Biomedical Structure Analysis Group (A0600)

Group Leaders: Prof. Dr. Michael F. Trendelenburg (acting)
Prof. Dr. Eberhard Spiess

A0601: Structural Analysis of Transcriptin Control
and Service support units:
Confocal Microscopy and Video-enhanced Microscopy
Injection
Analytical Electron Spectroscopic Imaging

Group leader:
Prof. Dr. Michael F. Trendelenburg

Senior research scientist:
Dr. Herbert Spring

Scientists:
Dr. Helmut Tröster (10/95 – 12/99)
Dr. Corinne Crucifix (10/98 – 11/00)
Dr. Karin Schwab (8/99 – 8/02)

Technical staff:
Roger Fischer

A0602: Invasion and Metastasis
and Service support unit:
Conventional Electron Microscopy

Group leader:
Prof. Dr. Eberhard Spiess

Postgraduate student:
Dipl. Biol. Felix Bestvater (11/97 – 11/00)

Technical staff:
Claudia Müller (2/99-9/99)
Anna Heckel-Pompey (Technician ½; 08/00)

As of January 1993 a central unit was established at the DKFZ to support structural analysis in the field of biomedical research by application of advanced microscopic techniques.

A - limited - service facility exists for the following areas: Confocal laser scanning microscopy, videomicroscopy, automated microinjection techniques and electron spectroscopic structure analysis at the nanometer range using advanced electron spectroscopic imaging (ESI).

Confocal microscopy and Videomicroscopy allow by combination of molecular and optical detection techniques and image digitalisation a versatile computer assisted process- ing of images often resulting in a 3D reconstitution. The consequence is a range of novel image information. Especially, the application of fluorescence confocal laser scanning microscopy leads to image information not only for the conventional two-dimensional images, but also allows to obtain images in the z-axis at a high resolution. By movement of the specimen in the optical axis of the microscope and by suppression of image information which is beyond the defined focus space, by the so called confocal aperture, sharp ‘optical’ sections of the specimen can be generated in the fluorescence mode without destroying the object. With a series of sections taken from a specimen, a computer assisted three dimensional reconstruction of the object at high resolution can be generated.

In combination with multiple fluorescence labelling and simultaneous detection, a three dimensional image can be obtained from the structural organization of intracellular compartments, in many cases the distribution of labelled structures per compartment can be quantitatively analyzed. In addition, this technique allows the observation of ‘thick’ microscopical specimen like tissue, egg cells and even whole embryos.

Outlook: Time-resolved live cell microscopy using GFP and additional constructs will become more and more important. Considering novel types of instrumentation the two-Photon-technology offers interesting new possibilities to obtain good resolution in relatively thick specimens thus we plan to add to the existing confocal microscope a two-photon-system. Automated microinjection is closely connected to microscopy. It allows a defined injection of cells with various biological probes. In combination with the techniques mentioned above, microinjection is an elegant means to study gene expression, cell regulation and transport mechanisms.

Expertise is also provided for electron microscopical specimen preparation and fixation procedures: In particular, projects can be supported in the fields of structural characterization macromolecular complexes and also for detailed structure / function investigations of cell regulation and transport mechanisms.

Starting in 1995, the group extended its activities into the field of electronmicroscopic spectroscopy with special emphasis towards application in biomedical research (supported by a grant from Federal Ministry of Science and Technology [BMBF], Bonn, Germany). In close cooperation with other divisions, experimental and analytical parameters of this novel technique are currently being optimized for molecular analysis of DNA/protein interactions, in particular, those of transcription complexes. At present, in close cooperation with other DKFZ divisions, probe configurations and parameters for spectroscopic analysis are progressively optimized in order to facilitate widespread application of this novel spectroscopic technique in molecular biomedicine.

In parallel to the above described specimen configuration work, the research is focused on novel concepts for specially designed molecular marker complexes for this type of probe detection method.
Service Support (A0601)
Confocal microscopy and video enhanced microscopy
H. Spring, M.F. Trendelenburg, A. Burzlaff
K. Schwaib, R. Fischer

For confocal laser scanning microscopy, a Zeiss LSM 510 UV microscope is used. This instrument is equipped with 4 Lasers. Thus a total of 6 wave length can be used for imaging. In addition to the laser scanning microscope, a special software program is available for 3D-deconvolution and also a high quality colour printer.

For video microscopy an Argus 20 systems (Hamamatsu Photonics, Japan) is available. This system is mainly used for the analysis of dynamic process in live cells.

Publications (* = external co-author)

Microinjection
R. Fischer, H. Tröster, M.F. Trendelenburg

An automated injection system (AIS, Zeiss) is used for injection into tissue culture cells. In addition, an Eppendorf microrinjection system is available for injections into tissue culture cells and small oocytes. Equipment and support is also provided for Xenopus oocyte and embryo injections.

Publications Microinjection (* = external co-author))

Organization of International DKFZ Advanced Practical Courses and of symposia on progress in microscopy and probe detection in biomedicine.

Concept and coordination:
M.F. Trendelenburg, H. Spring
A. Burzlaff, K. Schwaib, H. Tröster, R. Fischer


DKFZ Advanced Practical Courses
September 28. – October 2.1998
DKFZ-Advanced Course on Digital Microscopy and Fluorescence Techniques in Biology (Video Enhanced Microscopy, Multiparameter Fluorescence, Luminescence, Calcium-Measurement, Green Fluorescent Protein, FISH, Image Processing, 3-D- Procedures).

DKFZ Advanced Practical Courses
October 4-8, 1999
DKFZ-Advanced Course on Digital Microscopy and Fluorescence Techniques in Biology (Green Fluorescent Pro-
tein Technology in Live Cells, Multiparameter Fluorescence Calcium Imaging, Advanced Digital Microscopy, Confocal Microscopy, FISH, 3-D-Procedures)

**EMBO Practical Courses**
M.F. Trendelenburg, R. Fischer: Organization and Instruction on: Microinjection into Xenopus Oocytes and Eggs. EMBO Practical Courses on Microinjection and Probe Detection
EMBL Heidelberg June 1-6,1998 and EMBL Heidelberg, June 7-12,1999
H. Spring: Organization and Instruction on: Fluorescent live cell microscopy
EMBO Practical Course on Confocal and Multi-Photon Microscopy of Live Specimens
EMBL, Heidelberg: April 16-23,1999

**Analytical electron microscopy in cell and molecular biology**

Coordination: M.F. Trendelenburg and H. Spring
A. Haking, K. Richter, E. Spieß, H. Spring, M.F. Trendelenburg, H. Tröster

In cooperation with W.W. Franke, J. Kartenbeck (A0100); I. Grummt, R. Voit (A0300); J. Langowski (H0500); D. Werner (B0300); M. Wießler (B0300); M. Pawlita (F0200), all DKFZ; E. Delain (Inst. Gustave Roussy, Villejuif/ France); S. Fakan (Univ. Lausanne/Switzerland); P. Oudet, P. Schultz (Univ. Strasbourg/France); H. Kohl (Univ. Münster).

Instrumentation: ZEISS > LEO >EM 912 Omega, equipped with Slow Scan-CCD Camera, Electron Spectroscopic Imaging System, special image analysis software, EELS detector, and components for cryo-electron microscopy.

The principle of electron spectroscopic imaging (ESI) consists in the inelastic deflection of electrons at the energetically defined inner shells of atoms. Basically defined by the relation between the energy loss of the incoming beam electron and the energy gain of the electron located at the inner shell of the atom. If this principle is applied to electron microscopic specimens containing defined elements of a required concentration, element-characteristic absorption spectra can be recorded (see Fig. 1 for details). Using these elementspecific absorption spectra, quantitative data considering the specific element within the specimen can be obtained. The main aim of this project (supported by ZEISS/LEO Elektronenmikroskopie, Oberkochen and Bundesministerium für Bildung und Forschung, Bonn) is to optimize application parameters for specific element detection in cell and molecular biology.

The main application of this type of analytical electron microscopy in biomedical research is a quantification of phosphorus signals contained in DNA and RNA samples. This holds for both types of specimen, e.g. spread preparations of isolated materials as well as nuclear structures in thin sections. An additional set of spectroscopic analyses is carried out on boron and/or halogenlabelled biomolecules.

**Topics of research and development**

I. Quantitation of parameters required for electron spectroscopic imaging (ESI) and electron energy loss spectroscopy (EELS). As specimen we use spread DNA / Protein complexes and non-contrasted ultrathin sections through fixed cells for high resolution detection of phosphorus. Similarly the quantification for spectroscopic detection of phosphorus, constructs containing other elements will be coupled to biomolecules. As further area of research are ESI analyses on spread DNA/Protein complexes using specific lipid membranes for orientation of spread DNA complexes. An important application of the technique is cryo-electronmicroscopy of vitrified samples.

II. Design of an optimized procedure for ESI detection of Phosphorus (P)-content within DNA / RNA probes. Viral particles containing DNA or RNA which had been adsorbed on a supporting membrane for ESI-EM analysis contain on are rage 3-5 P-Atoms / nm² (depending on the type of specimen) As a consequence, unspecific background signals have to be subtracted, in order to quantify the specific P-signals. Since quantification of P-signals is of out most general importance for spectroscopic analysis of biomedical samples. Thus, we successfully designed a special standard-P-reference specimentype in order to quantify P-signal detection [2-6, 8, 9].

III. Quantification of viral genomes

In regard to the rapid progress in the design and application of viral vectors in molecular gene therapy, we developed a fast screening system for quantitation of the phosphorus content of viral genom sizes by application of element specific imaging (ESI). The principal aim

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**Fig. 1:** Principle and application of electron spectroscopic imaging in biomedical research
A: Beam path of the analytical microscope for inelastic imaging.
B: Principle on inelastic scattering on specimen atoms.
C: D: Phosphorus-specific EM-spectroscopic imaging using co-adsorbed TYMV and TMV viruses.
D: Image taken at 150 eV, e.g. the maximum of the phosphorus P-L2,3 edge. In addition to the phosphorus information, this image also contains 'unspecific' background signal contributions.
D: Calculated image representing the P-map signal distribution within TYMV and TMV virus particles.
of this approach is the precise determination of the detection of differences in the order of 500-1000 bp of assembled viral genome, e.g., for purified viral samples as deceived from gradient purification [2-6, 8, 9].

IV. A new possibility for elementspecific spectroscopic imaging of gold-labelled probes

Among the predominantly used EM-labelling techniques is the nanogold-coupled immuno-labelling technique the most frequently used one. Experience from the inspection of a large variety of samples however, indicates that often the precise position and number of probe-reacted nanogold particles cannot always be clearly determined, since the conventional detection of nanogold particles largely depends on the “density” of the underlying structure in the contrasted ultrathin sections with the new principle using our ESI detection method, smallest nanogold particles can be detected, irrespective of the “density” of the background [1, 3, 5].

V. A particular impact of ESI EM analysis is to be expected for a localization of boron clusters directly in the BNCT (boron neutron capture therapy) treated patient tumors [10]. An major progress in this type of analysis shall be reached following the introduction of more concentrated boron probes [11, 12] into the BNCT therapy schema. It is also of high interest to note that –recently– a modified BNCT technique could be proposed for therapy of rheumatic arthritis [13].

Publications

Analytical Electron Microscopy (* = external co-author)


Patents


Structural Analysis of Gene Structure and Function (A0601)


In cooperation with

Internal cooperation: Prof. A. Alonso (F0500), Dr. M. Schmidt-Zachmann (A0100), Prof. M. Wießler (C0300), Prof. W. Schlegel (E0400), Prof. G. Szczyzkiel (F0200), Dr. M. Pawlita (F0200)
External cooperation: Dr. N Angelier (CNRS/Univ. Paris, France), Prof. W. Ansorge, EMBL Heidelberg, Dr. P. Bucher (ISREC, Epalinges-Lausanne, Switzerland), Prof. J. C. Bulinski (Columbia Univ. New York, USA), Dr. S. Fakan (Univ. Lausanne, Switzerland), Prof. O.L. Miller (Univ. Virginia, USA), Prof. P. Oudet, Dr. P. Schultz, (CNRS, INSERM, Univ. Strasbourg, France), Prof. O.V. Zatsepina (Moscow State University, Moscow, Russia), Prof. S.A. Gerbi (Brown Univ. Providence, RI, USA).

1.) High resolution light microscopy of active gene domains in the cell nucleus and transcript transport into the cytoplasm

Among the most challenging prospects in structural chromatin research are the ‘gene gating’ concept as proposed by G. Blobel and the ‘loop-model’ of interphase chromatin. Both concepts strongly emphasize the importance of the spatial gene domain arrangements in the interphase cell nucleus. Due to the fact that transcriptionally active genes are mostly located in nuclear areas of very high electron density, a straightforward electronmicroscopic analysis of the in situ gene organization is difficult. Another limitation of a direct EM-visualization is due to the small dimension of the active gene structure. In order to investigate the in situ configuration and spatial arrangement of active genes in detail, we had developed novel concepts for light microscopic analysis of gene organization [1, 9]. Thus, it had been possible - for the first time - using video enhanced light microscopy at high magnification, to analyse the structural organization of ‘native’, i.e. fully hydrated transcriptionally active rRNA genes [1, 9].

Due to the rapid progress in microscopic instrumentation, we could use transmission X-ray microscopy for our studies in addition to photonic microscopy. Imaging of cellular structures is possible without a previous impregnation with heavy metal salts as required for conventional transmission electron microscopy of thin sections. Cellular organelles such as intact nucleoli can be analysed in a fully hydrated state using a considerably higher resolution than can be reached with optical microscopy [1, 2, 9].

A novel type of structural analysis of spread active genes is provided by scanning force microscopy. Using this instrument it is possible to obtain precise structural dimensions in the Z-axis [3].

In regard to the ultrastructural analysis and localization of defined nucleolar proteins we were able to work out a novel concept for ultrastructural localization - based on cooperation experiments with an AvH stipendiate O. Zatsepina (Moscow): By a strictly controlled hypotonic shock to live tissue culture cells, it could be demonstrated, that the normally very compact nucleolar structure considerably unravels into its subcomponents: Thus, physiologically spread, in situ decompacted nucleolar subunits can be analysed. Theses are then reacted with antibodies against nucleolar proteins [1, 4, 9]. The terms ‘physiologically spread nucleolar subunits’ was demonstrated to be justified, since after release of the shock treatment - cells continue to grow and nucleolar function is fully restored [1, 4].

2.) Structural analysis of regulation of transcription

Among the most thoroughly analysed eukaryotic genes are the Xenopus laevis ribosomal RNA genes. This holds in particular for the close interrelationship of structural and molecular investigations. Using the above mentioned videomicroscopic approach, we could successfully clear a major discrepancy between molecular and structural data regarding rDNA transcriptional regulation. The predominant model indicated, that transcription of the 40S precursor rRNA normally terminates at sequence blocks T1/T2 and not at the sequence T3. Using an elegant in vitro transcription assay it had been postulated, that transcription - also under in vivo conditions - exclusively terminates at position T3. This would implicate a constitutive transcription of 2-5 kb rDNA spacer sequence. Using a combination of data from videomicroscopic, electron microscopic, and S1 transcript analysis, we could show, that - under in vivo conditions -, exclusively the sequence region T1/T2 is used for efficient transcription termination for the in vivo situation [1, 9].

Our present investigations are focussed on three topics: 1. In situ detection of the topology of nucleolar rDNA during different states of transcription regulation [1, 4]. 2. In addition, the in situ localisation of the major components of pol I transcription complexes will be analyzed, e.g. RNA-Polymerase I and the ‘upstream binding factor’ (UBF) [1, 4]. Using the above mentioned structural data [1] we have also begun, to characterize in detail the fine structure of the primary rRNA transcripts [1, 9], using high resolution densitometry and computer-assisted image analysis. Due to the recent progress as obtained for phosphorus mapping by analytical electron spectroscopy imaging (ESI) we have started to apply this high resolution phosphorus detection technique also for analysis of P - distribution within nascent pre-rRNA transcripts [5, 6-10].

3.) Chromatin organisation, genome-integration and expression of injected genes during early embryogenesis.

Microinjection experiments of viral and nonviral DNA sequences into Xenopus oocytes and fertilized eggs provide appropriate experimental conditions for investigations on transcriptional control, chromatin assembly and gene replication. In addition to microinjection experiments, incubation of defined DNA samples in Xenopus egg extracts is a frequently used method for experimental analysis of chromatin reconstitution and factor requirement for DNA replication [Trendelenburg, M.F., Grunz, H.: Developmental Biology in Germany, Int. J. Develop. Biol. 40 (1996) 1-439]

Xenopus offers the unique possibility to compare different mechanisms of transcription regulation of ribosomal RNA...
genes in the momologous in vivo situation for both, the meiotic cell (e.g. the oocyte, which is arrested for a period of 2-3 month’s in the diplotene stage of meiotic prophase) as well as for the early embryo.

In regard to transcription regulation in the diplotene stage oocyte we carried out a detailed analysis of rDNA topology within intact oocyte nucleoli during premeiotic oocyte growth and differentiation in order to correlate changes of rDNA topology with the onset of the very high rRNA transcription rates characteristic for oocyte nucleoli: Using UV-laser scan microscopy, Transmission Electronmicroscopy and S1 Transcript analysis we could show that (i) a massive rearrangement of amplified rDNA occurs within individual nucleolar units and (ii), that this characteristic change in rDNA arrangement is directly correlated with the observed sudden onset of an extreme transcription rate of ribosomal RNA genes (Trendelenburg, M.F., Grunz, H.: Developmental Biology in Germany, Int. J. Develop. Biol. 40: 1996, 1-439; 1, 9). A completely different type of transcription regulation can be postulated for the early phase of embryogenesis, e.g. before „mid-blastula transition“. A continuous elongation of the cell cycle is established between mid-blastula transition and the early gastrula stage. Using well characterized antibodies against nucleolar proteins, including transcription factors [1, 4] combined with a recently consistently improved method of background substraction. Acta Microscopia (1999) 8, 133a, 2000.

Electron microscopy

E. Spiess

Electron microscopical techniques are restricted to conventional transmission electron microscopy. The following techniques can be applied: negative staining, DNA spreading, embedding, thin sectioning and immunocytochemistry.

Publications Electron microscopy, Service support (* = external co-author)


Modellexperiments in Tumor Cell Invasion and Metastasis (A0602)
Cathepsin B and Tumor Cell Invasion

E. Spiess, F. Bestvater

In cooperation with: Dr. Berend Werle, Med.Klinik und Polyclinik der Universität Heidelberg; Dr. W. Ebert, Thoraxklinik, Rohrbach, Heidelberg; Dr. Tamara Lah, National Institute of Biology, Ljubljana, Slovenia; Dr. J. Kos; KRKA, Ljubljana, Slovenia; Dr. Torsten Porwol, Dr. Helmut Acker, Max-Planck-Institut für Molekulare Physiologie, Dortmund; Dr. A.-R. Strohmaier, Nikon GmbH, Düsseldorf.

The aim of our studies is the understanding of the role of cysteine proteases, particularly cathepsin B, in tumor progression, tumor invasion and metastasis.

By investigation of clinical material cathepsin B was identified as a possible independent marker for prognosis in squamous cell carcinoma of the lung [1, 2, 3]. We applied confocal and wide field microscopy combined with computer assisted image restoration and 3-D reconstruction techniques as well as quantitative flow cytometry enzyme cytochemistry to analyze the spatial arrangement of cathepsin B in cells and its induced secretion [4, 5]. Cathepsin B-GFP chimeras were constructed and expressed in tumor cells to delineate in detail the sorting of the enzyme in living cells under conditions as close as possible to a natural invasive situation. Thus far, the results support the possibility of intra and extracellular participation of cathepsin B in invasion.

Publications (* = external co-author)