the nucleotide sequence of clone pHMP10A should help to clarify these controversial issues.

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Response: We reported the chromosomal localization and the translocation and rearrangement of the myeloperoxidase gene (*MPO*) in two patients with acute promyelocytic leukemia (APL) (*1*). This report has caused some concern among other groups who have localized the *MPO* gene to a more distal site (*2, 3*) on chromosome 17 and among those who have not demonstrated any molecular rearrangements of this gene using two different, independently isolated, *MPO* cDNA clones (*4*).

We too are eager to resolve these discrepancies. With regard to the chromosomal localization of *MPO*, in situ hybridization of the pHMP10A clone resulted in specific labeling of chromosome 17 at bands q11–q22. Hybridization of this clone and of the pHMP2E probe to metaphase cells from bone marrow aspirates from APL patients resulted in specific labeling of the normal chromosome 17 at bands q11–q22 and of the translocation derivative 15q+ at bands q12–q21. We concluded from these studies that *MPO* was located at q12–q21, distal to the breakpoint of the t(15;17) at band 17q11.2, and that it had been relocated to chromosome 15 as a result of this translocation. The translocation of *MPO* to the derivative 15q+ in an APL patient has also been shown by Liang *et al.* (*5*). As noted by Miller *et al.*, in situ hybridization experiments conducted by other investigators resulted in specific labeling of a more distal site on chromosome 17 at bands q22–q23 (*2, 3*); however, the localization of *MPO* to chromosome 17 with the use of a panel of somatic cell hybrids containing deleted or translocated segments of this chromosome resulted in the assignment of *MPO* to 17q21–q23 (*3*), which overlaps with our localization. Van Tuinen *et al.* (*3*) found that a hybrid which contained bands 17q21 to qter (including most or all of 17q21, as illustrated in figure 1 of their paper) was positive for *MPO* sequences. Discrepancies in the localization of genes to specific chromosome bands by investigators who use different techniques or different probes are not uncommon. It is possible that the more distal localization of *MPO* to chromosome 17 is correct (*2, 3*), in which case rearrangements of *MPO* in APL cells would likely be unrelated to the translocation breakpoint. This possibility was clearly stated in our report. Close proximity of *MPO* to the t(15;17) breakpoint is not requisite for rearrangements of this gene to occur. If *MPO* is located at 17q22–q23, why our probe hybridized to the adjacent proximal bands of this chromosome is unclear. Additional hybridization with the use of our *MPO* clones and other *MPO* probes may clarify this issue.

Regarding the rearrangements, our finding has been extended for one of the APL patients in our study from whom a lambda genomic library has been constructed. An abnormal clone has been isolated and characterized from the library of patient L2, whose bone marrow DNA exhibits a novel 3.1-kb Bgl II band in Southern blotting with our *MPO* clone, pHMP10A. Restriction enzyme mapping of this clone reveals extensive homology with a normal *MPO* genomic clone, except for a novel Bgl II site in which two Bgl II fragments of 3.1 and 4.5 kb replace the normal 7.6-kb fragment. Only the 3.1-kb and normal 7.6-kb fragments hybridize to our *MPO* cDNA probe. Both of the novel Bgl II fragments localize to chromosome 17 by somatic cell hybrid panels, and preliminary sequence data indicate that the novel Bgl II site may be the result of a single base change. Whether this represents a leukemia-related point mutation or a rare constitutional restriction fragment length polymorphism cannot be determined at this time because material from patients in remission is unavailable. We have, however, shown that restriction fragment length polymorphism is not present with Bgl II or Bam HI restriction enzyme recognition sequences (*6*). This is corroborated by the data presented by Donti *et al.*

We agree that rearrangement of the *MPO* gene in APL is a rare occurrence. Since publication of (*1*), 15 additional APL patients have been studied by Southern blot analysis. Fourteen of them showed no novel Bam HI or Bgl II fragments. One, however, showed a novel 18-kb Bgl II fragment, as well as a novel Bam HI doublet in the 5.5- to 6.0-kb range. None of these fragments was present when remission DNA from the same patient was studied. Thus, if patient L2 is omitted, two out of 19 M3 patients analyzed in this laboratory with the use of *MPO* clone pHMP10A as a probe showed novel leukemia-associated bands that disappeared upon remission. Our cDNA did not hybridize to any genomic fragments in normal or HL-60 DNA that were different from those reported by others. These recent data help resolve some of the questions raised, especially by Miller *et al.*, and suggest that the discrepancies noted are due to differences in patient material rather than in cDNA clones.

Regarding our cDNA clones, only pHMP10A was used for Southern blotting. We deliberately chose not to use pHMP2E for the exact reason mentioned by Miller *et al.*, that is, it extends 600 base pairs further 3′ than accountable by *MPO* coding sequences. This was not discussed at length in our paper. We have recently remapped clone pHMP10A. When compared with the map from Miller *et al.*, this clone actually ends just 3′ to the Kpn I site and does not contain the Bgl II site in question. Why earlier mapping experiments revealed this Bgl II site remains unclear. We can conclude, however, that clone pHMP10A is virtually identical to other published *MPO* cDNA sequences (*4, 7*). We believe it is important to reiterate one of the main points of our paper: two novel restriction fragments seen in leukemic (L1) DNA (with the use of Bam HI and Bgl II, not Hind III, as stated by Miller *et al.*) are absent from remission DNA when it is probed with a gene (*MPO*) that is tightly restricted both functionally and temporally within the myeloid lineage. This strongly suggests a leukemia-associated rearrangement. We have no explanation for the weak bands observed in the Southern blot of the remission sample from patient L1. In

any case their presence does not detract from the differences between the leukemic and remission samples of this patient. Miller *et al.* and Donti *et al.* suggest that the conclusions of our paper imply an understanding of the pathogenesis of APL or a direct association between rearranged *MPO* and critical DNA sequences involved in the t(15;17); this is not the case. Although questioned, it should be noted that only "one explanation for the presence of these novel bands in two of the M3 (APL) patients examined is that the breakpoint on chromosome 17 of the t(15;17) occurs within the *MPO* sequences. Alternatively, M3-associated rearrangements of the *MPO* gene such as submicroscopic deletions or inversions that are unrelated to the translocation breakpoint may have occurred" (*1*).

We were not aware of the publications by Chang *et al.* in *Leukemia* (*2*) or by van Tuinen *et al.* in *Oncogene* (*3*) at the time we submitted our report to *Science*. We regret any offense this may have caused. We thank Miller *et al.* and Donti *et al.* for their responses to this interesting story and look forward to future collaborations to resolve the current discrepancies.

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Relation of the Amyloid β Protein Precursor to Heparan Sulfate Proteoglycans

David Schubert *et al.* (*1*) present evidence suggesting that, on the basis of studies of PC12 pheochromocytoma cells, the amyloid β protein precursor (ABPP) is a heparan sulfate proteoglycan (HSPG) core protein. In the course of our previous studies of proteoglycans and other glycoconjugates in this neuronal cell line (*2–4*), we obtained data that raise several questions concerning this conclusion.

1) Schubert *et al.* state that they purified the secreted form of a neuronal HSPG from PC12 cells and that they used this purified HSPG to obtain tryptic peptides that were fractionated by high-performance liquid chromatography. A major peptide labeled with $^{35}SO_4$ (designated peptide 51) had an NH_2-terminal amino acid sequence that was almost identical to the deduced sequence for residues 18 through 44 of human ABPP. The authors conclude that this peptide must be derived from an HSPG core protein, as HSPGs are the only proteoglycans produced by PC12 cells, and that all tryptic peptides labeled with $^{35}SO_4$ should therefore be derived from this proteoglycan.

However, we previously reported (*2*) that chondroitin sulfate accounts for approximately 80% of the glycosaminoglycans secreted (in the form of proteoglycans) by the original line of PC12 cells (*5*), and we have recently found (*4, 6*) that chondroitin sulfate proteoglycans represent a smaller but still highly significant proportion (approximately 35%) of the proteoglycans secreted by the PC12 cells studied in two other laboratories (*1, 7*). The paper (*7*) cited as evidence that only one proteoglycan has been detected in PC12 cells reports an investigation specifically of HSPGs identified with monoclonal antibodies and does not address the question of whether other proteoglycans may also be present. Moreover, the reference cited by Schubert *et al.* in support of the statement that HSPGs are the major class of proteoglycans in nervous tissue clearly demonstrates that chondroitin sulfate, and not heparan sulfate, is the predominant sulfated glycosaminoglycan at all ages in brain (*8*).

Because we have found [on the basis of gel filtration and SDS-PAGE (polyacrylamide gel electrophoresis)] that the molecular size of the HSPG secreted by PC12 cells (110 to 135 kD) is the same as that recently reported for the ABPP (*9*), we do not think it is surprising that these would elute together on Sepharose CL-4B. Therefore, in the absence of electrophoretic and fluorographic evidence for the purity of the proteoglycan fraction isolated by Schubert *et al.* (*1*), we do not believe that one can exclude the possibility that peptide 51 was derived from copurified ABPP, rather than from a heparan sulfate proteoglycan.

2) Additional evidence for the conclusions of Schubert *et al.* is based on the finding that the ABPP-related protein was not found in the medium of mutant PC12 cells (F3), which do not bind certain monoclonal antibodies and are therefore said to lack a cell-surface HSPG (*10*). Although biochemical analyses of proteoglycans produced by the F3 mutant cell line have not been reported, we have found (*4, 6*) that both the F3 mutant and the parent cell line (B2) secrete a mixture of proteoglycans having the same composition (approximately 65% HSPG and 35% chondroitin sulfate proteoglycan). The ABPP should therefore have been easily detectable in the F3 cell media, if it was in fact derived from an HSPG, rather than from some other protein possibly lacking in these cells.

3) Schubert *et al.* also report (*1*) that immunoblots of a PC12 cell HSPG fraction were stained only weakly by polyclonal antisera to ABPP and not at all by a monoclonal antibody (*7*) reported to recognize a PC12 cell HSPG core protein. As both antibodies reacted with a 65-kD band after treatment with heparinase 2 (which partially degrades heparan sulfate chains), Schubert *et al.* conclude either that epitopes on a 65-kD protein core were masked by the presence of heparan sulfate or that the native proteoglycan does not transfer well to nitrocellulose. However, we have had no problems in the electrophoretic transfer of PC12 cell proteoglycans to nitrocellulose, and the monoclonal antibodies reported to recognize the HSPG protein core have not required prior removal of heparan sulfate chains for reactivity (*7*). Since flavobacterial heparan sulfate–degrading enzymes are known to contain contaminating protease activity, and since the enzyme digestions were apparently performed in the absence of protease inhibitors (*11*), an alternative explanation for the findings of Schubert *et al.* not addressed in their report is that the 65-kD immunoreactive band (whose sequence was not determined) may have been generated from a larger protein as a result of proteolysis.

4) Finally, we have attempted to immunoprecipitate an HSPG or its core protein from the medium of PC12 cells labeled with $^{35}SO_4$ using several rabbit antisera to synthetic peptides corresponding to sequences in the ABPP (*12*). These include an antiserum to residues 45 through 62 (SP 18) that immunostains a 100- to 110-kD component present in human cerebrospinal fluid (*13*).