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HOWARD HUGHES MEDICAL INSTITUTE

November 23, 2004

Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Department of Physics
Indiana University
Swain Hall West 117
Bloomington IN 47405-7105

RE: Jochen Genschel

Dear Sir/Madam:

This letter is to offer my strong endorsement to Jochen Genschel's application for a junior faculty position in your Department. Jochen is an outstanding young scientist -- I would rank him among the top 10% of the postdocs who have worked with me during my scientific career.

Jochen's initial work in the lab addressed the functions of the two human mismatch recognition activities, MutSα (MSH2•MSH6 heterodimer) and MutSβ (MSH2•MSH3 heterodimer). He was the first to directly demonstrate the presence of MutSβ in human cells where he showed that intracellular levels of MSH6 and MSH3 play an important role in the partitioning of MSH2 between MutSα and MutSβ complexes, with about 85% of nuclear MSH2 being present in the MutSα MSH2•MSH6 heterodimer. Jochen also established the mismatch repair specificities of MutSα and MutSβ. He showed that MutSα supports repair of all eight base-base mispairs and insertion/deletion mismatches in which one strand contains 1 to 8 unpaired nucleotides. By contrast, MutSβ supports correction of insertion/deletion heterologies of 2-8 unpaired nucleotides, is inactive on base-base mispairs, and only weakly active on insertion/deletion mismatches with one unpaired nucleotide. These observations were the basis of subsequent work implicating *MSH6* defects in hereditary cancer predisposition. At the time these studies were done, *MSH2*, *MLH1*, and *PMS2* defects had been implicated in the development of hereditary nonpolyposis colon cancer, but *MSH6* defects had not, a paradoxical finding given the broad mismatch specificity of the MutSα complex. However, Jochen's specificity studies suggested that MSH6-deficient tumors would have been missed by the screen for mismatch repair deficient tumors in use at the time, which scored dinucleotide repeat instability. Jochen's findings predicted that dinucleotide repeats would be stable in *MSH6* mutant cells, but mononucleotide repeats would not. This prediction was confirmed by a number of laboratories, who showed that *MSH6* mutations cause familial colon cancer and demonstrated that such tumors are characterized by mononucleotide repeat instability.

A collaborative study by Jochen and Jim Drummond, another postdoc in the lab, dramatically demonstrated the significance of the partitioning of MSH2 between the MutSα and

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MutSβ complexes. Previous work by the Shimada group had demonstrated that the human *MSH3* gene is adjacent to the *DHFR* locus and is co-amplified with *DHFR* in methotrexate-resistant cells. There were also hints in the literature that methotrexate-resistant cell lines were genetically unstable, but the basis of this observation was unexplained. In a beautiful set of experiments Jim and Jochen demonstrated that amplification of the *DHFR-MSH3* region leads to dramatic overproduction of the MSH3 polypeptide and sequestration of virtually all of the cellular MSH2 into the MSH2•MSH3 MutSβ complex. This occurs at the expense of the MSH2•MSH6 MutSα heterodimer, which as noted above is essential for the repair of base-base mismatches. As a consequence of this phenotypic MutSα deficiency, base substitution mutation is elevated several hundred-fold in such cell lines.

Jochen's most impressive accomplishment has been his work on the nature of the excision step of human mismatch repair. Although we and others identified a number of activities involved in the reaction, there has been a distinct lack of information concerning the identity of excision activities required for repair. Jochen addressed this question by devising a clever, rapid assay to score mismatch provoked excision. Using this method and a traditional fractionation approach, he demonstrated that the human homolog of yeast EXOI is required for excision directed by a strand break located either 5' or 3' to the mismatch, results that have been subsequently confirmed by others using EXOI knockout mouse cells. During the course of this work, Jochen demonstrated that the 5' to 3' hydrolytic activity of EXOI undergoes a dramatic, mismatch-dependent activation in a pure system comprised of only heteroduplex DNA, MutSα, MutLα, EXOI, and the single-stranded DNA binding protein RPA. MutLα is not essential for this effect, but enhances the mismatch dependence of the reaction. As observed in nuclear extracts of human cells, excision directed by a 5'-strand break in this four-protein system initiates at the strand break, proceeds toward the mismatch, and terminates at a set of sites about 150 nucleotides beyond the mispair. The molecular events responsible for excision termination in this manner has been a puzzle in the mismatch repair field, but Jochen's analysis of the pure system has suggested a simple mechanism by which this occurs. EXOI initiates poorly at a single strand break, and MutSα facilitates this reaction in a mismatch-dependent fashion. Upon MutSα-promoted helix entry, the 5' to 3' hydrolytic activity of EXOI degrades the incised strand of the heteroduplex by a highly processive mechanism that removes thousands of nucleotides. However, if RPA is present, the gap produced by ongoing EXOI hydrolysis is filled by the single-stranded binding protein. RPA gap filling behind the excision complex promotes dissociation of MutSα, EXOI, or both proteins, resulting in termination of processive hydrolysis. Since the RPA-filled gap is also a poor substrate for EXOI entry, additional rounds of excision require the mismatch-dependent assistance of MutSα. Hence, MutSα-dependent, iterative reloading of EXOI continues until the mismatch is removed, at which point excision terminates. These studies have provided the basis for other work in the lab that has led to the initial reconstitution of mismatch-provoked 3' to 5' excision in a purified system, as well as preliminary reconstitution of the overall mismatch repair reaction. In my view Jochen's EXOI studies represent an outstanding accomplishment.

Jochen is an enthusiastic young man who exudes a love for science. He is extremely well read, has broad scientific interests, and displays a level of commitment that is rarely seen these days.

Sincerely yours,

Paul Modrich
James B. Duke Professor of Biochemistry