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13. Compound **1** was prepared earlier [F. Nakatsubo, I. D. Reid, T. K. Kirk, *Biochem. Biophys. Res. Commun.* **102**, 484 (1981)]. Specific activity = 2.4×10^9 dpm/mmole.
14. Cultures were grown as described (*9*), except that they were buffered at *p*H 4.3 with 10 m*M*, 2,2-dimethylsuccinate. [Ligninolytic activity appears as a part of idiophasic metabolism, triggered by nitrogen starvation (*3*).] Replicate cultures were pooled and centrifuged (10,000*g*; 15 minutes) and the mycelial pellet was discarded. The supernatant was concentrated about tenfold by means of a 10,000-dalton filter, and then freed of any residual cells or spores by passage through a 0.45-μm filter (T = 4°C throughout). This preparation contained 80 to 100 μg of protein per milliliter.
15. Products were extracted with a mixture of chloroform and acetate (1:1). Radioactive products were isolated by TLC and quantified by scintillation spectrometry (*9*). TLC procedures: C.-L. Chen, H.-m. Chang, T. K. Kirk, *Holzforschung* **36**, 3 (1982). Unlabeled standards for compounds **4** and **5** were prepared as described for related compounds (*9*).
16. This compound was prepared from 3-methoxy-4-hydroxy-α-(2-methoxyphenoxy)-β-hydroxypropiophenone, labeled uniformly with ^{14}C in the propiophenone ring [L. L. Landucci, S. A. Geddes, T. K. Kirk, *Holzforschung* **35**, 67 (1981)], by ethylation with ethyl iodide and K_2CO_3 in *N*,*N*-dimethylformamide, and subsequent reduction with $NaBH_4$. Specific activity was 7.2×10^8 dpm/mmole.
17. T. K. Kirk and H.-m. Chang [*Holzforschung* **28**, 217 (1974)] isolated and characterized this sample. It was chosen here for its high phenolic hydroxyl content [0.44 hydroxyls per monomer (C_9) unit], indicative of a large number of end groups.
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19. About 2 mg of each lignin was methylated with $^{14}CH_3I$ and K_2CO_3 in *N*,*N*-dimethylformamide. Specific activities: spruce, 1.9×10^8 dpm/mg; birch, 1.1×10^8 dpm/mg.
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21. A column (2.5 by 43 cm) of Bio-Gel P-100 was eluted with 100 m*M* sodium acetate, *p*H 4.5 and calibrated with glucose oxidase, serum albumin, ovalbumin, cytochrome c and vitamin B_{12}.
22. The K_M probably reflects formation of **3** via two competing reactions; therefore, the value of 55 μ*M* is not a true K_M. Nevertheless, the results demonstrate saturation kinetics.
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* On assignment from Department of Wood and Paper Science, North Carolina State University, Raleigh 27650.

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Transcriptional Enhancer Elements in the Mouse Immunoglobulin Heavy Chain Locus

Abstract. *Two regions in the immunoglobulin heavy chain locus were tested for their ability to enhance transcription of the SV40 early promoter. A portion of the intervening sequence between the heavy chain joining region (J_h) and the constant region of the μ chain (Cμ) can enhance transcription when it is cloned either 5′ or 3′ to the SV40 early promoter. The region between Cα and the alpha switch site, which occurs 5′ to the translocated c-myc oncogene in many murine plasmacytomas, does not show transcriptional enhancer activity in this assay.*

Efficient transcription from certain eukaryotic viral promoters requires the presence of positive regulatory sequences that have been called enhancer sequences (*1*). Enhancer sequences have been found in several papovaviruses and retroviruses (*2*). Although there is no striking sequence similarity among enhancers from various viruses, they generally have several properties in common: (i) they act on promoters only in *cis*, (ii) they can act from a location either 3′ or 5′ to the promoter, (iii) they usually work in either orientation with respect to the promoter, and (iv) they can act on heterologous promoters. The activity of certain mammalian promoters, such as the rabbit β-globin promoter, is significantly increased in the presence of a viral enhancer sequence (*3*). It is not yet known whether DNA sequences similar in function to the viral enhancers also occur in mammalian DNA and whether they might play a role in gene regulation. Several lines of evidence discussed below have led us to ascertain whether enhancer-like elements are located at two particular sites in the immunoglobulin heavy chain gene region.

During ontogeny of antibody-producing B lymphocytes, functional heavy chain genes are formed as a result of a DNA rearrangement called VDJ joining (*4*). Unrearranged variable (V) gene segments contain the necessary 5′ sequences for transcription but are not actively transcribed even in fully differentiated B cells (*5*). Regulatory regions 5′ to the V gene cap (initiation) site remain unaltered during B-cell development (*6*). Thus, enhancer elements located between J_h (heavy chain joining region) and Cμ (constant μ chain region), brought into functional proximity with V_h promoters after VDJ joining, could activate V_h transcription. Two observations support this suggestion: (i) Cμ gene segments are transcribed in lymphoid cells in the absence of VDJ joining (*7*, *8*) and (ii) a pre-B-cell lymphoma line which suffered a deletion in the J_h-Cμ intron shows a decrease in Cμ transcripts (*8*).

Most murine plasmacytomas and human Burkitt's lymphomas have characteristic chromosomal alterations which involve translocation of c-myc (the cellular homolog of the avian myelocytomatosis virus transforming gene) to the immunoglobulin heavy chain locus (*9*). In murine plasmacytomas, the c-myc gene is often translocated a few kilobases 5′ to the nonexpressed Cα gene segment (*10*). Translocation leads to an increase in c-myc transcripts, many of which are initiated at aberrant sites (*11*). Since enhancer sequences associated with the Cα gene segment could be responsible for this altered c-myc transcription, we set out to determine if such elements were located 5′ of Cα.

We used a convenient vector system (*12*) to test for enhancer activity in the immunoglobulin heavy chain locus. The pA10CAT-2 vector contains the SV40 early promoter directing transcription of the bacterial chloramphenicol acetyltransferase (CAT) gene but does not contain a functional SV40 72-bp enhancer sequence. Since CAT activity is normally absent from mammalian cells, the enhancer activity of fragments cloned into the vector may be quantified by measuring CAT activity in transfected cells. A positive control, pSV2-CAT, contains the SV40 enhancer sequence.

Portions of the J_h-Cμ intron and of the 5′ flanking region of the Cα gene, which were cloned into pA10CAT-2, are illustrated in Fig. 1b. The clones were transfected into COS cells (SV40 transformed monkey kidney line constituitively expressing T antigen), and the CAT activity was determined. The J_h-Cμ intervening sequence contained on a 7.6-kb Bam HI fragment is capable of enhancing transcription from the SV40 early promoter when it is cloned 3′ to the CAT gene in the "antisense" orientation (Fig. 2a). The immunoglobulin sequence had 60 percent activity of the SV40 enhancer element, which represents a 20-fold increase over the enhancer-deleted negative control.

Since there is a low amount of CAT gene transcription from the pA10CAT-2 vector lacking an enhancer, it was possible that the high levels of CAT activity observed with immunoglobulin sequence cloned into pA10CAT-2 were the result of preferential replication of these vectors to levels 20-fold higher than pA10CAT-2 itself. Therefore, we determined the approximate copy number of the various vectors in the extrachromosomal DNA fraction (Hirt supernatant) (*13*) of transfected cells by probing quantitative dot blots (*14*) with a pA10CAT-2 probe. Although this experiment does not distinguish between replicated and nonreplicated molecules, nor can we be sure that all of the copies detected are

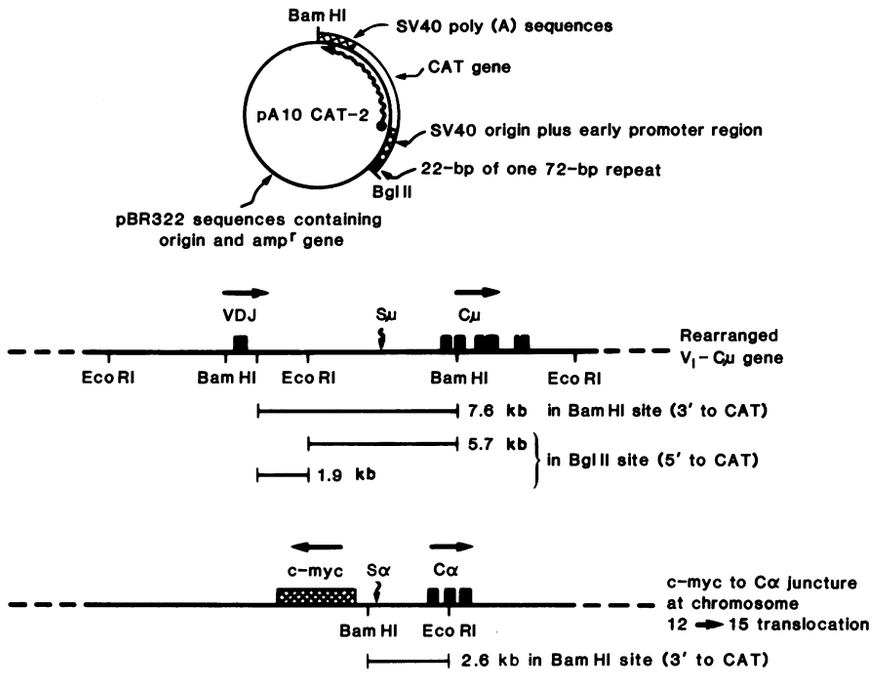

Fig. 1. The vectors used in the assay for transcriptional enhancing activity. (A) The vector pA10CAT-2. (B) The bars beneath the Cμ and Cα genomic clones indicate the regions subcloned into pA10CAT-2 either 5′ (Bgl II site) or 3′ (Bam HI site) to the CAT gene. Arrows above the exons indicate the 5′ to 3′ direction of transcription. Sμ and Sα indicate μ and α switch regions.

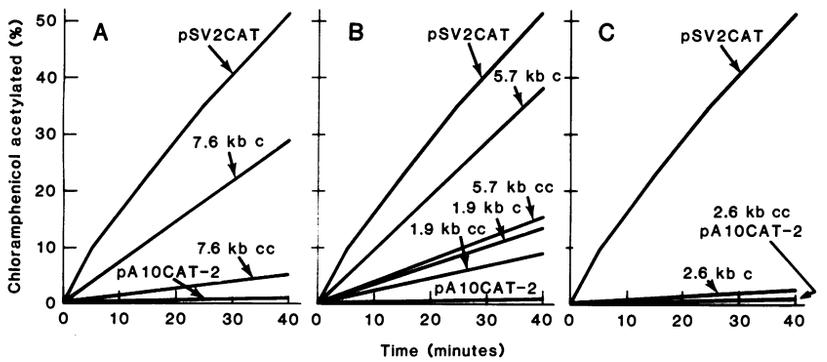

Fig. 2. CAT enzyme activity in transfected cells. Approximately 44 hours after transfection by the calcium phosphate coprecipitation technique, cells were harvested and extracts were assayed for enzyme activity (*12*). The rate curves shown for the individual clones represent the average of six transfections normalized to the activity observed for pSV2CAT, which contains both endogenous SV40 enhancers. The orientations of the fragments from the Cμ and Cα clones are indicated: "c" is antisense and "cc" is sense with respect to the CAT gene as diagrammed in Fig. 1. (A) The entire region between J_h and Cμ cloned in both orientations; (B) J_h-Cμ region, divided at the Eco RI site, cloned in both orientations; (C) region 5′ to Cα cloned in both orientations.

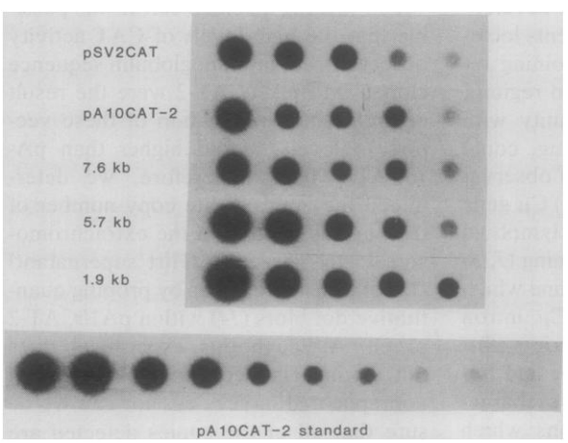

Fig. 3. Vector DNA recoverable in Hirt supernatants (*13*) of transfected cells. DNA from Hirt extracts was serially diluted 1:2 and transferred to nitrocellulose and probed with pA10CAT-2. The first dot in each set represents DNA from approximately 2×10^3 cells. For comparison, 3 ng of pA10CAT-2 plasmid DNA was serially diluted 1:2 and likewise probed.

functional templates for transcription, the results (Fig. 3) show that the immunoglobulin clones are not present at significantly higher levels than either the positive or negative control vectors. Therefore the high CAT enzyme activity (Fig. 2) is due to enhanced transcription of the CAT gene and not to copy number effects.

The J_h-Cμ region was divided at the Eco RI site, and each portion was cloned separately 5′ to the CAT gene in pA10CAT-2. Both fragments had enhancer activity (Fig. 2b); the 1.9-kb fragment 5′ of the Eco RI site had lower activity than the 5.7-kb fragment 3′ of the Eco RI site cloned in the antisense orientation. The simplest interpretation of these data is that there is more than one region of enhancer activity in the J_h-Cμ intron, one located between J4 and the Eco RI site and a second located 3′ to the Eco RI site. It may also be that a single enhancer region spans the Eco RI site. A 2.5-kb Bam HI–Eco RI fragment, containing the region of mouse chromosome 12 that is translocated 5′ to the c-myc gene in plasmacytoma M603, was also tested in pA10CAT-2 for enhancer activity. This fragment had no enhancer activity in either orientation (Fig. 2c).

The orientation of the 7.6- and 5.7-kb inserts relative to the CAT gene had a strong influence on the amount of enhancement observed. The active orientation of the 5.7- and the 7.6-kb fragments is the same relative to the CAT gene in their respective 5′ and 3′ positions. This fact is inconsistent with the possibility that a strong promoter in the J_h-Cμ region masks the enhancer activity in one orientation. However, since the cloned fragments are large, we may be observing a distance rather than an orientation effect. Cloning smaller segments of the region is necessary to clarify this question and to identify the location and number of enhancer elements in the J_h-Cμ region.

The J_h-Cμ sequences give less enhancement than the SV40 72-bp repeat (Fig. 2). In addition, we have been unable to demonstrate enhancer activity in the J_h-Cμ region with CV1 cells, the nontransformed parent line of COS cells (data not shown). These findings may reflect a requirement by the J_h-Cμ sequences for tissue-specific cellular components in a manner similar to the specificity found for viral enhancers (*2*). In COS cells increased numbers of templates or altered template structures resulting from replication may allow detection of enhancers that interact weakly with heterologous cellular factors.

The J_h-Cμ sequence as the first exam-

ple of a cellular enhancer element adjacent to a known gene strengthens the hypothesis that enhancer elements may be important in regulation of cellular genes. In agreement with our results, Banerji *et al.* have also observed that portions of J_h-Cμ enhance transcription of the SV40 early promoter (*15*). It is difficult to extrapolate from viral systems to B cells where the J_h-Cμ sequences may not activate V_h promoters. However, cellular promoters from human, rabbit, and chicken can be enhanced with viral sequences (*5, 16*), and cellular DNA with enhancer activity has been detected previously (*17*). Furthermore, that V_h gene activation is mediated by sequences on the same chromosome located distant from the Vh promoter is entirely compatible with the properties of viral enhancers. Thus, the sequences identified in our study may act in situ as enhancers for immunoglobulin promoters and may explain how V_h gene transcription is activated on VDJ joining. This model is supported by the work of Gillies *et al.* (*18*) who have shown that specific deletions in the J_h-Cμ intron result in decreased transcription from a V_h promoter when the deleted gene is transfected into myeloma cells.

No transcriptional enhancer element was detectable by our assay between Cα and the alpha switch site—the region located 5′ to the translocated c-myc oncogene in many murine plasmacytomas. It is a formal possibility that a mouse lymphoid cell specific enhancer element does exist in this region but was not detected. However, this seems unlikely for two reasons. First, our system provides a very sensitive test for enhancers because small amounts of CAT enzyme are measurable and because COS cells amplify the number of templates. Second, we have used this system to identify enhancer activity in the J_h-Cμ region, where it has also been observed by others with different cells and promoters. The J_h-Cμ enhancer, like its viral counterparts, is able to act on various promoters in different cell types. Thus, some regulatory mechanism other than chromosome 12 enhancer elements is probably responsible for aberrant transcription of translocated c-myc genes. This would be consistent with the recent findings of Erikson *et al.* (*19*) who have shown that increased c-myc transcription occurs in all Burkitt's lymphoma lines regardless of the proximity of the translocation juncture to the c-myc gene or of the region in the heavy chain locus to which the c-myc gene is translocated. Possibly different chromatin structure or the removal of negative regulatory sequences is responsible for the altered transcription of the translocated c-myc.

MARK MERCOLA
XIAO-FAN WANG
JORY OLSEN
KATHRYN CALAME
Department of Biological Chemistry and Molecular Biology Institute, University of California, Los Angeles 90024

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Chromosomal Mosaicism Confined to the Placenta in Human Conceptions

Abstract. *Placental and fetal tissues from 46 human pregnancies were cultured and cytogenetically analyzed in an attempt to document the existence of chromosomal mosaicism confined strictly to tissues of extraembryonic origin. In two gestations in which chromosomal mosaicism was found, it was expressed exclusively in placental chorionic cells and was not detected in cells derived from the embryo proper. This demonstration of confined chorionic mosaicism may have implications for the understanding of the fetoplacental unit and for prenatal diagnosis.*

The number and distribution of chromosomally abnormal cells in a mosaic conceptus has been thought to reflect the relative viability of the cells in the early cleavage embryo in which a nonlethal nondisjunctional event first occurred. Cell lineage now appears to be equally important (*1*), and therefore a single random nondisjunctional event occurring at a very early stage could produce mosaicism in the placenta or the fetus, but not necessarily in both, since only three or four cells in a mammalian blastocyst are selected as progenitors of the embryo proper (*2*). There should be many instances of mosaicism in which cells of only one line are selected for the embryo; since most of the remaining cells will become the placenta, the incidence of mosaic placentas should be higher than the incidence of mosaic fetuses. To test this prediction, we analyzed matched chorion, amnion, and cord blood samples from 46 human conceptuses. In the two instances in which chromosomal mosaicism was found, it was expressed exclusively in placental chorionic cells and was not detected in cells derived from the embryo proper.

Table 1. Chromosomal mosaicism in placental cultures from 46 gestations.

Category	Number of cultures		
	Total	One cell line	Two cell lines
Normal term male infants	9	9	0
Aneuploid infants and fetuses	6	6	0
Infants with IUGR	31	29	2

Chromosomal mosaicism confined to chorionic tissue has not been previously described. Although mosaicism has been