Promoters with the octamer DNA motif (ATGCAAAAT) can be ubiquitous or cell type-specific depending on binding affinity of the octamer site and Oct-factor concentration

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ABSTRACT
Immunoglobulin (Ig) gene promoters contain the octamer sequence motif ATGCAAAAT which is recognized by cellular transcription factors (Oct factors). Besides the ubiquitous Oct-1 factor, there is also a group of related factors (Oct-2 factors) encoded by a separate gene. The Oct-2 gene is regulated in a cell-type specific manner, and the protein is present in large amounts in B lymphocytes. We have previously shown that simple composite promoters of an octamer/TATA box type are poorly active in non-B cells but are strongly responsive to ectopic expression of Oct-2A factor, a major representative of the lymphocyte Oct-2 factors. In the present study we have tested the activity of a number of composite promoters and natural Ig promoters, and their response to Oct-1 and Oct-2 factors. Unexpectedly, we find that octamer/TATA promoters with a high affinity octamer site direct ubiquitous expression. By contrast, promoter constructions that behave in a B cell-specific manner tend to have a weak octamer binding site. These promoters are responsive to ectopic expression of additional Oct-factor, irrespective of whether it is Oct-1 or Oct-2. Using natural Ig promoters rather than composite promoters, we find that an IgH promoter is well transcribed in non-B cells via the ubiquitous Oct-1 factor, while Ig kappa and Ig lambda light chain promoters require additional Oct factor for maximal expression. It seems therefore likely that during B cell differentiation, Ig heavy chain promoters can be activated by Oct-1, before the appearance of Oct-2 factors. Oct-2 factors then would serve to boost the expression from Ig light chain promoters, which are known to be activated only after successful heavy chain gene rearrangement.

INTRODUCTION
When immunoglobulin gene promoters are linked to a reporter gene and tested for transcription by transfection into various cell types, they are preferentially active in B cell lines (1–4). All of these promoters contain a conserved sequence motif upstream of their TATA box, the so-called octamer site ATGCAAAAT (5, 6). In mutagenesis experiments, this octamer sequence was found to be an essential component of the immunoglobulin promoters. Therefore, the octamer sequence was an excellent candidate sequence for conferring B-cell specific transcription. Indeed, several groups have shown that a promoter construction consisting of a single octamer sequence in front of a TATA box is preferentially transcribed in B cells (7–9). A group of related octamer-binding transcription factors has been identified, which occur in B lymphocytes (10–14) as well as in a number of other cell types (15–17). In B-cells, the most prominent of these is a factor designated Oct-2A (also called NF-A2, OTF-2 or oct-B2; reviewed in ref. 18).

Additionally, a ubiquitous octamer-binding factor has been found in all mammalian cells analyzed thus far (19–23). This factor, Oct-1 (also called NF-A1, OTF-1, NFIII, OBP100, oct-B3) has been shown to have the same affinity for a given octamer sequence as Oct-2 (10, 24). Oct-1 activates a group of housekeeping genes including the histone H2B and UsnRNA genes that also contain an octamer sequence (25, 26). Furthermore, Oct-1 has been shown to be involved in adenovirus DNA replication (27, 28).

The cloning of the cDNAs coding for the Oct-2A and Oct-1 factors has facilitated the studies of B-cell specific transcription (9, 29–32). In particular, it was shown that expression from a B cell-specific promoter construction, consisting of an octamer sequence upstream of a TATA box, could be dramatically enhanced by ectopic overexpression of Oct-2A in non-B cells (9, 33, 34). The simplest explanation of these findings was that the Oct-1 and Oct-2 factors have different functions: Oct-2 was thought to activate promoters, like immunoglobulin promoters, consisting of just an octamer sequence in front of a TATA box/cap site, while Oct-1 was supposed to function only in the context of more complex promoters, such as the histone H2B promoter, which contain several other upstream factor binding sites in addition to the octamer sequence (reviewed in ref. 35). A recent result of W. Herr and his colleagues supported this idea. They found that in vivo a composite promoter construction was strongly activated by Oct-2, but not by Oct-1 factor (36). Other
investigators, however, found that when assayed in vitro, natural immunoglobulin promoters can be activated by both Oct-1 and Oct-2 (37, 38).

We addressed the issue of B-cell specific expression of immunoglobulin promoters with a set of defined promoter constructions containing octamer sequences, including flanking nucleotides, from either immunoglobulin heavy chain, immunoglobulin kappa light chain, or histone H2B genes. Unexpectedly, a segment containing the octamer sequence from either an immunoglobulin heavy chain or a kappa light chain promoter confers expression in non-B (HeLa) cells. A promoter with a histone H2B-related octamer site is active in B-cells but essentially inactive in HeLa cells, as we reported previously (9, 33). DNA-binding studies show that the affinity of a given promoter for Oct-factors (Oct-1 or Oct-2) correlates well with its transcriptional activity in HeLa cells. Furthermore, we show that the transcription of weak promoter constructs but not strong ones, can be stimulated in HeLa cells by additional Oct-factor expressed from a cotransfected cDNA. Most interestingly, the activity can be potentiated by Oct-2 and even by Oct-1. When analyzing natural immunoglobulin promoters, we also find that the expression of a Ig kappa light chain promoter can be stimulated in non-B cells by overexpression of Oct-2 or Oct-1. By contrast, an Ig heavy chain promoter is virtually non-responsive to either Oct-1 or Oct-2, which may have implications for the different timing of Ig heavy and light chain expression during B lymphocyte differentiation.

MATERIALS AND METHODS

Construction of plasmids
Plasmids IgH-O(E) and H2B-O are identical to OCTA(1) and OCTA(2) described in Müller et al., 1988. IgH-O(P) and Igx-O were constructed by inserting the corresponding doublestranded oligonucleotides (see below) into the SacI and SalI sites of the OVEC(S) vector (39). For Igx-000 an oligonucleotide containing three tandem octamer sites was inserted similarly. The Oct-1 expression vector (pEV-oct-1) was constructed in the following way: the oct-1 cDNA was cut out from vector pBSoct-1+ (32) with HindIII, the ends were filled in with Klenow enzyme and the fragment was cut with BamHI. This fragment was inserted into the BamHI/Smal site of expression vector pEV3S1 (40). The Oct-2 expression vector (pOEV1(+)) is described in Müller et al. (9).

Transfections and RNA analysis
Namalwa cells were transfected by the DEAE-dextran procedure with 4μg test plasmid and 1μg of reference plasmid (12). HeLa cells were transfected by the calcium phosphate coprecipitation procedure (39) with 10μg reporter plasmid, 1μg reference plasmid and 9μg sonicated salmon sperm DNA as carrier. In the cotransfection experiments 5μg of Oct-2 or Oct-1 expression vector and 4μg sonicated salmon sperm DNA were used. After incubation of the cells for about 40 hours, cytoplasmic RNA was extracted (12). Twenty micrograms of RNA were used for hybridization to a radioactive complementary strand RNA probe (spanning positions -37 to +179 of the vector), generated by SP6 RNA polymerase. Hybridization was done at 37°C overnight. Hybridization products were digested with RNase A (6μg/ml) and RNase T1 (10U/ml) at 37°C for 60 min and separated on a 6% polyacrylamide/7M urea gel. All bands were cut from the gel and quantitated by scintillation counting. The signals of the bands derived from the reference gene were used to normalize for variability in transfection efficiency.

Preparation of nuclear extracts and bandshift experiments
Nuclear extracts from HeLa and Namalwa cells were prepared as described by Schreiber et al. (14). For bandshifts the Ddel-Hinfl fragment (nucleotides position 518–566) from the IgH enhancer (14) was used. 16 fmol of 32P-end-labelled fragment were incubated with 3.5μg HeLa nuclear extract in the presence of no specific competitor DNA or increasing amounts (3, 9, 27, 81, 240, 720, 2160, 6480 fmol) of doublestranded oligonucleotides:

- Igx-O
- TCTGAGACTTAAATTTGCTACACCCTGAAAGCAGGAG
  TCGAGAGCTCTGAAATTATTTAATTTCCGGTCCTCAGCT
-IgH-O(P)
- CGAGAAATATGCAAATTTGGCAGGACTTAATAATTTGCATACCCTGAAGGAG
  TCGAGCTCTTATACGTTAGTTAACCAGCT
-IgH-O(E)
- CGAGCCGGGGAATTTGCAATTCTTCTTACTAG
  TCGAGCTCCGGGCCCCATTAAACGTAAAGATGATGCTAAGCT
-H2B-O
- CTTAATTTCAGTTGGGATATAAAGGCGAGGCGACTGCA
  TCGGATATACAGGTAAACGCCGATATTTTCCGGCTCTGAG

A different competitor DNA containing the H2B octamer element in the middle of the oligonucleotide gave a similar result as the H2B-O competitor (data not shown). Reactions were performed as described in Kemler et al. (41). The bandshift competition experiments were quantified by cutting out all retarded complexes and the corresponding free probe and scintillation counting.

RESULTS

Octamer/TATA box promoter constructs can be ubiquitously expressed
Several groups reported that a composite promoter consisting of an octamer element placed upstream of the TATA box of a reporter gene is sufficient for its tissue-specific transcription (7–9). We constructed promoters containing the octamer element derived from different genes and compared their transcriptional activity in B-cells to non-B cells. Figure 1A shows the constructs used in this analysis. The octamer sequence in construct H2B-O was derived from the promoter of the ubiquitously expressed histone H2B gene. Upon insertion of the binding sequence in the test promoter, upstream flanking nucleotides were maintained while the nucleotides downstream of the octamer motif differed from the histone H2B promoter. Nevertheless, for the sake of simplicity this construct is called H2B-O. Plasmids IgH-O(P) and Igx-O contain the octamer motif and flanking nucleotides from the promoters of the tissue-specific immunoglobulin heavy chain and light chain genes, respectively. The octamer element from the kappa promoter was also inserted in three tandem copies (Igx-000). Furthermore, the octamer motif from the IgH enhancer, which occurs in the inverted orientation, was used in the construct designated IgH-O(E). As a negative control we used the vector OVEC(S) which contains a TATA box but no upstream binding site (39). All these plasmids carry the simian virus 40
Enhancer downstream of the globin gene; the plasmid OVEC-REF was cotransfected as an internal control. All constructs were introduced into B-cells (Namalwa) or non-B cells (HeLa) and RNase mappings were performed to measure the amount of globin mRNA transcribed from both the test and control genes. As shown in Figure 1B and 1C (lane 1) the vector OVEC(S) was poorly expressed in both the B-cell line and the non-B cell line. All the other constructs were equally well transcribed in Namalwa cells, irrespective of the origin of the octamer element (Fig. 1B, lanes 2–6). In HeLa cells, as previously demonstrated, the construct with the histone H2B-related octamer sequence (H2B-O) was virtually inactive (Fig. 1C, lane 2; designated OCTA(2) in Müller et al. (9)). Also construct Igx-O(E) was only weakly expressed in HeLa cells (lane 6) and is therefore B-cell specific. Surprisingly, in this same cell line, the Igx-derived and the Ig-kappa derived octamer promoters were well expressed (lanes 3 and 5). In HeLa cells, three copies of the kappa-derived octamer motif (Igx-OOO) resulted in a stronger promoter than one copy (compare lanes 3 and 4). However, both promoters were equally well transcribed in B-cells. From these findings we conclude that a single copy of the octamer motif of the kappa promoter is sufficient for high level activity in B-cells. In contrast, three copies of the site are required in HeLa cells.

The transcriptional activity of these composite promoters in HeLa cells is dependent on an intact factor binding site, since mutation of the octamer motif (ATCAGTCGCC) abolished transcription (data not shown).

Figure 1. Octamer containing promoter constructs can be ubiquitously or tissue-specifically transcribed. (A) Plasmids used for the transfection experiments. The Oct-1 and Oct-2 expression vectors are described in Materials and Methods. Reporter plasmids are based on the OVEC vector (39). They contain the β-globin TATA box and the SV40 enhancer downstream of the globin gene. OVEC(S) has no upstream factor binding site. In H2B-O the octamer sequence was derived from a human histone H2B promoter (25), but the 3' flanking sequences are different. Igx-O(P) contains the octamer motif from the murine IgH 104-2 IgH promoter (74). In construct Igx-O(E) the octamer sequence was taken from the murine IgH enhancer (59). Constructs Igx-O and Igx-OOO contain the octamer element from the MOPC-41 kappa light chain promoter (43) in one or three copies, respectively. (B, C) RNase mappings of cytoplasmic RNA from Namalwa cells and HeLa cells transfected with the constructs shown in (A). β init indicates the position of the correctly initiated RNA and ref indicates the position of the reference signal from OVEC-REF. Lane M, size marker (HpaI-cleaved pBR322).

Figure 2. Promoter activity in HeLa cells correlates with the affinity for Oct-factor. (A) Autoradiograph of gel retardation competition experiments. An endlabeled fragment containing the octamer element from the IgH enhancer (14) was incubated with HeLa nuclear extract in the presence of increasing amounts of unlabeled competitor oligonucleotides (as indicated above each lane). Only the retarded DNA-protein complexes are shown. (B) Schematic representation of the competition experiments. Percentage (1.0=100%) of retarded DNA molecules is plotted against amount of competitor oligonucleotide used (half-logarithmic scale).

The activity of octamer/TATA promoters in HeLa cells depends on the affinity of the octamer site for factor

A possible explanation for the results shown in Figure 1 is that the promoter activity is dependent on the affinity of the octamer site for factor. We therefore tested the various octamer motifs for binding affinities in competition experiments by the gel retardation assay. A radioactively labelled oligonucleotide containing the octamer sequence from the IgH enhancer (14) was incubated with HeLa cell nuclear extract in the presence of increasing amounts of unlabelled competitor DNA. As competitor we used doublestranded oligonucleotides of approximately the same length which contained the octamer site from either the constructs H2B-O, Igx-O, Igx-O(P), or IgH-O(E), (Figure 2A). All DNA-protein complexes were cut from the gel and quantitated by scintillation counting. The results of this analysis are schematically shown in Figure 2B. By comparing the amount of competitor needed to reduce the signal of the retarded complex by 50%, we found that the oligonucleotides Igx-O(P) and Igx-O bound Oct-1 with the highest affinity. In contrast, oligonucleotide H2B-O showed the lowest affinity for Oct-factor, as 30 times more competitor was needed to achieve a 50% signal reduction. Oligonucleotide IgH-O(E) showed low, but significantly better affinity than H2B-O.
These results show a correlation between the strength of the promoter in HeLa cells and the affinity of the octamer element for Oct-proteins. The promoter constructs IgH-O(P) and Igx-O, which are strongly expressed in HeLa cells, contain octamer sequences which display the highest affinity for factor in the titration experiment. In contrast, construct H2B-O is very poorly expressed in HeLa cells and the corresponding octamer motif shows low affinity for HeLa cell derived Oct-factors.

Overexpression of Oct-1 also activates octamer/TATA promoters in HeLa cells

The finding that some of our octamer/TATA promoter constructs are expressed in non-B cells suggests that the ubiquitous Oct-1 factor has the capacity to activate such promoters. We tested this directly by cotransfection experiments. As shown in Figure 3A (lane 2), the H2B-O promoter construct was activated in HeLa cells by ectopic overexpression of Oct-2 factor (9,33). Moreover, Oct-1 transactivated the same lymphoid-specific promoter construct in HeLa cells (Figure 3A, lane 3). Quantitation of the signals revealed a 5-fold stimulation by Oct-1 and a 10-fold activation by Oct-2. A similar result was obtained with the non-B cell lines CV-1 and Cos7 (data not shown). The promoter constructs IgH-O(P), Igx-O and Igx-OOO which were well transcribed in HeLa cells, were not further activated by ectopic Oct-1 or Oct-2 factor (Figure 3B).

To determine whether the close octamer-TATA spacing (5bp) accounted for the activation of the H2B-O construct by Oct-1, we analyzed the B-cell specific promoter construct IgH-O(E), where the octamer element is separated from the TATA box by 20 bp. This corresponds well with the distance found in IgH promoters (42). As shown in Figure 3C, construct IgH-O(E) was also stimulated by Oct-1 factor (compare lanes 1 and 4). Bandshift experiments, performed with extracts from the transfected cells showed that the amounts of Oct-1 and Oct-2 protein produced from the expression vector were approximately the same (data not shown).

Nevertheless, it could be argued that these experimental conditions saturate the cell with Oct-factor and, as a consequence, any differences in the ability of Oct-1 and Oct-2 to activate transcription may be obscured. Therefore, we performed titration experiments with increasing amounts of Oct-1 or Oct-2 expression vector (2.5μg, 5μg or 10μg). This experiment, seen in Figure 3C, shows that the activation by Oct-1 and by Oct-2 was linear with increasing amounts of expression vector. Even at low factor concentration, Oct-1 could stimulate this B-cell specific promoter construct. The stimulation was very similar to the one observed with Oct-2 at the same vector concentration. Again, bandshift experiments with extracts from the transfected cells confirmed that comparable amounts of Oct-1 and Oct-2 had been produced (data not shown).

Oct-1 and Oct-2 preferentially activate a natural Ig light chain promoter, rather than an Ig heavy chain promoter

Even though the B cell-specific constructs H2B-O and IgH-O(E) are similar to natural Ig promoters in that they contain a single octamer element and a TATA box, they are still composite promoters. Therefore, we wanted to know how natural Ig heavy and light chain promoters would respond to ectopic Oct-2 factor, and in particular to additional Oct-1 factor.

We first tested the MOPC-41 kappa light chain promoter (43) containing 400 bases upstream of the transcription start site. As shown in Figure 4A, this promoter was indeed responsive to cotransfected Oct-1 (lane 2) and Oct-2 factors (lane 3) in HeLa cells. Quantitation of the signals revealed a 3-fold stimulation by Oct-1 and a 6-fold stimulation by Oct-2 factor. The activation by Oct-factors was also seen in the absence of the downstream SV40 enhancer (data not shown). The same result was obtained with the V105 Ig kappa promoter (44) and the Igλ light chain promoter (3; data not shown). The transcriptional activation of these light chain promoters was not dependent on the cell-type, since the same observation was made with the non-B cell line CV-1 (data not shown).

The analysis of the 70ZH+ heavy chain gene promoter, including 280 bp upstream of the transcription start site (45), yielded a surprising result (Figure 4B): This promoter, which would at least be expected to respond strongly to Oct-2 factor, was only marginally activated both by Oct-1 (lane 2) and by Oct-2 (lane 3). In both cases, the stimulation was only about 2-fold. Another IgH promoter (104–2; ref. 42) containing the IgH TATA box and 68 bp upstream of the start site, including the octamer element, was also not responsive to elevated Oct-1 and Oct-2 protein (T. Enzler and W. Schaffner, unpublished results).
**DISCUSSION**

**Correlation between ubiquitous activity of octamer containing promoters and affinity of binding site**

We have studied the tissue-specific expression of various promoter constructions, all containing an octamer motif derived from different genes. The four octamer sites of our promoter constructs have different affinities for octamer factor even though all of them perfectly match the consensus site ATGCAAAATNA, or its complement TNATTGGCAT. Therefore, flanking nucleotides must favor or disfavor tight interactions. This is not so surprising in light of the finding that octamer sites that deviate from the core consensus can nevertheless bind well to Oct-factor if they are surrounded by favorable flanking sequences (46). We find that the B-cell specificity of our composite promoters is strongly dependent on the affinity of the octamer motif for factor. The two high affinity sites (derived from IgH and Ig kappa promoters) are likely saturated in HeLa cells by ubiquitous Oct-1 factor and thus direct constitutive expression. In contrast, the two lower affinity sites (based on the octamer sequences found in a histone H2B promoter and the IgH enhancer) direct transcription in HeLa cells only if Oct-factor levels are elevated. The distance between the octamer element and the TATA-box varies among the four analyzed promoter constructs. However, the octamer-TATA distance does not influence tissue-specificity as shown by both Wirth et al. (7) and by us (P. Künzler, P. Matthias and W. Schaffner, unpublished results). It was also shown that the B-cell specificity is independent of the orientation of the octamer motif.

We feel that the results obtained by others with B-cell-specific octamer/TATA promoter constructions (7, 8) can also be explained by the concentration/affinity concept. Wirth et al. (7) constructed a B-cell-specific promoter by inserting the octamer element in a polylinker in front of the β-globin gene. In light of the demonstrated ability of octamer proteins to bind to some non-consensus octamer sequences (41, 46–49), the observation that a mutation in any of the first 7 bp of the octamer element abolished protein binding (10), suggests that this construction contains a low affinity binding site that does not tolerate changes. Dreyfus et al. (8) inserted an octamer sequence in the promoter of the mouse Renin-1 gene. This lead to high expression from the promoter in B-cells, but not in fibroblasts (Dreyfus et al. (8)). Although no binding studies with this promoter have been published, it might well be that a weak binding site was created by the insertion of the octamer motif. Another octamer motif containing promoter with B-cell specificity was described by W. Herr and his colleagues (36, 50). These authors analyzed a promoter which contained six tandem copies of the octamer element from the simian virus 40 enhancer (ATGCAAAAGCA) which has been shown by others to be a rather low affinity site (27).

**Oct-1 can activate B-cell specific promoters**

Perhaps most surprising is the finding that we cannot discriminate qualitatively between activation by Oct-2 and Oct-1. Any of our promoters—composite or natural—that is activated by Oct-2 is also responsive to Oct-1 factor, although to a lesser extent (Figures 3 and 4). A similar observation was made, when studying the role of octamer factors in adenovirus replication. Oct-2 was found to stimulate DNA replication as efficiently as Oct-1 (31). However, Oct-1 and Oct-2 are not functionally equivalent, since Oct-1 was reported to interact with the herpes simplex virus transactivator protein VP16, whereas Oct-2 does not (47, 52, 53).

Our results concerning the ability of Oct-1 to activate a B-cell-specific promoter construct are in apparent contrast to those of W. Herr and his colleagues, who have observed a strong activation of their promoter with Oct-2 but not with Oct-1 factor (36). This most likely reflects differences between the promoter constructions used. As mentioned above, Tanaka and Herr had used multiple copies of a weak octamer site derived from the SV40 enhancer. However, the failure of Oct-1 to activate this particular promoter construction cannot be merely dismissed as a result of insufficient Oct-1 expression from the cDNA clone, because Oct-1 was found, by means of a repression assay, to bind to a similar promoter in vivo. The reason why one set of promoter constructions responded to Oct-1 while another did not remains to be elucidated.

We have shown in a model system that cell type-specific transcription could be explained by the concentration of factor and affinity of the corresponding DNA site. Although there may be cases where this principle is sufficient to explain the transcriptional activity of a given promoter (see below), it is most likely overly simplistic to explain all Ig gene transcription. The situation is often quite complex due to the presence of IgH and Ig kappa subfamilies with characteristic promoter structures. For example, the Vx19 promoter has a weak, mutated octamer site (ATGCAAAgGA). Efficient transcription of this promoter requires the interaction with a neighboring site, referred to as X·Y (CTTCCTA; 54). Similarly, it has been shown that a ubiquitous protein, binding to the so-called N-element in the IgH promoter can, in conjunction with Oct-factor, promote transcription in B-cell extracts (55). And one must keep in mind that Oct-2A is only one member of a family of proteins resulting from alternative splicing (14, 56; L. Staudt; T. Leanderson, personal communications). One might envisage a role for the different factors where one, but perhaps not another Oct-2 factor could cooperate with an additional upstream factor bound to a particular Ig promoter.

In view of the conflicting results obtained with composite promoter constructs, we considered it of paramount importance to test the response of natural Ig promoters to Oct-1 and Oct-2 factors. We show that two naturally occurring Ig kappa light chain promoters and an Ig lambda light chain promoter can be stimulated in non-B cells by overexpression of Oct-2 and, remarkably, also by Oct-1. Recently, Junker et al. (57) have shown by fusion experiments between B cells and fibroblasts that the loss of Oct-2 expression correlated with downregulation of transcription from an Ig kappa promoter. Transcription could be partially restored by transfection of an Oct-2 cDNA. Endogenous Oct-1, still present in the hybrids was not sufficient to activate this promoter. However, it remains to be seen if the same effect could not be achieved by transfecting the Oct-1 cDNA.

The kappa light chain promoter we used in our analysis was derived from the MOPC-41 gene, whose octamer sequence and flanking nucleotides are identical to those present in the Igx-O promoter construct. The natural Ig kappa promoter from MOPC-41 shows intermediate activity in HeLa cells and is well activated by Oct factor. Composite promoters which contain the same high affinity octamer site (Igx-O and Igx-OOO) perform quite well in HeLa cells and can only be marginally stimulated by additional Oct-1 and Oct-2 factor. This probably means that the promoter constructions with an Ig kappa octamer linked to the globin TATA box happens to be particularly well suited for expression in HeLa cells, perhaps due to the β-globin TATA-
box, which is more efficient than the TATA-box from the Ig\(_\kappa\) promoter (L. Xu, M. Thali and W. Schaffner, unpublished observation).

In light of our results showing that Oct-1 and Oct-2 have similar activation potentials, one might ask why Oct-2 is necessary at all. We offer the following explanation. In contrast to an Ig kappa promoter, an Ig heavy chain promoter is expressed at intermediate level in HeLa cells and responds only very weakly to overexpressed Oct-1 and Oct-2 protein in these cells. It seems that Oct-factors are not limiting for IgH promoter transcription in HeLa cells. This probably also means that maximal transcription from an IgH promoter depends on other B cell-specific components (or on removal of a negative factor in HeLa cells). Nevertheless, Oct-1 factor at natural concentration should be able to activate IgH transcription in B cell precursors (see also the in vitro results of LeBowitz et al. (37) and Johnson et al. (38)). We therefore speculate that during B-cell differentiation, Ig heavy chain promoters are activated by Oct-1 factor before the appearance of Oct-2 factors. These would serve to boost the expression of Ig light chain promoters, which are activated only on removal of a negative factor in HeLa cells. Nevertheless, Oct-1 factor at natural concentration should be able to activate IgH transcription in B cell precursors (see also the in vitro results of LeBowitz et al. (37) and Johnson et al. (38)). We therefore speculate that during B-cell differentiation, Ig heavy chain promoters are activated by Oct-1 factor before the appearance of Oct-2 factors. These would serve to boost the expression of Ig light chain promoters, which are activated only after successful rearrangement and expression of Ig \(\mu\) heavy chain, at the switch from pre-B to B cells (58).

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