



Miscellaneous Research Groups

There are four miscellaneous research groups at the institute, headed by Erich Lanka, Rudi Lurz, Richard Reinhardt, and Enzo Russo.

Phage & Conjugation Group



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Sabine Krause (10/96 – 9/99, DFG)

Ralf Eisenbrandt (10/96 – 9/99, DFG)

Jan Deneke (7/99 – 6/02, DFG)

Christian Rabel (12/99 – 11/02, DFG)

Gunnar Schröder (1/00 – 12/02, DFG)

Isabel Pasch (1/02 – 6/02, Combinature)

Undergraduate students:

Renate Kühn (3/98 – 9/98)

Tobias Reick (12/98 – 9/99)

Stefan Ehrentraut (4/02 – 4/03)

Technician:

Marianne Schlicht

Guest scientists:

Dr. A. Marika Grahn (2/99)

Dr. Ramón Díaz Orejas (10/02 – 12/02)

Dr. Beth Traxler (9/98)

Enzymology of bacterial conjugation & bacteriophage & plasmid replication

Two topics were pursued in recent years on both of which we continued working for more than two decades. The work started in the former department of Heinz Schuster. Beside our primary effort to unravel the enzymology of bacterial conjugation, bacteriophage and plasmid replication we have vigorously increased our input towards *structural biology*. Currently we collaborate with two NMR groups and three crystallographer groups. Although these collaborations adsorb a good deal of our working power the payback is reasonably good in terms of the number of publications (1, 5, 8, 10, 17, 24, 28). Currently, the structure of KorB is being solved as a DNA-protein complex to a resolution of 2 Å. The protein is encoded by IncP plasmids exerting dual roles as a ParB analogue and a global transcriptional repressor for replication, maintenance and conjugative transfer genes. The preparation of a manuscript is in progress describing structural properties of a partitioning protein for the first time.

Horizontal gene transfer and type IV secretion

In the model system for bacterial conjugation, the broad host range P-type plasmids, we localised the 12 plasmid-encoded components of the mating pair formation (Mpf) complex to the cell envelope. Hence, the proteins seem to bridge inner and outer membrane in the Gram-negative organism *E. coli* (22). Conjugative junctions were found in between cell envelopes of donor and

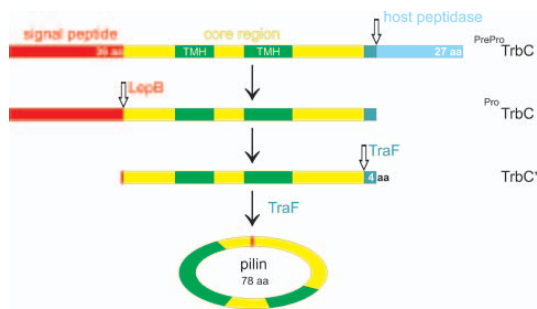


Figure 1: Maturation cascade of the RP4 pilin. *TrbC*, 145 residues in length, is represented by a bar. Defined sections of *TrbC* are marked: signal peptide (red), core region (yellow), trans-membrane helix (olive), carboxy-terminal end (light blue) and tetra-peptide, the leaving group (blue). *TraF* is a RP4-encoded specialized protease that catalyses the circularization of *TrbC** by the formation of a new peptide bond between the N and C terminus.

recipient in mating cells in analogy to the F-system (26). The nature of these junctions still remains a mystery because the components which make up these electron dense contact zones of mating cells are unknown. One of the major roles of the Mpf complex is the assembly and erection of conjugative pili on the cell surface. According to current models, pili are needed to establish the first contact of the donor to the recipient cell. Our work concentrated on the dissection of the 12 Mpf components that are essential for the maturation process of the pre-pilin. The pilin, a ribosome-synthesized protein, consists of a circular structure that is formed after several proteolytic steps, in the last of which the circularization is catalysed by the formation of a new peptide bond. The catalysis is due to a novel mechanism exerted by an IncP-encoded signal peptidase-like enzyme, that in principle resembles the reverse reaction of proteolytic cleavage (Figure 1) (13, 14, 21, 30).

Pilus assembly and substrate secretion (Type IV secretion) are most likely energy consuming processes as indicated by three potential NTP hydrolysing enzymes that are present among the transfer components. In other words the proteins contain distinct motifs for interaction with nucleotides. The prediction proved to be valid for at least one protein class, analogues of the VirB11 protein family. These hexameric proteins (24, 25) associated with the cytoplasmic side of the inner membrane (22), hydrolyse preferentially ATP (25). Structural analyses suggest that they function as chaperons although the specific substrate remains to be discovered (5, 28). The two other proteins, a VirB4-like protein and the potential DNA-transporter, a TraG-like protein, also called coupling protein, bind NTPs but do not hydrolyse them (4, 6, 15, 23). Both proteins may alter their conformation upon binding and release of NTPs, or they might hydrolyse NTPs in the presence of other proteins. The challenge now is to continue and extend the dissection of Mpf, to put the 12 component system together and define the role of each of the components in form of a realistic model.

Our collaborative efforts also focussed on the discovery of new conjugative transfer systems because it became clear that certain questions may only be solved by applying not only one system but introducing new ones as well (7, 16, 18, 34). There is still no structure for a relaxase, the key component in DNA processing. Thus we are looking for systems with smaller enzymes since they may prove as better crystallisation substrates as the ones which have been tried already.

Helicases, primases and protelomerases

Origin binding (17), DNA strand separation by replicative helicases (3, 8, 19, 27, 35) and the telomere resolution reaction in the generation of linear genomes (2, 11, 12, 20) were our topics in phage and plasmid replication. Since the replicative hexameric helicase of the broad host range plasmid RSF1010 is the smallest known helicase that is independent of a helicase loader and its structure is known we use the system for searching and assaying potential inhibitors of the DNA strand separation activity (8, 19, 35). Enzymatic trials with a set of polyketide compounds are in progress. The analysis of the structure-function relationship of the enzyme re-

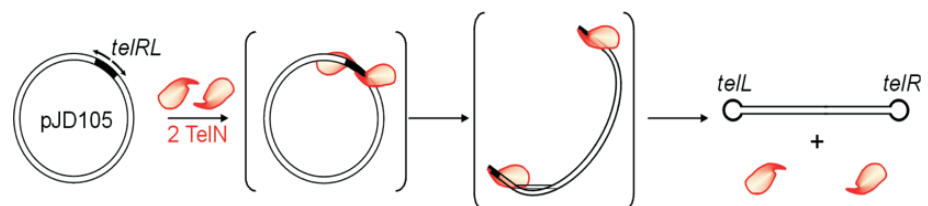


Figure 2: Scheme for N15 telomere resolution. The telomere resolution site *telRL* (black segment) is recognized by *TelN* (red) sequence specifically. In a concerted action cleavage and covalent bond formation yield hairpin ends on the linear DNA molecule.



vealed that the extraordinary stability of the oligomeric subunit arrangement is due to an eye-hook principle (8).

A fascinating topic in phage biology is the discovery of the enzyme involved in the generation of linear DNA with covalently closed hairpin ends. This topic has a direct connection to the long standing continuing interest in the transient formation of covalent protein-DNA linkages similar to those we have described previously in relaxases. The enzyme processes a 56-bp palindrome that might prove to contain a 14-bp stretch of Z-DNA (Figure 2) (2, 11, 20).

We look forward to collaborate with the *Ultrastrukturnetzwerk* (USN) to be set up at the Institute. The analysis of some of our structures by cryo electron microscopy is likely to yield additional conformations that may not be obtained by crystallization.

The group produced 35 peer-reviewed publications in the six-year evaluation period, six of which are review articles (9, 13, 27, 29, 31, 33). In addition, invitations to the major international meetings in the field underline the creative scientific potential of the small group. The work was sponsored by the Deutsche Forschungsgemeinschaft and by grants of the European Commission. We also have a close connection to a small innovative Biotech company.

General information

Publications 1998 -2003

1. Dostál L, Khare D, Bok J, Heinemann U, **Lanka E** & Welfle H (2004). *RP4 repressor binds to the major groove of the operator DNA – a Raman study*. *Biochemistry* 43 (in press)
2. Hertwig S, Klein I, **Lurz R**, **Lanka E** & Appel B (2003). *PY54, a linear plasmid prophage of Yersinia enterocolitica with covalently closed ends*. *Mol Microbiol* 48: 999-1003
3. Lemonnier M, **Ziegelin G**, Reick T, Muñoz Gómez A, Diaz Orejas R & **Lanka E** (2003). *Bacteriophage P1 Ban protein is a hexameric DNA helicase that interacts with and substitutes for Escherichia coli DnaB*. *Nucleic Acids Res* 31: 3918-3928
4. **Rabel Ch**, Grahn M, **Lurz R** & **Lanka E** (2003). *The VirB4 family of proposed traffic NTPases: common motifs in plasmid RP4 TrbE protein are essential for conjugation and phage adsorption*. *J Bacteriol* 185: 1045-1058
5. Savvides SN, Yeo H-J, Beck MR, **Blaesing F**, **Lurz R**, **Lanka E**, Buhrdorf R, Fischer W, Haas R & Waksman G (2003). *VirB11 ATPases are dynamic hexameric assemblies: new insights into bacterial type IV secretion*. *EMBO J* 22:1969-1980
6. **Schröder G** & **Lanka E** (2003). *TraG-like proteins of type IV secretion systems: functional dissection of multiple activities of TraG (RP4) and TrwB (R388)*. *J Bacteriol* 185: 4371-4381
7. Strauch E, Goelz G, Knabner D, Konietzny A, **Lanka E** & Appel B (2003). *A cryptic plasmid of Yersinia enterocolitica encodes a conjugative transfer system related to regions of CloDF13 Mob and IncX Pil*. *Microbiology* 149:2829-2845
8. **Ziegelin G**, Niedenzu T, **Lurz R**, Saenger W & **Lanka E** (2003). *Hexameric RSF1010 helicase RepA: alanine-scan of single amino acid residues proposed to play key roles in the protein's function*. *Nucleic Acids Res* 31:5917-5929
9. Baron C, O'Callaghan D & **Lanka E** (2002). *Bacterial secrets to secretion: EuroConference on the biology of type IV secretion*. *Mol Microbiol* 43: 1359-1365
10. Delbrück H, **Ziegelin G**, **Lanka E** & Heinemann U (2002). *A SH3-like domain is responsible for dimerization of the repressor protein KorB encoded by the promiscuous IncP plasmid RP4*. *J Biol Chem* 277: 4191-4198
11. **Deneke J**, **Ziegelin G**, **Lurz R** & **Lanka E** (2002). *Phage N15 telomere resolution: target requirements for recognition and processing by the protelomerase*. *J Biol Chem* 277: 10410-10419
12. Heinrich J, Schultz J, Bosse M, **Ziegelin G**, **Lanka E** & Moelling K (2002). *Linear closed mini DNA generated by the prokaryotic cleaving-joining enzyme TelN is functional in mammalian cells*. *J Mol Med* 80: 648-654 (published ONLINE August 28, 2002)
13. Kalkum M, Eisenbrandt R, **Lurz R** & **Lanka E** (2002). *Tying rings for sex*. *Trends Microbiol* 10: 382-387
14. Lai E-M, Eisenbrandt R, Kalkum M, **Lanka E** & Kado CI (2002). *Biogenesis of T-pili in Agrobacterium tumefaciens requires precise VirB2 propilin cleavage and cyclization*. *J Bacteriol* 184: 327-330

15. **Schröder G**, Krause S, Traxler B, Zechner EL, **Lurz R**, Yeo H-J, Waksman G & **Lanka E** (2002). *TraG-like proteins of DNA transfer systems and of the Helicobacter pylori type IV secretion system: the inner membrane gate for exported substrates?* J Bacteriol 184:2767-79
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17. Yeo H-J, **Ziegelin G**, Korolev S, Calendar R, **Lanka E** & Waksman G (2002). *Phage P4 origin-binding domain structure reveals a mechanism for regulation of protein activity by homo and heterodimerization of winged helix proteins.* Mol Microbiol 43: 855-867
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23. Hamilton CM, Lee H, Pei-Li L, Cook DM, Piper KR, Beck von Bodman S, **Lanka E**, Ream W & Farrand SK (2000). *TraG and its homologs from pTiC58 and RP4 confer relaxosome specificity to the Ti plasmid conjugal transfer system.* J Bacteriol 182:1541-1548
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27. Waksman G, **Lanka E** & Carazo JM (2000). *Helicases as nucleic acid unwinding machines.* Nature Struct Biol 7:20-22
28. Yeo, H-J, Savvides S, Herr AB, **Lanka E** & Waksman G (2000). *Crystal structure of the hexameric traffic ATPase of the Helicobacter pylori type IV secretion system.* Mol Cell 6: 1461-1472
29. Zechner EL, de la Cruz F, **Eisenbrandt R**, Grahn AM, Koraimann G, **Lanka E**, Muth G, Pansegrau W, Thomas CM, Wilkins BM & Zatyka M (2000). *Conjugative-DNA transfer processes.* In *The Horizontal Gene Spread*. Thomas CM, ed., Harvard Academic Publishers GmbH, Amsterdam, pp. 87-174
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32. Ziemienowicz A, Görlich D, **Lanka E**, Hohn B & Rossi L (1999). *Import of DNA into mammalian nuclei by proteins originating from a plant pathogenic bacterium.* PNAS USA 96: 3729-3733
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Teaching

Project students of the Freie Universität Berlin, the Ernst-Moritz-Arndt-Universität Greifswald and the Fachhochschule Lausitz

Theses

Christian Rabel: *Enzymologie der bakteriellen Konjugation: Hydrolysieren Vertreter der VirB4-Proteinfamilie während der Pilusbio-genese Nukleosid-Triphosphate?*, PhD Thesis, Technische Universität Berlin, 2003

Gunnar Schröder: *TraG-Like Transporter Proteins of Type IV Secretions Systems*, PhD Thesis, Freie Universität Berlin 2003

Jan Deneke: *Das Tyrosinintegrase-Analog TelN katalysiert die telomere resolution im Bacteriophagen N15*, PhD Thesis, Freie Universität Berlin, 2002

Susanne Schneiker: *Das konjugative Hg-Resistenzplasmid pSB102 aus der bakteriellen Gemeinschaft der Luzernerhizosphäre: Isolierung, Sequenzierung und Sequenzinterpretation*, PhD Thesis, Universität Bielefeld, 2001 (E. Lanka as an external advisor)

Ralf Eisenbrandt: *Macromolecular Export Systems, Identification of Conjugative Pilins and their Modification*, PhD Thesis, Technische Universität Berlin, 1999

Markus Kalkum: *Massenspektrometrische Methoden für die biochemische Proteomforschung: Identität, Primärstruktur und Prozessierung funktioneller Proteine der bakteriellen Konjugation*, PhD Thesis, Freie Universität Berlin, 1999 (E. Lanka as an external advisor)

Sabine Krause: *Die Transferproteine TraG und TrbB des konjugativen Plasmids RP4: Strukturelle und funktionelle Gemeinsamkeiten zu analogen Proteinen anderer Transportsysteme*, PhD Thesis, Freie Universität Berlin, 1998

Stefan Ehrentraut: *Spezifische DNA-Bindung von TelN erfordert N- und C-terminale Domänen*, Diploma Thesis, Fachhochschule Lausitz, Senftenberg, 2003

Tobias Reick: *Das Ban-Protein des Bacteriophagen P1, eine DnaB-ähnliche replikative Helikase*, Diploma Thesis, TU Berlin, 1999

Renate Kühn: *Gerichtete Mutagenese in der Nukleotidbindungsstelle des Genprodukts B (TrbB), einer essentiellen Komponente des konjugativen Transferapparates des Plasmids RP4*, Diploma Thesis, TFH Berlin, 1998

Stefan Ehrentraut: *Inhibitoren für die konjugative Helikase TraI (R1)*, Project Thesis, Fachhochschule Lausitz, Senftenberg 2001

External funding

EU, BIO4-CT98-0106: *Novel Strategies for the Design of Helicase Inhibitors*, 10/98-9/00

EU, BIOTECH 970099: *MECBAD Mobile Elements' Contribution to Bacterial Adaptability and Diversity*, 1/98 – 9/01

EU, QLRT-1999-31624: *COINS Discovery of a New Class of Bioactive Compounds: Bacterial Conjugation Inhibitors*, 4/01 – 5/03

EU, QLRT-1999-30634: *DNA REPLICATION INHIBITORS Replication Initiation Proteins as New Targets for Bacterial Growth Inhibitors*, 9/00 – 8/03

INTAS, 96-1492: *Evolution of Self-Transmissible Genetic Elements: Replication Mechanisms and Control of Phage-Plasmids N15 and P4*, 1/98 – 1/01

DFG, La 672/3-4: *Die Aktivitäten des Replikationsinitiator-Proteins des E. coli Satellitenphagen P4*, 9/99 – 8/01

DFG, La 672/6-1: *DNA-Transfer durch bakterielle Konjugation*, 1/00 – 12/02

DFG, La 672/8-1: *Replikation linearer Plasmid DNA aus Gram-negativen Bakterien*, 9/03–8/05

Industrial co-operation

Combinature Biopharm AG, Berlin

Organization of scientific events

The 6th International Workshop on P2, P4 and Related Bacteriophages, 23.-26.10.1998, Hamack-Haus, Berlin

Workshop on Helicases as Molecular Motors in Nucleic Acid Strand Separation, 20.-22.11.1999, Instituto Juan March de Estudios e Investigaciones, Madrid, Spain

MECBAD Workshop on Conjugation Systems Viewed as Protein Secretion Pathways, 21.-24.5.2000, Schloß Ringberg

3rd Symposium of the EU-Concerted Action on Mobile Genetic Elements' Contribution to Bacterial Adaptability and Diversity, 21.-25.9.2001, Hamack-Haus, Berlin