fibrin-Sepharose is 1/100 of that of unconjugated urokinase, at both 2.5 and 15 hours. The control conjugate does not differ significantly from unconjugated urokinase. These results have been reproduced in separate experiments with three different conjugate preparations. Urokinase-antifibrin conjugate enhances the rate of release of peptides from fibrin-Sepharose, and this effect is unimpaired by fibrinogen at a physiologic concentration (Fig. 2). β-Peptide inhibits fibrinolysis of urokinase-antifibrin conjugate, whereas it has no effect on the fibrinolytic rate of unconjugated urokinase or control conjugate. In the presence of β-peptide (1.5 mg/ml) during the incubation of urokinase with fibrin-Sepharose, 82 percent of the increase in fibrinolysis is blocked. Taken together, the data in Fig. 2 and the β-peptide inhibition of increased fibrinolysis are consistent with the specificity of antibody 64C5 (*4*).

Thus a monoclonal antibody specific for fibrin is able to target the plasminogen activator urokinase to fibrin and, by virtue of enhanced local concentration, increase the efficiency of plasmin lysis by a factor of 100. The antibody is sufficiently fibrin-specific that physiologic concentrations of fibrinogen do not interfere with enhanced fibrinolysis. Fibrinolytic effectiveness is not increased by the coupling of urokinase to a monoclonal antibody of irrelevant specificity, and it is diminished by a peptide representing the epitope recognized by the fibrin-specific antibody. If these effects can be demonstrated in vivo, the dose of urokinase required for effective lysis of a thrombus would result in only minimal fibrinogenolysis, the major impediment to the widespread application of this form of therapy.

Note added in proof: Preliminary reports of a similar nature have been reported (*13*).

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The Role of the c-*mos* Gene in the 8;21 Translocation in Human Acute Myeloblastic Leukemia

Abstract. *The human* c-mos *proto-oncogene is located on chromosome 8 at band q22, close to the breakpoint in the t(8;21) (q22;q22) chromosome rearrangement. This translocation is associated with acute myeloblastic leukemia, subgroup M2. The* c-myc *gene, another proto-oncogene, has been mapped to 8q24. The breakpoint at 8q22 separates these genes, as determined by in situ hybridization of* c-mos *and* c-myc *probes. The* c-mos *gene remains on the 8q− chromosome and the* c-myc *gene is translocated to the 21q+ chromosome. Southern blot analysis of DNA from bone marrow cells of four patients with this translocation showed no rearrangement of* c-mos.

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The cellular homolog (c-*mos*) of the transforming sequence (v-*mos*) of the Moloney murine sarcoma virus has transforming activity when linked to a viral long terminal repeat (LTR) and transfected into NIH 3T3 cells (*1*). Transcriptional activation of c-*mos* has also been observed in non–virally induced mouse plasmacytomas XRPC-24 (*2*) and MOPC-21 (*3, 4*) after insertion of an LTR (from an intracisternal A-type particle) at the 5′ end of the c-*mos* gene coding region. Apart from these two cell lines, transcription of c-*mos* has not been observed in various normal or malignant cells (*5, 6*) and c-*mos* has not been associated with spontaneously developing malignancies. The human c-*mos* gene has been cloned from human genomic libraries (*7–9*) and assigned to chromosome 8 by Southern blot hybridization to DNA from somatic cell hybrids. By means of in situ hybridization between a human c-*mos* probe and human meiotic chromosomes, the gene was localized to band q22 (*10*); this location is proximal to the locus of the c-*myc* gene, which had previously been assigned to band q24 (*10*). The c-*myc* gene is frequently rearranged and is transcriptionally activated in the t(8;14) (q24;q32) translocation of Burkitt lymphoma after juxtaposition with the immunoglobulin gene sequences (*11*). The chromosomal band to which c-*mos* has been mapped is also the site of one of the breakpoints in the t(8;21)(q22;q22) of acute myeloblastic leukemia (AML) (*12, 13*). We have begun a study of this translocation to explore the possibility that c-*mos* may be activated by the rearrangement.

The 21q+ chromosome (which contains the distal portion of chromosome 8) from malignant cells of a patient having the M2 subtype of AML (AML with maturation) and a t(8;21) carries the translocated c-*myc* gene but lacks the c-*mos* gene (*14*). We now provide evidence from two additional cases that the c-*mos* gene remains on the 8q− derivative chromosome after the t(8;21), whereas the c-*myc* gene is translocated to the 21q+ chromosome. To detect possible rearrangements of c-*mos* due to the translocation breakpoint, we have also analyzed the genomic DNA from the malignant cells of four AML patients with a t(8;21).

DNA probes were derived from (i) a human genomic clone containing c-*mos* coding and flanking sequences within a 2.7-kilobase (kb) Eco RI insert in pBR322 (*8*) and (ii) a complementary DNA clone containing part of the second and all of the third exon of the c-*myc* gene in a 1.03-kb insert cloned in pBR322 (*15*). The ^{3}H-labeled probes were prepared from the complete plasmids by nick-translation and were used for in situ hybridization (Table 1 and Fig. 1). When the two probes were hybridized to normal metaphase cells from peripheral blood lymphocytes, specific labeling of

Table 1. The statistical analysis of the distribution of observed number of grains (O) over chromosomes 8 and 21 and the expected number (E) if grain distribution was according to chromosome length after in situ hybridization with c-*mos* and c-*myc* probes. Numbers in parentheses after the probe designation represent the number of cells analyzed. Cells were from a normal control (phytohemagglutinin-stimulated peripheral blood lymphocytes), or from two AML patients (bone marrow cells) with a t(8;21) (q22;q22). The χ^2 values corresponding to probability levels below 0.005 are italicized. The technique for the in-situ hybridization experiments was as described (*23*).

Chromosome		Normal						Patient 1						Patient 2					
Number	Relative length (%)	c-*mos* (50)			c-*myc* (50)			c-*mos* (100)			c-*myc* (83)			c-*mos* (41)			c-*myc* (23)		
		O	E	χ^2	O	E	χ^2	O	E	χ^2	O	E	χ^2	O	E	χ^2	O	E	χ^2
8	2.39	12	5.1	*9.3*	22	7.5	*27.8*	23	5.0	*64.8*	12	4.7	*11.3*	6	1.8	*9.8*	5	1.4	*9.3*
8q−	1.79							18	3.8	*53.1*	4	3.5	0.07	7	1.4	*22.4*	3	1.1	3.3
21	0.85	3	1.8	0.8	2	2.7	0.17	4	1.8	2.7	1	1.7	0.3	2	0.7	2.4	0	0.5	0.5
21q+	1.44							4	3.0	0.3	11	2.9	*22.6*	0	1.1	1.1	5	0.8	*22.1*
Total*		107			158			211			198			77			59		

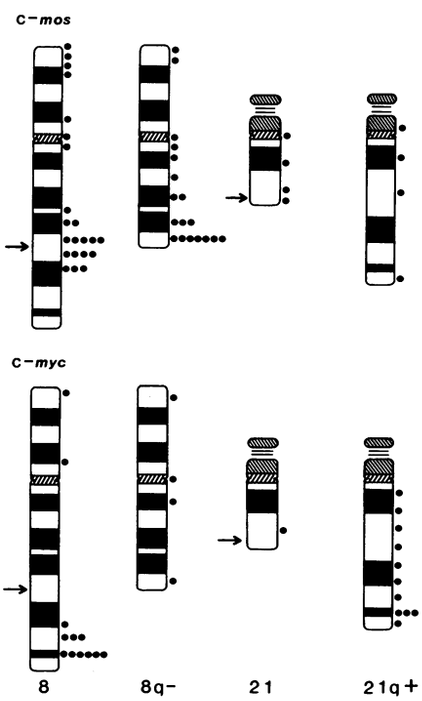
*Total number of grains.

chromosome 8 was observed (χ^2, $P < 0.005$). There was no significant labeling of chromosome 21 or of any other chromosomes. Of the 50 metaphase cells examined from the hybridizations with c-*mos*, 9 cells (18 percent) showed label on one or both chromosomes 8 at bands q22 or q23. These labeled sites represented 8.4 percent of all labeled sites. For c-*myc*, 12 of 50 cells (25 percent) showed label on one or both chromosomes 8 at band q24; this represents 7.3 percent of all labeled sites.

Of 100 metaphase cells with a t(8;21) examined from patient 1-[46,XY,t(8;21)(q22;q22)] that were hybridized with c-*mos*, 15 cells showed label on 8q21.3 to 8q23 of the normal chromosome 8, and 10 were labeled on 8q21 to 8q22 of the rearranged homolog (Fig. 1, top). These sites represented 7.1 percent (15/211) and 4.7 percent (10/211) of all labeled sites, respectively. Both the normal chromosome 8 and the 8q− chromosome were significantly labeled (χ^2, $P < 0.005$). In contrast, neither the normal chromosome 21 nor the 21q+ chromosome showed significant labeling. Analysis of 83 metaphase cells hybridized with the c-*myc* probe showed significant labeling of both the normal chromosome 8 at band q24 (10/198, 5 percent of all labeled sites) and at the corresponding band on the 21q+ chromosome (6/198, 3 percent) (Fig. 1, bottom). The normal chromosome 21 and the 8q− were not significantly labeled. Similar results were obtained from hybridizations of metaphase cells from a second patient with this translocation [46,XY,t(8;21)(q22;q22)(91 percent)/46,XY,t(8;21),del(9)(q13q31) (9 percent), Table 1].

Thus, in metaphase cells with the t(8;21), the c-*mos* probe hybridized specifically to both the normal and rearranged chromosomes 8, whereas the c-*myc* probe hybridized to the normal chromosome 8 and to the 21q+ chromosome. These results indicate that c-*mos* is located proximal to the 8q22 breakpoint in the t(8;21) and remains on the 8q− chromosome. As anticipated by the earlier localization of c-*myc* to 8q24 (*10*) and by the results obtained with the somatic cell hybrids retaining the 21q+, this proto-oncogene is translocated to the derivative chromosome 21.

To detect rearrangements of the c-*mos* gene at the molecular level, we prepared Southern blots from the Bam HI- and Bgl II-digested DNA of bone marrow cells from four AML patients with a t(8;21) or human placental DNA; the DNA was hybridized with ^{32}P-labeled c-*mos* DNA. In each patient, only germline-sized fragments were detected with these two enzymes (Fig. 2). These results do not exclude the possibility of rearrangements related to the chromo-



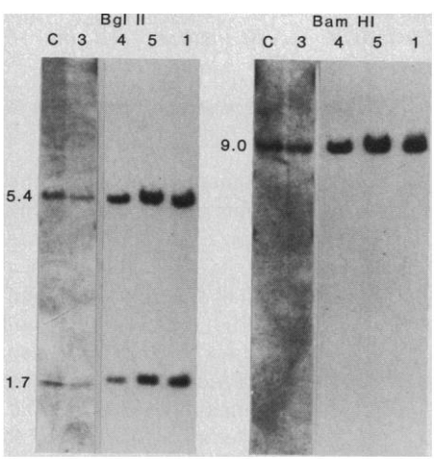


Fig. 1 (left). Distribution of labeled sites on chromosomes 8 and 21 and on the translocation derivatives 8q− and 21q+ after hybridizations of c-*mos* and c-*myc* probes to metaphase cells from patient 1, an AML patient with a t(8;21)(q22;q22). Clusters of grains (filled circles) were observed around band 8q22 on the normal chromosome 8 and close to the breakpoint junction on the 8q− chromosome in hybridizations with the c-*mos* probe. In hybridizations with the c-*myc* probe, clusters of grains were noted on the distal region of the long arm of the normal chromosome 8 at band q24 and on the homologous region on the 21q+ chromosome. Arrows show the position of the breakpoints on the normal chromosomes. The ^{3}H-labeled probes were prepared by nick-translation of the complete plasmids to specific activities of 3.6×10^7 (c-*mos*) and 7.7×10^7 (c-*myc*) count/min per microgram of DNA. Fig. 2 (right). Southern blots of DNA from bone marrow cells from four AML patients with a t(8;21). DNA was extracted, digested, separated by electrophoresis, and hybridized to the probe as previously described (*14*). The ^{32}P-labeled probe used was prepared by nick-translation of the c-*mos* gene–containing plasmid to a specific activity of $\sim 10^8$ count/min per microgram of DNA. Human placental DNA was used as a control (C). Patient 3 has been described (*14*) and cells of patient 1 were used for in situ hybridization here. The size of the DNA fragments is in kilobase-pairs.

somal breakpoint occurring outside the c-*mos*–containing sequences encompassed by the 5′-side Bam HI site (5.0 kb upstream of the c-*mos* coding sequence) and the 3′ Bgl II site (5.2 kb downstream of the c-*mos* coding sequence).

Our results indicate that the c-*mos* gene remains on the 8q− chromosome after the t(8;21) in AML. Analysis of complex translocations involving three chromosomes (8, 21, and a third chromosome) has demonstrated that the junction on the 8q− created by the movement of material from chromosome 21 is conserved, whereas the chromosome that donates material to 21 is variable (*16, 17*). The observation that there is a conserved junction in the three-way translocation variants in the t(8;21) of AML, in the t(15;17) in acute promyelocytic leukemia, and in the t(9;22) in chronic myeloid leukemia suggests that the genes or DNA sequences that are relevant to the development of these neoplasias are located in such conserved junctions (*17*). In each of the t(9;22) complex variants that have been analyzed, the c-*abl* gene is translocated to the conserved junction on the 22q− chromosome (*18*). The function of the c-*abl* gene is clearly altered in CML, resulting in transcripts of an abnormal size (*19*) and in protein products of abnormal size and function (*20*).

The association of the c-*mos* gene with the conserved junction of the t(8;21) may be fortuitous, but it is compatible with the hypothesis that this gene is involved in the oncogenic process for this type of leukemia. The finding that no rearrangement of c-*mos* was found in the Southern blot analysis does not exclude this hypothesis, because in other translocations, the breakpoints may be far from the affected genes. For example, in the t(9;22), the breakpoint on chromosome 9 is located from 14 kb to more than 100 kb upstream of the c-*abl* gene (*21*). Moreover, the Daudi line of Burkitt lymphoma cells, which has a t(8;14), has an activated c-*myc* gene, although no rearrangement has been detected within a 25-kb Bam HI fragment (*22*).

Note added in proof: After completion of this manuscript we learned that transcription of c-*mos* was detected in mouse testis, ovary, and embryos (*24*).

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Noninvasive Study of High-Energy Phosphate Metabolism in Human Heart by Depth-Resolved ^{31}P NMR Spectroscopy

Abstract. *Phosphorus-31 nuclear magnetic resonance (NMR) spectra showing the relative concentrations of high-energy phosphate metabolites have been recorded noninvasively from the human heart in vivo. Spectral data were spatially localized by combining a pulsed magnetic field gradient with surface NMR excitation-detection coils. The location of the selected spectral region was determined by conventional proton NMR imaging immediately before examination by phosphorus-31 NMR spectroscopy.*

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Current noninvasive clinical techniques for assessing injury in heart disease utilize the anatomical information provided by x-ray computed tomography, ultrasound imaging, γ cameras, emission tomography, and proton (^{1}H) nuclear magnetic resonance (NMR) imaging techniques. Limited information on relative perfusion and glucose and fatty acid metabolism in the heart is also available from γ cameras and emission tomography by administration of isotopically labeled pharmaceuticals (*1*). An advantage of metabolic probes in general is that physiologic changes affecting heart function are virtually immediately detectable and can precede anatomic changes by days or more. Such probes are therefore better suited to monitoring therapeutic response.

Recent applications of natural-abundance phosphorus-31 (^{31}P) NMR to the detection of high-energy phosphate metabolism in vivo have provided valuable diagnostic information about various muscular and cerebral disorders in adults and infants without the use of ionizing radiation (*2*). ^{31}P NMR studies of isolated perfused rodent hearts have revealed transient metabolic response to drug therapies (*3, 4*), cardiotoxic chemotherapeutic agents (*5*), and global and regional ischemia (*6*) in times as short as 1.5 minutes after onset (*7*). In most cases, pathology is detected by ^{31}P NMR spectroscopy as disturbances in the ratios of the high-energy metabolites phosphocreatine (PCr) and adenosine triphosphate (ATP) as well as inorganic phosphate (P_i).

The in vivo ^{31}P heart studies have been extended in situ by enclosing the heart in an NMR coil either permanently implanted or in open-chest animals (*8–10*); by using catheter NMR coils inserted through a peripheral blood vessel (*11*); and by restricting the NMR spectrometer sensitivity to the heart by magnetic field profiling and using an external NMR coil with the surface musculature of the subject surgically removed (*12*). This work was limited to rodents and dogs, and the techniques were rendered invasive by the necessity of ensuring adequate spatial localization of the ^{31}P