoter (Fig. 2A, lanes 9, 11, and 12). Only anti-Oct-1 antibody could supershift the complex in U937 nuclear extracts, but not anti-Oct-2 antibody (Fig. 2A, lanes 13 and 14). B) A-B nuclear extracts demonstrated a similar pattern of competition and supershifts for Oct-1 binding, and in addition an Oct-2 complex was observed which could be specifically competed by unlabeled oligonucleotides and supershifted by anti-Oct-2 antibody (Fig. 2A, lanes 15–22). Nonimmune antibody could not supershift the Oct-1 and Oct-2 complexes (data not shown). These results show that in the B cell line BJ A-B, both Oct-1 and Oct-2 bind to the PU.1 promoter octamer site probe, as well as that of the PU.1 site probe, as well as that of the upper autoradiograph in Fig. 2A, lane 13. The legend above indicates the source of protein, competitor, and antibody used. The upper autoradiograph indicates the source of protein, competitor, and antibody used.

The Transcription Factors PU.1 and Sp1 Bind to the PU.1 Promoter at bp +20 and -40, Respectively, in B Cells—We have previously shown that in myeloid cells, PU.1 binds to a site in the 5′-untranslated region of the PU.1 promoter (at bp +20), and that this site is functionally important, leading to an autoregulatory loop in myeloid cells (9). Since PU.1 is also expressed at high levels in B cells, we asked whether PU.1 from B cell extracts also binds to this site. As shown in Fig. 2B, PU.1 from the B cell line BJ A-B binds to the PU.1 site to form a complex which comigrates with that formed by in vitro translated PU.1, indicating that the complex does not contain other proteins, specifically NF-EM5, which can interact with PU.1 at sites found in B cell enhancers (12). This complex can be competed efficiently with wild type but not mutant PU.1 oligonucleotides, indicating a specific interaction. Interestingly, although this site is functionally important (see below), PU.1 appears to bind with slightly lower affinity to this site compared to the CD11b PU.1 promoter site (Fig. 2B, lanes 4 and 6). This was also observed when using nuclear extracts from myeloid cells (9). Most of the binding activity is supershifted with an antibody specific for PU.1, which does not react with the related ets family member Spi-B (50). Therefore, although both PU.1 and Spi-B bind to this site (9), and both are expressed in BJ A-B cells (50), it appears that most of the binding activity to this site in B cells represents PU.1 and not Spi-B.

Sp1 bound to bp -40 of the PU.1 promoter, as shown in Fig. 2C. The binding complex could be formed in nuclear extracts from both B cells and myeloid cells, competed out specifically (Fig. 2C, lanes 3, 4, 9, and 10) and abolished specifically with anti-Sp1 antibody (Fig. 2C, lanes 6 and 12).

The Octamer Site Is Protected in Vivo—Since in vitro DNA-protein binding experiments may not reflect binding occurring on promoters embedded in chromosomal structures in vivo (42, 46), in vivo footprinting analysis was performed to determine whether DNA-protein interactions occurred in a B cell specific manner. Fig. 3 shows in vivo footprinting of the noncoding strand of the highly conserved region of the bp -120 to +102 PU.1 promoter. The human B cell line BJ A-B repeatedly showed a protected region located at bp -51, which lies in the center of the octamer binding sequence, ATGCAAAC (57, 58). This protection was not observed in the myeloid cell line U937. One hypersensitive band is located at bp +21, which is located just 3′ of the PU.1 core site AGGAA (59), and one protected band at bp +28 is noted in U937 cells (lane 3). No obvious change was observed at the -40 region, which contains a Sp1 site. The epithelial cell line HeLa showed neither protected nor hypersensitive bands (Fig. 3, lane 4) compared to that produced by deproteinized genomic DNA (Fig. 3, lane 1). A weakly protected band was also observed at bp -76 in BJ A-B and U937 cells. We did not observe any protected or hypersensitive bands on the coding strand in BJ A-B, U937, or HeLa cells (data not shown).

A Mutation in the Octamer Site Which Abolishes Oct-1 and Oct-2 Binding Reduces PU.1 Promoter Function—The results shown above indicate that the octamer site in the PU.1 promoter binds Oct-1 and Oct-2 in vitro, and is protected in vivo in...
footprinting was visualized using the ligation-mediated-PCR procedure.

B cells. In addition, deletion of a small region including this octamer binding site results in a large decrease of promoter activity in B cells. In order to determine the effects of specific mutations in the context of the entire promoter, a 6-bp mutation in the octamer site was introduced into the PU.1 promoter. Similar results were observed in the murine B cell line K-46 (data not shown). In the epithelial cell line HeLa, none of the mutants caused a significant decrease of wild type luciferase activity (Fig. 4). In contrast, in the myeloid cell line U937, the PU.1 mutant, rather than the octamer mutant, led to the most dramatic decrease (9).

Sp1 Can Transactivate the PU.1 Promoter—In gel shift experiments, Sp1 bound to a site at bp -40 of the PU.1 promoter, and mutation of this site led to a 36% decrease in luciferase activity (Fig. 4). To demonstrate that Sp1 can transactivate the PU.1 promoter, we cotransfected PU.1 promoter constructs with a Sp1 expression vector (pPacSp1) into Drosophila Schneider (S2) cells, which, unlike most mammalian cells, lack endogenous Sp1. When the wild type mPU-334 luc construct was cotransfected with the pPacSp1 expression vector, luciferase activity increased 35-fold, compared to a 9-fold transactivation if the Sp1 site was mutated (Fig. 5). These results indicate that Sp1 can bind to and activate the PU.1 promoter. The lesser increase of activity using a construct with a mutation in the Sp1 binding site is frequently observed with other Sp1 dependent promoters (60) and may reflect transactivation mediated by undetectably weak binding of Sp1 to the mutant site; by a mechanism which does not involve DNA binding, as has been observed with myb (61); or by binding of Sp1 to another location in the promoter. In any case, these results show that most of the observed Sp1 transactivation is mediated through specific binding to the site at bp -40.

Oct-2 is Expressed at Low or Undetectable Levels in Myeloid Cells—In myeloid cells, PU.1 itself is the most significant factor for PU.1 promoter function (9), but the octamer site is most important in B cells (Figs. 18 and 4). In order to understand this difference, we investigated the pattern of expression of factors interacting with the octamer site. Oct-2, unlike Oct-1, is expressed primarily in B cells, but its expression pattern in myeloid cells has not been extensively described (22, 57). Therefore, in order to understand why the octamer site was so
critical in B cells, we used EMSA to determine Oct-2 expression in myeloid cell lines. A 51-bp oligomer derived from the immunoglobulin heavy chain enhancer (51) was used as a probe. As shown in Fig. 6A, Oct-1 complexes are found at high levels in nuclear extracts of all cell lines tested (Fig. 6A, lanes 2–17). Oct-2 complexes were present at high levels in BJ A-B nuclear extracts only, with trace amounts in THP-1, KG-1a, and M1 myeloid lines. No Oct-2 complexes were observed in Mono Mac 6 or U937 myeloid lines, the T lymphoblastic line RPMI-8402, or in HeLa cells (Fig. 6A). An anti-Oct-2 antibody reacted with the Oct-2 complexes but not the Oct-1 complexes (Fig. 6A, lanes 3, 7, 15, and 17). These results indicate that Oct-2 is expressed at trace or undetectable levels in myeloid cells.

The Octamer Coactivator Bob1/OBF-1/OCA-B Is Not Expressed in Myeloid Cells—In order to further determine the mechanism of why the octamer site was relatively more important for PU.1 promoter function in B cells versus myeloid cells, we examined the expression and function of the Oct-1/Oct-2 coactivator protein, Bob1 or OBF-1. This protein, which does not bind to DNA directly, has been shown to increase the ability of octamer proteins to activate octamer-dependent promoters (33, 34). The octamer coactivator has been reported to be expressed exclusively in cells of B cell origin, but not in non-B cells such as T cell lines or HeLa cells (33, 34). In order to determine whether the coactivator is expressed in myeloid cells, we performed Northern blot analysis using a human Bob1 cdNA probe. As shown in Fig. 6B, the Bob1 probe hybridizes to a 3.2-kb mRNA species in human BJ A-B cells and slightly smaller species in murine K46, A20, and Sp2 B cell lines, and not in HeLa or Jurkat T cells, consistent with previously published results (33, 34). We observed no hybridization to the multipotential line LyD9, human promyelocytic/promonocytic lines U937 and HL-60, the human monocytic line Mono Mac 6, or to primary murine peritoneal macrophages, indicating that Bob1/OBF-1/OCA-B is not expressed in myeloid precursors or mature macrophages.

Octamer Coactivator Mediates Oct-1/Oct-2 Transactivation of the PU.1 Promoter—We next asked the question of whether the Oct-1/Oct-2 coactivator could augment the ability of octamer proteins to activate the PU.1 promoter. For these experiments, wild type mPU-334 and octamer mutant PU.1 promoter luciferase constructs were transfected along with combinations of Oct-1, Oct-2, and Bob1/OBF-1 expression constructs into HeLa cells, which contain significant endogenous levels of Oct-1 but not Oct-2. As shown in Fig. 7A, transfection of the PU.1 promoter into HeLa cells gave low but detectable reporter gene activity, and this activity was decreased 50% by introduction of a mutation in the octamer site. The residual activity possibly reflects the fact that HeLa cells contain significant
levels of both Oct-1 and Sp1, two activators of PU.1 promoter activity. Introduction of the Oct-1 expression vector did not augment wild type promoter activity, but addition of Oct-2 increased promoter activity 2.4-fold, as did transfection of Bob1 alone, presumably through its augmentation of Oct-1 activity. Cotransfection of both Oct-2 and Bob1 led to a dramatic increase in wild type activity, which was not observed in the presence of a mutation in the octamer site of the promoter (Fig. 7A). These results indicate that Bob1 can significantly stimulate PU.1 promoter activity, particularly in concert with Oct-2. Cotransfection of Oct-1 and Bob1 did not increase promoter activity greater than that of Bob1 alone, perhaps due to the significant endogenous levels of Oct-1 in HeLa cells. Similar experiments were performed in the promonocytic U937 cell line, which expresses neither Oct-2 nor Bob1. As shown in Fig. 7B, transient expression of the PU.1 promoter/luciferase construct with an expression vector for Oct-2 led to a 2.5-fold increase of PU-334 activity, higher than that observed with Oct-1 or Bob1 alone. Cotransfection of Bob1 with either Oct-1 or Oct-2 expression vectors with the PU-334 construct showed a further increase of promoter function. Cotransfection of Bob1 and Oct-2 increased the PU-334 promoter activity 5.7-fold, over twice the transactivation observed with Bob1 and Oct-1.

We considered how B cell-type specificity is mediated by the octamer site, since Oct-1 is expressed ubiquitously (22). One potential mechanism which could explain octamer mediation of B cell type specific regulation is that a special cofactor expressed only in B cells augments the function of Oct-1 or Oct-2. Our data support a model in which a B cell specific octamer cofactor augments the ability of octamer proteins to stimulate the PU.1 promoter, and this occurs specifically in B cells because the octamer cofactor Bob1/OBF-1/OCA-B is highly expressed in B cells and expressed at very low or undetectable levels in myeloid cells (Fig. 6B). In B cells, the octamer cofactor may augment the physical interaction between Oct-1 or Oct-2 and the TATAA binding protein, TBP (66). In myeloid cells, which express Oct-1 but very little or no Oct-2 (Fig. 6A) and no octamer cofactor, the TBP binding function mediated by Oct-1/OCT-2 and Bob1/OCBA can be substituted by binding of PU.1, since PU.1 can also bind to TBP in vitro (67, 68).

Although many cells, including myeloid cells, express Oct-1, Oct-2 is more restricted to B cells (Fig. 6A). We also showed that the octamer coactivator is expressed in B and not myeloid cell lines (Fig. 6B). In the case of the PU.1 promoter, expression of Oct-2 alone increases activation of the PU.1 promoter in HeLa cells and U937 cells to a degree greater than that of Oct-1, and this increase is augmented by the addition of the coactivator Bob1 (Fig. 7). These results indicate that Oct-1 can be itself activate the PU.1 promoter, but that this activation is weaker than that mediated by Oct-2, whose effect is further augmented by B cell-specific expression of the octamer coactivator.

In contrast, we observed no decrease of luciferase activity when the PU.1 promoter was transfected into Oct-2 knockout B cell lines (30), compared to that observed in matched heterozygote (Oct-2+/−) lines (data not shown). These findings imply
In B lymphocytes:

![Diagram of B lymphocyte transcription factors]

In macrophages:

![Diagram of macrophage transcription factors]

Note: GTF — general transcription factors

Fig. 8. Model for the differential activation of the PU.1 promoter in B lymphocytes and myeloid cells. We propose that, in B cells, the major activation of the general transcription factors (GTF), including RNA polymerase II and associated general factors, is via the octamer site binding Oct-1 or Oct-2, with postulated interactions of the octamer coactivator Bob1 (identical to OBF-1 and OCA-B) between octamer site binding Oct-1 or Oct-2, with postulated interactions of the octamer coactivator Bob1/OBF-1/OCA-B with the GTF. PU.1 and Sp1 may play lesser roles in activating the promoter. In myeloid cells, due to lack of Oct-2 and Bob1, the PU.1 site may play a more significant relative role. Our transactivation studies in Schneider cells implied that PU.1 can activate Sp1 and Oct-1 but either trace amounts or no Oct-2 and no Bob1 (see Figs. 1B and 7B).

that B cell-specific expression of Oct-2 is by itself not essential for PU.1 promoter function and, depending on the cell type, Oct-1 and/or Oct-2 can support PU.1 expression. The differences in the abilities of Oct-1 and/or Oct-2 to activate the PU.1 promoter reflect differences in expression of octamer proteins, octamer coactivators, and other transcription factors (Sp1, PU.1) which interact on the PU.1 promoter regions. In addition, differences in modifications of these proteins, such as phosphorylation of Oct-1 and Oct-2 during the cell cycle (44, 69), may also play an important role.

Our transactivation studies in Schneider cells implied that the Sp1 site at the PU.1 promoter region is involved in PU.1 regulation. In vitro gel shift assays also demonstrated that Sp1 from nuclear extracts from B, T, and myeloid cells can specifically form a complex with an Sp1 site oligonucleotide from the PU.1 promoter. Since the Sp1 and octamer sites are close to one another in the PU.1 promoter, the octamer proteins binding at bp −55 may interact with Sp1, which binds to an adjacent site at bp −40. This idea is supported by our data showing that if the octamer site has been deleted, further deletion of the Sp1 site does not lead to further loss of activity (Fig. 1B, compare −48 to −39 deletion). However, mutation of the Sp1 site in the presence of an intact octamer site (Fig. 4, Sp1 mut) leads to a significant loss in promoter activity. Cooperative interactions between Oct-1 and Sp1 have been described in other RNA polymerase II promoters (70). Cooperation between adjacent octamer and Sp1 sites could perhaps explain the functional importance of the octamer site in myeloid cells (9), in which the octamer coactivator is not expressed.

In the case of the PU.1 promoter, we were not able to observe a tertiary complex with Sp1 and Oct-1, even when large amounts of recombinant proteins were added to the binding reaction (data not shown).

PU.1 is also likely to be involved in activation of the PU.1 promoter in B cells. Mutation of the PU.1 site in the PU.1 promoter reduced promoter activity to 36% of wild type, close to the magnitude of that observed with mutations of the octamer site, but significantly less than that observed in myeloid cells. We sought but did not find evidence of any physical interactions between Oct-1 and either Oct-1 or Oct-2 in vitro (data not shown). Interestingly, it has been previously shown that Oct-1, Oct-2, and Ets-1 itself can positively regulate the Ets-1 promoter (71) in a manner that is similar to that which we have now described for the regulation of the related family member PU.1.

In summary, our studies have suggested an explanation of some of the mechanisms underlying the differences in activity of the PU.1 promoter in myeloid versus B cells (Table I). In B cells, the presence of both Oct-1 and Oct-2 and the octamer coactivator Bob1/OFB-1/OCA-B may mediate activation of the promoter, presumably by interactions with general transcription factors. Conversely, in myeloid cells, which express Oct-1 but either trace amounts or no Oct-2 and no Bob1 (Fig. 6), the octamer site is not as significant for promoter activity as is the PU.1 site. Overall, the PU.1 promoter appears to be approximately 5 fold stronger in the B cell lines tested than in myeloid cells (Fig. 1B). Our transactivation studies suggest that this 5 fold difference might be largely accounted for by the action of Oct-2 and/or Bob1/OFB-1 (Fig. 7). The actual role of the OBF-1/Bob1 coactivator in PU.1 expression in B cells may eventually be determined by investigating PU.1 expression in B cell lines expressing Bob1 “knockout” mice. Finally, an important question yet not addressed by these and our previous studies (9) is how PU.1 is activated in early multipotent cells (7, 50), and this shall be the focus of future studies of PU.1 gene regulation.

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