Curriculum Vitae

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Date of birth: December 18 1965
Place of birth: Hamburg, Germany

Positions

Research and Teaching Assistant (August 1992 – April 1996)
Medical School Hannover, Germany
Biophysical Chemistry
Laboratory of Prof. Dr. Günter Maass

Research Associate (May 1996 – present)
Duke University Medical Center
Department of Biochemistry
Laboratory of Dr. Paul Modrich

Education

Diploma in Biochemistry (October 1986 – June 1992)
Medical School Hannover, Germany
Grade: *Magna cum laude*
Advisor for diploma thesis: Prof. Dr. Günter Maass
Co-advisor for diploma thesis: Prof. Dr. Claus Urbanke

Doctorate of Philosophy in Biochemistry (August 1992 – May 1995)
Medical School Hannover, Germany
Grade: *Summa cum laude*
Advisor: Prof. Dr. Günter Maass
Co-advisor: Prof. Dr. Claus Urbanke

Fellowship

Postdoctoral research fellowship from the Deutsche Forschungsgemeinschaft, Germany (May 1996 – April 1998)

Scientific interest and research experience

My primary interest is to understand the nature of DNA replication, repair and recombination in terms of the molecular function of the activities involved.

Graduate work

Biophysical and genetic studies on the function of bacterial single-stranded DNA binding (SSB) proteins. Isolation of the *ssb* gene of *Pseudomonas aeruginosa*. Subcloning of three other *ssb* genes. Overproduction, purification, biochemical and biophysical analysis of seven SSB proteins. Site directed mutagenesis for deletion analysis of *E. coli* SSB protein.

Postdoctoral work

Isolation and characterization of known and unknown components of the human DNA mismatch repair system. These studies involve protein purifications from HeLa nuclear extract, *E. coli* and baculovirus-based expression systems as well as the preparation of f1-phage-derived DNA substrates. Based on this work, a mechanism for mismatch-provoked excision was established and the excision reaction reconstituted in vitro.

References

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List of publications

Dzantiev, L., Constantin, N., Genschel, J., Iyer, R. R., Burgers, P. M. and Modrich, P. (2004) A defined human system that supports bidirectional mismatch-provoked excision. *Mol. Cell* **15**, 31-41

Genschel, J. and Modrich, P. (2003) Mechanism of 5'-directed excision in human mismatch repair. *Mol. Cell* **12**, 1077-1086

Genschel, J., Bazemore L. R. and Modrich, P. (2002) Human exonuclease I is required for 5' and 3' mismatch repair. *J. Biol. Chem.* **277**, 13302-13311

Genschel, J., Curth, U. and Urbanke, C. (2000) Interaction of *E. coli* single-stranded DNA binding protein (SSB) with exonuclease I. The Carboxy-Terminus of SSB is the recognition site for the nuclease. *Biol. Chem.* **381**, 183-192

Genschel, J., Littman, S., Drummond, J. T. and Modrich, P. (1998) Isolation of hMutS from human cells and comparison of the mismatch specificities of hMutS and hMutS. *J. Biol. Chem.* **273**, 19895-19901

Drummond, J. T., Genschel, J., Wolf, E. and Modrich, P. (1997) DHFR/MSH3 amplification in methotrexate-resistant cells alters the hMutS:hMutS ratio and reduces the efficiency of base-base mismatch repair.
Proc. Natl. Acad. Sci. U. S. A. **94**, 10144-10149

Webster, G., Genschel, J., Curth, U., Urbanke, C., Kang, C. and Hilgenfeld, R. (1997) A common core for binding single-stranded DNA: Structural comparison of the single-stranded DNA-binding proteins (SSB) from *E. coli* and human mitochondria. *FEBS Lett.* **411**, 313-316

Genschel, J., Litz, L., Thole, H., Roemling, U. and Urbanke, C. (1996) Isolation, sequencing and overproduction of the single-stranded DNA binding protein from *Pseudomonas aeruginosa* PAO. *Gene* **182**, 137-143

Curth, U., Genschel, J., Urbanke, C. and Greipel, J. (1996) *In vitro* and *in vivo* function of the C-terminus of *Escherichia coli* single-stranded DNA binding protein. *Nucleic Acids Res.* **24**, 2706-2711

De Vries, J., Genschel, J., Urbanke, C., Thole, H. and Wackernagel, W. (1994) The single-stranded-DNA-binding proteins (SSB) of *Proteus mirabilis* and *Serratia marcescens*. *Eur. J. Biochem.* **224**, 613-622

Teaching experience and philosophy

Teaching experience

Medical School Hannover (1992 – 1996)
Lecture 'Mathematical problems in Biophysical Chemistry' accompanying the lecture 'Biophysical Chemistry for Biochemistry students'. (1h per week, weekly scored tests, 1 written examination per semester, 30 students, both winter and summer semester)

Supervision of students during the practical course 'Biophysical Chemistry'. (8 x 2 days, all day, 30 students, winter semester)

Supervision of rotation students during their practical laboratory work (6 weeks, all day, 2 students)

Duke University Medical Center (1996 – present)
Supervision of rotation students (graduate students of the Department of Biochemistry) during their practical laboratory work. (6 weeks, all day, 2 students)

Teaching philosophy

Although I did not have a lot of opportunity to teach students at my current position I have always felt that it is very important to educate young students. I have spent considerable time here at Duke training young graduate students and post-docs with the intention of enabling them to conduct their own project independently. A student should be given the chance to develop his/her own project with the teacher in an assisting position, mainly pointing out difficult steps and those aspects of the project that are critical for the project's success.

With respect to lecturing at the basic or advanced level, I would like to follow the same principle, providing students with as much information as they need but the same time ensuring that they learn how the individual pieces of information are interconnected. In my opinion, this is necessary to give students a concept of how science actually works.

My academic training is very broad, including not only biochemistry, but also biophysical chemistry, molecular biology and organic chemistry. I am confident that I am able to teach these subjects as well as other courses within the scope of life sciences.

Previous and current work: The biochemistry of human DNA mismatch repair

My postdoctoral work has addressed the mechanism of DNA mismatch repair in human cells. DNA mismatch repair contributes significantly to genetic stability by reducing the overall mutation rate up to 1000-fold. Deficiency in components of the human system has been implicated in the development of hereditary and spontaneous forms of cancer.

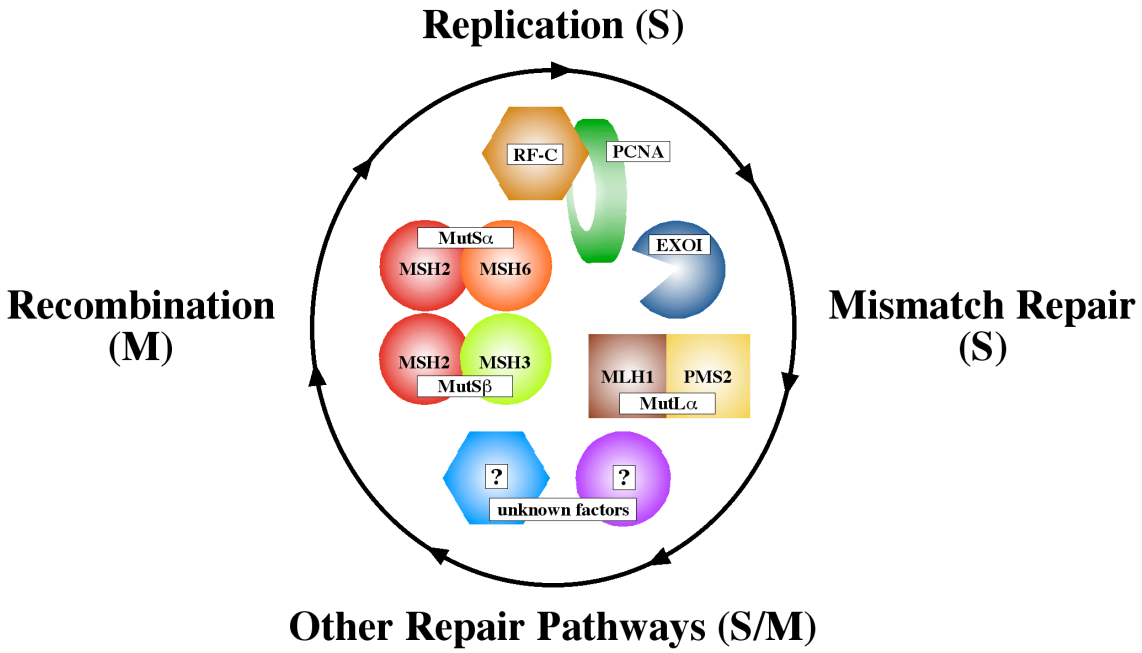

Fig. 1 Mismatch repair proteins are involved in DNA repair pathways at various stages of the cell cycle.

The mismatch recognition factors MutS and MutS

My first project dealt with mismatch recognition in human cells. The human genome encodes five homologs of the *E. coli* mismatch recognition activity MutS. Three of their yeast counterparts are clearly involved in mismatch repair: MSH2, MSH3, and MSH6. MSH2 and MSH6 form a heterodimer purified as the mismatch recognition activity MutS from HeLa nuclear extract by J. Drummond in the laboratory of Dr. Modrich.

There was evidence that MSH3 also forms a heterodimer with MSH2, designated MutS. I identified a mismatch recognition activity in MSH6 deficient cells and demonstrated that it was indeed human MutS. Immunodepletion of MSH3 confirmed this result. J. Drummond and I then showed that MutS is easily purified from a cell line over-producing MSH3, designated HL60-R. In this cell line, almost all MSH2 is sequestered by MSH3 to form MutS and very little MutS activity can be detected. Nuclear extracts from this cell line are proficient in the repair of small insertion/deletion mismatches but rectification of base-base mispairs is strongly reduced. Subsequently, I determined the substrate specificity of the two human mismatch recognition factors in collaboration with S. Littman, also a colleague in the Modrich laboratory. MutS

initiated mismatch repair on both base-base mispairs and short insertion/deletion mismatches while MutS only directed repair of the latter lesion type.

These results explain why mismatch repair deficiency was first considered of minor importance in MSH6 deficient tumors based on the analysis of microsatellite instability. Loss of MSH6 is associated with a dramatic increase in the overall mutation rate but the MutS present in these cells allows the repair of short insertions or deletions that are the molecular basis of microsatellite instability.

The excision step in human mismatch repair

My subsequent and current work focuses on the step following mismatch recognition, the excision step that removes the mispair. After the development of a suitable fractionation scheme using nuclear extracts, I was able to identify the first bona-fide excision activity involved in human mismatch repair, human exonuclease I (EXOI). This enzyme is a RAD2/XPG homologue and had been previously characterized as a strictly 5' directed exonuclease. Its preferred substrate is a 5' end in double-stranded DNA containing single-stranded gaps or strand breaks.

Purification of recombinant EXOI and immunodepletion experiments allowed me to conclude that EXOI is involved in mismatch-provoked excision directed by a strand break located either 3' or 5' to the mispair. This result suggests that EXOI may be essential for human mismatch repair and must therefore be regarded as a potential oncogene. All subsequent experiments depended on this key discovery.

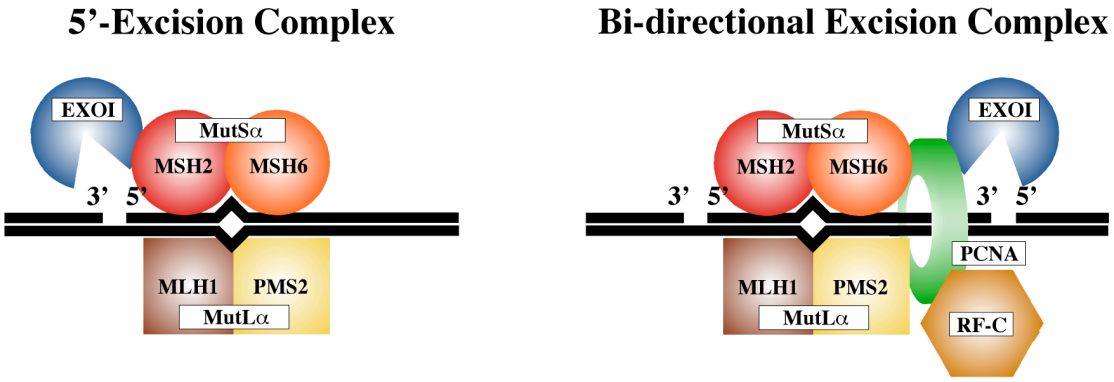

Fig. 2 Excision complexes involved human DNA mismatch repair.

I was able to demonstrate that 3' and 5' directed excision is performed by different excision complexes. Experiments in a purified system as well as with depleted nuclear extracts indicated that 5' directed excision has less stringent requirements than 3' directed excision. MutS and EXOI are sufficient for mismatch-provoked excision from a 5' end although MutL enhances the mismatch specificity of the reaction. For excision from a 3' end, all three factors as well as PCNA, the replication clamp, are required. Based on these data, I proposed the presence of two excision complexes in human cells (Fig. 2), a 5' specific excision complex consisting of EXOI, MutS and MutL and a larger bi-directional complex, which includes PCNA and RF-C, the protein complex that loads PCNA onto DNA.

A mechanism for controlled mismatch-provoked excision by EXOI

The simplicity of the 5' specific complex enabled me to address one of the most puzzling questions in mismatch repair, the control of excision tract length. When I added RPA, the human single-stranded DNA binding protein, to a reaction containing EXOI, MutS, and MutL I observed mismatch-specific excision comparable to that observed in HeLa nuclear extract. In addition, excision terminated distal to the mispair in a pattern very similar to that seen with HeLa nuclear extracts. Variation of the distance between the strand break and the mispair revealed excision intermediates where excision had not yet reached the mispair. Based on these results, I developed a model for excision control in mismatch repair (Fig. 3). Loading of EXOI at a 5' end is controlled by MutS and MutL, which are activated in an ATP-dependent manner by the presence of a mispair. EXOI excises approximately 200 nucleotides before it dissociates. RPA binds to the single-stranded DNA tract formed during excision. As long as the mispair persists, MutS and MutL facilitate re-loading of EXOI at the 5' terminus. When excision proceeds beyond the mispair, MutS and MutL change from an activating to an inhibitory function and prevent further excision by EXOI.

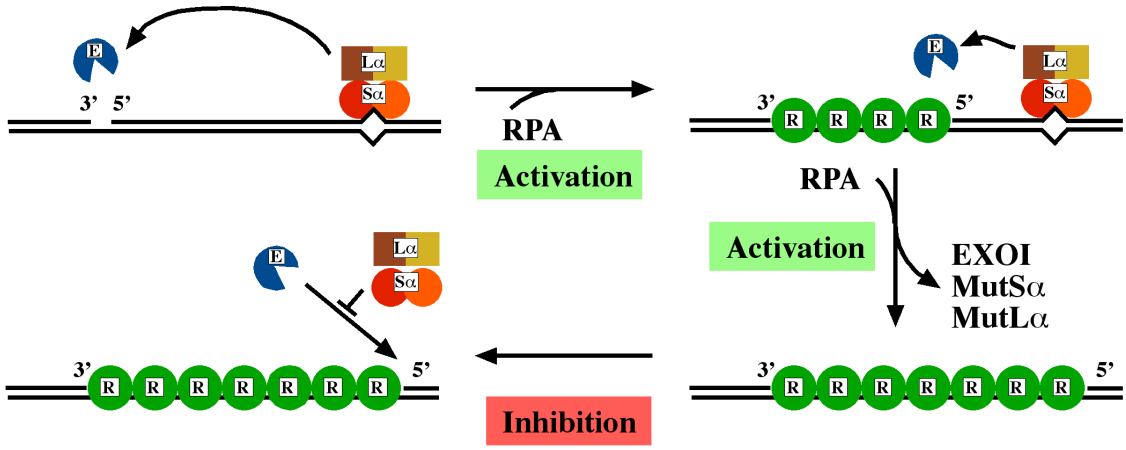

Fig. 3 Activation of multiple loading of EXOI (**E**) by MutS (**S**) and MutL (**L**) on a mismatch-containing substrate in presence of RPA (**R**).

Reconstitution of bi-directional mismatch-provoked excision

Most recently, I collaborated with Leonid Dzantiev and others in Dr. Modrich's laboratory to show that MutS, MutL, EXOI, PCNA, RF-C, and RPA constitute a minimal system for bi-directional mismatch-directed excision in vitro. In this system, EXOI is catalytically active in 3' as well as 5' directed excision, confirming my previous observations regarding the bi-directional function of EXOI. The major function of RF-C appears to be the loading of PCNA, which together with MutL activates a cryptic 3' exonuclease activity in EXOI. Thus EXOI is not only required for bi-directional excision but is also sufficient for this process in vitro.

However, 3' directed excision in the minimal system is uncontrolled and does not terminate after mismatch removal. Clearly other factors contributing to mismatch repair still have to be identified (Fig. 1).

Future research plan

The accurate replication of the human genome is extremely important to prevent genetic instability and cancer predisposition. Due to misincorporation and strand slippage by the replication machinery mismatched bases are formed at a considerable rate during replication. Human cells, as in fact all living organisms, depend on a specialized rectification mechanism, DNA mismatch repair, to substantially reduce the mutagenic potential of replication errors. Mismatch repair deficiency is correlated with both familial and sporadic forms of cancer, especially colon cancer.

The goal of this research project is to understand the mechanisms by which mismatched bases are recognized, how mismatch recognition is signaled to other repair factors and DNA damage signaling cascades, and how mismatches are effectively removed. This knowledge is then to be used to develop strategies to improve the efficacy of tumor therapeutic drugs since many of them such as cis-platin act through the formation of DNA-adducts recognized by mismatch repair.

1. Mismatch recognition

A number of factors involved in eukaryotic mismatch repair have been identified. There are two mismatch recognition factors, referred to as MutSα (MSH2-MSH6) and MutSβ (MSH2-MSH3). MutSα recognized all forms of mismatched bases, including non- Watson/Crick base pairs, small insertion and deletions resulting from strand slippage, as well as chemical adducts. So far MutSβ is only characterized as a mismatch repair factor specific for strand slippage lesions. Since such lesions are common in recombination intermediates, MutSβ may play an important role in DNA repair mediated through recombination. Since both MutSα and MutSβ share a common subunit and interact with the same proteins it is important to understand how these mismatch recognition factors can both contribute to genetic stability without inhibiting each another.

To date, even the mechanism by which these two proteins specifically recognize lesions is not well understood. I propose to produce these proteins in quantity and quality sufficient for crystallographic analysis, which will be done in cooperation with structural biologists. Furthermore, these proteins can be used to investigate strategies to potentiate the effect of DNA-damaging agents used as anti-cancer drugs. Even though MutSα recognizes lesions produced by these compounds it is known that the lesion is subsequently released in an ATP-dependent process. Since ATP is ubiquitous in the cell it stands to reason that it would be advantageous if this release could be inhibited reversibly during cancer chemotherapy. For this part of the project, a simple release assay based on the binding of fluorescence-tagged MutSα to immobilized DNA containing mismatched bases will be developed. In presence of a suitable compound, a challenge with ATP should not result in the release of MutSα but in its retention on the DNA, easily monitored by the fluorescence signal. This assay can be readily adapted for high-through-put screening of drug libraries. Here the crystallographic studies will also contribute because some of the screening may be done in *silico*.

2. Novel factors required for genetic stability and cancer prevention

Investigations into the nature of mismatch recognition and processing require an intimate knowledge of all factors involved in this process. As mentioned above, some activities required for mismatch repair have already been isolated. Not surprisingly, most of them are replication factors such as the replicative polymerase □, the replication clamp PCNA (proliferating cell nuclear antigen), and replication factor A (single-stranded DNA binding protein. We know very little about the factors, which actually perform the nucleolytic removal of mismatched bases. From my own work we know that exonuclease I is an important component of the excision machinery but no other excision factors have been identified to date. The problem is that many genes encoding proteins involved in DNA metabolic pathways are essential, especially in higher eukaryotes such as man or mouse. Saccharomyces cereviseae offers a way to identify new components of the DNA mismatch repair pathway. It is a unicellular eukaryote, on which most genetic work in DNA repair has been done. First a cell-free in vitro system for mismatch repair in yeast will be established. This work will also profit from recombinant forms of known factors, which will be used to complement extracts deficient in mismatch repair. Chromatographic fractionation of cell-free extracts and complementation of deficient extracts will then lead to the identification of new mismatch repair factors. Yeast strains harboring conditional mutations in DNA repair proteins will facilitate this process since they allow the preparation of extracts deficient in essential factors. Thus the integration of genetics and biochemistry in a lower eukaryote will be instrumental in the discovery of novel factors participating in DNA mismatch repair. Since yeast possesses homologs of all known human mismatch repair factors it is reasonable to expect that any finding in yeast mismatch repair will have great impact on our understanding of the same process in human cells.

3. Control of illegitimate recombination

DNA repair pathways such as mismatch repair rectify potentially mutagenic lesions in one of the two complementary DNA strands. More extensive damage involving both strands of the DNA (the simplest case is a double-strand break) requires homologous double-stranded DNA as a repair template. The repair process requires strand exchange between two double-stranded DNAs and is usually referred to as recombination. A large number of processes require recombination such as gene conversion or gene duplication. Recombination is also involved in the chromosomal rearrangements often observed in tumor cells. Mismatch repair is involved in the quality control of recombination. The above mentioned strand exchange leads to the formation of heteroduplex DNA containing one strand of each of the DNAs involved. If the sequence of these DNAs is slightly diverged, e.g. they present two different alleles of the same gene, mismatch recognition factors will bind to the mismatched bases in the heteroduplex DNA. It is generally assumed that mismatch recognition in recombination intermediates prevents

further processing of the intermediate. Thus mismatch repair serves as a powerful guardian against illegitimate recombination.

To date, essentially nothing is known about the exact mechanism of this process. The recombination intermediate should be covered with recombination factors such as RAD51 and others. This already presents the problem of how mismatch recognition can occur in the context of a complex protein/DNA filament. How mismatch repair proteins prevent further processing cannot be understood unless this problem is solved.

I propose two approaches to this problem. First, the development of an in vitro recombination assay in a cell-free system, beginning with a simple strand exchange assay based on circular single-stranded DNA and linearized double-stranded DNA. Cell-free extracts from a transformed chicken lymphoblast cell line, DT40, will be used as the basis for this assay. DT40 cells exhibit a hyper-recombination phenotype, which indicates that recombination factors are present in this cell line at elevated levels. In addition, the hyper-recombination phenotype of DT40 cells allows for a rapid generation of mutant variants. Thus the influence of mismatch repair deficiency on in vitro strand exchange can be readily investigated. As in the second part of the project, again recombinant proteins will be used to complement the experiments in the cell free system. Yeast recombination proteins have already been used successfully to investigate several aspects of DNA recombination. Thus it is feasible to use these factors in recombinant form to study the influence of mismatch repair proteins on strand exchange reaction between DNAs with diverged sequences.

The final goal of this project part again is to find ways to inhibit recombination in human tumor cells. It is possible that again simply blocking the release of mismatch recognition factors from recombination intermediates results in a potentiation of mismatch repair dependent suppression of illegitimate recombination.