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Expression of the J chain gene during B cell differentiation is inversely correlated with DNA methylation

(*Hpa* II/*Msp I* isoschizomers/immunoglobulin secretion/somatic cell hybrids/DNA sequence rearrangement/control of transcription)

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ABSTRACT **During B cell differentiation, transcription of the J chain gene is initiated. To determine the regulatory mechanism involved, we have analyzed the structure of the J chain gene in cell lines representing successive stages in B cell development. Comparison of restriction sites showed that the J chain gene does not require a rearrangement of DNA for expression; cleavage sites present in embryonic J chain DNA were preserved through all the subsequent differentiative steps. However, comparison of 5-methylcytosine contents showed that J chain gene expression correlates with a loss of methyl groups. The J chain gene was heavily methylated in cell types not expressing J chain (i.e., embryo and lymphomas representative of immature and mature B cells) and significantly less methylated in cell lines representative of antigen-stimulated lymphocytes synthesizing J chain. These changes in J chain gene methylation represent a specific differentiation-induced response. Analyses of the heavy chain C_μ and $C_{\gamma 2b}$ genes, which are expressed at earlier and later stages than the J chain gene, showed that the C_μ sequences were undermethylated in all cell types examined whereas the $C_{\gamma 2b}$ sequences were undermethylated only in cell lines expressing the IgG2b protein. The primary encounter of a B cell with antigen must therefore trigger events that effect J chain gene transcription through a mechanism involving loss of methyl groups from cytosine.**

The immunocompetent B lymphocyte responds to a primary antigenic stimulation by differentiating into a blast cell that assembles and secretes pentamer IgM antibody (1). The assembly process requires a small protein, the J chain, which functions to initiate IgM polymerization and is incorporated into the secreted product in a regular stoichiometry of one J chain per five monomer IgM subunits (2). By analyzing the J chain content of B cells before and after stimulation, it was found that J chain synthesis is induced as a consequence of the interaction of antigen with specific receptors on the B cell surface (3). Moreover, by comparing the expression of J chain-specific RNA in non-IgM- and IgM-secreting cell lines, J chain synthesis was found to be controlled at the level of transcription rather than at the levels of RNA processing and translation (4). Investigations of the mechanism for activating the J chain gene therefore should provide valuable clues as to how membrane receptor signals are transmitted to the nucleus and how they induce the expression of new gene products.

One possible mechanism of activation is gene rearrangement. A striking example occurs early in the development of the B cell when a translocation of variable (and D) gene segments is required for the expression of μ heavy chain and light chain (5–7). Recombinational events also occur late in the life history of the B cell; switches in the class of antibody secreted are accomplished by translocation of the heavy chain variable region gene complex to the 5′ side of constant region genes farther downstream (8–10). It would not be surprising, therefore, if J chain expression, which is associated with an intermediate stage in B cell differentiation, were effected by a similar rearrangement process.

A second mechanism proposed for transcriptional control involves DNA modification (11). The DNA of eukaryotic cells contains small but significant quantities of a modified base, 5-methylcytosine, which is found primarily in the dinucleotide 5′ CpG 3′ (12). Recent analyses of the extent of cytosine modification suggest that methylation of this base is inversely correlated with gene activity. Thus, in systems ranging from *Xenopus* ribosomal genes to integrated adenovirus, the transcriptionally active sequences have consistently been found to be less methylated than their nonexpressed counterparts (13, 14). Surveys of methylated sites in rabbit and human β-globin gene clusters indicate that transcriptional activation is associated with the loss of methyl groups from specific sites rather than with undermethylation of all the CpG sequences in the gene cluster (15, 16). Finally, inhibitors of methylation have been shown to induce differentiation in both erythroleukemic and embryonic cell lines (17, 18).

We have investigated which of these activation mechanisms leads to J chain gene expression by analyzing the DNA from cell lines representing different stages in B cell development. The possibility of gene translocation was evaluated by digesting the DNAs with various restriction endonucleases and comparing the patterns obtained after hybridization with a J chain cDNA probe. The possibility of gene modification was investigated by performing tandem digestions with the restriction endonucleases *Msp* I and *Hpa* II. These enzymes recognize and cut the same sequence, 5′ C-C-G-G 3′, but differ in their sensitivity to methylation of the inner cytosine residue (13–16, 19): *Hpa* II does not cleave DNA methylated at this position, whereas *Msp* I cleavage is independent of methylation at this residue. Therefore, digestion of a given DNA with *Msp* I can be used to determine the positions of the C-C-G-G sequences nearest the gene of interest, and digestion with *Hpa* II will indicate the state of methylation of those sequences. The cell lines used as sources of DNA were selected as representatives of specific differentiative stages on the basis of surface markers and immunoglobulin chain production (20).

MATERIALS AND METHODS

Cells and Culture Conditions. The MOPC 104E plasmacytoma was grown as an ascites tumor in Pristane-primed BALB/c mice. The B lymphomas WEHI 231 and 279.1, the myeloma cell line MPC 11, and a hybrid derived from the fusion of WEHI 231 and MPC 11, M×W 231.1.2, were obtained from W. Raschke (Salk Institute) (21, 22). MOPC 315 cells were adapted

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Abbreviation: kb, kilobase(s).

to culture by E. Siden (Massachusetts Institute of Technology). L929 fibroblast cells were provided by O. Martinez (University of California, Berkeley). These cell lines were maintained in Dulbecco's modified Eagle's medium containing 4500 mg of glucose per liter of medium and supplemented with 5% or 10% fetal calf serum. The WEHI 231 and 279.1 lines required the addition of 2-mercaptoethanol to 50 μM; 279.1 cells further required 0.1 mM asparagine in the medium. The B lymphoma 70Z/3 was the gift of P. Kincaide (23). The lymphomas L10A(6.2), X16C(8.4), and K46R were provided by R. Asofsky and K.-J. Kim (National Institutes of Health, Bethesda, MD) (24). These lines were maintained in RPMI-1640 with 10% fetal calf serum and 50 μM 2-mercaptoethanol.

DNA Preparations. High molecular weight DNA was prepared from cells and tissues essentially as described by Gross-Bellard *et al.* (25). Briefly, cells were harvested, washed in 100 mM NaCl/20 mM Tris·HCl, pH 7.5/1 mM EDTA, and lysed by the addition of an equal volume of the same buffer containing 0.2% $NaDodSO_4$ and proteinase K (Merck) at 100–500 μg/ml. The lysate was incubated at 60°C for 2 hr and then extracted three or four times with phenol saturated with the lysis buffer, twice with phenol/chloroform, 1:1 (vol/vol), and twice with chloroform. The DNA was precipitated with 3 vol of isopropanol and dissolved in 10 mM Tris·HCl, pH 8.0/1 mM EDTA. Ten-day BALB/c embryos were homogenized to single-cell suspensions in 100 mM NaCl/20 mM Tris·HCl, pH 7.5/10 mM EDTA, lysed, and processed as described above.

Restriction Enzyme Digestions, DNA Transfer, and Hybridization. The DNA preparations were digested with a 4- to 6-fold excess of restriction endonuclease (usually 2 units of enzyme per μg of DNA for 2–3 hr at 37°C) in the buffers recommended by the manufacturers (New England BioLabs or Bethesda Research Laboratory, Rockville, MD), and electrophoresed on 0.5–1.0% agarose gels in a Tris/borate/EDTA buffer (26). The DNA was denatured and transferred onto nitrocellulose filters (Schleicher & Schuell, BA85) according to the procedure of Southern (27) and hybridized by the method of Wahl *et al.* (28) using a cDNA clone of the J chain mRNA (4) labeled by nick-translation with [α-^{32}P]deoxynucleotides (New England Nuclear) (29). The filters were washed in 0.1% $NaDodSO_4$ and decreasing salt concentrations to 0.3× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M Na citrate, pH 7.4) at 63°C and then exposed at −70°C to Kodak X-Omat R film through DuPont Cronex Lightning-Plus intensifying screens. For analysis of the μ chain gene, a 10.5 kilobase (kb) *Eco*RI genomic fragment which included the sequences for the entire Cμ gene through the membrane exon and 4 kb of 5′ flanking sequence was nick-translated and used as a probe. For the γ_{2b} gene analyses, the probe used was a pMB9 plasmid containing a γ_{2b} cDNA insert (30). The insert included the 3′ untranslated region and most of the constant region of the γ_{2b} gene through $C_\gamma 1$ but lacked the variable region sequences.

The completeness of the *Msp* I and *Hpa* II digestions was monitored by adding 43 ng of λ DNA to the digestion mixtures at the start of incubation. After hybridization and autoradiography with the probes of interest, the filters were rehybridized with ^{32}P-labeled λ DNA, washed, and autoradiographed as above. The results showed that the λ DNA was digested to completion in all the reaction mixtures.

RESULTS

Translocation of J Chain Gene. To determine whether the J chain gene is activated by a translocation mechanism, the restriction pattern of embryo DNA was compared to that of more differentiated lymphoid cells. When the various DNAs were digested with restriction endonucleases that did not cut the J chain cDNA used as a probe, a single band of hybridization was found in each preparation (Fig. 1). The mobilities of the bands were identical whether the source of the DNA was undifferentiated embryonic tissue, a B lymphoma that does not express J chain, or an IgG-secreting plasmacytoma that synthesizes appreciable quantities of the J protein. Thus, digestion with *Eco*RI invariably yielded a J chain-specific 6.4-kb fragment, digestion with *Bam*HI yielded a 6.2-kb fragment, and digestion with *Hin*dIII yielded an 8.0-kb fragment. A similar conservation of restriction sites was observed when the DNAs were digested with *Taq* I which cuts at a single site within the J chain cDNA sequences (Fig. 2). In this case, two fragments, 3.5 and 1.5 kb, were generated from all the DNAs examined. These findings indicate that the J chain gene, unlike the immunoglobulin heavy and light chain genes, does not require a translocation of gene segments for expression.

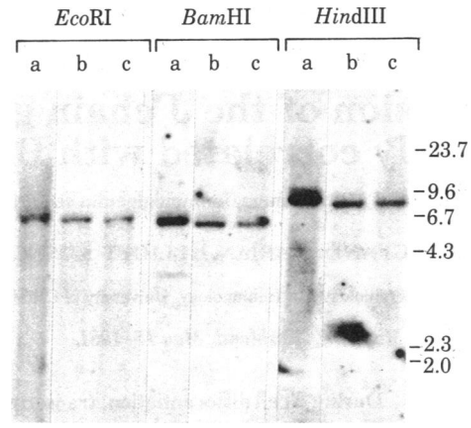

FIG. 1. Restriction patterns of J chain DNA. DNA (10 μg) from embryo (lanes a), B lymphoma WEHI 231 (lanes b), or myeloma MPC 11 (lanes c) was digested to completion with the indicated enzyme. The fragments were separated by electrophoresis through 0.5% agarose gels and hybridized to the J chain cDNA. The indicated fragment lengths (in kb) are those of λ(cI857s7) digested with *Hin*dIII.

Methylation and J Chain Gene Expression. The relationship between cytosine methylation and J chain gene expression was first investigated by using a set of cell lines that had been characterized in previous studies of J chain function (22). The WEHI 231 lymphoma was shown to have the properties of a relatively immature B cell that synthesizes monomer IgM as a membrane receptor but does not express J chain and, consequently, does not secrete pentamer IgM. However, pentamer IgM secretion could be induced by fusing this lymphoma with a cell line, MPC

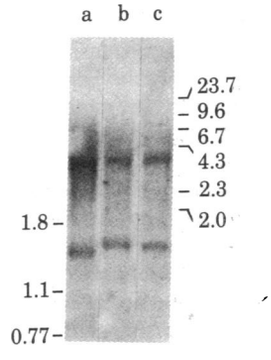

FIG. 2. *Taq* I restriction pattern of J chain DNA. DNA (10 μg) as in Fig. 1 was digested and fragments were separated on an 0.8% agarose gel and hybridized to the J chain cDNA. λ*Hin*dIII and simian virus *Hin*fI fragments (shown in kb) were used as standards.

11, that is representative of a fully differentiated plasma cell producing large quantities of IgG and J chain. Analyses of the hybrids indicated that pentamer IgM was expressed as a result of complementation between the synthetic capacities of the parental lines. Thus, these lines provided a useful system for probing changes in J chain gene methylation that might be associated with B cell differentiation to pentamer IgM secretion.

For the methylation studies, DNA from the parental and hybrid lines was digested with *Msp* I and *Hpa* II and the fragments containing J chain sequences were identified by the Southern blotting technique. DNA from a fibroblast line, L929, was used as a nonlymphoid control. Digestion with *Msp* I generated a single J chain-positive 7.5-kb fragment from all four DNA preparations (Fig. 3 *Left*), a pattern similar to that observed in the translocation experiments described above. Digestion with *Hpa* II, however, resulted in strikingly different restriction pattern (Fig. 3 *Right*). The J chain DNA sequences from the nonlymphoid line and the B lymphoma were located in very high molecular weight fractions, 12–15 kb in the case of the nonlymphoid L cells and >20 kb in the case of the B lymphoma. The relatively weak intensity of the high molecular weight bands compared to those seen in the *Msp* I autoradiogram may have been due to inefficient transfer of such large fragments to nitrocellulose. In contrast, the J chain DNA sequences from the hybrid and plasmacytoma cells were located in significantly lower molecular weight fragments, the smallest of which comigrated with the 7.5-kb fragment obtained by *Msp* I digestion. These data suggested that the gene is heavily methylated in J chain-negative lines, whereas the gene is relatively undermethylated in lines expressing J chain.

Further evidence that J chain expression is associated with gene undermethylation was obtained by analyzing a panel of cell lines representing successive stages in B cell differentiation to pentamer IgM secretion (Fig. 4). The panel included both lymphomas with the characteristics of immature B cells and those shown to have the properties of mature B cells (21–24). Also included in the survey were the WEHI 279.1 and MOPC 104E lines which represent the early and late stages, respectively, in the development of IgM-secreting plasma cells (20). The results of *Hpa* II digestion (Fig. 5) supported the data obtained in the previous analyses of the parent–hybrid cell system. In three of the four B lymphomas examined, the J chain sequences were again found exclusively in the high molecular weight (>20 kb) fraction. Again, in the IgM-secreting lines, the J chain sequences appeared predominantly in the undermethylated 7.5-kb fragment. In addition, two cell lines gave rise to intermediate patterns of hybridization. DNA from the L10A lymphoma contained a mixture of heavily (>20 kb) and partially (12–15 kb) methylated copies of the J chain gene and DNA from the WEHI 279.1 line contained a mixture of heavily methylated and undermethylated gene copies.

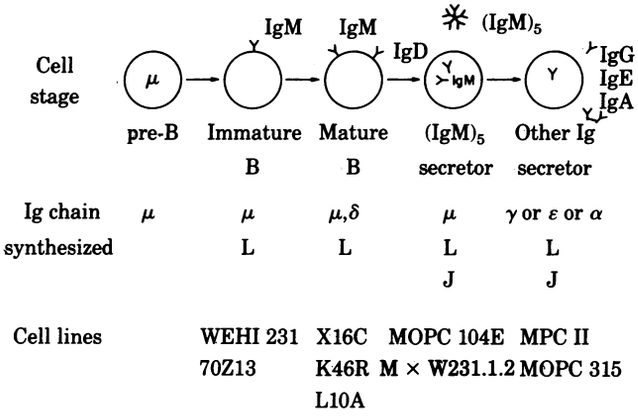

FIG. 4. Stages of B cell differentiation and representative cell lines. The assignments of cell lines to specific differentiative stages were made as described in ref. 20.

The methylation patterns correlated closely with the known phenotypes of the cell lines surveyed. In those cells that do not express the J protein, the J chain gene was found exclusively in the heavily methylated form with the exception of the L10A lymphoma. This line displays the blast appearance characteristic of a newly stimulated B lymphocyte, and thus the finding of some partially methylated J chain sequences is consistent with its more differentiated state. On the other hand, the completely undermethylated form of the J chain gene was seen only in those cells actively expressing J chain. Although partially and heavily methylated species were also present, the fact that the 7.5-kb *Hpa* II fragment was found exclusively in J-expressing cells suggested that the latter represents the transcriptionally active gene form. The presence of heavily methylated as well as completely undermethylated gene copies in the WEHI 279.1 line can be explained on the basis of its phenotype; the line has been shown to be a counterpart of an intermediate in the IgM secretion pathway that characteristically retains membrane IgM

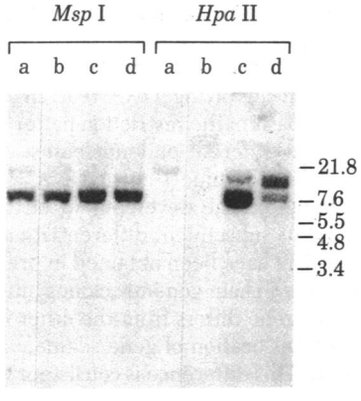

FIG. 3. Methylation of C-C-G-G sequences in J chain-specific DNA. DNA (7.5 μg) from fibroblast line L929 (lanes a), B lymphoma WEHI 231 (lanes b), fusion hybrid 231.1.2 (lanes c), and myeloma MPC 11 (lanes d) was digested and the fragments were electrophoresed on 0.7% agarose gels and hybridized with J chain cDNA insert. The indicated fragment lengths (wkb) are those of λ (cI857s7) digested with *Eco*RI.

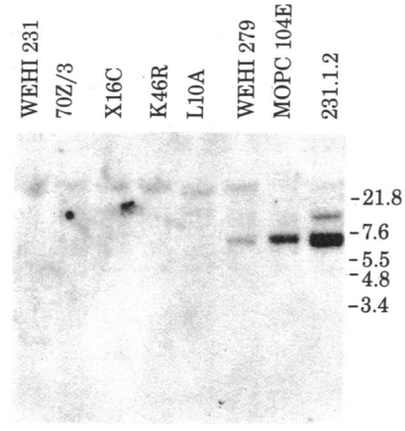

FIG. 5. J chain gene methylation in lymphoid cell lines. The DNA preparations were digested with *Hpa* II and the fragments were electrophoresed on 0.7% agarose gels and hybridized with the J chain cDNA insert.

but also synthesizes small amounts of J chain and pentamer IgM. However, the finding of partially methylated (10–12 kb) forms of the J chain gene in mature IgG-secreting cells and the IgM-secreting hybrid line is more difficult to explain. One possibility is that these species are transcriptionally active because the methylated cytosines are located in irrelevant positions. Another possibility is that these species represent unexpressed copies of the J chain gene that are present in the polyploid MPC 11 and 231.1.2 hybrid cells.

Methylation of the μ and γ Heavy Chain Genes. The methylation of several other Ig genes was investigated to determine whether the observed changes in the J chain gene are specifically associated with its expression or merely reflect a general modification of lymphoid DNA during B cell differentiation. The constant region genes of the μ and γ heavy chains were selected for study because they were known to have different patterns of expression compared to the J chain gene. As shown in Fig. 4, μ chain synthesis is initiated in the pre-B cell whereas γ chain synthesis is induced by a switchover event that occurs late in the life history of the B cell. In order to compare the gene methylation patterns, the DNA analyses were carried out on a panel of cells that represented the entire spectrum of differentiation.

The C_μ gene fragments generated by *Msp* I and *Hpa* II digestion are compared in Fig. 6. The hybridization patterns were more complex than those of the J chain gene because multiple 5′ C-C-G-G 3′ sequences occur within the C_μ gene (31), and the unexpressed C_μ gene can rearrange during differentiation (32). Despite their complexity, the hybridization patterns after *Msp* I and *Hpa* II digestion were identical for each DNA preparation examined. The identity of the *Msp* I and *Hpa* II patterns from embryo DNA indicated that the unrearranged μ constant region is undermethylated, and the patterns from the γ_{2b}-producing MPC 11 plasmacytoma suggested that extensively rearranged, nonfunctional, C_μ gene copies are also undermethylated. Furthermore, the extent of methylation of C_μ genes did not differ noticeably in non-IgM- and IgM-expressing cells. These findings were in marked contrast to the results of the J chain gene studies and they suggested that activation of the C_μ gene may not involve DNA modification.

The methylation pattern of the $C_{\gamma 2b}$ gene, on the other hand,

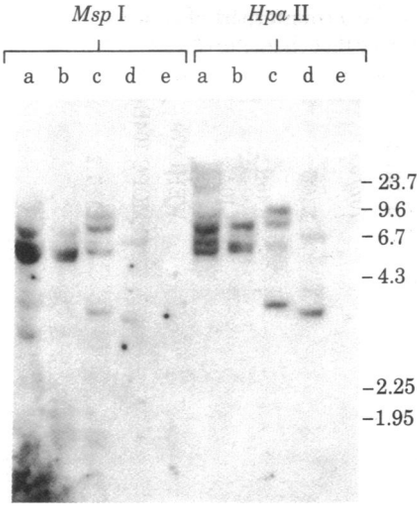

FIG. 6. Methylation of the C_μ gene in lymphoid cell lines. DNA (7.5 μg) was digested with *Msp* I or *Hpa* II; the fragments were separated on an 0.7% agarose gel and hybridized with the plasmid containing the C_μ gene. Lanes: a, embryo; b, WEHI 231; c, MOPC 104E; d, MPC 11; e, MOPC 315.

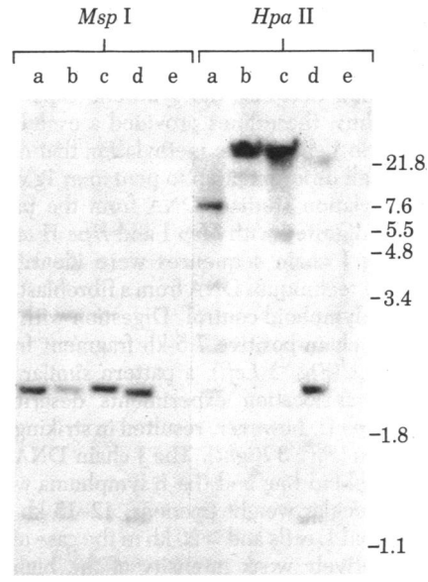

FIG. 7. Methylation of the $C_{\gamma 2b}$ gene in lymphoid cell lines. The DNA preparations were digested and electrophoresed as in Fig. 6 and hybridized with the $C_{\gamma 2b}$ cDNA insert. Lanes as in Fig. 6.

was found to be analogous to that of the J chain gene (Fig. 7). *Msp* I cleavage of the various DNAs yielded a strongly hybridizing 2.2-kb fragment and several small, less intensely hybridizing fragments some of which probably resulted from cross-hybridization with the C_γ genes (33, 34). The MOPC 315 preparation was the exception; no bands of hybridization were detected, indicating that the $C_{\gamma 2b}$ genes have been deleted from this line. *Hpa* II digestion of the same DNAs showed that the extent of methylation of the $C_{\gamma 2b}$ gene changed radically with its expression. In the DNA from IgG-negative lines, the γ_{2b} sequences were contained in high molecular weight fractions; only in the IgG2b-expressing MPC 11 line were the gene sequences found in the completely undermethylated 2.2-kb fragment. On the basis of these data, it would appear that the observed changes in the methylation of J chain and $C_{\gamma 2b}$ genes correlate specifically with their transcription.

DISCUSSION

By analyzing the structure of the J chain gene as a function of B cell differentiation, we have been able to distinguish among several possible mechanisms for regulating transcription of this gene. A mechanism involving DNA rearrangement could be eliminated on the basis of the restriction patterns obtained after digestion with five different endonucleases. All the cleavage sites identified in embryonic J chain DNA were found to be maintained throughout the development of the immunocompetent B cell and its subsequent differentiation to antibody secretion. Similar data have been obtained in preliminary restriction mapping of the J chain genomic clones (unpublished data). Thus, the J chain gene differs from the other immunoglobulin genes in that a translocation of gene segments is not required for its expression. This difference is consistent with the function of the J protein; because it plays a single structural role in polymer immunoglobulin assembly, there is no need for a recombination of genetic information.

The restriction patterns obtained after *Msp* I and *Hpa* II cleavages, on the other hand, support a mechanism of transcriptional control involving cytosine modification. The J chain gene was found to be heavily methylated in cell lines repre-

sentative of the early differentiative stages before the J protein is expressed. In contrast, at least one copy of the gene is completely undermethylated in lines that are counterparts of antigen-stimulated cells synthesizing J chain. Because these data were derived from homogeneous cell populations, they provide clear-cut evidence that methylation of the J chain gene is inversely correlated with its transcriptional activity. Moreover, the data suggest that very few CpG sequences need to be demethylated for J chain gene transcription. In the DNA restriction patterns from a panel of lymphoid lines, only three discrete partially methylated fragments were detected rather than the smear that would be expected if a large number of CpG sites were involved.

The correlation between J chain gene transcription and undermethylation was strengthened by comparing the restriction patterns of the J chain gene with those of the C_μ and $C_{\gamma 2b}$ genes. The C_μ gene was found to be unmethylated in every tissue and cell line examined, whether or not the μ heavy chain was expressed. In contrast, the $C_{\gamma 2b}$ gene remains heavily methylated through all the differentiative steps that precede its expression, including the stage at which J chain synthesis is induced. Once IgG2b synthesis is initiated, however, undermethylated forms of the gene are found. These results indicate that changes in immunoglobulin gene methylation occur with the expression of a specific gene product and do not represent a general differentiation-induced response. In addition, the results imply that the switch to IgG synthesis requires both a rearrangement of heavy chain DNA sequences and a modification of cytosine residues. The same dual mechanism may operate in μ chain synthesis. The finding that C_μ DNA lacks methylcytosine does not necessarily eliminate the possibility that methylation influences transcription of the μ chain gene. If CpG sites in the "upstream" variable region sequences are modified prior to translocation, such changes would not be detected by the probes used in the present studies.

The coupling observed between J chain gene transcription and cytosine modification has important implications for understanding the signaling of differentiation. It provides a biochemical clue as to how signals generated at the B cell membrane effect the expression of a specific gene. Moreover, differences in the methylation patterns of the parent and hybrid lines provide a system for pursuing this clue. The J chain genes in the nonproducing parent WEHI 231 are heavily methylated; those in the J chain-positive parent MPC 11 are either partially or completely undermethylated. Yet in the hybrid daughter, 231.1.2, no methylated copies of the gene can be detected, and nearly all copies of the gene are undermethylated. Because the hybrid cells appear to contain the full complement of parental chromosomes (22), these findings indicate that a product or products of the MPC 11-derived genes induced the removal of methyl groups from the unexpressed J chain genes on the lymphoma chromosomes. Thus, by comparing the nuclear contents of the parent and hybrid lines, it should be possible to identify the gene product(s) involved and further clarify the mechanism by which activation of the J chain gene is signaled.

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