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*).

However, no significant binding of proteins labeled with $^{35}SO_4$ (that is, <1% of the sample radioactivity) was obtained from either the proteoglycan-containing fraction itself (before or after heparitinase treatment) or from the two preceding fractions from the ion-exchange column. Moreover, recent studies indicate that the size of the ABPP is 110 to 135 kD (*9*), rather than the 65 kD that correspond to the HSPG core protein proposed by Schubert *et al.*

Although the ABPP may be secreted by PC12 cells, we believe there is as yet insufficient experimental support for the conclusion that it is a HSPG core protein.

Note added in proof: Schubert *et al.* have recently published a paper (*14*) in which they have significantly revised several of their original conclusions and confirmed a number of the points made above. These include the following. (i) Two tyrosine-sulfated proteins which bind to heparin and are detected by an antiserum to residues 175 to 186 of the predicted ABPP sequence (which they name the GID antigen) have the same molecular size range (115 to 140 kD) as the PC12 cell HSPGs (110 to 135 kD) and are found to copurify with HSPG when examined by SDS-PAGE. (ii) The protein sequenced in their purified HSPG preparation may be the GID antigen. Although these two components have now been separated by ion exchange chromatography on DEAE-cellulose (*14*), as evidence of homogeneity it was previously stated that "all of the sulfate-labeled material migrates as a single peak with heparan sulfate on a DEAE column" (*1*). (iii) The GID antigen is not secreted by the F3 mutant line of PC12 cells, which lacks a cell-surface HSPG (*10*), but secretes the same HSPGs as the parent cell line (*4*).

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6. PC12 cells obtained from L. A. Greene at Columbia University were cultured in Eagle's Basal Diploid Medium as described by Greene and Tischler (*5*). A line of PC12 cells (B2) which had been maintained by David Schubert (Salk Institute, San Diego, CA) was obtained from L. Reichardt (University of California, San Francisco), together with F3 mutant cells produced from the B2 parent line in his laboratory.

   Media from cells labeled with $^{35}SO_4$ were clarified by centrifugation and made to a concentration of 6*M* urea, 50 m*M* sodium acetate, 0.15*M* NaCl, 5 m*M* *N*-ethylmaleimide, and 0.2% Triton X-100, at *p*H 6.0, to which was added 5 m*M* of unlabeled sodium sulfate carrier. After several exchanges with the same solution on a Centriprep-30 membrane (Amicon), the medium (10-ml original volume) was made to 0.1*M* NaCl/0.5% CHAPS and applied to a 0.9 by 8 cm column of DEAE-Sephacel equilibrated with 50 m*M* sodium acetate (*p*H 6.0), containing 6*M* urea, 0.1*M* NaCl, and 0.5% CHAPS. Proteoglycans were eluted with the same buffer containing 0.06*M* NaCl after elution with 0.1*M* and 0.31*M* NaCl.
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Response: Although we were aware of the work of Gowda *et al.* on PC12, I do not believe that the properties of the PC12 clone that we were dealing with were the same as the properties of the clone they have used. Different PC12 clones have different properties. Since we were interested in sequencing a neuronal heparan sulfate proteoglycan (HSPG), our intention was to find a clone among our many nerve cell lines that secreted only one type of proteoglycan. The implication of the first part of the comment by Gowda *et al.* is that we did not distinguish heparan sulfate from chondroitin sulfate proteoglycan; the second part concerns some unpublished experiments that Gowda *et al.* believe are contradictory to our results. They conclude that it is possible that the precursor protein is a protein that copurified with the HSPG. This possibility was stated explicitly on page 225 of our report. However, all of the available data are consistent with the identity of the PC12 HSPG core protein, defined by us and by Matthew *et al.* (Gowda *et al.*'s reference 7), with the amyloid β-protein precursor (ABPP).

The following are my answers to specific comments.

1) We outlined in the text and in reference 14 of our original manuscript the results showing that only HSPG is secreted. The data were deleted from the text of the manuscript at the request of *Science* to conserve space. Most of these data have recently been published (*1*).

2) We have looked at the proteoglycans of the F3 variant in great detail. Although the F3 cells clearly secrete sulfated proteoglycans, some of which migrate in the position of HSPG on a DEAE column, the proteoglycan with the core protein of Matthew *et al.* was not present or was greatly reduced in amount [as initially shown by Inestrosa *et al.* (reference 10 in Gowda *et al.*)]. The sulfated proteoglycan that migrates in the position of HSPG on DEAE columns has very different characteristics from those of a typical HSPG. It is relatively insensitive to heparitinase and nitrous acid degradation and has a different size from that of the PC12 proteoglycan. The F3 variant does not secrete the antigen defined by the anti-ABPP antiserum used in our experiment, nor does it secrete a different but related form of the ABPP that is detected by a different antiserum that unambiguously (by means of comparison of cells transfected with ABPP gene and parent) reacts with the precursor molecule (*1*). We agree with Matthew *et al.* (reference 7 of Gowda *et al.*) that the PC12 HSPG is about 200,000 daltons. We obtained this value on both sizing columns in guanidine and as heparitinase-sensitive material on SDS gels. The 110- to 135-kD PC12 protein that Gowda *et al.* say is an HSPG on SDS gels appears in fact to be a tyrosine-sulfated form of ABPP which lacks sugar sulfate. This form of ABPP does not coelute on DEAE or sizing columns with PC12 HSPG. Data for these points are presented in (*1*).

3) The transfer systems for Western blots vary from laboratory to laboratory. That was undoubtedly why Matthew *et al.* (Gowda *et al.*'s reference 7) stained whole gels rather than transferred material. In the original version of our text, we stated that silver-stained patterns of secreted protein did not change with heparitinase digestion, suggesting, but not totally ruling out, that proteases were not present. We certainly considered this possibility. Furthermore, proteases alone could not have explained the experimental results, and heparitinase did not degrade the core of the 115- and 140-kD forms of ABPP (*1*).

4) It is implied by Gowda *et al.* that the PC12 cell lines tested were obtained from us