

Research Program B

Structural & Functional Genomics

Peter Lichter (coordinator)

dkfz. GERMAN
CANCER RESEARCH CENTER
IN THE HELMHOLTZ ASSOCIATION

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The Research Program on Campus



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Research Program: Structural & Functional Genomics

Divisions:

Molecular Biophysics
Sandor Suhai

Tumor Genetics
Manfred Schwab

Biophysics of Macromolecules
Jörg Langowski

Molecular Genetics
Peter Lichter

Molecular Genome Analysis
Stefan Wiemann

Functional Genome Analysis
Jörg Hoheisel

Theoretical Bioinformatics
Roland Eils

Signaling and Functional Genomics
Michael Boutros

Groups:

Cellular Biophysics
Matthias Weiss

Chip-based Peptide Libraries
Ralf Bischoff, Frank Breitling, Volker Stadler

Signal Transduction in Cancer and Metabolism
Aurelio Teleman

Molecular RNA Biology and Cancer
Sven Diederichs

Mechanisms of Leukemogenesis
Daniel Mertens

Microenvironment of Tumor Cell Invasion
Thomas Ludwig

Molecular Genetics of Breast Cancer
Ute Hamann

Functional Architecture of the Cell
Harald Herrmann-Lerdon

Genome Organization and Function
Karsten Rippe

Modeling of Biological Systems
Thomas Hoefer

Peter Lichter

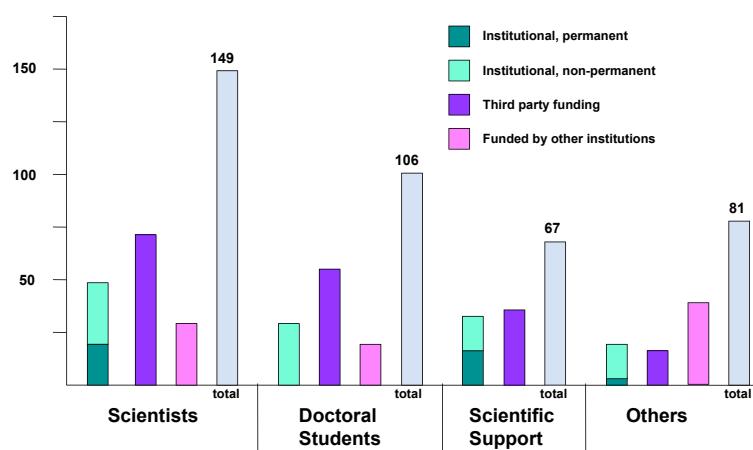
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Members (403)

September 30, 2008



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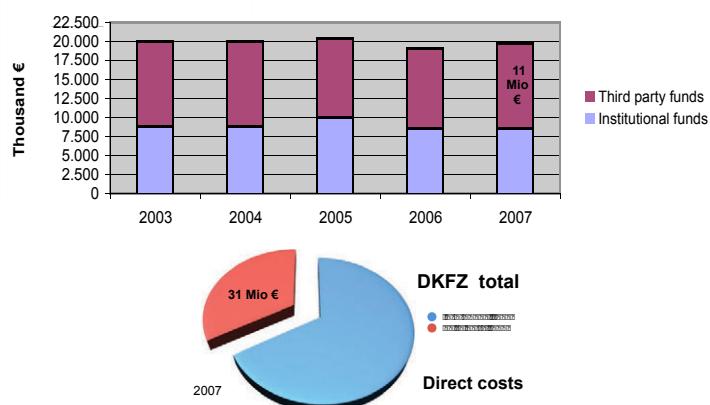


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Funding

Direct costs

Research Program „Structural and Functional Genomics“



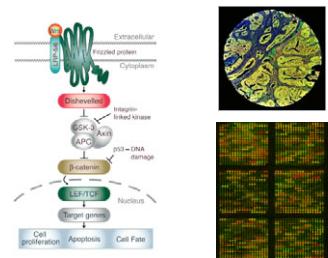
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Central Topics



Genes & Pathways

Oncogenomics

Quantitative Cellular & Molecular Biophysics



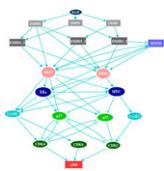
Bioinformatics & Systems Biology

Technology Development

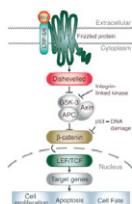


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Genes and Pathways



- Large scale functional screening
- Gene expression analysis
- In-depth functional characterization and animal models

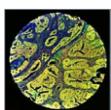


Aims:

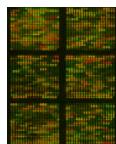
- Identification and characterization of full complements of pathways
- Pathways with relevance to tumor etiology and progression
- Regulatory mechanisms of gene expression (incl. non-coding RNA)
- Identification of targets for therapeutic intervention

Oncogenomics

Study of molecular variations in tumor cells on the level of the



- Genome, incl. Fragilome
 - Epigenome
 - Transcriptome, incl. microRNAs
 - Proteome
- to identify prognostic and predictive gene signatures



Aims:

- Identification of signatures for tumor cell subpopulations (e.g. stem cells)
- Optimization of prognostic and predictive gene signatures
- Translation of signatures (prospectively tested) in clinical routine
- Robust diagnostic tools (e.g. DNA vs. RNA)

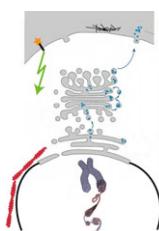
Quantitative Cellular & Molecular Biophysics

Interaction of proteins with DNA on multiple length and time scales

Chromatin conformation & nucleosomes

Interphase chromosomes & nucleoskeleton

Cell membranes: protein trafficking & environmental signaling



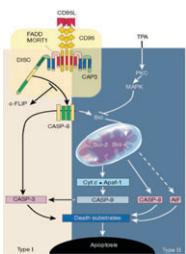
Aims:

- Physico-chemical principles of dynamic cellular self organization (chromatin domains, nuclear bodies, filaments, membrane traffic)
- Spatio-temporal descriptions of reaction-diffusion processes (incl. signaling pathways, DNA repair, epigenetic silencing)
- Assessment of dynamic processes from microseconds to hours
- Correlative electron and high resolution light microscopy

Bioinformatics & Systems Biology

Analysis of high throughput functional genomics data
in combination with disease specific information

Theoretical and computational modeling
to gain a systems biology view
of complex biological processes



Aims:

- Advancing functional genomics to a mechanistic understanding of tumor diseases
- Deciphering molecular function in a spatio-temporal cellular context
- Developing predictive models for therapeutic intervention (targets) in cancer
- Linking molecular cell biology models to medical applications

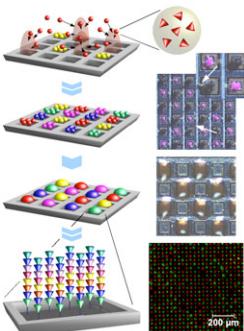
Technology Development

High throughput technologies

- Genome regulation via epigenetics
- Proteomics

Quantification of biomolecules

Single molecule detection



Aims:

- Genome-wide protein expression profiling methods
- Global and quantitative analysis of protein activities and interactions
- High affinity binders of pharmaceutical relevance
- Synthetic genes, proteins etc., synthetic biology
- Advancing single molecule detection for high-throughput assessment of biomolecular interactions *in situ* and *in vivo*

Strategic Collaborations & Alliances

Majority of funding through external sources



MolDiagPaCa
Proteome Binder
CancerPathways
RegGenomics
Chromatin Plasticity
Laminopathies
etc.



Nationales Genom-Forschungs-Netzwerk:
Coordination of 1/2 of all cancer networks

Helmholtz Alliance
SB Cancer
ForSys
MedSys
BIOMS

HZM - Mouse Clinic
MDC - Protein Structure

Association with Core Facilities

Genomics and Proteomics Core Facility: Bernhard Korn

- Genomics
 - Sanger Sequencing
 - "Next Generation" Sequencing
 - Expression Profiling
 - Genotyping
 - Clone Repository
- Proteomics & Structure Analysis
 - Peptide synthesis
 - Protein Interaction Screening
 - Protein Analysis, 2D (M. Schörlzer)
 - Protein Analysis MALDI, esIMS/MS
 - Mass Spectrometry: Small molecules and protein modifications
 - NMR

Light Microscopy: Matthias Weiss

- Sample preparations
- Wide-field microscopy
- Laser scanning microscopy
- Laser microdissection
- Microinjection



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Retreat - Kloster Schöntal



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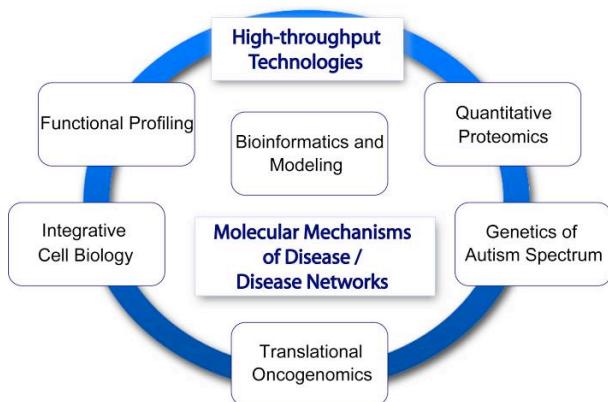
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Molecular Genome Analysis (B050)

Stefan Wiemann



Functional Profiling

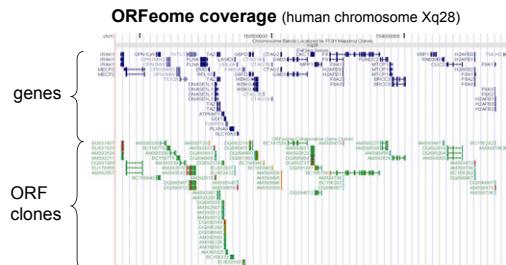
Resources

Applications

Projects

- Whole-genome RNAi ← with Michael Boutros (B110)
- RNAi Global / Dharmacon

- 12k human ORFs
International ORFeome Collaboration
 - 3,500 ORFs
 - 3,000 genes
 - 6,200 expression clones
 - clones @ www.orfeomecollaboration.org
 - sequences @ GenBank/EMBL/DDBJ



poster U. Tschulena

Functional Profiling

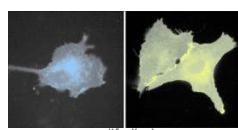
Resources

Applications

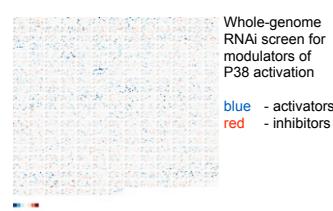
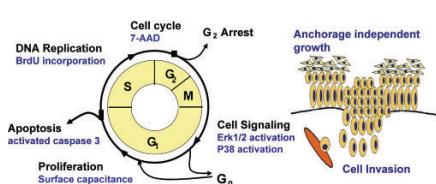
Projects

- Whole-genome RNAi
RNAi Global / Dharmacon
- 12k human ORFs
International ORFeome Collaboration

- Protein localization
 - Functional screens
 - RNAi - P38 signaling, cell detachment
 - Overexpression - apoptosis
- Genome Res 2004, Cancer Res 2005, EMBO J 2005, Oncogene 2008



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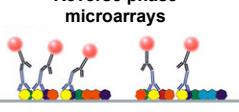
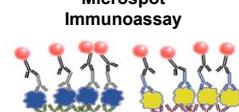
poster U. Tschulena

Functional Profiling

Resources	Applications	Projects
<ul style="list-style-type: none"> Whole-genome RNAi RNAi Global / Dharmacon 12k human ORFs International ORFeome Collaboration 	<ul style="list-style-type: none"> Protein localization Functional screens <ul style="list-style-type: none"> RNAi - P38 signaling, cell detachment Overexpression - apoptosis <p>with Sven Diederichs (B150) □</p>	<ul style="list-style-type: none"> Expansion of resources ORF, shRNA, miRNA Protein dynamics (subcellular localization & cellular phenotypes) Assay development NF_κB translocation, miRNA in signaling & cancer Identification of signaling network candidates
	<p>Data analysis</p> <p>Candidate validation (<i>in vitro</i>, <i>in vivo</i>, clinics)</p> <p>Systems biology</p>	
<p>poster U. Tschulena</p> <p>Stefan Wiemann Molecular Genome Analysis (B050)</p> <p style="text-align: right;">dkfz.</p>		

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Quantitative Proteomics

Technologies	Specifications	Projects
<p>Reverse phase microarrays</p>  <p>Proteomics 2007</p>	<ul style="list-style-type: none"> cost-effective tumor protein profiling: (1,000 tumor samples/ slide) > 80 RPPA validated ABs (different suppliers) 	<p>Tumor profiling</p> <p>J Pathol 2008a, J Pathol 2008b</p> <p>>GIST, colon, breast, prostate cancer, neuroblastoma</p>
<p>Microspot Immunoassay</p>  <p>Adv Bioch Eng Biotechnol 2008</p>	<ul style="list-style-type: none"> dose-dependent and dynamic effects of signal transduction (5% SD) sensitivity: pg/mL dynamic range: >10³ 	<p>Signaling networks</p> <p>PNAS 2007, Proteomics 2008</p> <p>>Quantitative protein profiling</p> <p>>RNAi based strategies, Receptor Tyrosine Kinase inhibition</p>
		<p>Systems biology</p> <p>Proteomics in press</p> <p>>Quantitative analysis of ERBB and ESR1 signaling in breast cancer cell lines</p> <p>>Validation of network models in clinical samples</p>
<p>poster U. Korf</p> <p>Stefan Wiemann Molecular Genome Analysis (B050)</p> <p style="text-align: right;">dkfz.</p>		

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Integrative Cell Biology

Signaling and Cell Cycle Regulation	Cell – ECM Interactions	Cell – Cell Interactions
<p>Computational simulations and experimental validations to elucidate therapeutic strategies</p> <p>PNAS 2007</p>	<p>siRNA screen for detachment → glycosaminoglycan degradation pathway</p> <p>BMC Bioinformatics 2008</p>	<p>Vmp1 as metastasis-relevant protein involved in the formation of cell-cell contacts</p> <p>Oncogene 2008</p>
<p>ERBB signaling network</p> <p>Mechanisms of targeted therapeutics</p> <p>Validation of results using patient samples</p> <p>Clinical tests for combinatorial therapies</p>	<p>Glycosaminoglycan degradation and signaling</p> <p>Influence on Integrin, TGFβ, Met signaling</p> <p>Test systems for combinatorial treatment using therapeutic antibodies and biogenic glycans</p>	<p>Vmp1 in ErbB2 signaling</p> <p>Vmp1 knockout mice</p> <p>Mouse models and tissue microarrays to test VMP1 as prognostic marker</p>
<p>posters Ö. Sahin, A. Hussain, D. Arlt</p> <p>Stefan Wiemann Molecular Genome Analysis (B050)</p> <p style="text-align: right;">dkfz.</p>		

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Genetics of Autism Spectrum

Patient samples	Projects
<ul style="list-style-type: none"> DNA (patients) 560 Lymphoblastoid cell lines 190 Complete trios (for association) 250 	<ul style="list-style-type: none"> Whole-genome screens (IMGSAC, AGP) <ul style="list-style-type: none"> - 2q, 7q, 11p, 15q, 16p Association studies (SNP/microsatellites) <ul style="list-style-type: none"> - Neuronal migration: Reelin (<i>RELN</i>) Mol Psychiatry 2003 Candidate gene screening (Sequencing) <ul style="list-style-type: none"> - Ribosomal protein L10 (<i>RPL10</i>) Mol Psychiatry 2006
Consortia/collaborations	
<ul style="list-style-type: none"> International Molecular Genetic Study of Autism Consortium (IMGSAC) Autism Genome Project (AGP) Nat Genet 2007 The Autism Simplex Collection (TASC) <p>► poster S. Klauck</p>	<p>➤ Functional <i>in vitro</i> modeling <i>RPL10</i> mutations L206M, H213Q in neuronal cell lines</p> <p>➤ Animal models <i>RPL10</i> knock-in mouse</p>
Stefan Wiemann Molecular Genome Analysis (B050)	dkfz. 22

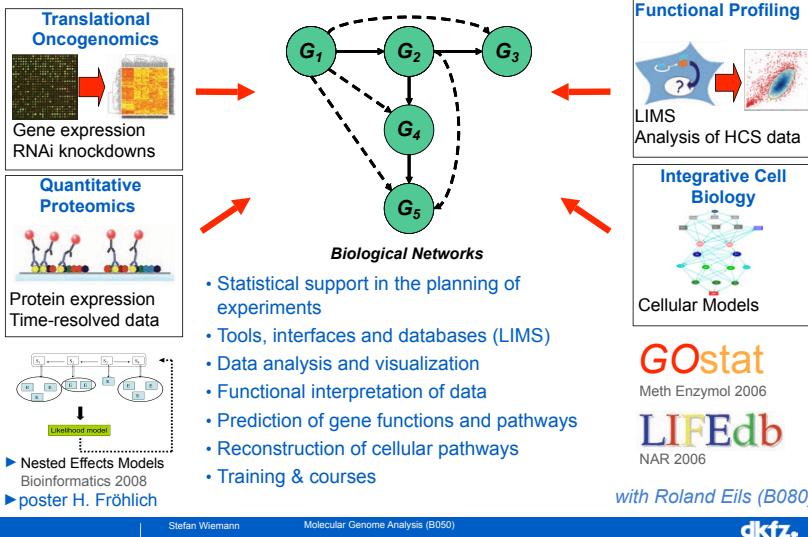
Translational Oncogenomics

Technologies	Clinically Oriented Research
Microarrays, qRT-PCR, IHC □with Peter Lichter (B060)	Signatures for cancer prognosis and progression
Isogenic cell lines	<p>➤ Prostate Europ. Urol. 2008</p> <p>➤ clinical study</p> <p>➤ splicing, miRNA</p> <p>Breast</p> <p>➤ <i>ESR1</i>/lymphocytes Breast Cancer Res Treat 2008</p>
Cellular and animal models	<p>➤ Lung Lung Cancer 2008</p> <p>➤ Endobronchial microsampling</p> <p>Kidney Clin. Can. Res. 2005</p> <p>➤ prediction of metastases</p>
Development	
► posters H. Sültmann, R. Kuner	
Stefan Wiemann Molecular Genome Analysis (B050)	dkfz. 23

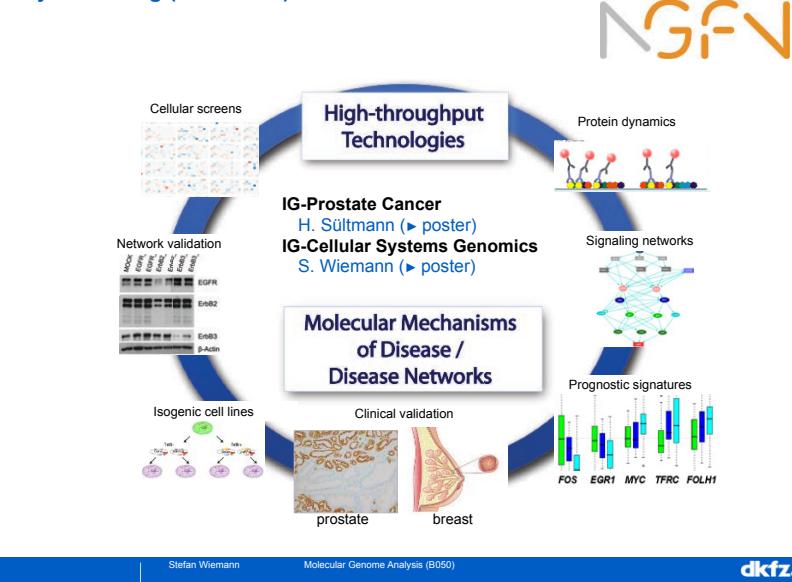
Translational Oncogenomics

Technologies	Functional Analysis
Microarrays, qRT-PCR, IHC	Tumor gene expression networks Breast cancer Bioinformatics 2008 ➤ Prostate cancer
Isogenic cell lines	Gene function analysis Melanoma, glioblastoma ➤ Prostate cancer
Cellular and animal models	Therapy targets and mouse models ➤ <i>Tmem45B</i> knock-out mouse
Development	<p>➤ Serum biomarker screening □with Ralf Bischoff (B120)</p> <p>➤ Signal amplification</p>

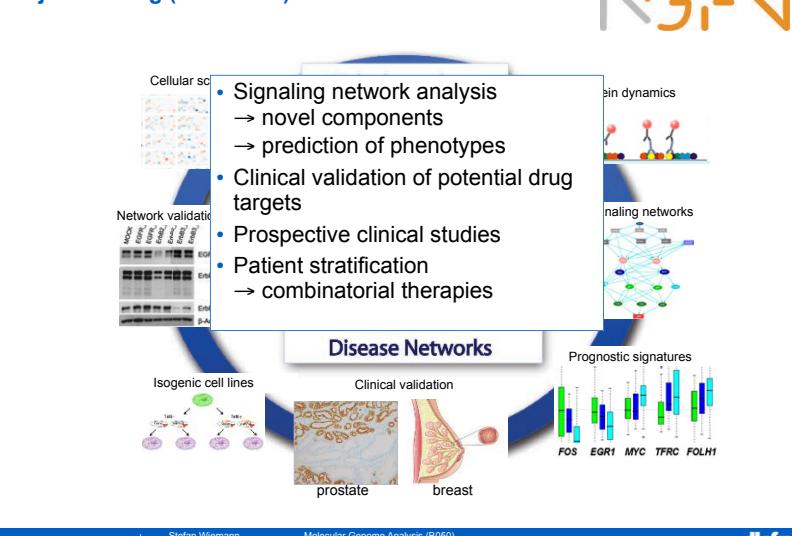
Bioinformatics and Modeling



Major Funding (2008-2013)



Major Funding (2008-2013)





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Tumor Genetics (B030)

1. Basic and Translational Neuroblastoma Research

- Diagnostic NB Chip in the Clinic (Poster #1)
- Systems Biology of MYC Oncoproteins (#2)
- Spontaneous Neuroblastoma Regression: A Goldmine for New Therapeutic Concepts (#3)
- CAMTA1, a New Tumor Suppressor on 1p36 (#4)
- miRNA functions in NB biology (#5)

2. Fragilome Project (#6-9)

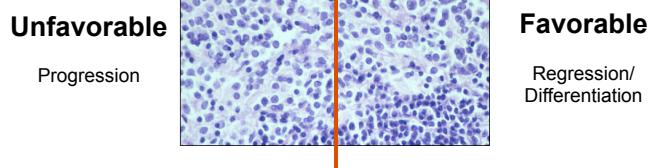
Head: Prof. Dr. M. Schwab
Group Leader: Dr. F. Westermann
Group Leader: Dr. L. Savelyeva



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Differential Diagnostics

Clinical Courses



Markers

	Amplified MYCN	Stage	Age at Diagnosis
	Error Prone		
Risk Prediction/Classification	High Risk	Intermediate	Low

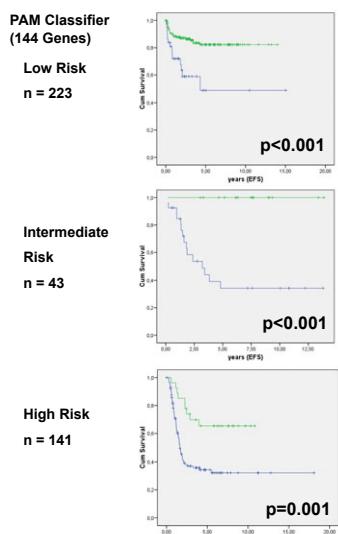
Risk Prediction/
Classification

Intensity of
Therapy

Multimodality
Treatment

Wait-and-See

Diagnostic NB Chip in the Clinic



- Customized NB Gene Expression Chip
 - Nature Med. 2001, Cancer Cell 2002
 - Cancer Res. 2004, Oncogene 2005
 - Clin Cancer Res. 2007
- Retrospective Study (German and International Samples, n=370)
 - JCO 2006, BMC Cancer 2007
 - Cancer Letters 2007, Clin Cancer Res. 2008
- Prospective Study since October 2004 (n=137)
 - >1000 Gene Expression Profiles
- NB Chip Integrated into the German NB Trial since February 2008
- Participation in FDA-initiated MAQC2 Study (Standards for HT Data Transfer into Clinic)

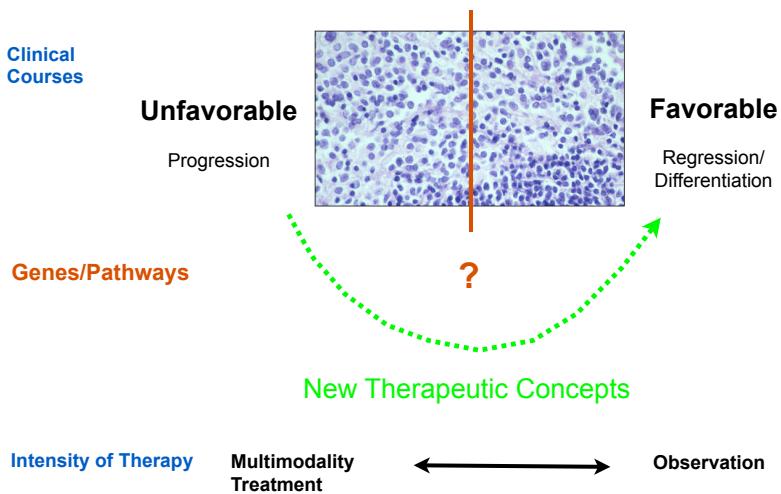
Collaboration with Theoretical Bioinformatics (Brors, Eils) and German NB Study Group (Fischer)

Frank Westermann Tumor Genetics (B030)

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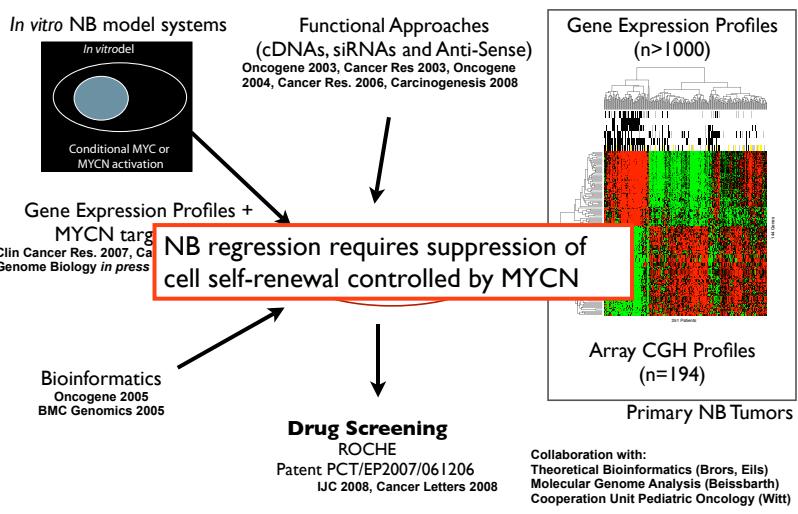
Progression versus Regression



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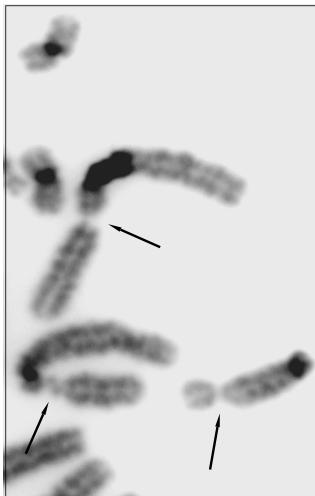
Progression versus Regression



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Fragile Sites



Common Fragile Sites

- ★ are seen in **all** individuals
- ★ specific loci on metaphase chromosomes
- ★ replication stress
- ★ result in DNA breaks
- ★ molecular basis is unknown

Common Fragile Sites (CFS) and Cancer

★ Well known:

Activation of CFS can result in deletions and translocations breakpoints, oncogene amplification via breakage-fusion bridge cycles, integration of viral DNA

★ New:

Breakage at CFS is an initiating event in the generation of DNA damage at very early stages of tumorigenesis and in premalignant lesions

Aims of the FRAGILOME project

★ Identification of the full repertoire of common fragile sites in the human genome

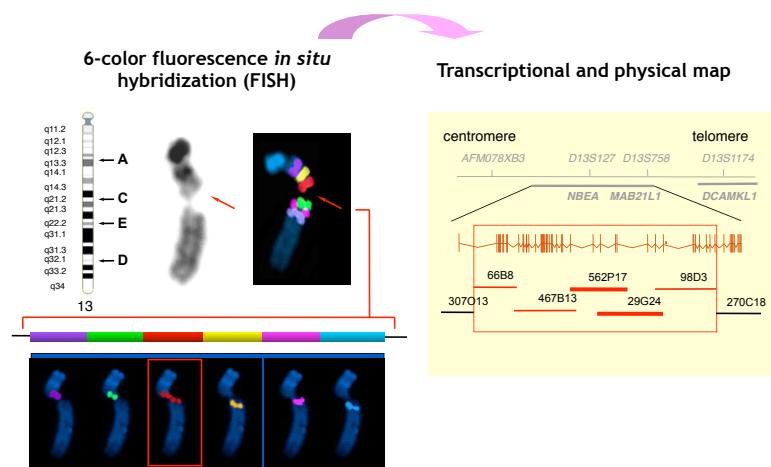
Total: ~120
Identified: 34 (20 in our department)
Unknown: ~ 90

★ Definition of significant genetic rearrangements caused by the activation of fragile sites during tumor development

Neuroblastoma
Breast cancer
Colon cancer

★ Development of new DNA biomarkers for diagnosis and individualized therapies

Current approach to identify CFS DNA sequences:



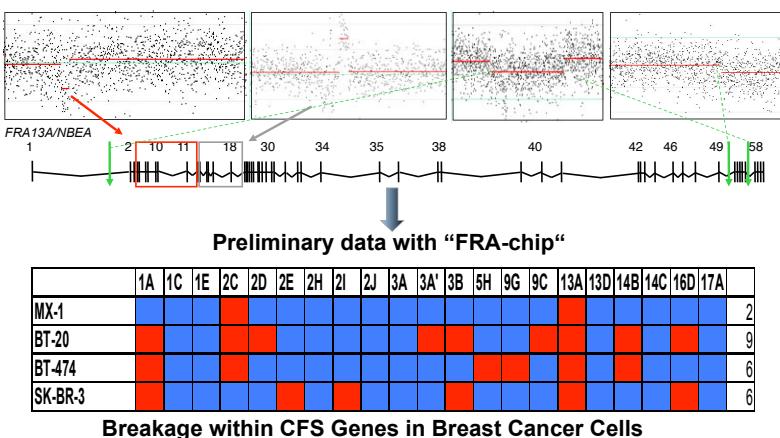
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Genetic Rearrangements at CFS in Tumor Cells

Fine-Tiling Custom CFS Array for CGH
(10bp spacing)



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Cooperations

Intramural

- Theoretical Bioinformatics (B. Brors, R. König, R. Eils)
- Molecular Genome Analysis (T. Beissbarth, U. Korf, S. Wiemann)
- Molecular Biophysics (A. Hotz-Wagenblatt, K.-H. Glatting)
- Modeling of Biological Systems (T. Höfer)
- Molecular Genetics (S. Pfister, P. Licher)
- Cooperation Unit Pediatric Oncology (O. Witt)

Extramural

- M. Fischer, F. Berthold (German NB Study Group, Univ. Köln)
- A. Eggert (Univ. Essen)
- M. Ellers (Univ. Marburg)
- F. Speleman (Univ. Ghent, Belgium)
- J. Khan (NIH, NCI, USA)
- K. Ushijima (NCI, Tokyo, Japan)
- ROCHE, Nutley, New Jersey, USA (P. Gillespie)
- RNAi Screening Facility, BioQuant (H. Erfle)
- EET Pipeline Consortium (EU, 8 Partners)

Grants

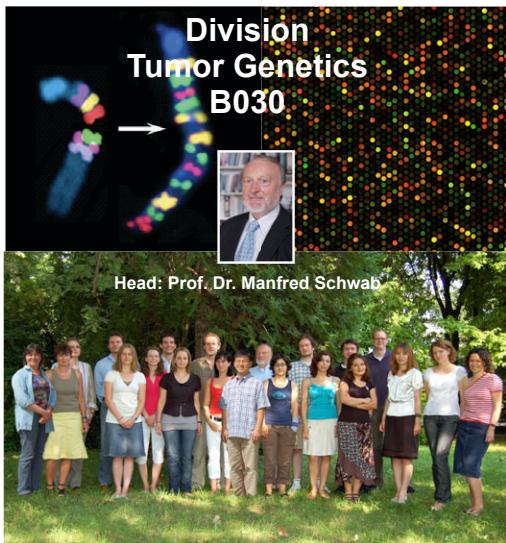
- NGFN2/NGFN^{Plus} (German NB Network)
- EU EET-Pipeline
- EU MYCN/c-MYC in Embryonal Tumors
- Deutsche Krebshilfe
- DFG (Spontaneous NB Regression)
- Helmholtz-Russia Cooperation
- Intramural DKFZ Funding

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Molecular Genetics of Breast Cancer (B055)

Ute Hamann

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

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Research Activities

Determination of the contribution of the *BRCA1/2* genes to hereditary breast/ovarian cancer in non-Caucasian populations

Search for genetic factors that are associated with

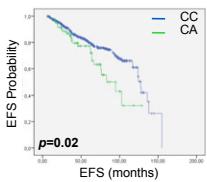
- Breast cancer risk and progression (K. Hemminki, J. L. Bermejo, DKFZ)
- Hormone therapy associated postmenopausal breast cancer risk (J. Chang-Claude, R. Eils, DKFZ)
- Hereditary breast and ovarian cancer risks

Identification of markers predictive of response to tamoxifen

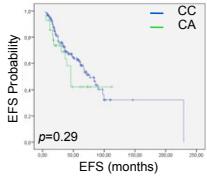
Genetic Variation of p21 and Response to Tamoxifen

- Tamoxifen can induce cell cycle arrest and apoptosis
 - A critical mediator of its effect is the cyclin dependent-kinase (Cdk) inhibitor p21
 - Nuclear p21 binds to cyclin-Cdk complexes to inhibit cell cycle progression
 - In contrast, cytoplasmic p21 can promote cell cycle progression and correlates with poor response to tamoxifen
- ↓
- p21 genetic variation may influence protein function and tamoxifen response*

Tam-positive group (n=521)
Median age at diagnosis (years): 65.4
Median follow up (months): 56.4
Event-free survival (EFS, months): 50.6



Tam-negative group (n=194)
Median age at diagnosis (years): 62.1
Median follow up (months): 42.2
Event-free survival (EFS, months): 35.6



Coding SNP (C>A) in *p21* resulting in a non-synonymous amino acid change

- CC carriers have a more favorable clinical outcome than CA carriers
- CC carriers may benefit from tamoxifen treatment

Ute Hamann

Molecular Genetics of Breast Cancer (B055)



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Research Activities

Determination of the contribution of the *BRCA1/2* genes to hereditary breast/ovarian cancer in non-Caucasian populations

Search for genetic factors that are associated with

→ Poster #1

- Breast cancer risk and progression (K. Hemminki, J. L. Bermejo, DKFZ)
- Hormone therapy associated postmenopausal breast cancer risk (J. Chang-Claude, R. Eils, DKFZ)
- Hereditary breast and ovarian cancer risks

Identification of markers predictive of response to tamoxifen

Investigation of the expression levels of estrogen receptor variants in early-(≤40 yrs) and late-onset (≥60 yrs) breast cancer → Poster #2

Proteome-wide approach to identify SUMOylated proteins in breast cancer (M. Schnölzer, DKFZ) → Poster #3

Ute Hamann

Molecular Genetics of Breast Cancer (B055)



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Outlook

Risk Factors

- Identification of further low penetrance genes in Caucasians
 - Identification of low penetrance genes and modifiers of hereditary breast cancer risk in the Hispanic population
 - Identification of novel modifiers of hereditary breast cancer using a genome-wide approach (K. Hemminki)
- ↓
- Prevention**

Prognosis

- Identification of genetic factors that influence prognosis and survival
 - Identification of expression profiles with prognostic value for early- and late-onset breast cancer using a microarray approach (J. Hoheisel)
- ↓
- Markers of clinical outcome**

Treatment Prediction

- Identification of intrinsic and tumor-associated factors for prediction of response to tamoxifen



Treatment optimization targeted intervention

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Molecular Genetics of Breast Cancer (B055)



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Thomas Dünnebier
Vesna Mandusic
Anna Jakubowska



Diana Torres
Muhammad U. Rashid



Antje Seidel-Renkert
Michael Gilbert
Ute Hamann



Ute Hamann

Molecular Genetics of Breast Cancer (B055)

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Thank you!

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Molecular Genetics of Breast Cancer (B055)

dkfz.

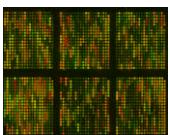
47

Molecular Genetics (B060)

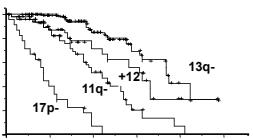
Peter Licher

Division Molecular Genetics

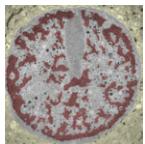
- Molecular mechanisms involved in tumor etiology and development



- Identification and validation of prognostic and predictive molecular markers



- Multidimensional structure of genomic information
Functional architecture of cell nucleus



- Technology development

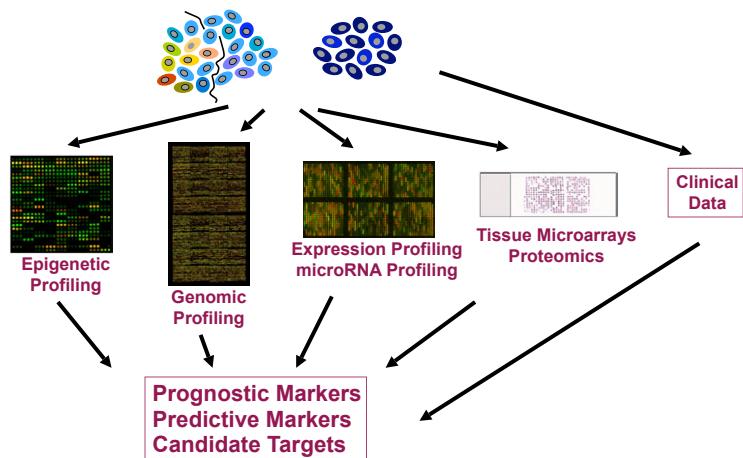
Peter Lichter

Molecular Genetics (B060)

dkfz.

49

Integrated Analysis of Molecular Alterations



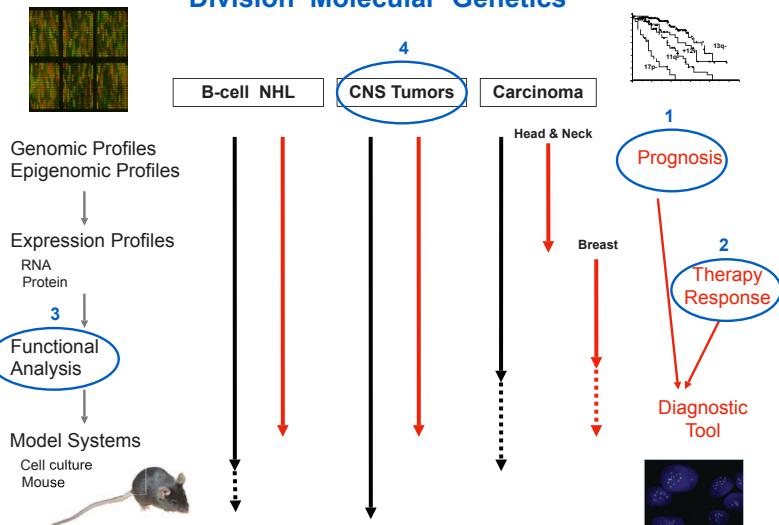
Peter Lichter

Molecular Genetics (B060)

dkfz.

50

Division Molecular Genetics



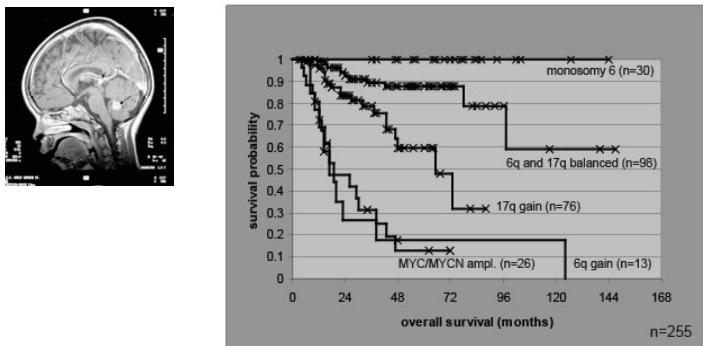
Peter Lichter

Molecular Genetics (B060)

dkfz.

51

1 Risk-adapted Stratification in Medulloblastoma



Pfister et al. 2008

Prospective Study, started July 2008

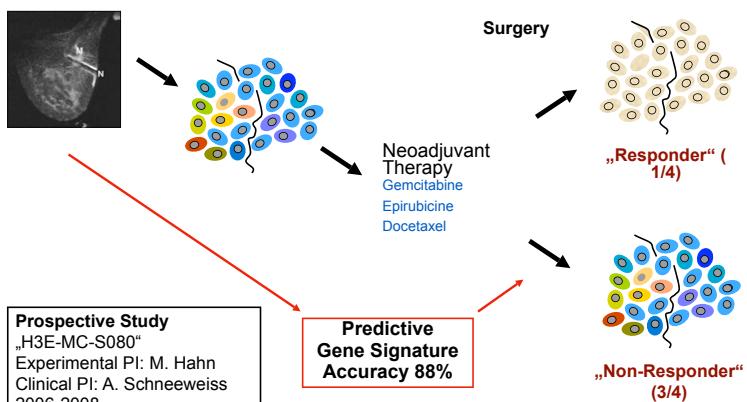
Peter Lichter

Molecular Genetics (B060)

dkfz.

52

2 Predictive Gene Signatures for Breast Cancer



Thuerigen et al. JCO, 2006

Peter Lichter

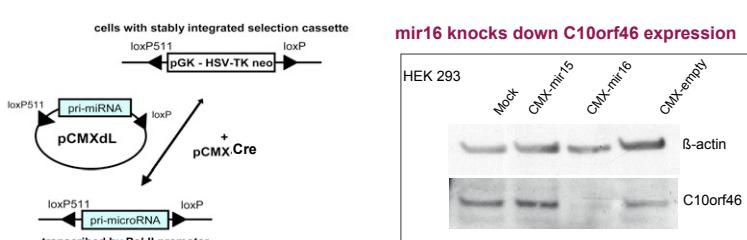
Molecular Genetics (B060)

dkfz.

53

3 Transgenesis-based Expression System for miRNAs

Expression of miRNAs in isogenic cell lines



C10orf46:
Cullin family of E3-ubiquitin ligases
component of the SCF complex
=> Protein degradation

Stable miRNA transgenes

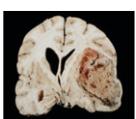
→ Identify miRNA targets on a proteome level

Peter Lichter

Molecular Genetics (B060)

dkfz.

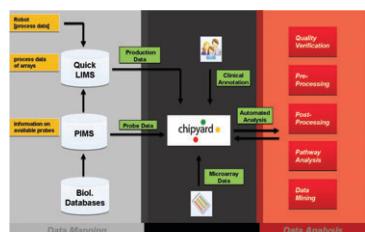
54



④ Glioma Core Collection (n=130)

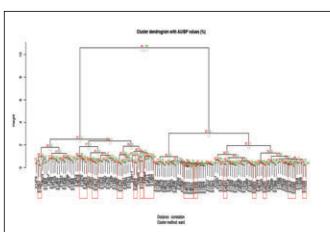
WHO grade	II	III	IV	IV
	12	21	13	84
Astrocytoma	Anaplastic Astrocytoma	Secondary Glioblastoma	Primary Glioblastoma	

ChipYard analysis platform



Peter Lichter
Molecular Genetics (B060)

Unsupervised clustering

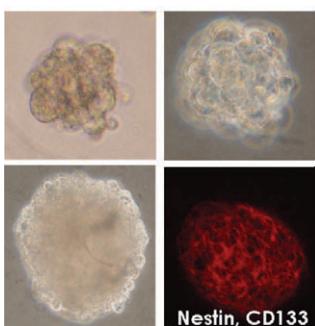


dkfz.

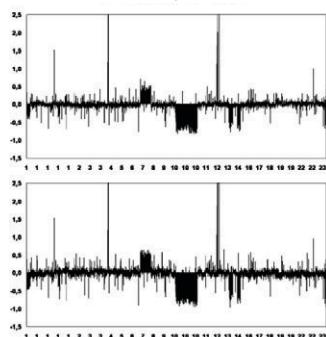
55

Chromosomal Aberrations in Spheroids and Primary Tumors are very Similar

array CGH



Glioblastoma neurospheres



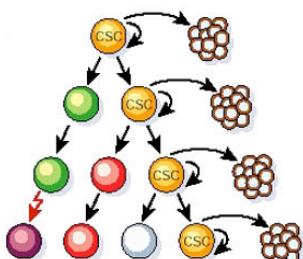
Primary tumor

Spheroid culture

Glioma: The Cancer Stem Cell (CSC) Concept

Glioma cells are heterogeneous

but: Genetic Homogeneity
even between CSCs & Tumors



- ⇒ Epigenetic Regulation?
- ⇒ Environmental Signals?
- (stem cell niche)

Peter Lichter

Molecular Genetics (B060)

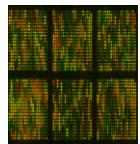
dkfz.

57

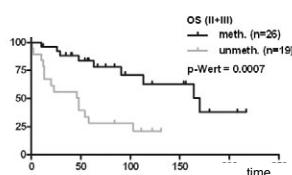
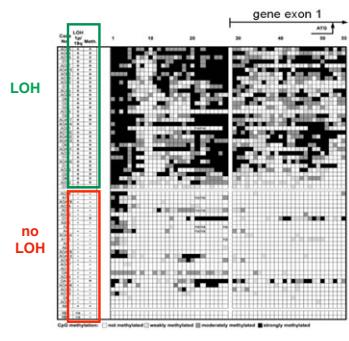


Oligodendrogloma

LOH 1p/19q
is associated with
longer survival time
due to better therapy response

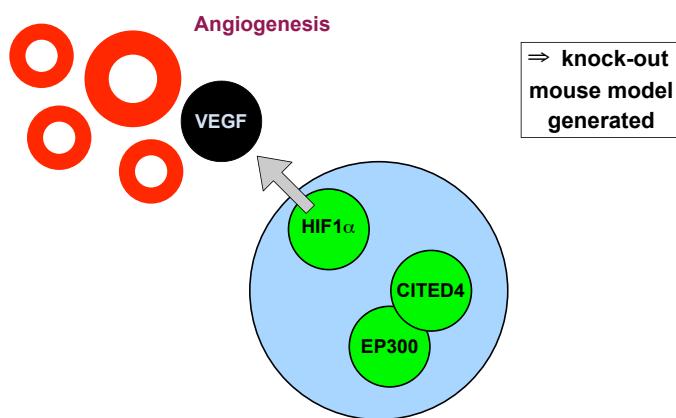


⇒CBP/P300 interacting
transactivator 4
(CITED4)



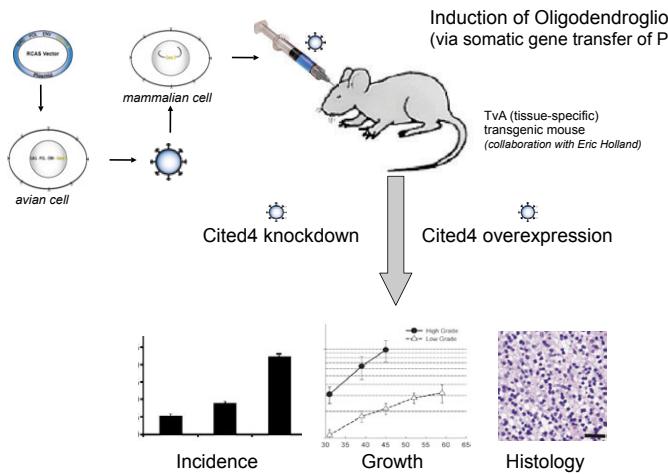
Univariate/multivariate analysis:
CITED4 promotor methylation is an
independent prognostic marker

CITED4: Model for Therapy Response



LOH 1p/19q, silencing by methylation

In vivo Analysis: Oligodendrogloma Mouse Model



Outlook

- Complementation of current integrated cancer studies by:

- microRNA profiling
- genome-wide methylation profiling (bisulfite sequencing)
- primary cancer genome sequence analysis (deep sequencing)

- Functional assays:

- identification of pathogenic microRNA targets
- functionally tested candidate genes and gene products
- identification of inhibitors for molecular interference

- Cancer stem cells:

- elucidation of symmetric and asymmetric cell division
- identification of stem cell specific targets

- Molecular signatures:

- prospectively tested prognostic and predictive signatures
- new diagnostic tools

- Nuclear architecture:

- assessment of the role of organizational principles such as molecular crowding

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Molecular Genetics (B060)



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Molecular Genetics (B060)

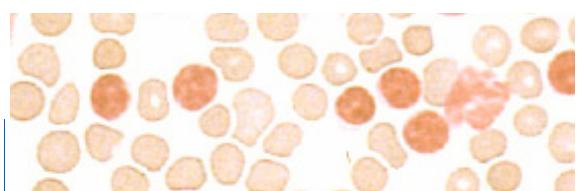


62



Mechanisms of Leukemogenesis (B061)

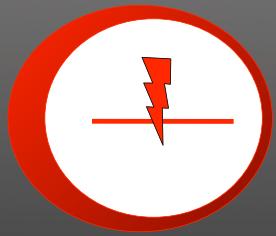
Daniel Mertens



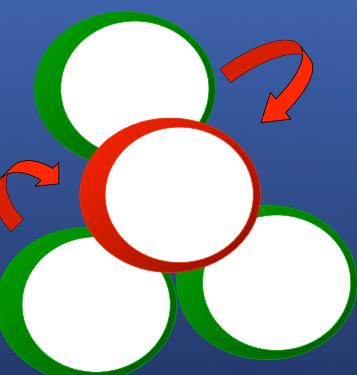
GERMAN
CANCER RESEARCH CENTER
IN THE HELMHOLTZ ASSOCIATION

63

Intracellular:
(Epi)genetic
Aberrations



Extracellular:
Microenvironmental
Effects



Daniel Mertens

Mechanisms of Leukemogenesis (B061)

dkfz.

64

1. (Epi)genetic Aberrations

Loss of a Critical Region in 13q14 is Leukemogenic



Daniel Mertens

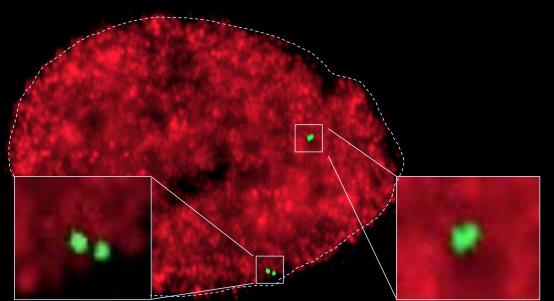
Mechanisms of Leukemogenesis (B061)

dkfz.
UNIVERSITÄT ULM
SCIENCE & MEDICINE

65

1. (Epi)genetic Aberrations

Critical Region in 13q14.2 Replicates Asynchronously



UNIVERSITÄT ULM
SCIENCE & MEDICINE
dkfz.

PNAS 2006

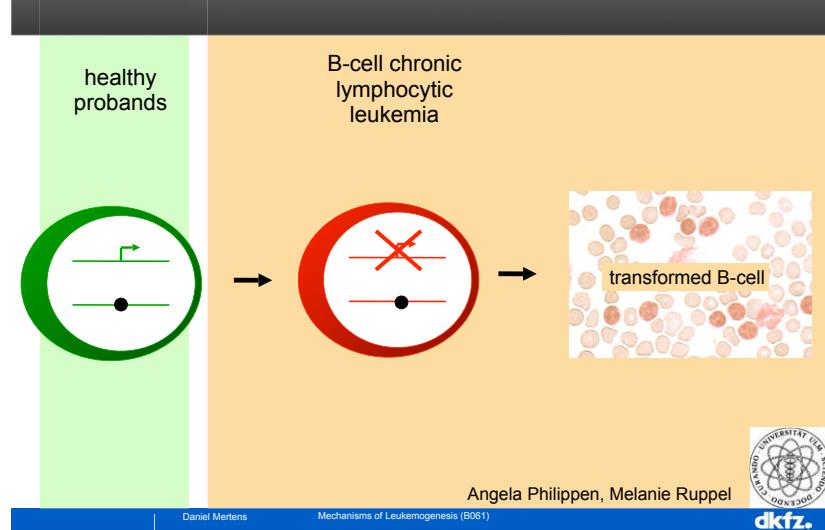
Daniel Mertens

Mechanisms of Leukemogenesis (B061)

66

1. (Epi)genetic Aberrations

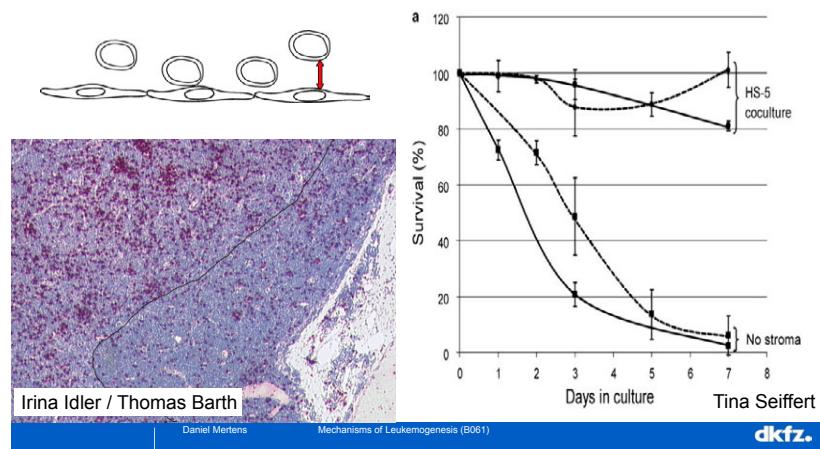
Loss of the Active Copy in Tumors?



67

2. microenvironmental interaction

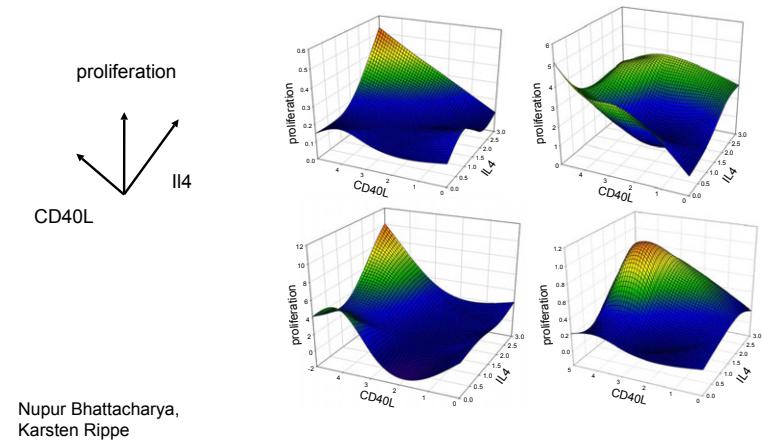
leukemic cell \leftrightarrow microenvironment



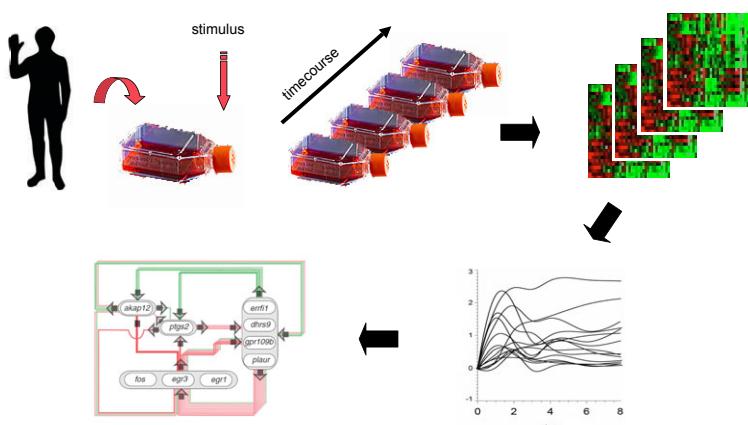
68

2. Microenvironmental Interaction

Model CLL Cell Response to Ligand Stimulation



Model Gene Networks from Transcriptome Dynamics



Nupur Bhattacharya, Hauke Busch, Roland Eils

Daniel Mertens

Mechanisms of Leukemogenesis (B061)

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70



71

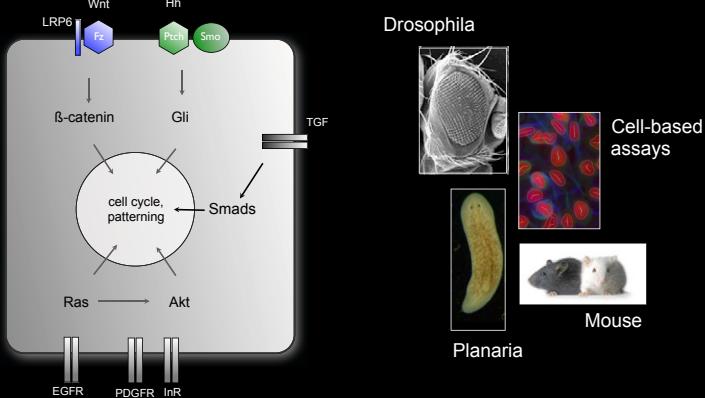
Signaling and Functional Genomics (B110)

Michael Boutros

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

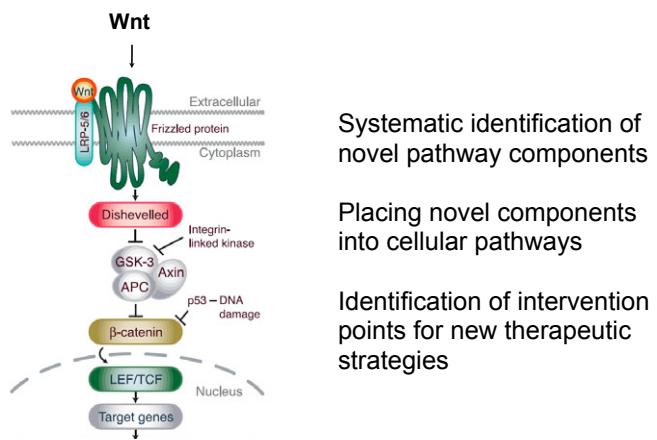
72

Aberrant control of conserved signaling pathways lead to developmental defects and cancer



73

Functional analysis of signaling networks



Systematic identification of novel pathway components

Placing novel components into cellular pathways

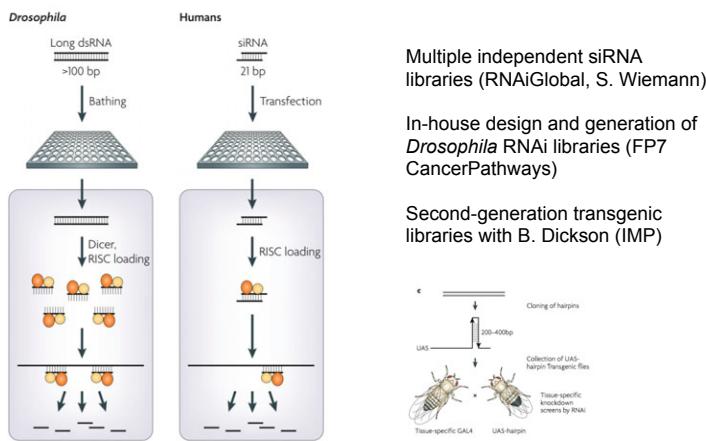
Identification of intervention points for new therapeutic strategies

Michael Boutros Signaling and Functional Genomics (B110)

dkfz.

74

Large-scale Functional Screens by RNAi



Boutros & Ahringer, Nat Rev Genet 2008

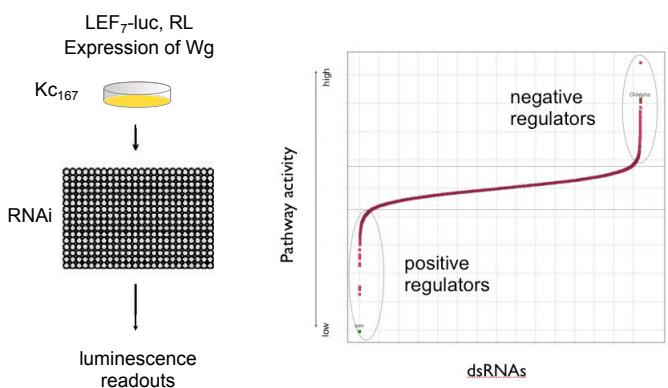
Michael Boutros

Signaling and Functional Genomics (B110)

dkfz.

75

Screening for factors required for Wg/Wnt signaling in *Drosophila*



Michael Boutros

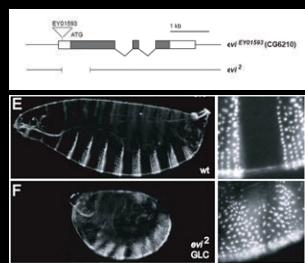
Signaling and Functional Genomics (B110)



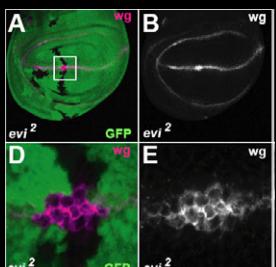
76

Evi/Wls is required for the secretion of Wnt growth factors

Evi has segment-polarity phenotypes identical to other core components



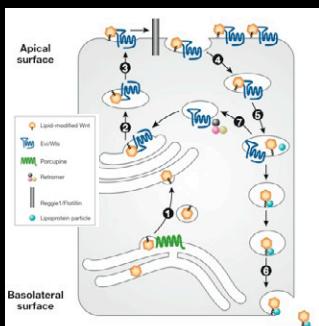
Wnt proteins are retained in the secreting cells



Bartscherer et al., Cell 2006

77

Wnt signaling is regulated at the level of secretion



Evi is a novel core component for Wg/Wnt signaling required for Wg secretion from *Drosophila* to human

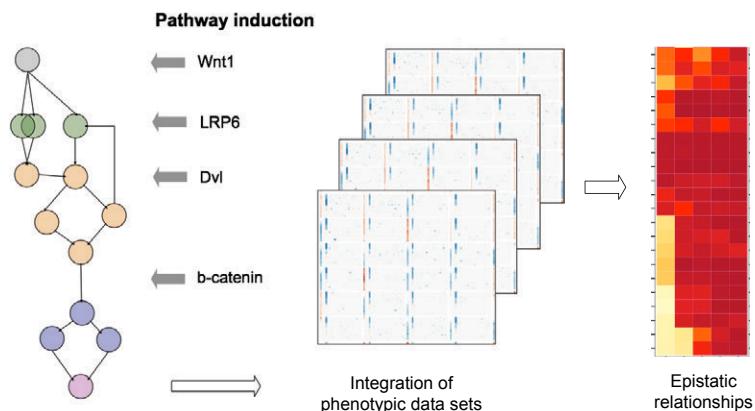
Evi requirement is specific for Wg/Wnt signaling
Required for all Wnts tested

Restricted expression, additional regulatory mechanism for Wnt

Bartscherer et al. EMBO report 2008

78

Reconstructing signaling networks by epistasis screens



Michael Boutros

Signaling and Functional Genomics (B110)



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On-going Wnt research projects

Animal models of novel signaling factors

Novel components and CRC linkage
(with K. Hemminki, Mol. Epidemiology)

Small molecule inhibitors of Wnt signaling
and interacting pathways

Michael Boutros

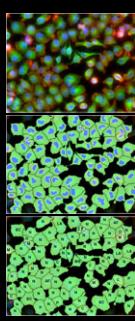
Signaling and Functional Genomics (B110)



80

Building Functional Signatures of Cellular Phenotypes

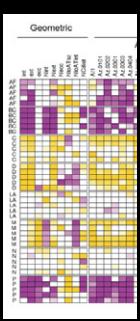
Multiparametric phenotypes



*multiplex reporter
deep-seq*

by RNAi or
compounds
perturbations
automated
computational
analysis

Single-cell
phenoprints



Phenotype proximity
to predict gene
function

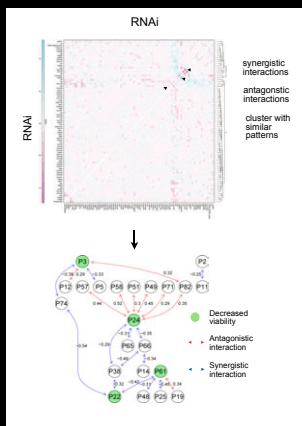


Fuchs et al., submitted

81

Systematic analysis of synthetic genetic interactions

- Synthetic genetic interaction studies to dissect pathways redundancy and identify networks
- Co-RNAi matrices of functional groups
- Enhancer/suppressor screens with small molecules



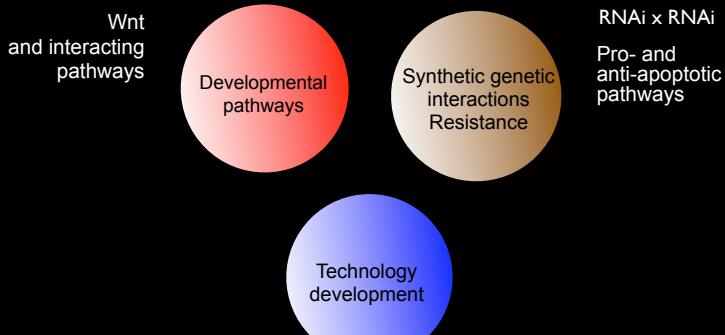
82

Summary

- Rapid functional screens to interrogate the genome for novel components of cellular pathways
- Identification of novel key components of signaling pathway implicated in cancer
- Cross-species approaches for the characterization of novel factors

83

Future research activities



Miniaturization of cell-based assays and novel analysis approaches
Bioinformatic analysis of HTS

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Acknowledgements



Funding and research networks
HFSP
National Genome Network
EU CancerPathways
EU Marie-Curie Excellence Team
Helmholtz Alliance Systems Biology
DFG

85

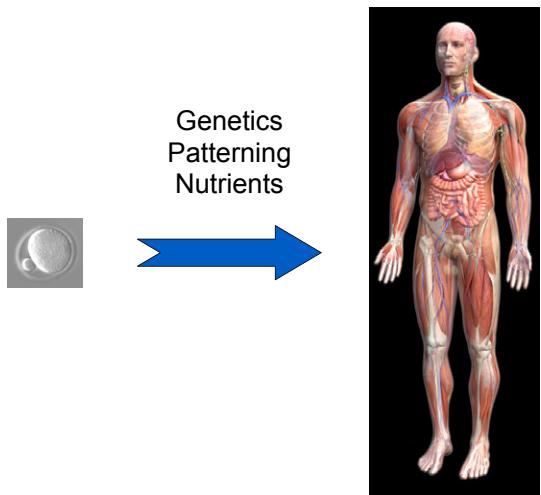
Signal Transduction in Cancer & Metabolism (B140)

Aurelio A. Teleman

dkfz. GERMAN
CANCER RESEARCH CENTER
IN THE HELMHOLTZ ASSOCIATION

86

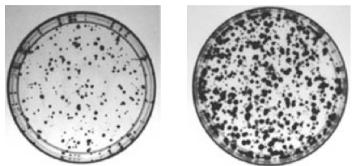
Lab focus: Tissue Growth Control



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Signaling pathways regulating tissue growth & cancer development

Pathway	Oncogene/tumor suppressor
Insulin / TOR	PTEN (melanomas, glioblastomas,...)
Hippo	Warts (soft-tissue sarcomas, ovarian tumors)
TGF-b/BMP	TGF-b receptor (gastrointestinal, ovarian..)



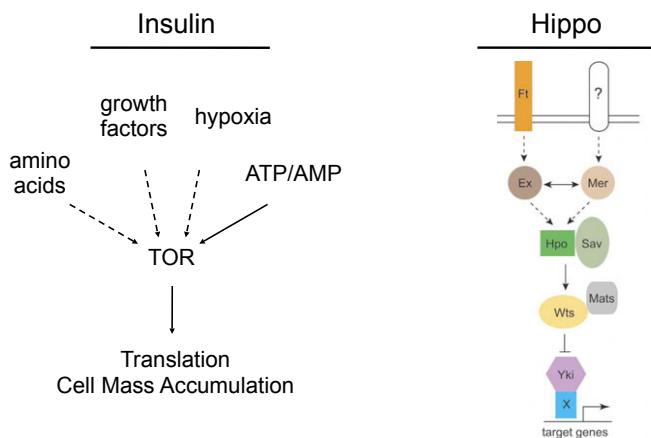
Aurelio Teleman

Signal Transduction in Cancer & Metabolism (B140)

dkfz.

88

Gaps in our knowledge - many missing components



Duoja Pan. Genes Dev 2008

Aurelio Teleman

Signal Transduction in Cancer & Metabolism (B140)

dkfz.

89

Drosophila as model system

- Highly conserved
 - Molecular pathway
 - Physiological output
- Powerful genetics
- Short generation time / small size



Aurelio Teleman

Signal Transduction in Cancer & Metabolism (B140)

dkfz.

90

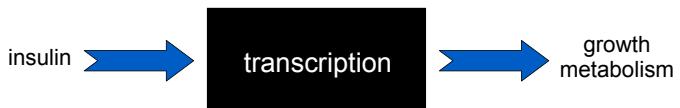
Past work

- Identified & characterized new components regulating insulin signaling

- Melted (Dev Cell 2005)
- mir-278 (Genes Dev 2006)



- Studied transcriptional output of insulin signaling



(Cell Metab 2008)

Aurelio Teleman

Signal Transduction in Cancer & Metabolism (B140)

dkfz.

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Future work / Goals

1. Insulin signaling

- how TOR activity is regulated
- new component identification via cell-based RNAi screens
- in vivo analysis of components identified in cell culture

2. Identification of components in other pathways regulating size

- Hippo
- Dpp (TGF- β)

Aurelio Teleman

Signal Transduction in Cancer & Metabolism (B140)

dkfz.

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B140 Group



Aurelio Teleman

Signal Transduction in Cancer & Metabolism (B140)

dkfz.

93

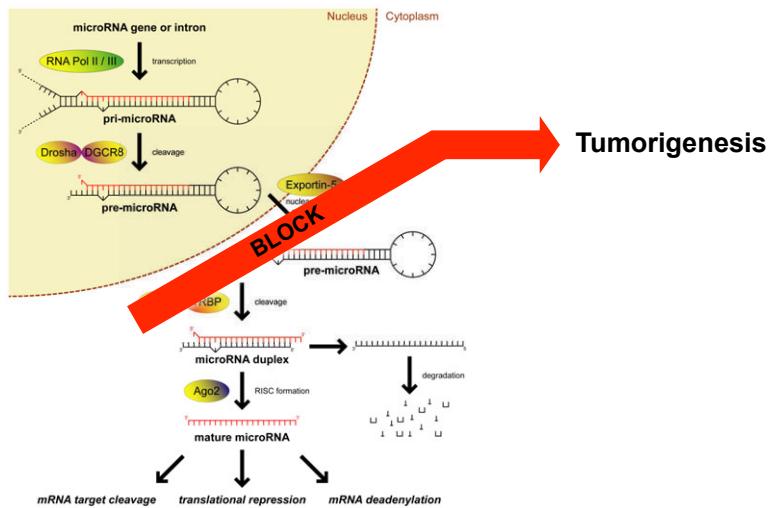
Molecular RNA Biology & Cancer (B150)

Sven Diederichs

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

94

Introduction: microRNA Biogenesis & Cancer



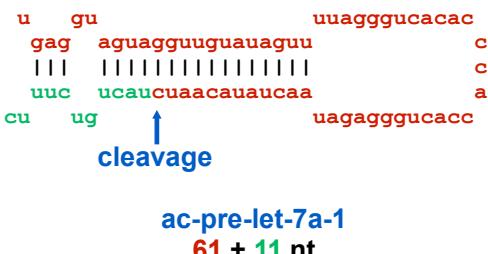
Sven Diederichs

Molecular RNA Biology & Cancer (B150)

dkfz.

95

Previous Research: A novel step in microRNA Processing



Diederichs & Haber, *Cell* 2007

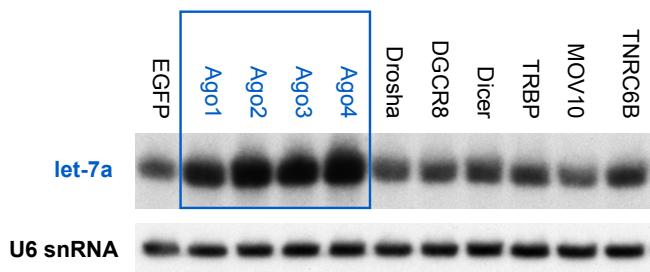
Sven Diederichs

Molecular RNA Biology & Cancer (B150)

dkfz.

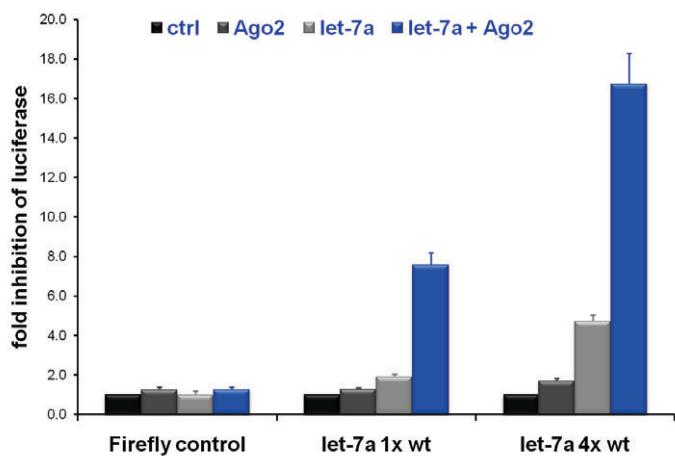
96

Previous Research: Novel Regulators of microRNA Expression



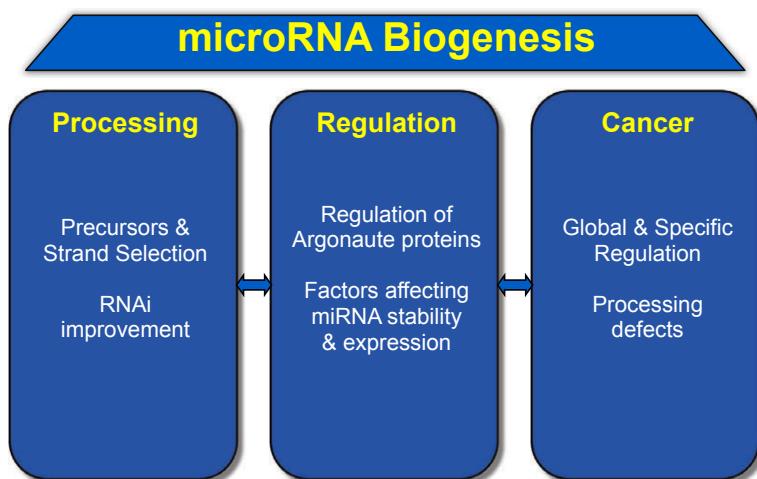
Diederichs & Haber, *Cell* 2007

Previous Research: Improving RNA interference



Diederichs et al., *PNAS* 2008

Future Perspectives: microRNA Processing, Regulation & Cancer



Integration at the DKFZ and Collaborations

• Internal: DKFZ, Structural & Functional Genomics

- Stefan Wiemann (B050)
- Michael Boutros (B110)

• External:

- Georg Stoecklin (DKFZ, Cell & Tumor Biology)
- Peter Schirmacher (Inst. of Pathology, University of Heidelberg)
- Christine Clayton (ZMBH, University of Heidelberg)
- Daniel Haber (MGHCC, Harvard Medical School)

B150

Sven Diederichs
Julia Winter
Sarina Keller
Maria Polycarpou
Pedro Simoni
Tony Gutschner

**...started fall 2008
at the DKFZ !**

Sven Diederichs

Molecular RNA Biology & Cancer (B150)

dkfz.

100

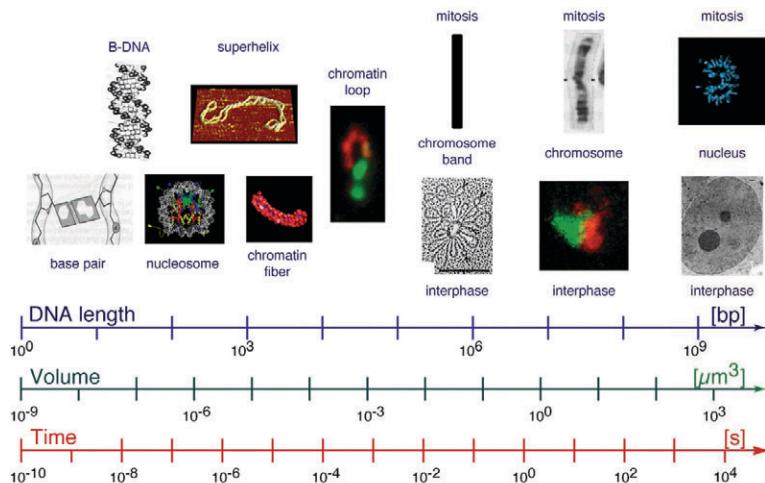
Biophysics of Macromolecules (B040)

Jörg Langowski

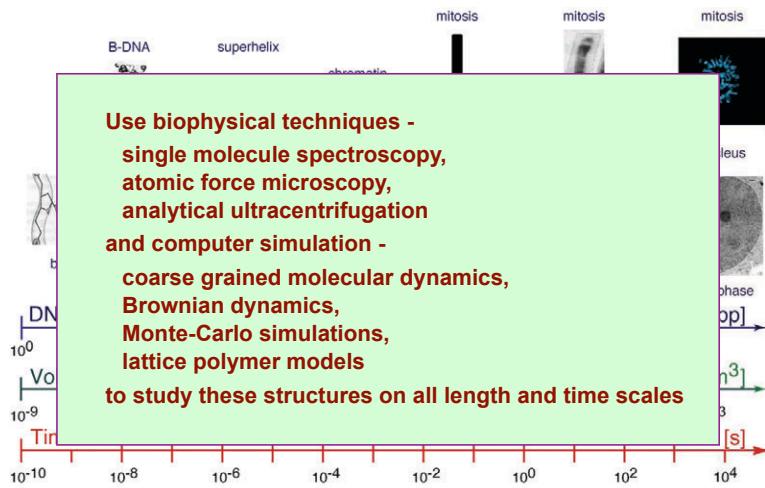
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101

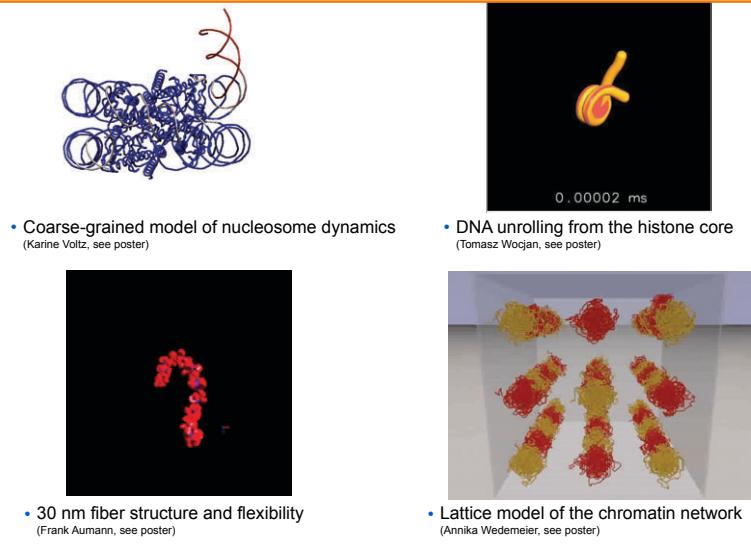
Dynamics of the genome – time and length scales



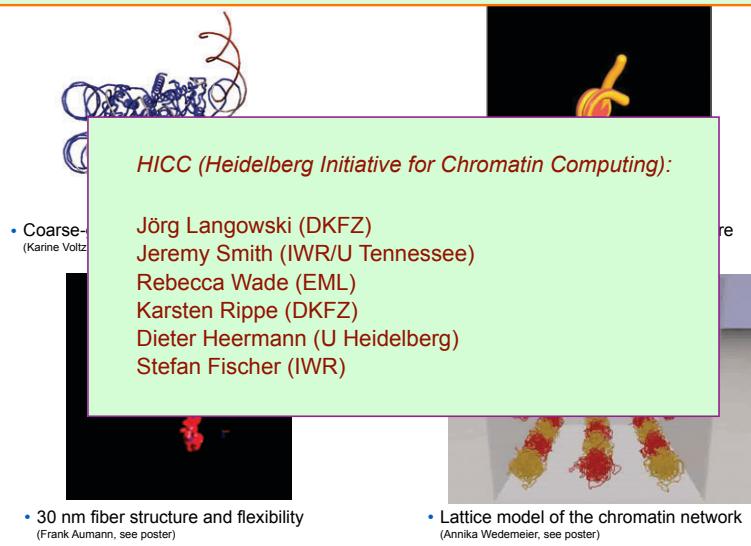
Dynamics of the genome – time and length scales



a multiscale view of genome structure

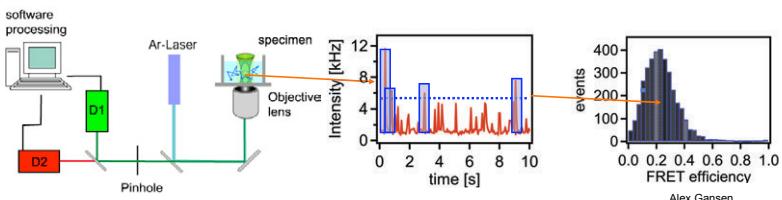
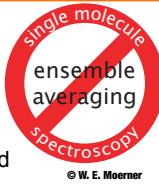


a multiscale view of genome structure



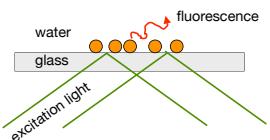
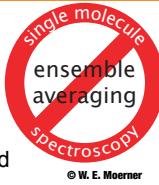
single molecule fluorescence spectroscopy

- Advantages:
 - single molecules can be detected at any dilution:
a molecule is a molecule!
 - no ensemble averaging:
molecules are *individually* analyzed, *subpopulations* separated
 - single molecules can be *followed over time* and in parallel
- Single fluorescent molecules are observed in the focus of a (homemade) confocal microscope, measure FRET, lifetime, anisotropy

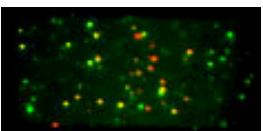


single molecule fluorescence spectroscopy

- Advantages:
 - single molecules can be detected at any dilution:
a molecule is a molecule!
 - no ensemble averaging:
molecules are *individually* analyzed, *subpopulations* separated
 - single molecules can be *followed over time* and in parallel
- TIRF (total internal reflection fluorescence): surface bound single fluorescent molecules are observed with a fast video camera (up to 2000 frames per s)



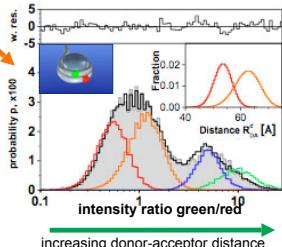
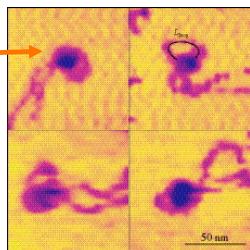
Example: TBP-induced bend on double-labeled DNA visualized by enhanced FRET
-green: no FRET / donor only
-red: acceptor-only
-yellow: FRET (protein bound)



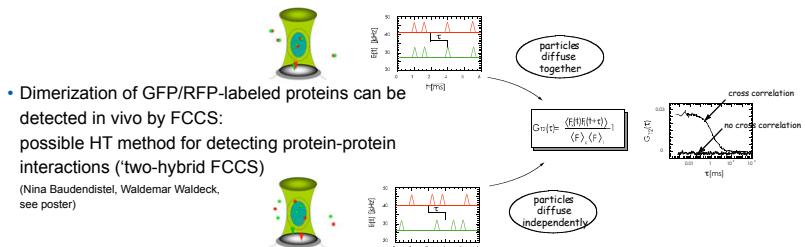
Aaron Hieb, see poster

chromatin structure and dynamics

- Atomic force microscopy of nucleosomes on superhelical DNA
(Malte Bussiek, see poster)
- Resolution of nucleosomal substrates by single molecule spectroscopy
(Alex Gansen, Katalin Tóth, with Claus Seidel / Düsseldorf, see poster)
- Effect of histone acetylation on linker DNA dynamics
(Alex Gansen, Katalin Tóth, see poster)



fluorescence (cross) correlation spectroscopy - FC(C)S: protein mobility and interactions in the living cell

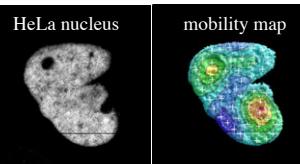


- Dimerization of GFP/RFP-labeled proteins can be detected in vivo by FCCS: possible HT method for detecting protein-protein interactions ('two-hybrid FCCS')

(Nina Baudendistel, Waldemar Waldeck, see poster)

- FCS of fluorescent probes in living cells for 'mobility imaging': proteins up to 108 kDa (eGFP tetramer) can freely access the chromatin network but their mobility varies (Nicolas Dross, see poster)

- The mobility of the actin-capping protein CapG is increased in highly invasive tumor cells (Malte Renz)



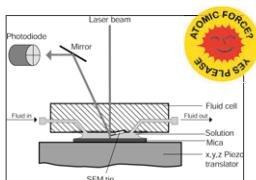
10/02/2008 | Seite Jörg Langowski Biophysik der Makromoleküle dkfz.

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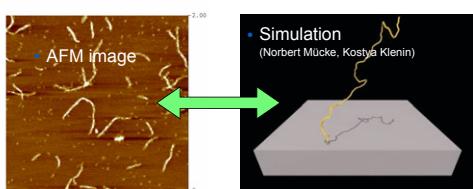
intermediate filament dynamics

(Norbert Mücke, Robert Kirmse, JL, with Harald Herrmann)

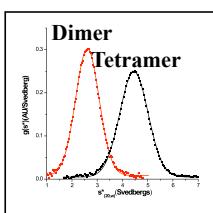
AFM - atomic force microscopy



IF proteins: vimentin, desmin, cytokeratins etc.: central role in maintaining stability of cytoskeleton



AUC - analytical ultracentrifugation



compare AFM images with computer simulations -> get structural parameters (Norbert Mücke, see poster)

AUC, stopped-flow kinetics -> get shapes, assembly mechanism (Mücke, Kirmse, see poster)

10/02/2008 | Seite Jörg Langowski Biophysik der Makromoleküle dkfz.

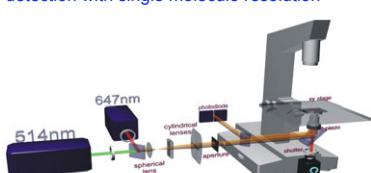
110

microarray detection with single molecule sensitivity

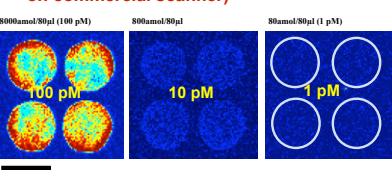
(coll. with Jens-Peter Kneymeyer, Hoheisel group, and Gerhard Schütz, Uni Linz)

Detector setup

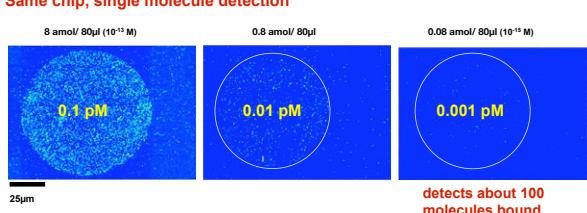
Dual wavelength laser excitation, CCD detection with single molecule resolution



Comparison (oligonucleotide sample on commercial scanner)



Same chip, single molecule detection



detects about 100 molecules bound

10/02/2008 | Seite Jörg Langowski Biophysik der Makromoleküle dkfz.

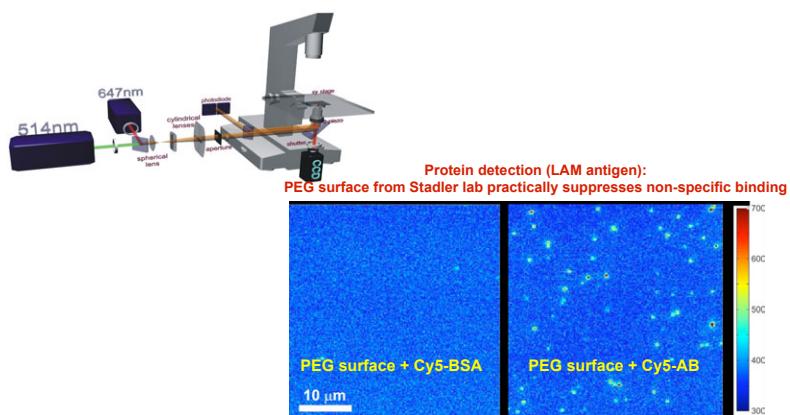
111

microarray detection with single molecule sensitivity

(coll. with Jens-Peter Kneimeyer, Hoheisel group, and Gerhard Schütz, Uni Linz)

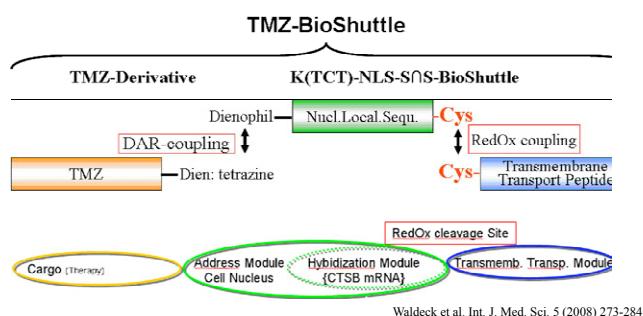
Detector setup

Dual wavelength laser excitation, CCD
detection with single molecule resolution



BioShuttle - a cellular delivery vehicle

(Waldemar Waldeck, with Klaus Braun, see poster)



Waldeck et al. Int. J. Med. Sci. 5 (2008) 273-284

- Active cargo is connected to address module and transmembrane transport peptide
- TMZ-Bioshuttle 10 times more active on glioma cell lines than simple temozolomide
- Other cargos tested: PNAs, plasmid vectors, gadolinium (MRI)

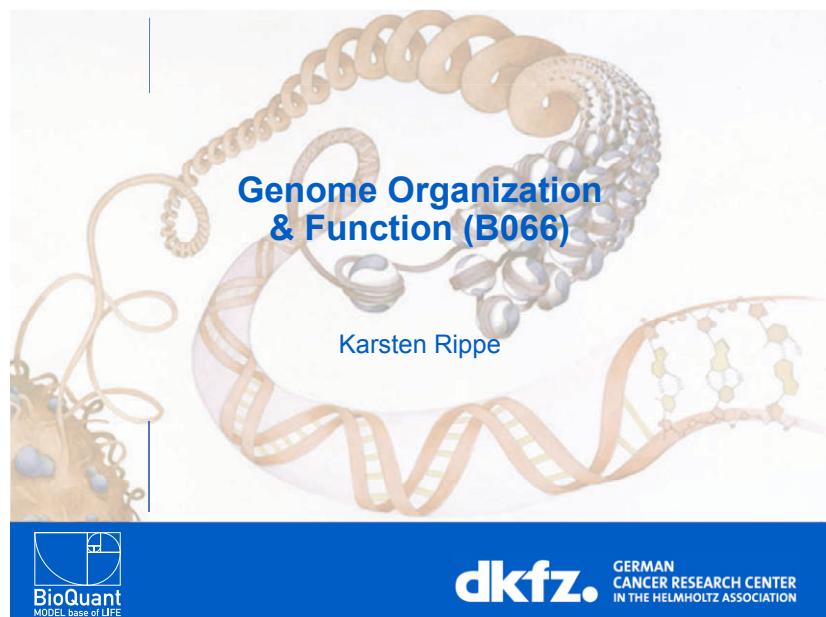
outlook

- Single molecule fluorescence
 - short-time (ms) solution dynamics with pulsed laser excitation
 - long-time (10s) dynamics of immobilized molecules
- Single molecule observation of ligand binding to microarrays
- Rapid scanning analysis of cell arrays for measuring protein-protein interaction in vivo ('two-hybrid FCS')
- Mobility Imaging:
using the mobility of fluorescent probes as an imaging parameter
- Single molecule mechanics of intermediate filaments by AFM
- 'Virtual cell nucleus':
Lattice model of a whole cell nucleus at nucleosome resolution

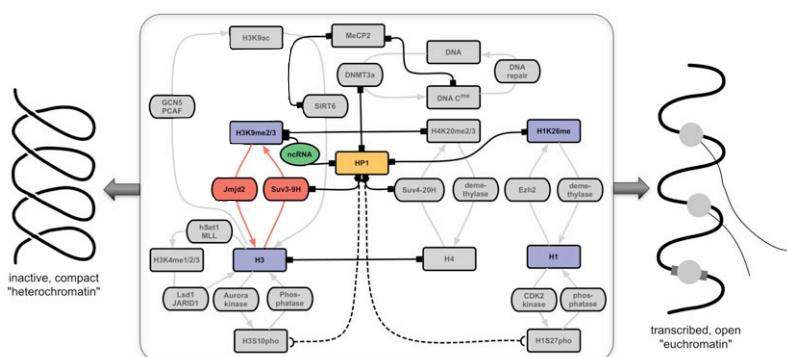
Our group



• Meet us in the afternoon....



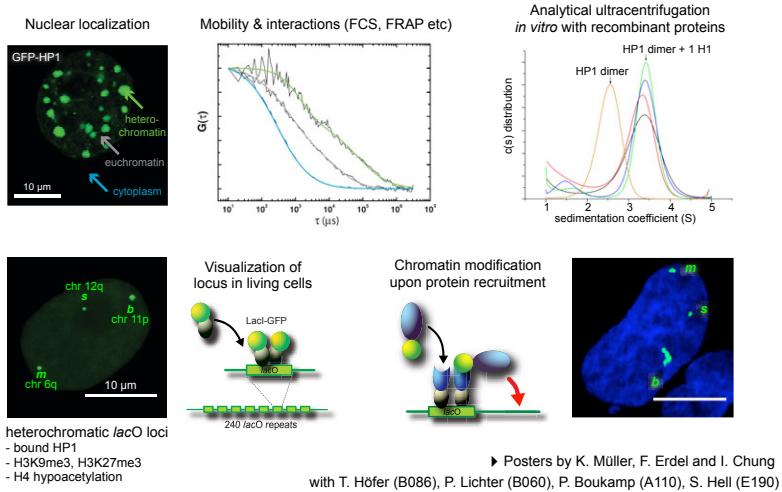
How do epigenetic networks and structural chromatin components establish and maintain (in)active chromatin states?



Experimental systems

- *in vitro* studies with recombinant proteins
- immortalized human/mouse cell lines (HeLa, U2OS, 3T3)
- mouse embryonic stem cells and tumor initiating cells

Dynamics and interactions of heterochromatin protein 1 (HP1), Suv39H1 histone methylase and linker histone H1



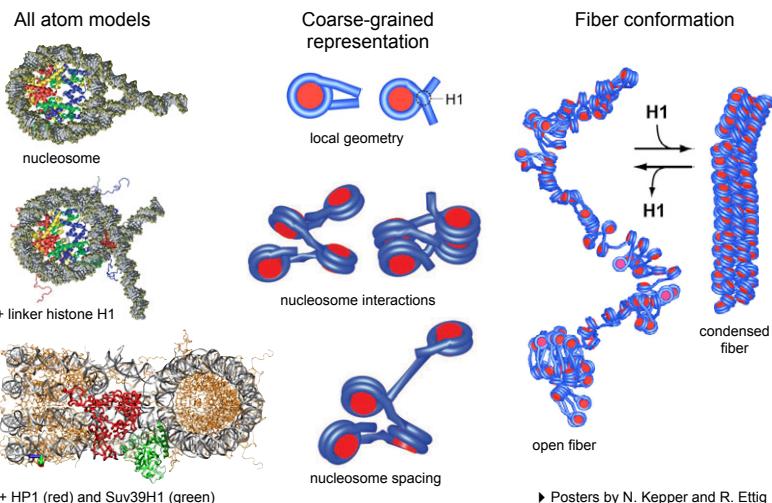
Karsten Rippe

Genome Organization & Function (B066)

dkfz.

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A model for fiber compaction due to the binding of linker histone H1



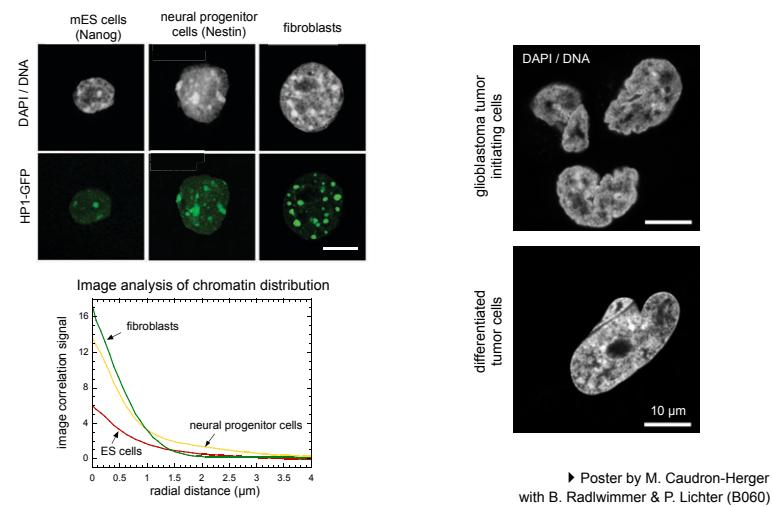
Karsten Rippe

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dkfz.

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Outlook: Heterochromatin formation during differentiation of mouse embryonic stem (ES) cells and tumor initiating glioblastoma cells



Karsten Rippe

Genome Organization & Function (B066)

dkfz.

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Genome Organization & Function Group



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Functional Architecture of the Cell (B065)

Harald Herrmann-Lerdon



dkfz. GERMAN
CANCER RESEARCH CENTER
IN THE HELMHOLTZ ASSOCIATION

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Intermediate filaments are hallmark structures of the cytoskeleton

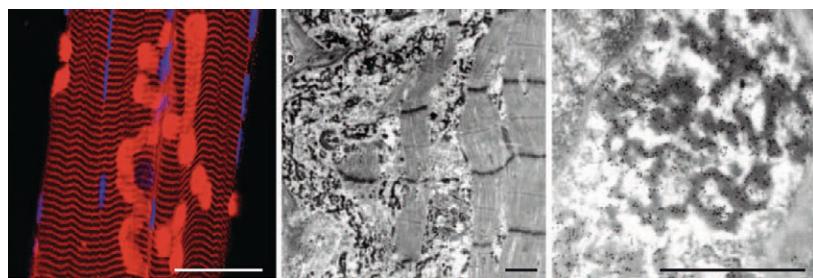
The human genome harbors 70 intermediate filament genes

More than 40 “Intermediate Filament Diseases” are known

DES (desmin): Muscular dystrophies and Dilated Cardiomyopathy

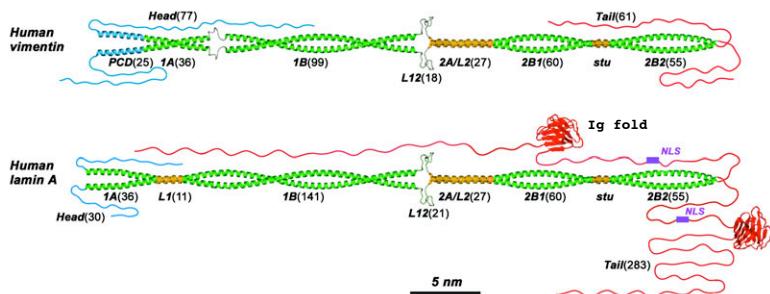
LMNA (lamin A): At least 14 different disease entities ranging from cardiomyopathy to premature aging

**Desmin aggregates are abundant in muscle fibers
as determined by immunological methods**



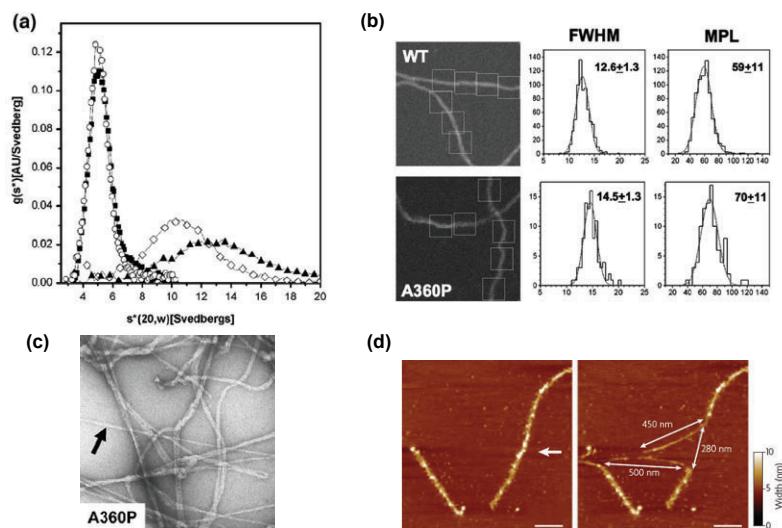
**How do “disease mutations” alter filament properties
such that the physiology of cells and tissues is affected ?**

Determination of the structure of IF coiled-coil dimers

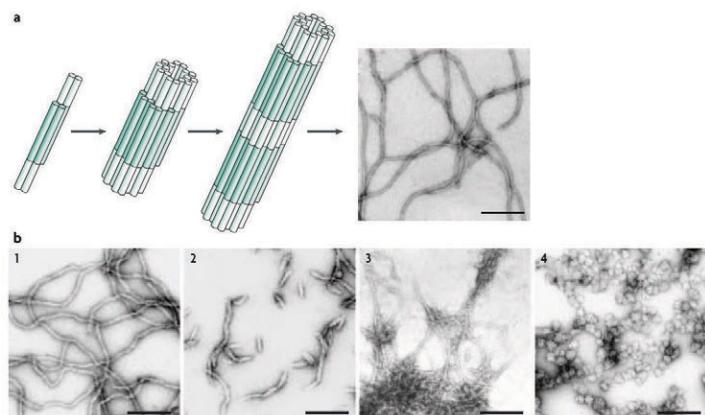


Biozentrum, University of Basel
Ueli Aebi, Larisa Kapinos

Pharmaceutical Sciences, University of Leuven
Sergei V. Strelkov, Stefan Nicolet

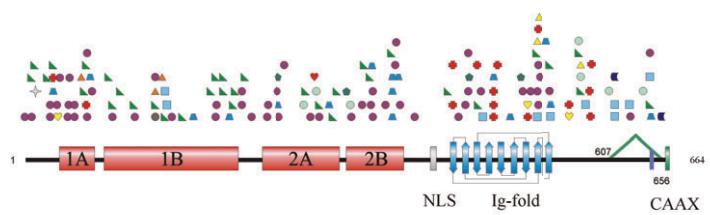


Desmin mutations interfere with filament assembly at various distinct stages



Cardiology, University of Heidelberg
Harald Bär, Helga Kleiner

Lamin A Disease Mutations (330 known by today)



- Restrictive Dermopathy
- Hutchinson Gilford Progeria Syndrome
- ▲ Atypical Werner Syndrome
- ▲ Mandibuloacral Dysplasia
- AD-Charcot-Marie-Tooth disease
- AR-Charcot-Marie-Tooth disease
- ▲ Limb Girdle muscular dystrophy 1B
- Familial Partial Lipodystrophy
- Congenital Muscular Dystrophy
- ▲ Dilated Cardiomyopathy
- Emery-Dreifuss muscular Dystrophy
- LDHPC
- SEIP
- Myopathy and Neuropathy, Insulin Resistance
- Cardiocutaneous progeria syndrome

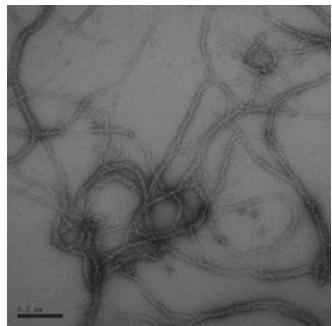
Ongoing projects:

Assembly of nuclear lamins.

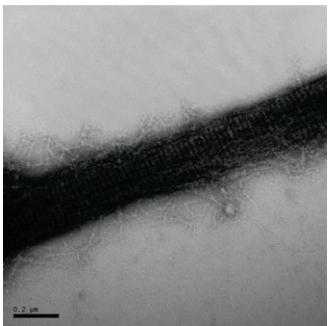
Biophysical characterization of “mini-lamins”.

How do lamin mutations influence nuclear architecture
and - eventually - gene expression programs ?

In vitro assembly of recombinant lamin A



10 nm filaments



paracrystals

Cellular Biophysics (B085)

Matthias Weiss

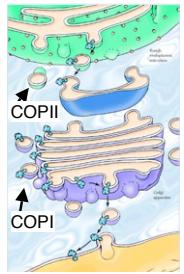
Cellular Biophysics in a nutshell

The team

Matthias Weiss
Gernot Guigas
Kristian Boye
Jed Szymanski
Ulrich Schmidt
Marcel Hellmann
Diana Morozova
Jens Kühnle
Felix Kapahnke
Nina Malchus
Claudia Kalla

Mission statement

Quantitative understanding of membrane traffic in the secretory pathway

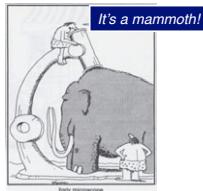


Tools

Theory & simulations

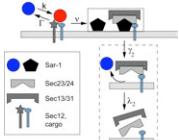
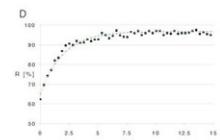
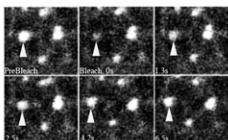


Advanced light microscopy



Spatiotemporal dynamics of exit sites in the endoplasmic reticulum

Fluorescence Recovery After Photobleaching + kinetic modeling



Cargo & sterols regulate COPII turnover on ER membranes

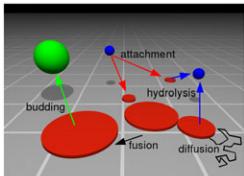
Forster & Weiss, et al., Curr. Biol. 16, 173 (2006)
Runz, Miura, Weiss & Pepperkok, EMBO J. 25, 2953 (2006)



A model for the self-assembly of ERES

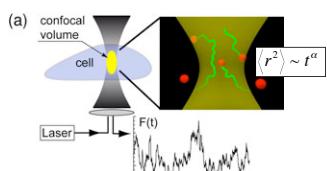
Explains spatial ERES arrangement, dynamic variation of ERES and secretory flux

Heinzer, Woerz, Kalla, Rohr & Weiss, J. Cell Sci. 121, 55 (2008)
Farhan, Weiss, Tani, Tagaya, Kaufman & Hauri, EMBO J. 27, 2043 (2008)



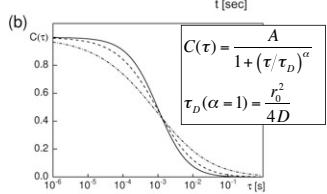
Diffusive transport in intracellular fluids

Fluorescence Correlation Spectroscopy



Proteins move by *subdiffusion* on membranes, in cytoplasm & nucleus
Due to macromolecular crowding and protein-protein interactions (→ chaperones)

Weiss et al., Biophys. J. 84, 4043 (2003)
Weiss et al., Biophys. J. 87, 3518 (2004)
Weiss & Nilsson, Trends Cell Biol. 14, 267 (2004)
Guigas, Kalla & Weiss, Biophys. J. 93, 316 (2007)
Guigas, Kalla & Weiss, FEBS Lett. 581, 5094 (2007)



Anomalous diffusion dramatically alters gradient/pattern formation, protein complex formation, and target finding

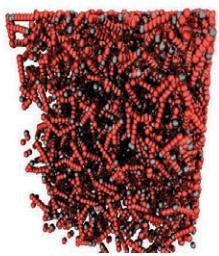
Weiss, Phys. Rev. E 68, 036213 (2003)
Guigas & Weiss, Biophys. J. 94, 90 (2008)

probe volume ~1 fl, particle conc. >1nM

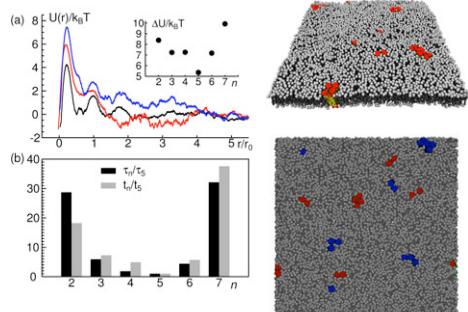
Beyond the diffraction limit – Mesoscopic membrane simulations

Coarse-grained molecular dynamics – faster, less information on Å-scale

Self-assembly of membranes



Oligomerization of membrane proteins due to hydrophobic mismatching



Guigas & Weiss, Biophys. J. 95, L25 (2008)
Schmidt, Guigas & Weiss, Phys. Rev. Lett. 101, 128104 (2008)

Matthias Weiss

Cellular Biophysics (B085)

dkfz.

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Perspective

Quantitative (systems-) understanding of the

- regulated formation of ER exit sites and COPII vesicles
- protein sorting into COPI/II vesicles at the ER and the Golgi
- biogenesis of the Golgi apparatus
- spatiotemporal dynamics of protein and membrane traffic

Matthias Weiss

Cellular Biophysics (B085)

dkfz.

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Protease Metabolites in Cancer (B041)

Thomas Ludwig

Membrane Type 1 Matrix-MetalloProteinase (MT1-MMP, MMP-14)

• membrane tethered transmembrane domain protease

- spatial restriction of proteolytic activity
- proteolytic cascade pacemaker

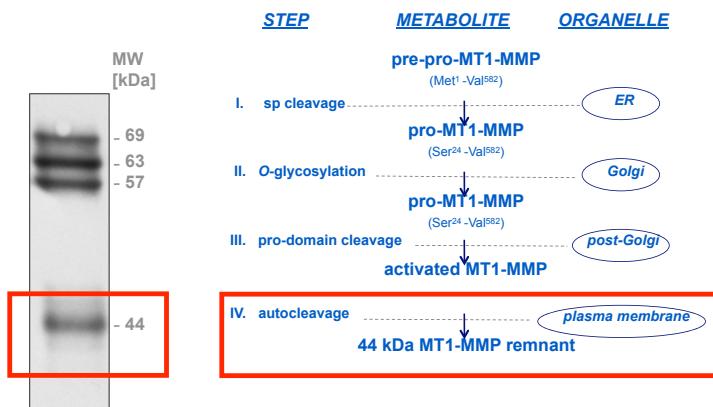
• potent regulator of cellular microecology

- i.e. essential for postnatal development
- abundant in highly invasive tumors

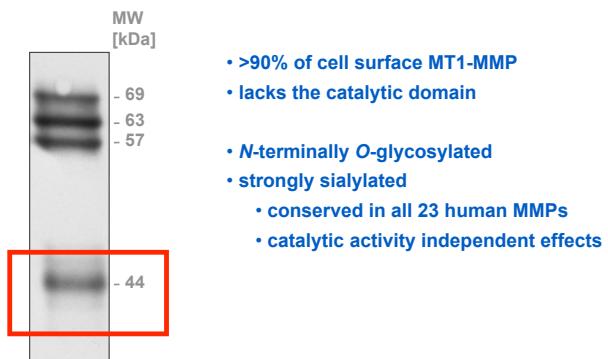
• protease inhibitors failed in clinical trials

REGULATION OF MT1-MMP?

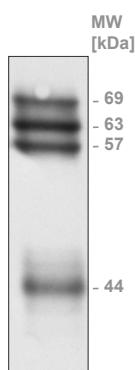
METABOLIC PROCESSING OF MEMBRANE TYPE 1 MATRIX-METALLOPROTEINASE (MT1-MMP)



RATIONALE



AIMS



- systematic analysis of metabolite
 - function
 - regulation
 - trafficking
- effects on local proteolytic activity
- modulation of biophysical parameters
 - cell adhesion
 - cortical cell stiffness
 - cytoskeletal traction

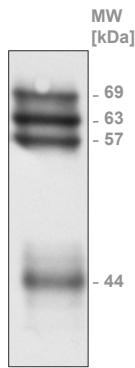
Thomas Ludwig

Microenvironment of Tumor Cell Invasion (B041)



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STRATEGY



- synthetic constructs
 - expression of individual metabolites
 - distinct epitope tags
 - mutants
 - catalytically inactive
 - glycosylation deficient
- distinct, stably expressing cell lines
 - MV3
 - COS
 - CHO
 - [...]

Thomas Ludwig

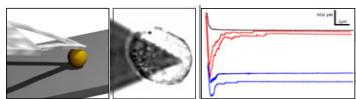
Microenvironment of Tumor Cell Invasion (B041)



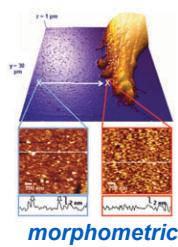
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ATOMIC FORCE MICROSCOPY

single cell force spectroscopy

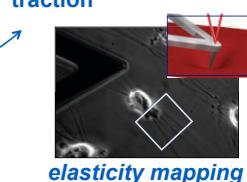


adhesion



morphometric imaging

proteolytic activity



elasticity mapping

traction

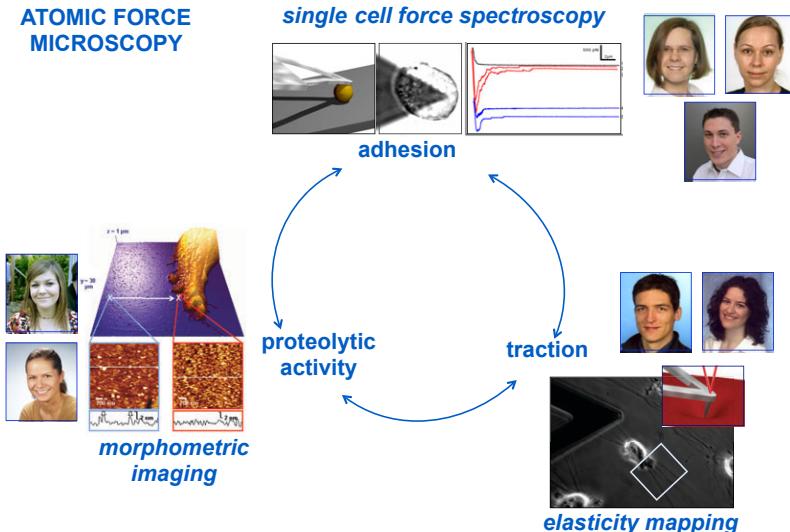
144

Thomas Ludwig

Microenvironment of Tumor Cell Invasion (B041)



ATOMIC FORCE MICROSCOPY



Thomas Ludwig

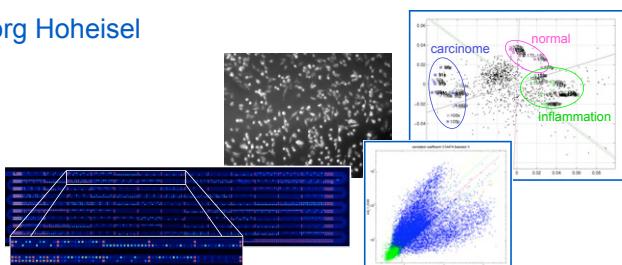
Microenvironment of Tumor Cell Invasion (B041)



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Functional Genome Analysis (B070)

Jörg Hoheisel



DKFZ
FUNCTIONAL
GENOME ANALYSIS

dkfz.

GERMAN
CANCER RESEARCH CENTER
IN THE HELMHOLTZ ASSOCIATION

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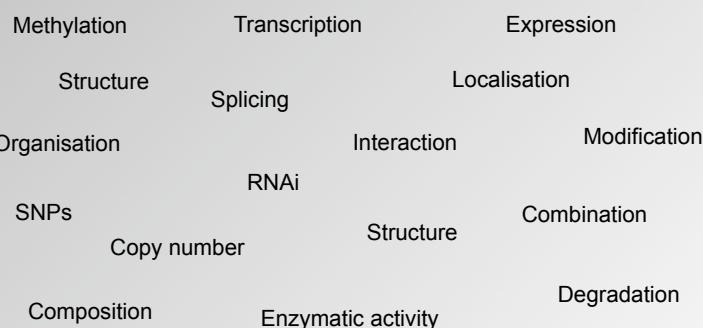
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Basic Level of Functional Analysis

DNA → RNA → Protein ➡



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Studying Pancreatic Cancer

WP1: Epidemiology / ESPAC
 WP2: Genetic profiles
 WP3: Transcriptional profiling
 WP4: Protein expression
 WP5: Epigenetics
 WP6: Molecular imaging
 WP7: Prospective clinical trials

NGFN

- Mouse models
- Kinase networks
- Protein interactions
- miRNAs as therapeutic targets
- Stroma activation
- Metastasis & local recurrence
- Protein secretion

- Genetically engineered mouse models for preclinical validation
- Therapeutic patient stratification

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Epigenetic Measurements

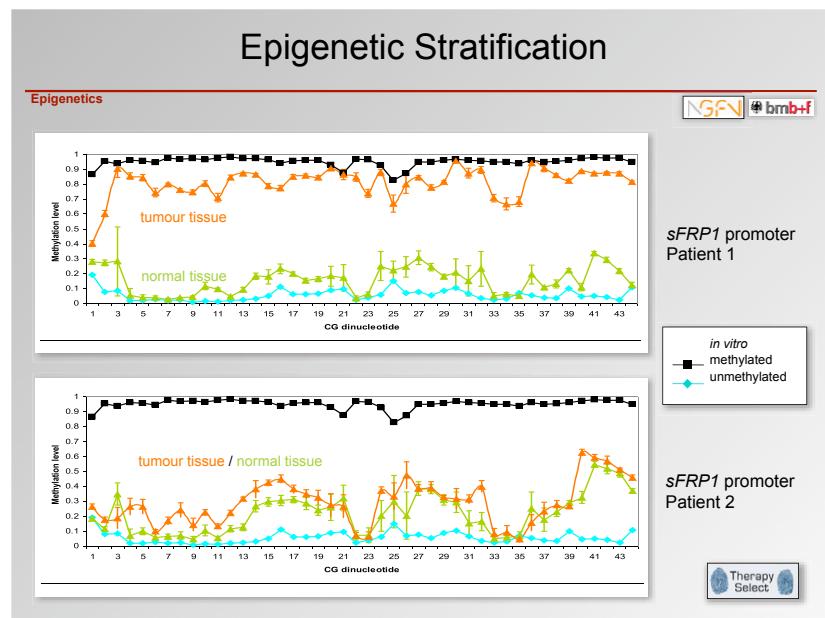
Epigenetics

NGFN **bmbf**

positive control

methylated unmethylated

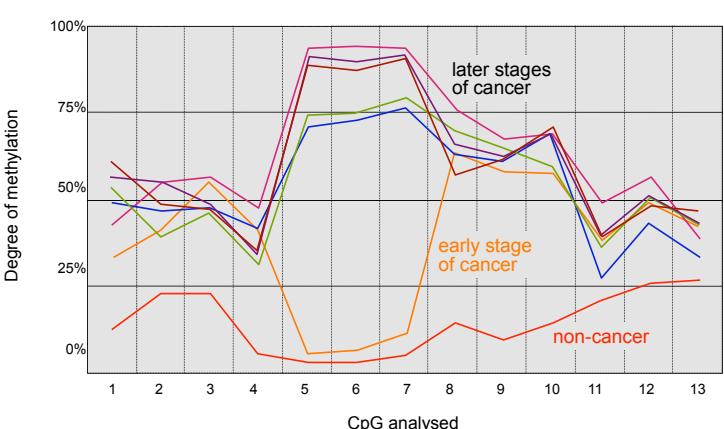
152



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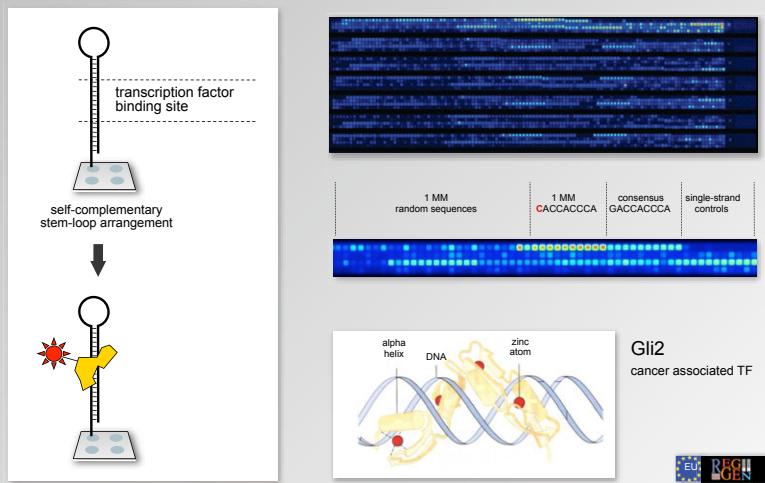
Epigenetic Diagnosis

Epigenetics



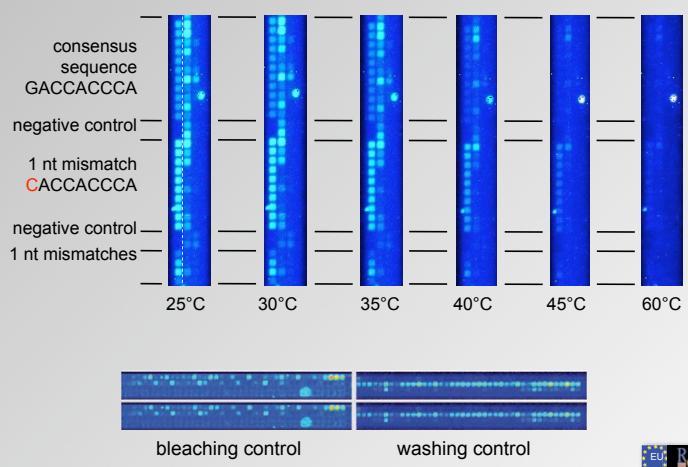
154

Transcription Factor Binding



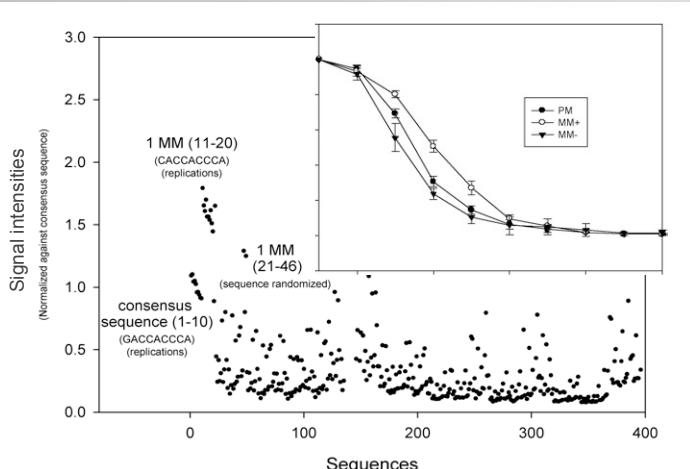
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Dissociation Behaviour of Gli2



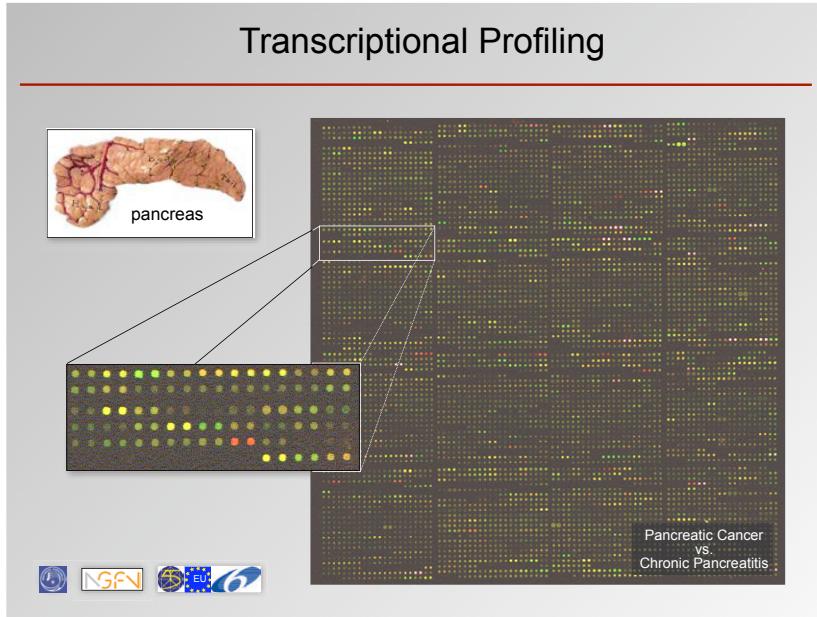
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Binding Strength of Gli2



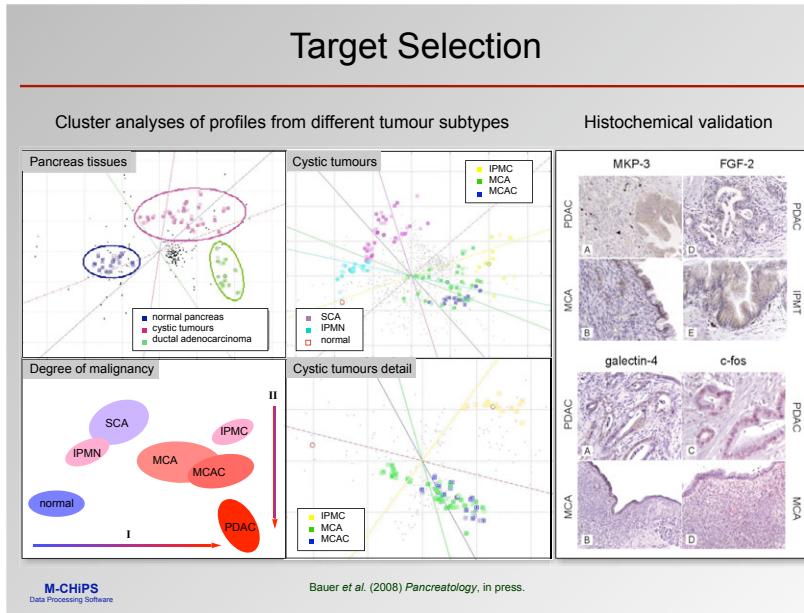
157

Transcriptional Profiling



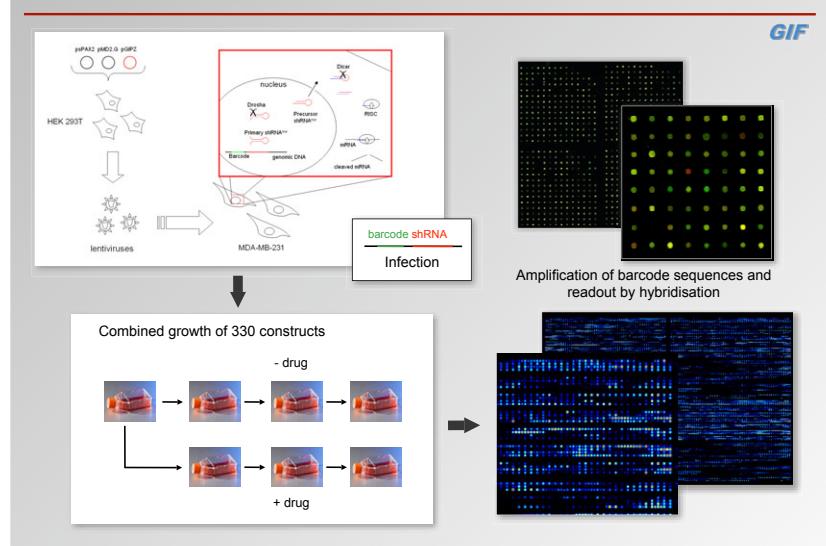
158

Target Selection



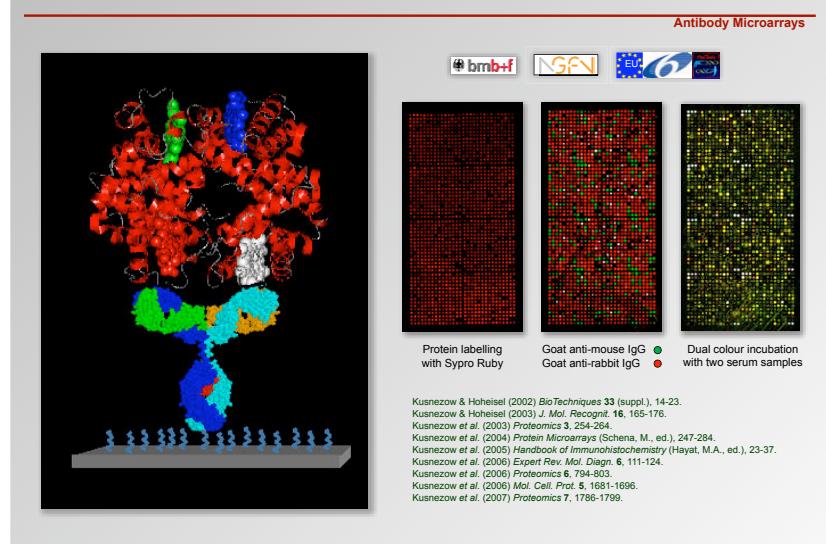
159

Simultaneous shRNA Screening



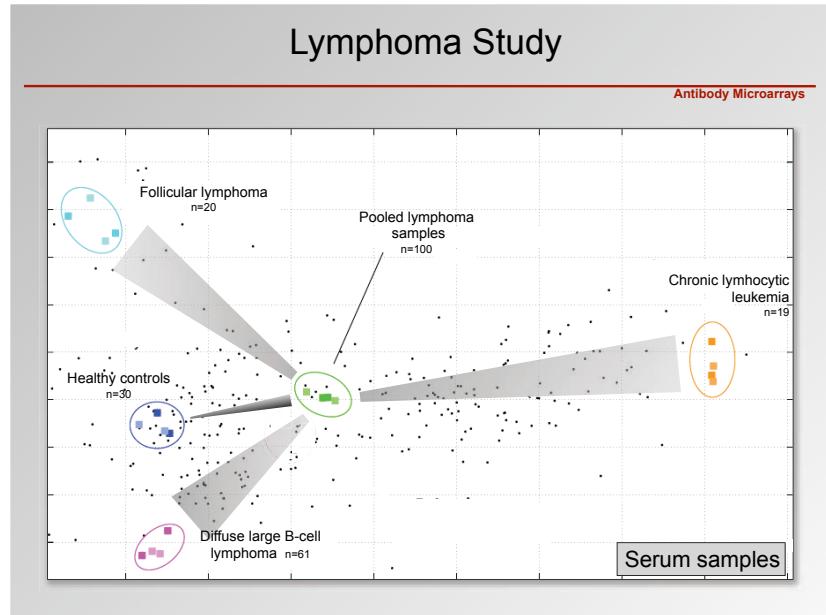
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Complex Antigen-Antibody Interactions



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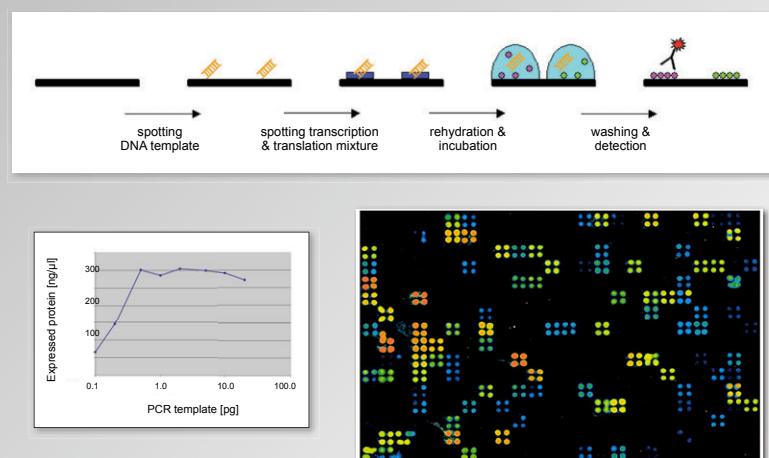
Lymphoma Study



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In Situ Protein Synthesis

Synthetic Biology

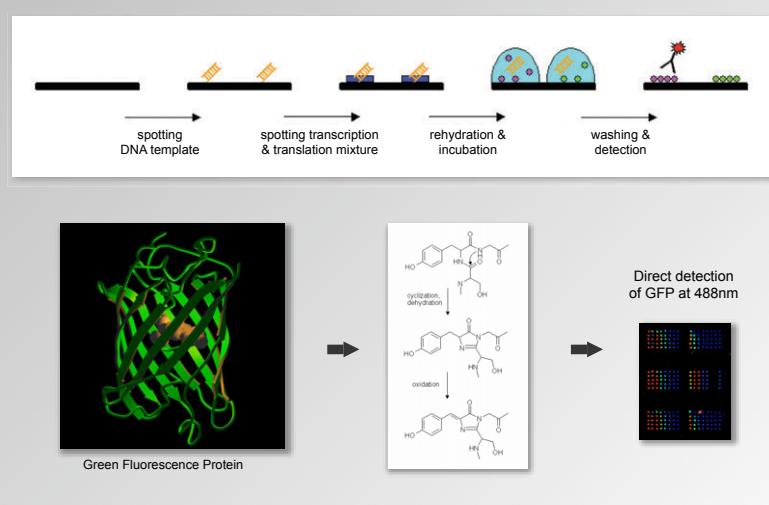


Angenendt et al. (2006) Mol. Cell. Prot. 5, 1658-1666.

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In Situ Protein Synthesis

Synthetic Biology

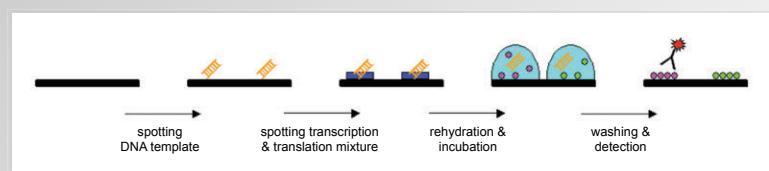
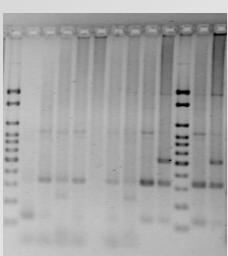


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Presentation of an Individual Proteome

Synthetic Biology

- F • Forward and reverse primer at surface
- R • Annealing of template RNA (or DNA)
- Extension of forward primer (reverse transcription)
- Removal of template
- Detection of extension reaction
- Initial PCR
- Subsequent amplification



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Multiparametric Analysis

Combining optical, physiological and molecular analyses

IDES **pO₂** sensor **TUM** **dkfz.**

Applied Biosystems **bmb+f** **drop-tc**

Genotyping: Gene-specific primer anneals to L-DNA, extension and labeling with dATP, dGTP, dCTP, dTTP, and gene-specific primer.

Transcript profiling: Gene-specific primer anneals to mRNA, extension and labeling with dATP, dGTP, dCTP, dTTP, and gene-specific primer.

ZIP-code approach: L-DNA universal platform, Scanning.

L-form and **"normal" D-form** DNA structures are shown with chemical formulas for L-DNA (R = H) and D-RNA (R = OH).

Chemical structures:

- L-form:** A deoxyribose sugar ring with a hydroxyl group (OH) at the 2' position, labeled 'B' at the 1' position.
- "normal" D-form:** A deoxyribose sugar ring with a hydroxyl group (OH) at the 3' position, labeled 'B' at the 1' position.
- Left-turning helix:** L-DNA (R = H) and L-RNA (R = OH).
- right-turning helix:** D-DNA (R = H) and D-RNA (R = OH).

Hoheisel (2006) *Nature Rev. Genet.* 7, 200-210.
Hauser et al. (2006) *Nucleic Acids Res.* 34, 5101-5111.

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Non-Human Studies

Hydractinia echinata

Model for stem cell research

Recovery phenotype
Control
Mild depletion of stem cells
Strong depletion of stem cells

Trypanosoma brucei

Insect host
Mammalian host

Proliferative
Quiescent

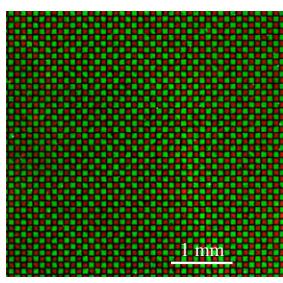
Short, stumpy form (blood)
Long, slender form (blood)

Cryptobiosis in Tardigrades

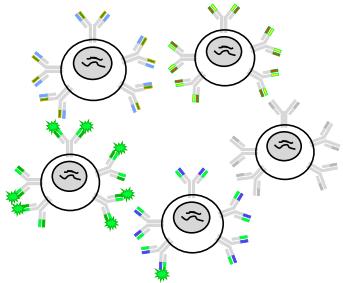
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Division of Functional Genome Analysis, DKFZ, Heidelberg





High-Density Peptide Arrays



Library of Human Hybridomas

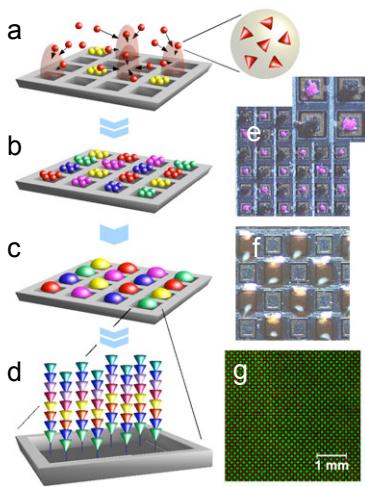
Chip-Based Peptide Libraries (B120)

Frank Breitling, Ralf Bischoff, Volker Stadler

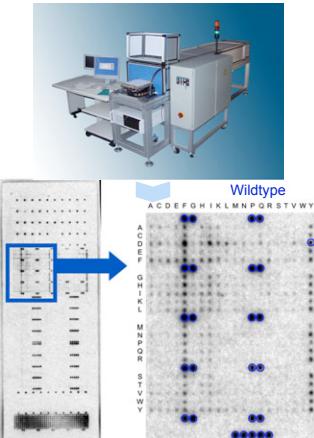
dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

169

Particle-based combinatorial synthesis of peptide arrays



Science 2007, 318, 1888



Angew. Chem. Int. Ed. 2008, 47, 7132
Stifterverband Science Prize 2008

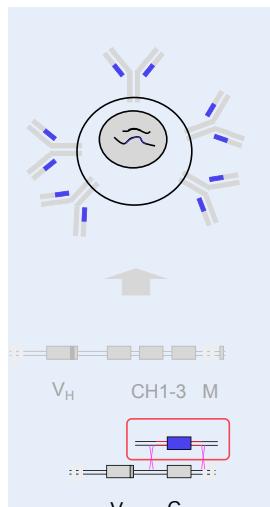
Frank Breitling, Ralf Bischoff & Volker Stadler

Chip-based Peptide Libraries (B120)

dkfz.

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Easy method to chimerize any mouse hybridoma

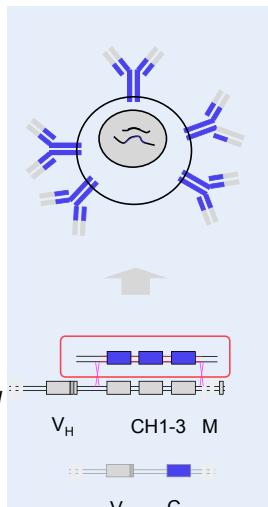


Only one chimeric DNA is needed to chimerize any mouse IgG1 gene by homologous recombination

Already done:

- anti-EpCAM
- anti-L1

(licensed to Medigene; therapeutic against ovarian carcinoma)



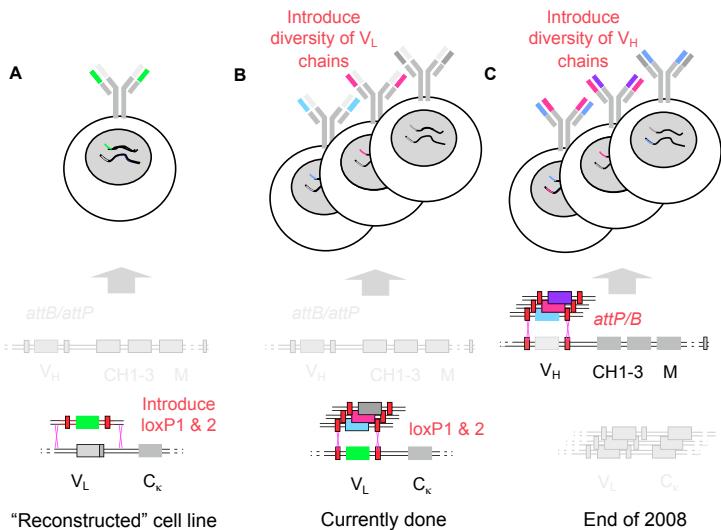
Frank Breitling, Ralf Bischoff & Volker Stadler

Chip-based Peptide Libraries (B120)

dkfz.

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Human hybridoma library by homologous & specific recombination



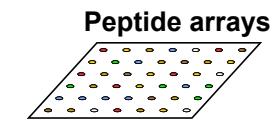
Frank Breitling, Ralf Bischoff & Volker Stadler

Chip-based Peptide Libraries (B120)

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Applications



High affinity D-peptides as

- Therapeutics
- Antibiotics
- Scientific tools

Diagnostic peptides

- Read out antibody diversity
- MHC-presented, “T-cell arrays” (Encke, Momburg)
- Rationally designed vaccines

Targets, e.g.
EpCAM (Moldenhauer), L1,
Mesothelin (Märten),
E6 (Hoppe-Seyler),
Fas (Apogenix),
EGFR (Alonso)

Patient's sera
(Pfreundschuh, Encke)

Human hybridoma library



Human antibodies as
- Therapeutics
- Diagnostics
- Scientific tools
- Screen for stem cell antigens

Human antibodies as
- Reverse engineered protecting antibodies

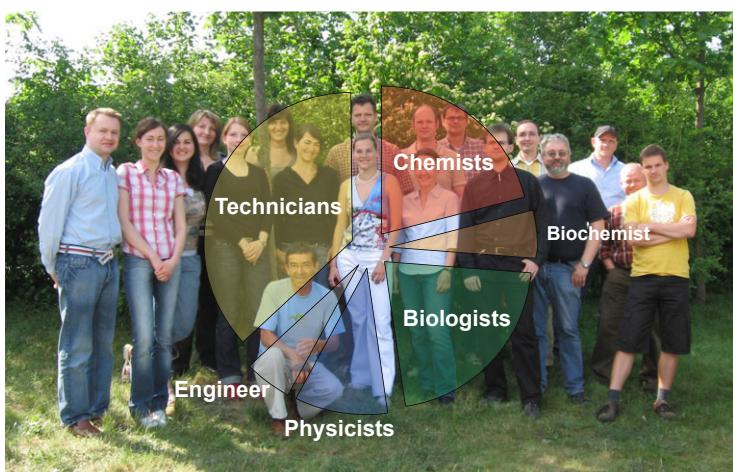
Frank Breitling, Ralf Bischoff & Volker Stadler

Chip-based Peptide Libraries (B120)

dkfz.

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Team



Frank Breitling, Ralf Bischoff & Volker Stadler

Chip-based Peptide Libraries (B120)

dkfz.

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Modeling of Biological Systems (B086)

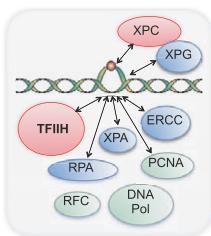
Thomas Höfer



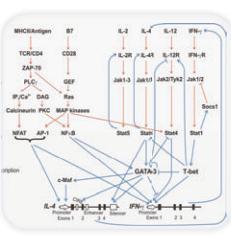
PREDICTIVE MODELING OF REGULATORY NETWORKS

Molecular interaction → functional dynamics

Chromatin-associated regulatory networks



Differentiation and proliferation decisions



EXPERIMENT
THEORY

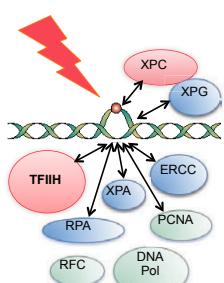
Common methodology for modeling and simulation of spatio-temporal dynamics



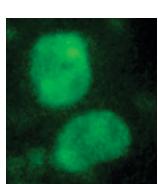
Biochemical networks
Nonlinear dynamics
Reaction-diffusion
Stochastic processes
Parameter estimation

CHOREOGRAPHY OF NUCLEOTIDE EXCISION REPAIR

UV radiation chemotherapy



Life-cell imaging
(80% of components)

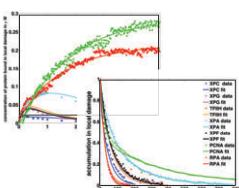


Kinetic modeling & simulation

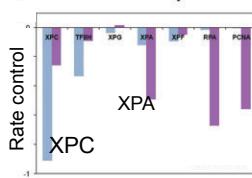
$$\frac{dc_i}{dt} = f_i(\bar{c}; \bar{p}) \\ i = 1, \dots, 220$$

~30 proteins

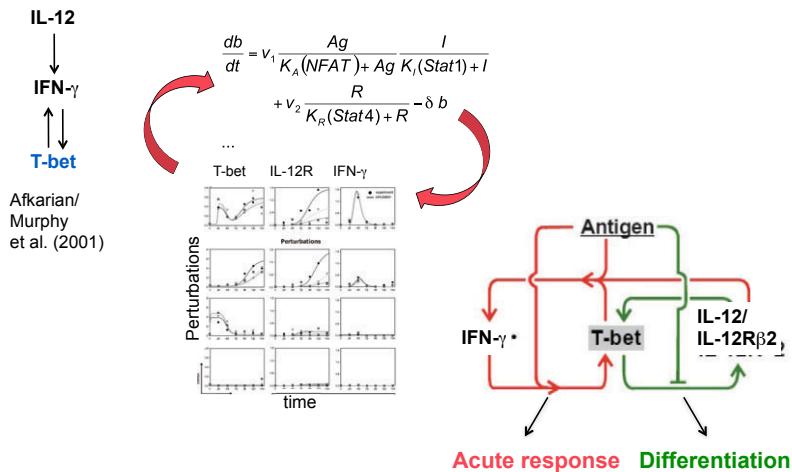
Complex dynamics



Prediction: control of NER



DYNAMICS OF T CELL DIFFERENTIATION



Thomas Höfer

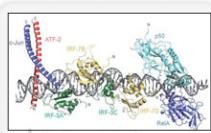
Modeling of Biological Systems (B086)

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PERSPECTIVES

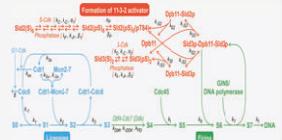
Chromatin-associated processes



DNA repair
Heterochromatin
regulation

Van Driel/Vermeulen (NL)
Rippe, Grummt, Eils (DKFZ)

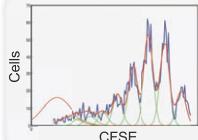
Regulatory networks



Mitogenic signaling
DNA replication

Westermann/Schwab (DKFZ)
Klingmüller (DKFZ)
Alberghina (Milan)

Cellular decisions



T-cell activation
& differentiation

Radbruch/Löhning (Berlin)
Rao (Boston)
Acuto (Oxford)

Thomas Höfer

Modeling of Biological Systems (B086)

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Anneke Brümmer
Carlos Salazar
Edda Schulz
Gesa von Bornstaedt
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Domenico Prellino
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Dorothea Busse
Michael Flossdorf
Florian Lamprecht
Kristof Kally-Kullai

Funding: EU-FP7, BMBF, DFG, Helmholtz Association

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Modeling of Biological Systems (B086)

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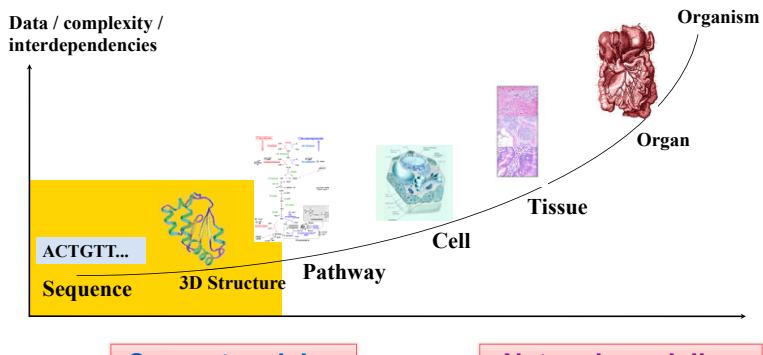
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Theoretical Bioinformatics

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Data integration, analysis and modeling



Roland Ells

Theoretical Bioinformatics (B080)

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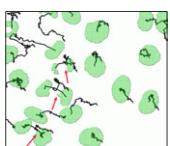
Biomedical Computer Vision Group (Karl Rohr)



Computer-based analysis and quantification of biological and medical images as a prerequisite for modelling in systems biology

Segmentation and Quantification

- RNAi high-throughput cellular screens (e.g., mitosis)
- Subcellular structures, structures in medical images



Tracking and Classification

- Analysis of object motion, object classification
- 2D and 3D multi-cell time-lapse images

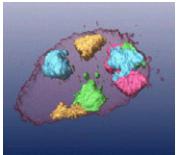


Image Registration

- Shape normalization by elastic registration

Cellular Organization

- Chromosome dynamics*, protein movement**

HT/HC analysis of cell arrays

*Cell 2003, **Nature Cell Biology 2006

Roland Ells

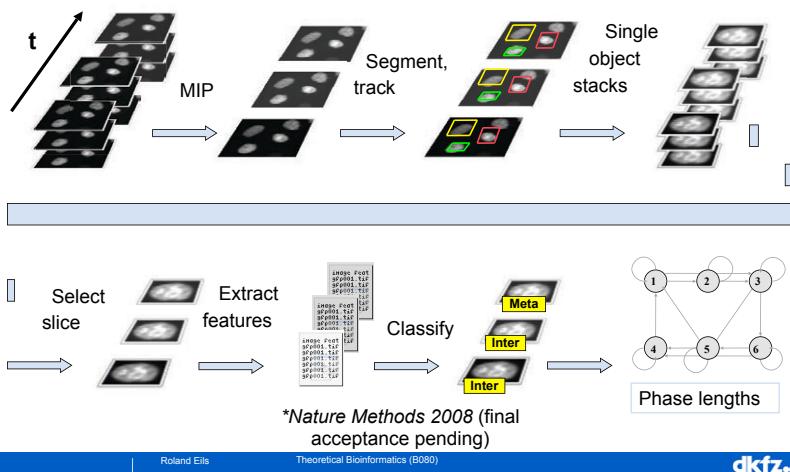
Theoretical Bioinformatics (B080)

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Large-scale image analysis of cell arrays* (~100 mill. images)

Tracking and Classification: Cell cycle analysis, Delay assay



Roland Ells

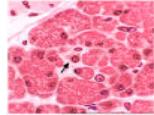
Theoretical Bioinformatics (B080)

dkfz.

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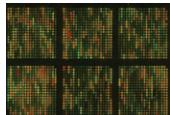
Computational Oncology

$n = 10 - 1000$



Phenotype

$n = 1.000 - 30.000$



Genotype



Predictive analysis
- prognosis
- therapy response

Correlation with phenotype
Most important variables?
Predictive patterns?

- aCGH (genomics) Finding genetic aberrations
- Epigenetic profiling Finding different DNA modifications
- Expression arrays (transcriptomics) Finding expressed genes
- Mass Spectrometry (proteomics) Finding proteins

*JCO 2006

**BMC Cancer 2007

***Clin Canc Res 2008

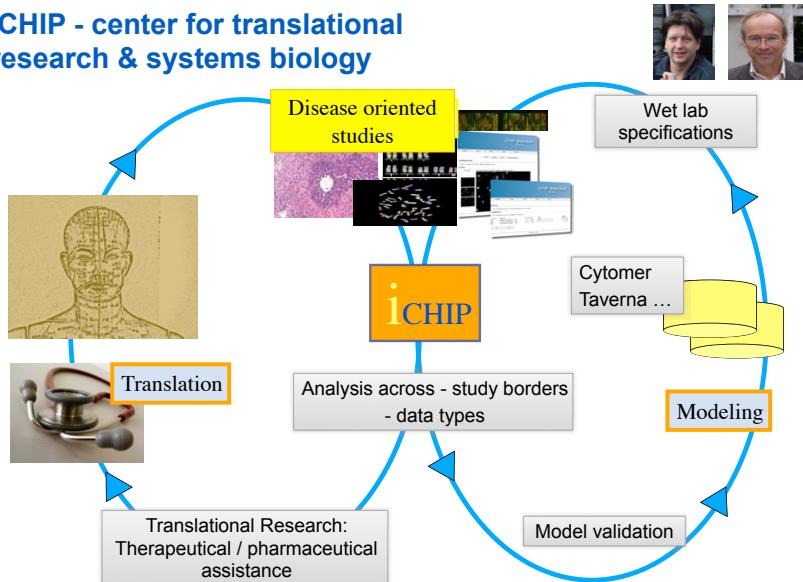
Roland Ells

Theoretical Bioinformatics (B080)

dkfz.

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iCHIP - center for translational research & systems biology



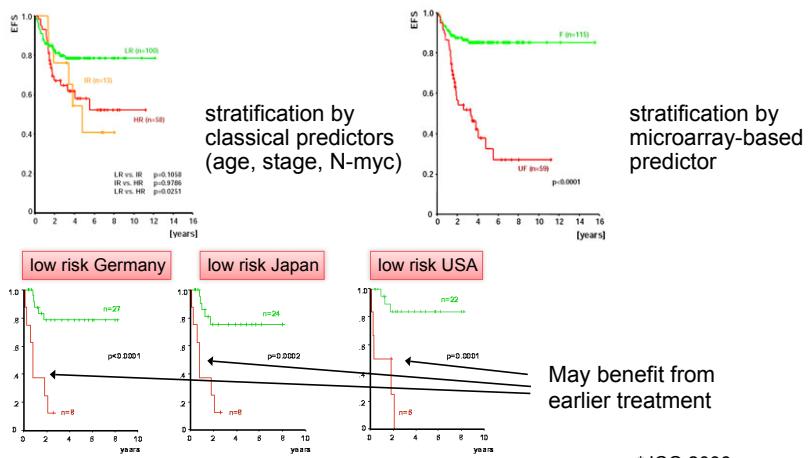
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Improved Prediction of clinical outcome by gene expression signature in neuroblastoma*



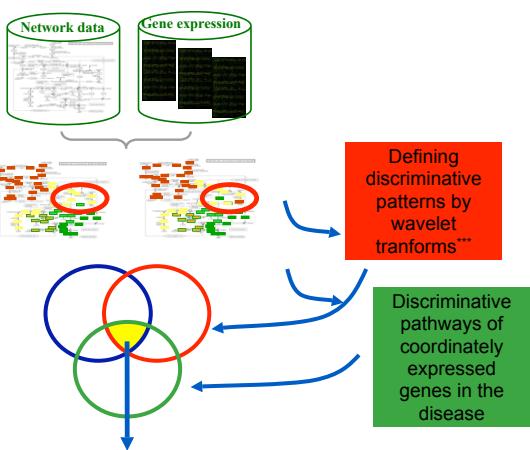
Roland Ells

Theoretical Bioinformatics (B080)

dkfz.

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Network Modelling Group



*Plaimas et al., BMC Systems Biology, 2008; ** Fatumo et al., Infection, Genetics and Evolution, 2008; ***König et al., BMC Bioinformatics, 2006

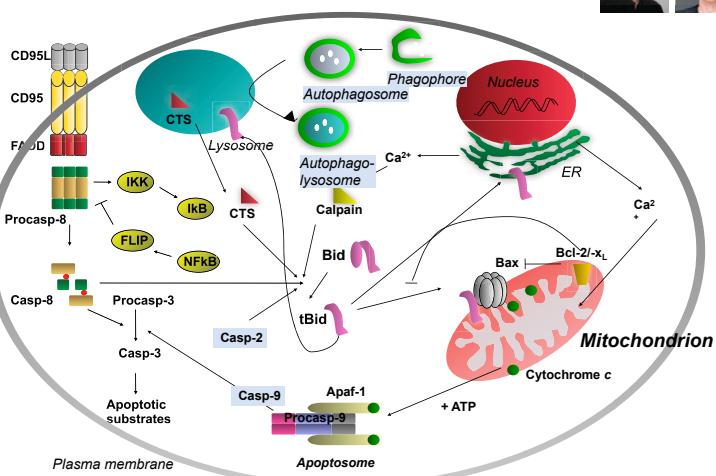
Roland Ells

Theoretical Bioinformatics (B080)

dkfz.

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Systems Biology of Cell Death



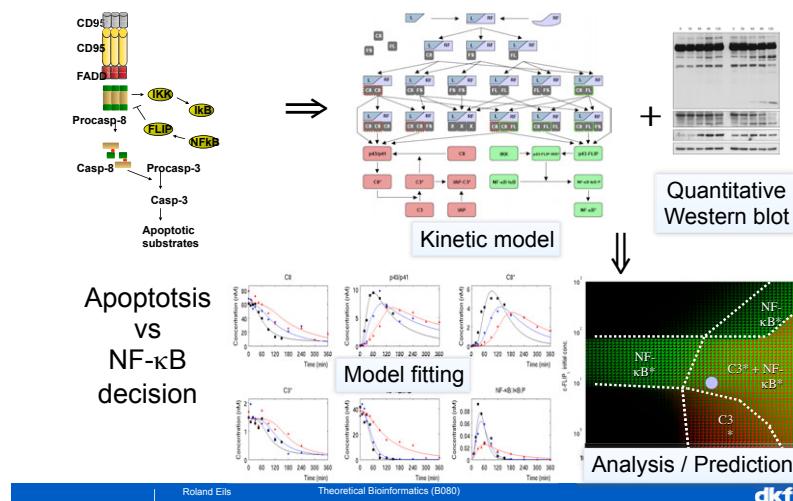
Roland Ells

Theoretical Bioinformatics (B080)

dkfz.

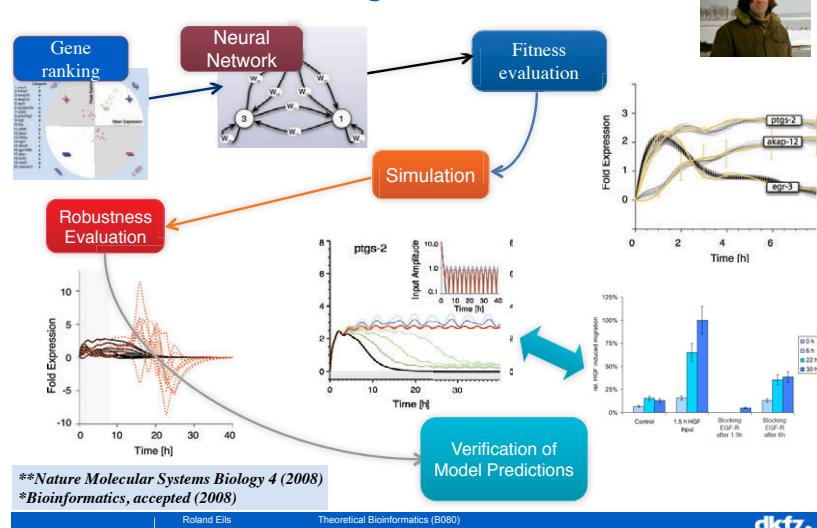
189

Apoptosis regulation* and alternative pathway induction by CD95 (*JCB 2005)



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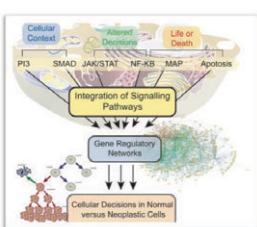
Modeling Gene Regulatory Networks* Controlling Cell Migration**



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Outlook

Medical Systems Biology Systems Biology of Complex Diseases



Synthetic Biology
From *understanding* to the *design* of biological systems



dkfz.

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