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Cell-type-specific transcription of an immunoglobulin κ light chain gene *in vitro*

(nuclear extract/B-cell factor/decanucleotide element)

JUNKO MIZUSHIMA-SUGANO AND ROBERT G. ROEDER

Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021-6399

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ABSTRACT **We have established a cell-free system, derived from a human B-cell lymphoma, in which immunoglobulin κ light chain gene promoters are both accurately transcribed and regulated in a cell-type-specific manner. Thus, accurate transcription from the T1 κ light chain gene promoter was much more efficient in B-cell extracts than in HeLa cell extracts, whereas control promoters (adenovirus major late and histone H2B) were transcribed equally well in either extract. More important, the increased κ light chain gene transcription in B-cell extracts was dependent upon upstream sequences (containing the conserved decanucleotide element) previously shown to be necessary for B-cell-specific transcription *in vivo*; in contrast, removal of these sequences had no effect on the low level of κ transcription in HeLa extracts. The maximal level of upstream sequence-mediated transcription was dependent upon template topology. These studies show that there is at least one B-cell-specific factor that stimulates transcription from purified DNA templates, and they further suggest that the *in vivo* action of the factor(s) on other components of the transcription machinery is direct rather than indirect (e.g., via the maintenance of an open chromatin structure). The cell-free system described here should facilitate both purification and functional studies of the B-cell-specific factor(s).**

The expression of immunoglobulin genes is restricted to cells of the B lineage and is controlled at one level by developmentally regulated DNA rearrangements leading first to heavy chain expression (pre-B cells) and subsequently to light-chain expression (B cells) (reviewed in ref. 1). Expression is regulated at a second level both by tissue-specific enhancer elements (reviewed in refs. 2–7) and by tissue-specific promoter elements (2–7), as shown by transfection analyses with mutated genes. Although not delineated precisely, the tissue-specific promoter element(s) lies upstream of the "TATA box" and appears to contain a decanucleotide (or octanucleotide) that is conserved in all human and mouse κ and λ light chain genes and (in inverse orientation) in corresponding positions of heavy chain genes (8, 9). An understanding of the developmental regulation of these genes, and ultimately cell commitment or determination events, would be greatly facilitated by identification and functional analysis of the factors that must operate through these sequences.

Although electrophoretic mobility-shift assays have been used to detect proteins that bind in a site-specific way to these (10) and other genes, a more powerful approach that allows both the detection and the functional analysis of transcription factors, whether or not they bind stably to DNA, is the use of cell-free systems in which cloned genes are both accurately transcribed and regulated. Such reconstituted systems have led to the identification of a number of factors that appear to be used by most RNA polymerase II-transcribed genes (11), as well as gene-specific factors that operate through upstream sequences or enhancer elements (reviewed in ref. 12).

In a few instances the *in vivo* regulation of RNA polymerase II promoter function has been reproduced (at least in part) *in vitro*. This has been shown by enhanced transcription of specific genes in response to viral immediate-early proteins (13), factors present in the S phase of the cell cycle (14), and a heat shock-induced factor (15) and by repressed transcription of a simian virus 40 promoter by viral large tumor (T) antigen (16). Except for a report of immunoglobulin μ heavy chain constant-region gene (C_μ) enhancer function with a heterologous viral promoter (17), there have been no studies demonstrating the *in vitro* function of genetic elements that modulate developmental regulation.

We describe here a B-lymphocyte-derived, cell-free system that mediates both accurate transcription and a high level of B-cell-specific transcription of a κ light chain gene that is dependent upon DNA sequences previously implicated in tissue-specific promoter function *in vivo*.

MATERIALS AND METHODS

Cell Culture and Extract Preparation. HeLa cells were maintained in suspension in Joklik's modified Eagle's medium containing 5% bovine calf serum. Namalwa cells, a generous gift from I. Tamm and P. Sehgal (The Rockefeller University), were grown in suspension (to 10^6 cells per ml) in RPMI 1640 medium supplemented with 10% fetal bovine serum. Nuclear extracts were prepared as described (18) and had protein concentrations of about 6 mg/ml. Whole-cell extracts were prepared as described (19) and had protein concentrations of about 16 mg/ml.

Plasmids. Plasmids containing the κ light chain gene T1 cloned into pSVgpt vectors (8) were generously provided by H. Zachau. Plasmid pLX31 containing the κ light chain gene MOPC41 (20) was generously provided by C. Queen and D. Baltimore.

***In Vitro* Transcription and RNA Analysis.** For transcription of the κ light chain genes, *in vitro* reactions were carried out as described (18); reaction mixtures (25 μl) contained 0.5 μg of DNA and 90 μg of extract protein. After RNA purification, transcripts were mapped with complementary SP6 RNA probes as described (21). After overnight hybridization at 45°C, the hybrids were treated with RNase T_2 (31 units/ml) and analyzed by electrophoresis in polyacrylamide gels in the presence of 7 M urea. After autoradiography, labeled transcripts were quantified by excision and scintillation counting. For transcription of the adenovirus type 2 major late (Ad2 ML) and human histone H2B promoters in circular plasmids, the protocol of Sawadogo and Roeder (11) was employed. Reaction conditions were as described above except that

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Abbreviation: Ad2 ML promoter, adenovirus type 2 major late promoter.

[α-^{32}P]UTP was used in place of [α-^{32}P]GTP, unlabeled GTP was omitted, and the Mg^{2+} concentration was either 3 mM (H2B) or 7 mM (Ad2 ML).

RESULTS

Accurate Transcription of κ Light Chain Genes in Lymphoid-Cell Extracts. Namalwa, a lymphoblastoid cell line derived from an African Burkitt lymphoma biopsy sample (22), was used as a B-cell source. Plasmids that contain the murine T1 and MOPC41 κ light chain genes and are actively transcribed *in vivo* were used as templates. Both genes have been characterized with respect to promoter-region sequences and *in vivo* initiation sites (4, 8, 9, 23). As summarized in Fig. 1, each gene contains the moderately conserved pentadecanucleotide (pd) sequence at −90 (relative to the cap site), the highly conserved decanucleotide sequence (dc) element at −60, and a TATA motif (8); the most highly conserved portion of the decanucleotide element is the octanucleotide ATTTGCAT (9).

Closed circular (supercoiled) templates containing the MOPC41 κ light chain gene were transcribed in nuclear extracts prepared from Namalwa cells by standard procedures (18). Accurately initiated transcripts, indicated by a 140-nucleotide signal, were scored with SP6-generated probes according to the scheme in Fig. 1 (*Lower*). The data in Fig. 2*A* show that the MOPC41 gene was accurately transcribed over a range of nuclear extract concentrations. The transcript was RNA polymerase II-dependent, as demonstrated by its extinction in the presence of 1 μg of α-amanitin per ml (data not shown). The band marked by the arrowhead results from minor vector-initiated readthrough (rt) transcripts, which have also been observed *in vivo* (20).

The optimal Mg^{2+} and template concentrations were found to be 6 mM and 20 μg/ml, respectively (data not shown), while the optimal input of nuclear extract was 12–15 μl (60–90 μg of protein). Whole-cell extracts from Namalwa cells also mediated accurate transcription of the MOPC41 κ light chain gene (Fig. 2*A*, lane 5), but quantitation of the transcripts indicated that the nuclear extracts were about 3-fold more active (normalized to protein input) under the conditions employed.

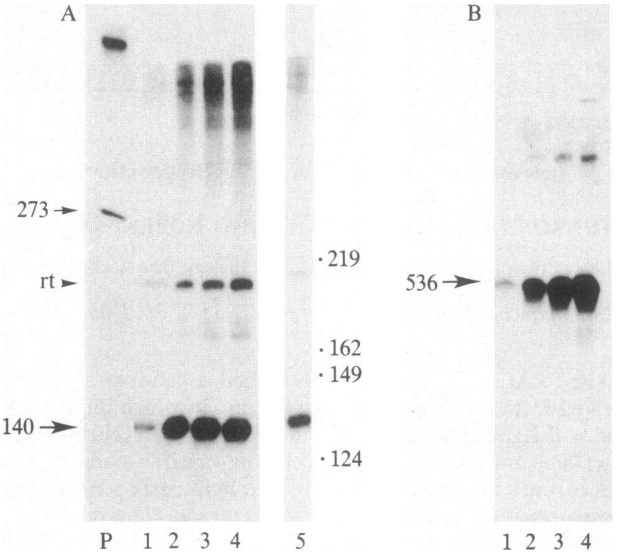

FIG. 2. Transcription of the MOPC41 κ light chain gene and the Ad2 ML promoter in B-cell extracts. (*A*) An intact (supercoiled) plasmid containing the MOPC41 κ gene was transcribed in reaction mixtures containing 4, 8, 12, or 15 μl of nuclear extract (lanes 1–4) or 6 μl of whole-cell extract (lane 5) from Namalwa cells. Transcripts were analyzed with the probe indicated in Fig. 1 *Lower*. Large arrow indicates accurately initiated transcripts, and the small arrow, the probe, analyzed separately in lane P. Readthrough (rt) transcripts are indicated by the arrowhead. Positions and lengths (in nucleotides) of markers (not shown) are indicated at right. (*B*) An Ad2 ML promoter-containing plasmid (pSmaF) was cut at the *Sma* I site (+536) and transcribed in reaction mixtures containing 4, 8, 12, or 15 μl of Namalwa cell nuclear extract (lanes 1–4, respectively). Position of the accurately initiated 536-nucleotide run-off transcript is indicated. All lanes are from the same gel and autoradiograph (which was spliced to eliminate irrelevant data).

Fig. 2*B* shows the results of transcription of a truncated plasmid containing the well-characterized Ad2 ML promoter

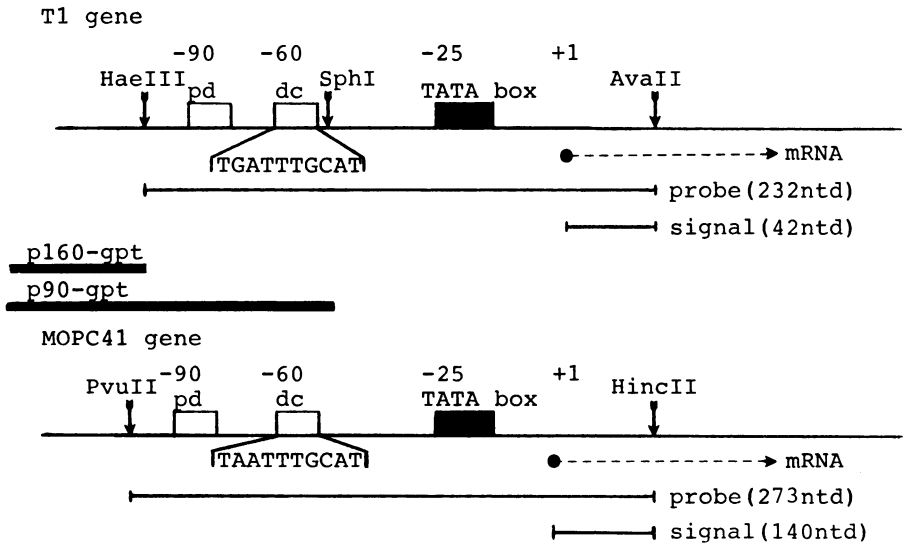

FIG. 1. Structure of κ light chain gene promoters. The diagrams indicate, for both the T1 (*Upper*) and MOPC41 (*Lower*) κ genes, the locations of the pentadecanucleotide (pd), decanucleotide (dc), and TATA elements relative to the transcription initiation (cap) site at +1. These elements are in virtually identical positions (see refs. 8, 9 for references) in the two genes. The location of the pd element in the MOPC41 gene was obtained from C. Queen (personal communication). The uniformly labeled probes indicated were prepared by cloning the *Hae* III–*Ava* II fragment (170 base pairs) of the T1 gene and the *Pvu* II–*Hin*cII fragment (231 base pairs) of the MOPC41 gene into the pGEM1 plasmid and transcription with SP6 and T7 RNA polymerases, respectively. On the basis of the known *in vivo* start sites (for references, see refs. 8 and 23), accurately initiated T1 and MOPC41 transcripts should give signals at 42 and 140 nucleotides, respectively, with the probes. Black bars show the regions deleted in plasmids p160-gpt and p90-gpt, with 5′-deletion endpoints at −131 and −57, respectively (8). ntd, Nucleotides.

in a Namalwa-derived nuclear extract. The 536-nucleotide signal (run-off product) is indicative of a high level of accurate initiation at this promoter, as previously observed with HeLa cell extracts (18). Human histone H4 and H2B promoters were also efficiently and accurately transcribed in the Namalwa nuclear extract (data not shown, see also below). Thus, this system supports initiation for a broad range of promoters.

B-Cell-Specific κ Light Chain Gene Transcription Directed by an Upstream Regulatory Region. To determine the generality of the Namalwa extract for transcribing other immunoglobulin genes, and to investigate the possible effects of upstream regulatory sequences, the T1 κ light chain gene was similarly analyzed in nuclear extracts according to the scheme in Fig. 1 (*Upper*). Plasmid p870-gpt contains the 5.5-kilobase *Eco*RI–*Bam*HI fragment, which includes the entire transcribed region and 845 base pairs of upstream sequence and is actively transcribed in transfected lymphoid cells but not in nonlymphoid cells (2, 8). Like the MOPC41 κ plasmid, the closed circular form of p870-gpt was accurately transcribed in the Namalwa nuclear extract (Fig. 3, lane 10) as evidenced by the 42-nucleotide signal (large arrow). The κ gene in p870-gpt was also accurately transcribed in a HeLa nuclear extract (lane 3), but at one-fifth the level observed in the Namalwa extract (the range in other experiments, when normalized to protein concentrations, was one-third to one-seventh). No transcripts were observed in control experiments without exogenous templates (lane 7 and 14), indicating that the specific signals derived from the products of exogenous templates and not from endogenous RNAs. As further controls, the efficiencies of transcription of circular templates containing the Ad2 ML (11) and human histone H2B (24) promoters were compared in HeLa vs. Namalwa extracts. As shown in Fig. 4, the ML template was

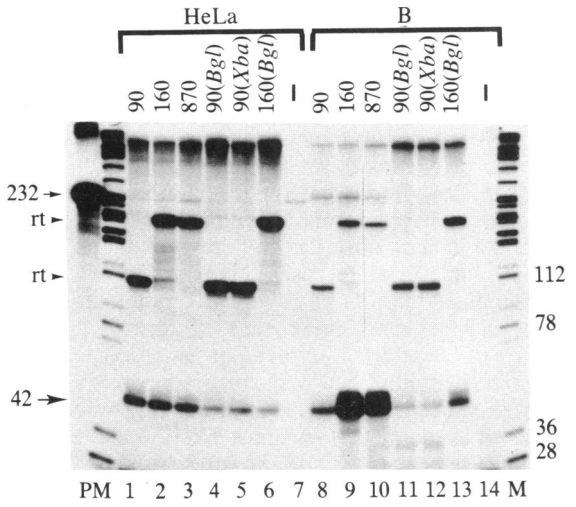

FIG. 3. Transcription of the T1 κ light chain gene in nuclear extracts from HeLa and B cells. Intact supercoiled plasmids (lanes 1–3 and 8–10) or restriction endonuclease-digested plasmids (lanes 4–6 and 11–13) containing different portions of the T1 κ gene were transcribed with HeLa (lanes 1–7) nuclear extract (96 μg of protein) or Namalwa (lanes 8–14) nuclear extract (90 μg of protein). The p90-gpt, p160-gpt, and p870-gpt plasmids contain, respectively, 82, 156, and 870 base pairs of κ gene sequence upstream of the ATG at position at +26 (see text and ref. 8). The linearized templates were digested with *Bgl* II or *Xba* I, as indicated. No templates were added in lanes 7 and 14. Transcripts were analyzed with the probe indicated in Fig. 1 *Upper*. Large arrow indicates the position of transcripts resulting from accurate initiation. Small arrow indicates the position of the probe (analyzed in lane P), and the small arrowheads indicate readthrough (rt) transcripts (see text). Markers (*Hpa* II-digested pBR322 plus pBR322 cut with *Sal* I, *Ava* I, and *Pst* I) are shown in lanes M.

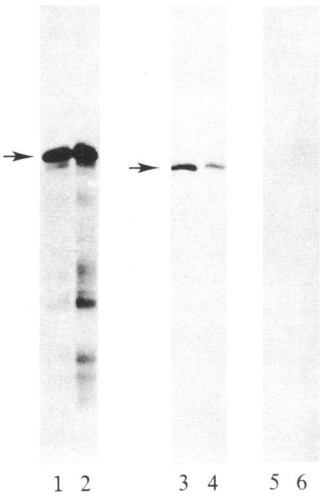

FIG. 4. Transcription of Ad2 ML and human histone H2B promoters in nuclear extracts from HeLa and B cells. Supercoiled plasmid pML(C_2AT) containing the ML promoter (−400 to +10) attached to a 400-base-pair cassette (C_2AT) containing no guanosine residues in the nontranscribed (RNA) strand was transcribed with nuclear extracts from HeLa cells (lane 1) and Namalwa cells (lane 2). A similar construct, containing the human histone H2B promoter (−174 to −18) attached to the C_2AT cassette (C. Fletcher, N. Heintz, and R.G.R., unpublished work), was transcribed in nuclear extracts from HeLa cells (lane 3) and Namalwa cells (lane 4). A control plasmid, pC_2AT, containing the C_2AT cassette, but no eukaryotic promoter, was transcribed in nuclear extracts from HeLa (lane 5) and Namalwa (lane 6) cells. Arrows indicate transcripts resulting from accurate initiation from these two promoters and elongation (in the absence of GTP) at the ends of the cassettes. Extract protein amounts were the same as in Fig. 3.

transcribed with equal efficiencies in the two extracts (lanes 1 and 2), whereas the H2B gene was transcribed about twice as efficiently in the HeLa extract (lanes 3 and 4) (see figure legend for details of the assay). Thus, the Namalwa extracts appear to reproduce in part the elevated cell-type specific transcription of κ light chain genes seen *in vivo*.

To further investigate this suggestion and to define the genetic basis for the elevated transcription of κ light chain genes in Namalwa extracts, a series of 5′-deletion mutants in the T1 κ gene were analyzed. Previous studies have shown that both p870-gpt (5′ deletion to −845) and p160-gpt (5′ deletion to −131) were actively transcribed in transfected plasmacytoma cells, whereas p90-gpt (5′ deletion to −57) (see Fig. 1 *Upper*) was not (note that the decanucleotide element extends from −59 to −68). As shown in the further analyses in Fig. 3, transcription of p160-gpt was indistinguishable from that of p870-gpt, showing a comparable high level of accurate initiation in Namalwa extracts (lanes 9 and 10) and a comparable low level in HeLa extracts (lanes 2 and 3). In contrast, with p90-gpt, accurate transcription in Namalwa extracts was reduced by a factor of 7 relative to that observed with p160-gpt and p870-gpt (lane 8 vs. lanes 9 and 10), whereas transcription in the HeLa extract remained at the low level observed with p160-gpt and p870-gpt (lane 1 vs. lanes 2 and 3). Further, the low level of upstream sequence-independent transcription (from the κ promoter) was roughly equivalent in the HeLa and Namalwa extracts. These results indicate that some of the upstream sequences located between −131 and −57 are indispensable for the increased B-cell-specific transcription of the κ light chain gene *in vitro* and that the factors which operate through these sequences are uniquely present (or active) in the B-cell extracts. Most significantly, the results obtained here with promoter mutants coincide with those observed *in vivo* (2, 8), although the relative level of upstream sequence-independent transcrip-

tion is apparently greater *in vitro* than *in vivo*. This most likely reflects a greater permissiveness of *in vitro* systems because of the loss of other *in vivo* constraints and has been observed for other genes (25, 26). At the same time, that the low levels of upstream sequence-independent transcription (from the κ promoter) are roughly the same for the HeLa and Namalwa extracts also argues that the major difference between these extracts, with respect to factors necessary for κ promoter function, is the B-cell-specific factor(s).

Also indicated in Fig. 3 are readthrough transcripts (designated by arrowheads) that originate in vector sequences. Since the probe contained 130 nucleotides of upstream sequence that was not present in p90-gpt (see Fig. 1), read-through transcripts from this plasmid are shorter than those from p160-gpt and p870-gpt. These transcripts were also observed *in vivo* (2, 8). It may also be noted, as a further internal control, that although more specific transcripts were observed with p160-gpt than with p90-gpt (above), the levels of readthrough transcripts were very similar (Fig. 3, lanes 8 and 9).

B-Cell-Specific Transcription Is More Evident with Circular Templates. To determine possible effects of template topology on the level of B-cell-specific transcription, plasmids p90-gpt and p160-gpt were linearized with *Bgl* II (which cuts 780 bp downstream of the cap site) and transcribed (Fig. 3). Again, in Namalwa extracts a higher level of specific transcripts was observed with p160-gpt than with p90-gpt (lanes 11 and 13), whereas identical levels of transcripts were observed with these plasmids in HeLa extracts (lanes 4 and 6). However, the magnitude of the difference (factor of 3) with the linearized templates in Namalwa extracts was not as great as that observed with the circular templates. This reflects a greater drop (factor of ≈5) in the upstream sequence-dependent transcription (lane 9 vs. lane 13) than in basal-level (upstream sequence-independent) transcription (lane 8 vs. lane 11) in going from circular to linear templates. These results suggest the importance of DNA conformation for the efficient function of the B-cell-specific factor(s) with the upstream sequence elements.

Increased Accumulation of κ Light Chain Gene Transcripts in B-Cell Extracts Is Not Due to Increased Stability. Transcripts synthesized in Namalwa extracts with plasmid p160-gpt were purified and incubated under transcription conditions in the presence of both Namalwa and HeLa extracts (with α-amanitin to block additional transcription), as well as in a control buffer. As shown in Fig. 5, the previously synthesized and accurately initiated transcripts were equally stable in the two nuclear extracts. These studies suggest that the higher level of κ transcripts observed in Namalwa extracts (by comparison with HeLa extracts) reflects a higher level of transcription and not RNA stabilization as a result of a B-cell-specific factor. This argument is consistent with the finding that the elevated level of B-cell transcripts is dependent upon upstream sequences that are without effect in the HeLa extract.

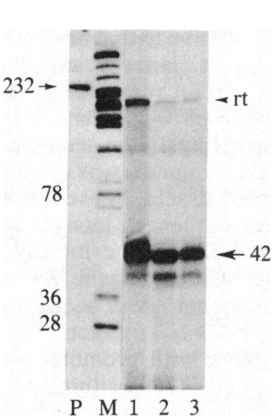

FIG. 5. Stability of κ transcripts in B-cell and HeLa cell nuclear extracts. The RNA synthesized in a Namalwa nuclear extract with a p160-gpt template was purified and aliquots were incubated with 25 μl of buffer [20 mM Tris·HCl, pH 7.9/20% (vol/vol) glycerol/0.5 mM dithiothreitol/0.2 mM EDTA/0.1 M KCl/0.5 mM phenylmethylsulfonyl fluoride] (lane 1), HeLa nuclear extract (lane 2), or Namalwa nuclear extract (lane 3) at 30°C for 1 hr under the same conditions used for transcription but with 1 μg of α-amanitin per ml. After incubation, the RNA was purified and analyzed as in Fig. 3.

DISCUSSION

Although the DNA sequences required for the regulated expression of immunoglobulin genes in the B-cell lineage are being defined by recombinant-DNA and gene-transfer techniques, equally crucial questions regarding the nature and mechanism of action of the cellular factors that must operate through these sequences remain to be answered. For the latter purpose, we have established a B-cell-derived cell-free system in which purified immunoglobulin genes are both accurately transcribed and regulated in a cell-type-specific manner.

***In Vitro* Transcription of Immunoglobulin Genes Is Dependent Upon Upstream Promoter Sequences and a B-Cell-Specific Factor(s).** The B-cell lymphoma (Namalwa cell)-derived nuclear extract was shown to mediate accurate transcription from two murine κ light chain genes, as well as from several control genes (Ad2 ML and histone H2B and H4). Two further observations indicate that the tissue-specific promoter regulation observed *in vivo* has been reproduced, at least qualitatively, *in vitro*. First, the κ gene was transcribed up to 7-fold more efficiently in B-cell extracts than in HeLa cell extracts, whereas control genes were transcribed equally or slightly more efficiently in HeLa extracts. Second, the elevated level of transcription observed in B-cell extracts was dependent upon upstream sequences (in the −131 to −57 region) previously shown to be essential for tissue-specific transcription in cultured lymphoid cells (2, 4, 8, 23); in contrast, the removal of these sequences had no effect on the basal level of transcription observed in HeLa extracts. These observations indicate that there is at least one factor responsible for the upstream sequence-dependent transcription of the immunoglobulin gene that is present (or active) only in B-cell extracts. That a basal level of accurate κ light chain gene transcription was observed in HeLa cells does not detract from the interpretation or significance of these results, since some permissiveness in the cell-free systems is expected on the basis of previous results showing low levels of accurate transcription with only the TATA regions (25, 26). Moreover, low levels of accurate initiation from light chain promoters (driven by "promiscuous" enhancers) have been observed in nonlymphoid cells in some cases (6), while in other cases this possibly was not eliminated because mRNA turnover was not analyzed. This system thus provides the means to further analyze the B-cell-specific factor(s) involved in immunoglobulin light chain (and, probably, heavy chain) transcription. Although the upstream sequences involved in this regulation have not been carefully defined, they most likely include the decanucleotide element (2–8) and the internal highly conserved octanucleotide (below), but not the upstream pentadecanucleotide element (23).

Relationship of Immunoglobulin Promoter Elements (and Interacting Factors) to Those Present in Other Genes. Although the decanucleotide element (and an interacting factor) has been presumed to be the major determinant of tissue specificity in immunoglobulin promoters, several observations argue against such a simplistic view. First, the conserved octamer is also present in other genes whose expression is not restricted to lymphoid cells including: the promoter of histone H2B genes (reviewed in ref. 24 and 27) and the enhancer of U1 and U2 small nuclear RNA genes (reviewed in refs. 27 and 28). Second, although Singh *et al.* (10) reported a nuclear factor that bound to the decanucleotide elements of a κ light chain variable-region gene (V_κ) promoter and a μ heavy chain constant-region gene enhancer, this factor was present in both HeLa and B cells.

Similarly, Sive and Roeder (27) demonstrated that a HeLa nuclear factor which bound to the human H2B octanucleotide also interacted with related elements in the V_κ promoter and the C_μ and U2 enhancers; they also showed that point mutants in the H2B octanucleotide inhibited both binding and transcription. Given these results, and the different modes of regulation of the various genes that contain these elements as functional units, it seems clear that there must be other factors and/or promoter elements involved in the control of these genes. Several possibilities may be considered. (*i*) Transcription of these genes may involve a common octanucleotide-binding factor, whose interactions and function are influenced by other specific factors that bind an adjacent region; candidates would include the histone-specific "hexamer" site in the H2B gene (24), the G+C-rich cluster in the U2 gene (28), and as yet unidentified sites in the immunoglobulin promoters. (*ii*) Transcription of these genes may employ a common octanucleotide-binding factor whose function in different genes is modulated by tissue-specific posttranslational modifications (outside of the DNA binding domain). (*iii*) These genes may employ a related set of factors (distinct gene products) with different functional activities but with similar binding activities that are not readily distinguished in simple DNA-binding (electrophoretic mobility-shift) assays. The present study suggests that the HeLa factor does not function on the κ promoter, although previous studies indicated an interaction of the HeLa factor (in comparable nuclear extracts) with the κ promoter (10, 27) and that the HeLa factor both interacted with the H2B octanucleotide and stimulated transcription (24, 27). The resolution of these fundamentally important questions regarding the role of the octanucleotide-binding factors, and the possible involvement of other specific factors and/or promoter sequences, awaits both a more detailed analysis of promoter structure and the direct isolation and characterization of the factors necessary for the function of the genetic elements.

Mechanism of Action of the B-Cell-Specific Factor(s). Although we have little detailed information of the mechanism by which the inferred B-cell-specific factor(s) operates, several points may be made. First, assuming that the decanucleotide element and its associated factor are involved, the proximity of that element to the TATA box raises the possibility of a direct interaction of the upstream sequence-binding factor(s) with the TATA box-binding factor (transcription factor TFIID), as has been shown for the upstream factor that activates the Ad2 ML promoter (26). Second, the effect of the upstream sequence is much greater with supercoiled than with linear templates, suggesting that some aspect of DNA topology (superhelical density and/or circularity) is important for function *in vitro* as well as *in vivo* (reviewed in ref. 29). Third, the B-cell-specific effect on the promoter is not dependent upon the presence of the enhancer (data not shown), in agreement with the *in vivo* studies demonstrating promoter function with heterologous enhancers (2–7). Finally, the effect of the upstream promoter element has been observed with purified DNA in a low-salt nuclear extract that should not have the capability of assembling a potentially repressive chromatin structure (by virtue of the absence of core histones). Consequently, the present results strongly suggest that the B-cell-specific factor(s) does not simply play an indirect role in promoter activation, for example by marking the gene and keeping it accessible to other factors in an otherwise repressive chromatin structure (discussed in ref. 29), but rather that it plays a more direct role in stimulating transcription by other initiation factors and RNA polymerase II. Of course, the B-cell-specific factor(s) might still, like the 5S rRNA gene-specific transcription factor TFIIIA, both play a direct role in initiation (30) and promote assembly of an active chromatin state (31). The presence of DNase I-hypersensitive sites in the upstream regions of κ promoters (32) is consistent with these possibilities. The identification of B-cell-specific factors and studies of their mechanism, regulation, and possible roles in gene commitment events remain important and increasingly tractable problems, given the *in vitro* system described here.

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