CONTENT

Newsletter of the German Society for Cell Biology

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Cell and Developmental Biology are meeting for good

This issue is devoted to the joint international meeting of the German Society for Cell Biology and the Society for Developmental Biology. This year, the conference replaces the two regular annual meetings of both societies at a decisively international level. In the planning, we remembered the good vibrations during the last joint meeting in Bonn in 2003, the additional value of having life sciences presented from two different but very related angles, and we actually wondered why we did not continue this successful strategy earlier. In organizing the individual sessions of the meeting program, we realized again how close we are in both "disciplines" with respect to the methods used, scientific aims and the conceptual thinking. Cells, groups of cells, communication of cells with each other in organizing higher order systems, organisms, are the topics of both fields. Sure enough, we have quite a number of colleagues who are for good reasons member of both societies. This experience led us to propose that in the future, both societies should work together more closely, at the conference level, with respect to a common newsletter and the webpages. The boards of both societies principally agreed to further this cooperation, and it is now our members task to organize events always such that the sister society is considered.

In this issue, we present and introduce the Schleiden lecturer of this year, Thomas Cremer from München, and we indeed hope many of you will come to his lecture on Saturday March23rd here in Heidelberg. Moreover, you will find a research profile by Barbara Conradt, München, which wonderfully exemplifies the "merger" of cell and developmental biology in research, and a perspective article by Jochen Wittbrodt, where he elaborates on how cellular developmental biology will possibly have to proceed in the future. Last but not least, Volker Gerke, Thomas Magin and Manfred Schliwa contribute an article that reflects and honours the scientific career of Klaus Weber, former director of the Max-Planck Institute for Biophysical Chemistry in Göttingen, on the occasion of his 77th birthday in April this year.

In this issue, the latest version of the program is found on page 4 and 5, and both of us hope that it will inspire the last hesitating member to decide to come and attend the meeting. Note that you still can register directly at the meeting, and in particular that registration for a single day is possible. For Saturday, featuring the Schleiden Lecture, the plenary session Stem Cells" and the "Open Symposium on Quantitative Biology", no registration will be needed.

Hoping to see you in Heidelberg in spring !!!

Harald Herrmann (DGZ) and Jochen Wittbrodt (GfE)

DGZ Member Meeting 2013

We are inviting all members to attend our next member meeting that will take place on

Thursday, March 21, 2013 12:30 – 13:30

at the International Joint Meeting (36th DGZ annual meeting) in Heidelberg
Universität Heidelberg, Hörsaalzentrum Chemie + Bioquant, Im Neuenheimer Feld 252, room: Kleiner Hörsaal

Agenda:
1. Confirmation of the minutes of the last year's DGZ member meeting
2. The president's annual report
3. Financial report
4. The auditors' report
5. Approval of the executive board
6. Annual membership fee
7. Change of the DGZ bylaws
8. „Other"

Joint International Meeting of the German Society for Cell Biology (DGZ) and the Society for Developmental Biology (GfE)

March 20 – 23, 2013, Heidelberg

Organized by Harald Herrmann (DGZ) and Jochen Wittbrodt (GfE)

Wednesday, March 20

08:00 – 20:00 Registration

09:00 – 10:30 **Talk & Question Time: DFG Funding Opportunities for all Career Stages**
- Dr. Dorette Breitkreuz, German Research Foundation (DFG), Programme Director, Life Sciences 2
- Dr. Astrid Klingen, German Research Foundation (DFG), Programme Officer, Life Sciences 2
- Review Board Members of the German Research Foundation (DFG)

11:00 – 13:00 **Student Symposium**: The Abstract Highlights

14:00 **Opening and Plenary Session PS1**

14:00 – 17:00 **Plenary Session PS1: Cell Polarity**
Chair: Jiri Friml (Gent, Belgium)

Invited Speakers:
- Carl-Philipp Heisenberg (Klosterneuburg, Austria)
- Erez Raz (Münster)
- Jochen Rink (Dresden)
- Marja Timmermans (Cold Spring Harbor, USA)

17:00 - 18:00 **DGZ Awards**
- Walther Flemming Medal
- Binder Innovation Prize
- Werner Risau Prize

18:00 - 19:00 **Carl Zeiss Lecture**
Robert A. Weinberg (Cambridge, USA)

19:00 **Welcome Reception – Posters for Display**

Thursday, March 21

09:00 – 12:00 **Symposia 1 – 4**

09:00 – 12:00 **Symposium S1: The Nuclear Envelope: Barrier & Transport Functions**
Chair: Jörg Großhans (Göttingen)

Invited Speakers:
- Amnon Harel (Haifa, Israel)
- Vivian Budnik (Worcester, USA)
- Ulrike Kutay (Zürich, Switzerland)

09:00 – 12:00 **Symposium S2: Non-coding RNA in Development and Disease**
Chair: Sven Diederichs (Heidelberg)

Invited Speakers:
- Judy Lieberman (Boston, USA)
- Maite Huarte (Pamplona, Spain)
- Stefan Hüttelmaier (Halle)

09:00 – 12:00 **Symposium S3: Ubiquitin-related Proteins**
Chair: Frauke Melchior (Heidelberg)

Invited Speakers:
- Madelon M. Maurice (Utrecht, Netherlands)
- Stefan Jentsch (Martinsried)
- Richard D. Vierstra (Madison, USA)

09:00 – 12:00 **Symposium S4: Epigenetics**
Chair: Sylvia Erhardt (Heidelberg)

Invited Speakers:
- Sandra B. Hake (München)
- M. Cristina Cardoso (Darmstadt)
- Dirk Schübeler (Basel, Switzerland)

12:00 **Lunch**

12:00 - 13:00 **Lunch Symposium 1**
Carl Zeiss Microscopy GmbH

12:30 – 13:30 **DGZ Member Meeting**

13:30 – 16:30 **Symposia 5 – 8**

13:30 – 16:30 **Symposium S5: Centrosomes**
Chairs: Ralph Gräf (Potsdam) and Oliver Gruss (Heidelberg)

Invited Speakers:
- Andrew Fry (Leicester, UK)
- Monica Bettencourt Dias (Oeiras, Portugal)
- Ingrid Hoffmann (Heidelberg)

13:30 – 16:30 **Symposium S6: Cell Metabolism**
Chair: Eckhard Lammert (Düsseldorf)

Invited Speakers:
- William Martin (Düsseldorf)
- Nils-Göran Larsson (Köln)
- Pierre Maechler (Geneva, Switzerland)

MEETING INFORMATION

13:30 – 16:30 **Symposium S7: Vesicular Transport**
Chair: Karin Schumacher (Heidelberg)
Invited Speakers:
– Peter Robin Hiesinger (Dallas, USA)
– Juan Ramón Martínez Morales (Sevilla, Spain)
– Anne Spang (Basel, Switzerland)

13:30 – 16:30 **Symposium S8: Evolution of Morphogenesis**
Chairs: Steffen Lemke and Alexis Maizel (Heidelberg)
Invited Speakers:
– Nicholas Gompel (Marseille, France)
– Patrick Lemaire (Montpellier, France)
– Miltos Tsiantis (Oxford, UK)

17:00 – 18:00 **Distinguished Lecturer**
Maria Leptin (Heidelberg)

18:00 – 21:00 **Poster Session 1**

Friday, March 22

09:00 – 12:00 **Symposia 9 – 13**

09:00 – 12:00 **Symposium S9: Primary Cilia & Signaling**
Chair: Achim Gossler (Hannover)
Invited Speakers:
– Gislene Pereira (Heidelberg)
– Hiroshi Hamada (Osaka)
– Heiko Lickert (München)
– Heymut Omran (Münster)

09:00 – 12:00 **Symposium S10: Biomechanics of Cells**
Chair: Jochen Guck (Dresden)
Invited Speakers:
– Franziska Lautenschläger (Paris, France)
– Eric M. Darling (Providence, USA)
– Sirio Dupont (Padua, Italy)

09:00 – 12:00 **Symposium S11: Cortical Development**
Chair: Orly Reiner (Rehovot, Israel)
Invited Speakers:
– Wieland B. Huttner (Dresden)
– Joseph LoTurco (Storrs, USA)
– Michael Frotscher (Hamburg)

09:00 – 12:00 **Symposium S12: Advanced Microscopic Methods**
Chair: Paul Walther (Ulm)
Invited Speakers:
– Ernst H.K. Stelzer (Frankfurt)
– Shigeki Watanabe (Salt Lake City, USA)
– Sonja Welsch (Einhoven, The Netherlands)

09:00 – 12:00 **Symposium S13: Lateral Gene Transfer & Evolution of Symbiosis**
Chair: Thomas Bosch (Kiel)
Invited Speakers:
– Angela E. Douglas (Ithaca, USA)
– Giles Oldroyd (Norwich, UK)
– Tal Dagan (Düsseldorf)

12:00 **Lunch**

12:00 – 13:00 **Lunch Symposium 2**
ibidi GmbH

12:00 – 15:00 **Poster Session 2**

12:30 – 13:30 **GfE Member Meeting**

15:00 -18:00 **Plenary Session PS2: The Nucleus and the Genome**
Chair: Peter Lichter (Heidelberg)
Invited Speakers:
– Bas van Steensel (Amsterdam, The Netherlands)
– Andrew Belmont (Urbana, USA)
– Ana Pombo (London, UK)
– Karsten Rippe (Heidelberg)

18:00 – 18:30 **Nikon Young Scientist Awards of the DGZ**

18:30 – 19:00 **PhD Students Awards of the GfE**

19:00 – 19:45 **Frontiers in Science Lecture**
Reinhard Jahn (Göttingen)

20:30 **Get Together**

Saturday, March 23

08:30 – 09:30 **Matthias Schleiden Lecture**
Thomas Cremer (Martinsried)

09:30 **The Open Symposium: Quantitative Biology – Where do we stand?**
– Ueli Aebi (Basel, Switzerland)
– Roland Eils (Heidelberg)
– Josef Käs (Leipzig)
– Yitzhak Rabin (Ramat-Gan, Israel)
– Jochen Wittbrodt (Heidelberg)

09:30 – 12:30 **Plenary Session PS3: Stem Cells**
Chair: Andreas Trumpp (Heidelberg)
Invited Speakers:
– Timm Schröder (Neuherberg)
– Marieke Essers (Heidelberg)
– Bruce Edgar (Heidelberg)
– Oliver Brüstle (Bonn)

12:30 **Closing Ceremony**

Nuclear architecture and function studied in space and time: origins, current state and perspectives

Thomas Cremer

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From Matthias Schleiden's spontaneous cell generation to the chromosome theory of heredity

Although several scientists, including Antoni van Leeuwenhoek (1632-1723), Jan Evangelista Purkinje (1787-1869) and Robert Brown (1773-1858) may claim priority for the discovery of the cell nucleus, Matthias Schleiden (1804-1881) was the first, who in his seminal contribution "Beiträge zur Phytogenesis" proposed a theory about its formation and function in plant cells (Schleiden, 1838). In the introduction Schleiden noted that each higher plant is an aggregate of self-contained individuals, known as cells, and then described how nuclei and cells are generated de novo. The first step is the spontaneous formation of "Kernchen", also called "Kernkörperchen", or in current nomenclature a nucleolus. Around this "Kernchen", a "Kern" (nucleus) is formed, and around the nucleus a new cell. Because of its capability to "build" a cell Schleiden called the nucleus "Zellenbildner" or cytoblast. In his textbook for students, published in two volumes in 1845 and 1946, Schleiden described the de novo generation (generatio spontanea) of yeast cells in filtered currant juice as one of his favorite and as he felt most convincing examples (Figure 1). Schleiden had no doubt that the filtered current juice contained no cells in the beginning and that the cells he observed after a few days had formed spontaneously in a "cytoblastem" containing necessary basic compounds, such as a nitrogen source. Schleiden had no qualms whatsoever that his theory was firmly proven by direct observation. In higher plants new cells according to Schleiden typically formed inside already existing cells (Figure 2).

In Schleiden's view Figure 2e shows a free cytoblast with a "Kernkörperchen" (nucleolus). In Figure 2f this free cytoblast had formed a complete cell. Figures 2g-n represent stages of a gradual dissolution of the old nucleus and the wde novo formation of two new nuclei, while the cytoplasm and cell wall remain intact. Figures 2o-q finally show the formation of new cells around the new nuclei. The wall of the old cell is still visible. Theodor Schwann (1810-1882) was fascinated by Schleiden's theory of the generatio spontanea of cells and the role of the nucleus in this process. In his book "Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachsthum der

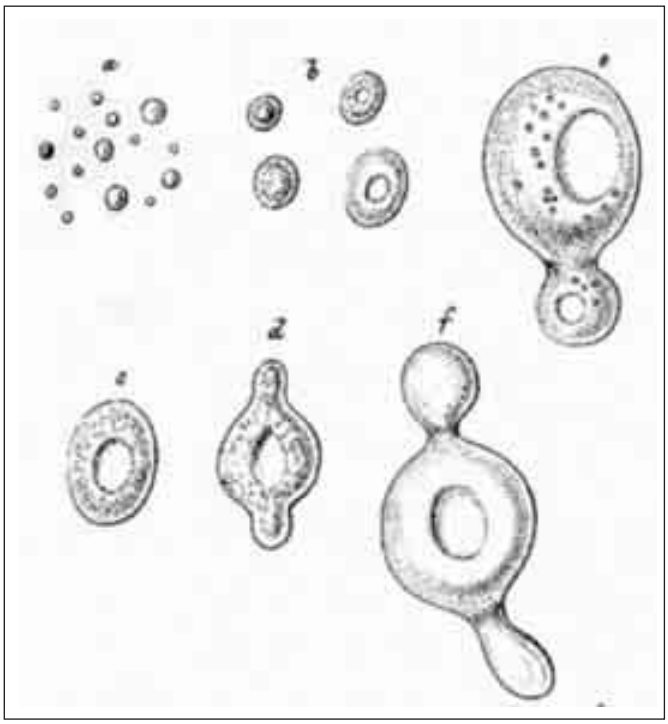
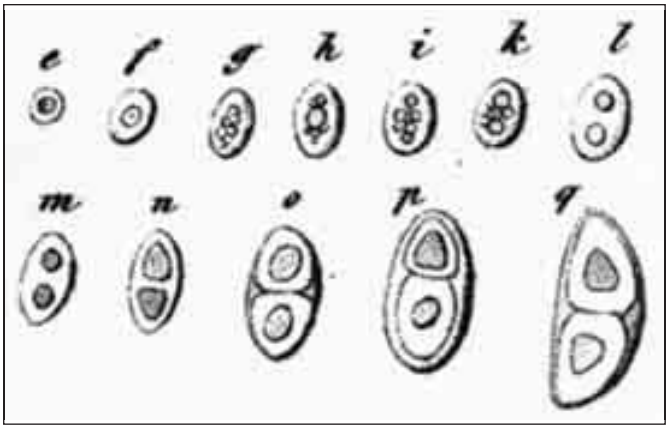


Figure 1. De novo formation of yeast cells in currant juice (Schleiden, 1846). "I finely grinded currants, pressed the juice through a cloth, diluted it with water and filtered it through filter paper. The fluid was clear and transparent. Under the microscope it showed no trace of grains, only some fine, water-clear oil drops." .. "After 24 hours the whole fluid opalised and now numerous grains became visible under the microscope (a). At the second day these grains had further multiplied and transition stages could be found from grains towards fully established yeast cells." (b, c). At the third and fourth day fermentation was observed and one could see how daughter cells formed at the edge of mother cells (d, f).



De novo formation of cells in Borrera ciliaris (Schleiden, 1846) At face value it may seem to today's readers that this figure depicts the universally valid way, how a mother cell forms two daughter cells. A mitotic event followed by cell division, however, is clearly not at all what Schleiden wanted to demonstrate in this sequence of events (for details see text).

Tiere und Pflanzen" Schwann claimed that his studies had proven Schleiden's theory for animal cells as well - „die Untersuchungen von Schleiden klärten den Bildungsprozess aufs herrlichste auf" (Schwann, 1839). Although being entirely wrong in its details, the Schleiden-Schwann cell theory had the great merit to show that both plants and animals are composed of cells with common fundamental features, including the cell nucleus. What is still fascinating today, is not so much that their theory was soon refuted as the last vestige of the long lasting, powerful generatio spontanea theory, but the fundamental shift to a fully materialistic view of the role of the nucleus as the bearer of heredity, which occurred within the next few decades. This shift resulted in the first chromosome theory of heredity proposed by August Weismann (1834-1914) already in the eighties of the 19th century and culminated in Theodor Boveri's (1862-1915) and Walter Sutton's (1877-1916) theory published in the early 20th century (reviewed in Cremer, 1985). Today's students may no longer know the names of the cytologists of this time, who described mitosis as part of indirect cell division, as well as meiosis and fertilization. This now seemingly self-evident textbook knowledge about nuclei and chromosomes formed the necessary structural basis for the still ongoing search for molecular mechanisms.

Rise, fall and resurrection of chromosome territories

The first studies of nuclear architecture were carried out at the turn from the 19th to the 20th century. The existence of chromosome territories (CTs) as fundamental building blocks was proposed by Carl Rabl (1853-1917), Eduard Strasburger (1844-1912) and Theodor Boveri, who coined this term in 1909 (reviewed in (Cremer and Cremer, 2006a). The concept of a territorial organization of chromosomes in the cell nucleus later fell into oblivion. Notably, despite all the important contributions of electron microscopy (EM) (for review see Rouquette et al. 2010) culminating in the discovery of nucleosomes (for review see (Olins and Olins, 2003)), individual CTs could not be distinguished. In the 1970th this failure prompted the statement "that discrete interphase chromosomes are absent" (Wischnitzer, 1973). In retrospect, the reason for this failure can be explained by the numerous contacts established between neighbouring CTs, which result in a higher order chromatin network expanding throughout the nucleus (Albiez et al., 2006; Markaki et al., 2010). As an example, Figure 3 shows a 3D reconstruction obtained from a rat hepatocyte nucleus based on a combination of ultramicrotomy and scanning electron microscopy known as serial block face scanning electron microscopy (SBFSEM) (Rouquette et al., 2009). While one recognizes the 3D network of chromatin clusters and bundles pervaded by wide chromatin free space, called the interchromatin compartment (IC), which occupies between 30 and 40% of the total nuclear volume (see below), individual CTs cannot be identified. For this reason experimental proof for a territorial organization of chromosomes in the cell nucleus became only possible with the development of methods for the visualization of individual CTs in the late 20th century (reviewed in (Cremer and Cremer, 2006b). Compelling experimental evidence for CTs as a fundamental principle of higher order chromatin organization was first obtained with the help of laser-uv-microbeam experiments (Zorn et al., 1979; Cremer et al., 1982a;) and thereafter by the direct visualization of individual CTs with in situ hybridization approaches (Manuelidis, 1985; Schardin et al., 1985; Lichter et al., 1988; Pinkel et al., 1988).

From chromosome territories to nuclear organization: the chromosome territory – interchromatin compartment (CT-IC) model

Using confocal laser scanning microscopy (CLSM), we studied 3D nuclear organization in a variety of cell types and species, ranging from mammals (Cremer et al., 2001; Tanabe et al., 2002; Bolzer et al., 2005; Küpper et al., 2007; Neusser et al., 2007; Koehler et al., 2009) to birds (Habermann et al., 2001), hydra (Alexandrova et al. 2003) and ciliates (Postberg et al. 2008). These studies have demonstrated evolutionary conserved principles of nuclear organization, as well as species specific characteristics, in particular with respect to the macronucleus in ciliate species. Current data support an evolutionary conserved, non-random, radial nuclear organization with gene poor chromatin positioned closer the nuclear envelope than gene dense chromatin in somatic cell types, although important exceptions were found for specific, terminally differentiated cell types (Solovei et al., 2009; Solovei et al., 2013). Figure 4A provides an example for the distinctly different arrangement of gene dense and gene poor chromosomal subregions in HSA 12 CTs from a human lymphocyte nucleus.

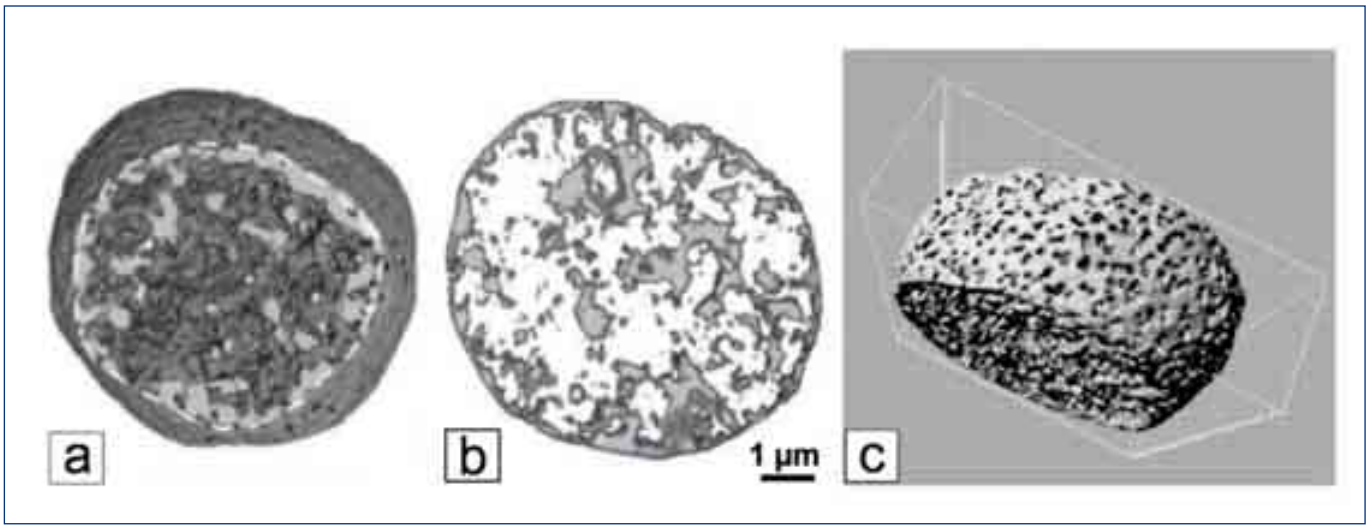


Figure 3. 3D topography of specifically stained DNA and the interchromatin compartment in a rat hepatocyte nucleus studied with serial block face scanning electron microscopy (SBFSEM)(adapted from (Rouquette et al., 2009).
(a) 3D reconstruction of the DNA (grey) of the entire nucleus. The top part facing the viewer is removed to allow direct insight into the nuclear interior. At face value this insight gives the impression as if this nucleus would be nearly completely filled with chromatin.
(b) 3D reconstruction of a 250 nm thick nuclear slice, however, demonstrates the extension of the interchromatin compartment (IC; white), which is essentially chromatin free and pervades between chromatin clusters (grey).
(c) 3D reconstruction of a several µm thick midpart shows numerous little holes in the peripheral layer of heterochromatin. These holes reflect IC-channels expanding to the nuclear pores (Schermelleh et al. 2008; Markaki et al. 2012). For further details see supplementary movies S1 and S2 in (Rouquette et al., 2009).

Non-random proximity patterns or neighborhood arrangements of CTs and chromosomal subregions down to individual genes do apparently also exist, but it should be emphasized that such arrangements are not rigidly deterministic, but allow for extensive cell-to-cell variability (Bolzer et al., 2005; for reviews see (Cremer and Cremer, 2010; Cremer et al., 2012). The interior of CTs consists of separate chromosome arm and band domains (Dietzel et al., 1998), which in turn are built up from interconnected clusters of megabase-sized chromatin domains (~1 Mbp CDs) (Zink et al., 1998; Walter et al., 2003; Albiez et al., 2006). Live cell imaging in space and time (4D) revealed that ~1 Mbp CDs typically show constrained Brownian motions, although a possible transition from random to directed movements could be detected (Bornfleth et al., 1999). While movements of CTs were locally constrained during interphase, 3D proximity patterns of CTs changed from one cell cycle to the next (Walter et al., 2003; Strickfaden et al., 2010). Major changes of higher order chromatin arrangements were observed during terminal cell differentiation (Solovei et al., 2004; Solovei et al., 2009). Occasionally, however, we also observed major changes of chromatin proximity patterns in interphase nuclei in correlation with complex, rotational movements of nuclei (Strickfaden et al., 2010).
In parallel to the experimental studies summarized above, models of CT organization and the functional nuclear architecture have been developed (Cremer et al., 1993; Munkel et al., 1999; Cremer et al., 2000; Cremer and Cremer, 2001; Cremer et al., 2006; Lanctot et al., 2007; Cremer and Cremer, 2010; Rouquette et al., 2010; Cremer et al., 2012). Figure 4D summarizes the essential features of the chromosome territory – interchromatin compartment (CT-IC) model. According to this model the IC interacts functionally with the PR and provides a highly organized and spatially contiguous compartment of its own. It starts at nuclear pores, pervades the peripheral nuclear layer of heterochromatin with IC-channels and then expands with larger and smaller branches between and within CTs, where IC-channels pervade the space between ~1 Mbp CDs. Narrow IC-channels may be invaded by small chromatin loops allowing direct contacts between CDs in cis and trans, whereas wider IC-lacunas contain splicing speckles and nuclear bodies. The IC may serve as a system for the preferential diffusion of macromolecules or complexes, such as RNPs (Mor et al., 2010). Theoretical and experimental evidence argues that ~1 Mbp CDs may be built up from a series of interconnected ~100 kbp CDs).

The light microscopic revolution and its impact on studies of nuclear architecture

Progress in the exploration of nuclear architecture during the last decades would not have been possible without the major advancements of 3D microscopy based on the invention of confocal laser scanning microscopy (CLSM) and new concepts, which started to challenge the widely held view of the Abbe limit of light microscopic resolution as a principle barrier limi-

ting this resolution to about 200 nm (for a historical review see Cremer and Masters, 2013). In line with electron microscopic evidence (Rouquette et al., 2009), 3D structured illumination microscopy confirmed that nuclear chromatin forms a three-dimensonal network of irregularly shaped chromatin clusters built up from megabase-sized chromatin domains (Markaki et al., 2010; Markaki et al., 2012). Chromatin clusters are built up from a dense chromatin core and a peripheral zone, called the perichromatin region (PR). The PR and its role as the functional compartment for the transcription of genes, co-transcriptional splicing, DNA replication and – at least to some extent - repair was first discovered in a series of seminal electron-microscopic (EM) studies performed by Stanislav Fakan and his collaborators since the 1970ies (reviewed in Rouquette et al., 2010) and later confirmed by super-resolution fluorescence microscopy (Markaki et al., 2010; Markaki et al., 2012). Figure 4B shows an example for the formation of nascent DNA and RNA in the PR. The functional importance of the PR was further demonstrated by the finding that RNA polymerase II and H3K4me3, a histone modification typical for transcriptionally competent, chromatin, are enriched within the PR, whereas H3K27me3 and other modifications typical for silent chromatin are enriched in the compact interior of chromatin clusters (Markaki et al., 2010; Markaki et al., 2012; and our unpublished data).

Ongoing developments of super resolution fluorescence microscopy are steadily narrowing the resolution gap between light microscopy and electron microscopy (for reviews see (Rouquette et al., 2010; Schermelleh et al., 2010). Various approaches of localization microscopy have made it possible to generate images based on many thousands of individually localized fluorophores tagged to macromolecules of interest - similar to the paintings from pointillists like George Seurat (1859 - 1891) in the late 19th century (for review see Cremer, 2012). Localization microscopy provides a means to pinpoint the 3D localization of nucleic acids and proteins of interest, for example individual histones and RNA Polymerase II (Markaki et al., 2010). Currently, it has become possible to perform localization microscopy with a 3D resolution in the order of 10 to 30 nm. The principal limitation of localization accuracy depends on the need of a sufficient number of photons emitted from an individual fluorophore. Correlative microscopy based on the sequential use of different microscopic approaches for single cell studies can help to use the special advantages of each approach (Hübner et al. 2013). Current advancements of super-resolution fluorescence microscopy open avenues to live cell studies with unprecedent resolution (for review see Cremer, 2012). It is hardly an exaggeration to expect that these developments will revolutionize structure-function analyses performed in individual cells. In the context of the cell nucleus they will allow quantitative studies of differences in the chromatin organization of individual, transcriptionally silent and active genes.

High-throughput biochemical strategies for 3D studies of the genome

High-throughput biochemical strategies have confirmed and expanded microscopic evidence for chromatin domains as evolutionary conserved building blocks of higher order chromatin organization. These strategies allow the genome wide identification of contact frequencies between genomic sites (Lieberman-Aiden et al., 2009; Dixon et al., 2012; Nora et al., 2012), as well as the genome wide identification of DNA interactions with proteins in cell populations (e.g., (Guelen et al., 2008), for review see (de Wit and de Laat, 2012). The average size of the domains may vary between species and cell types between some 100 kbp and 1 Mbp. The combination of these biochemical strategies and advanced microscopy is indispensable to further uncover nuclear structure and function. The advantage of biochemical strategies lies in their capacity to provide an overview of average changes of contact frequencies between genomic sites in cis and trans, as well as DNA-protein, RNA-protein and protein-protein interactions. Microscopic approaches allow to study the cell-to-cell variability of structure-function relationships. It appears "that the rules that dictate genome structure and function inside the cell ... are probabilistic not determinstic" (de Wit and de Laat, 2012). Studies of the nuclear topography of DNA, RNA and proteins at the single cell level will remain indispensable to further explore and better understand these rules.

Perspectives for future studies

A functional cell nucleus requires a higher order chromatin organization fit for (a) the local 'opening' and 'closure' of chromatin domains, (b) large- and small-scale chromatin movements and (c) the structural transformation of chromosome territories (CTs) into mitotic chromosomes to allow the proper segregation of chromatids from a mother cell to its two daughters. A nuclear architecture with distinct CTs and chromatin domains with a 'fractal' organization (Mirny, 2011) arguably helps to minimize detrimental chromatin fiber/loops entanglements and likely provided an important selective advantage already at the roots of eukaryote evolution and possibly even for prokaryotes. Such an organization may have been of particular importance at early stages of evolution, when highly efficient repair systems necessary to deal with large numbers of knotted chromatin fibers/loops were not available. A more general argument takes into account that evolution should favor individuals able to use their resources in a more efficient way for the production of viable offspring. For this reason individuals with a genome organization, which helped to reduce the frequency of such entanglements a priori, had likely a selective advantage compared with individuals, whose fitness was more dependent on the repair of breaks resulting during the resolution of knotted chromatin fibers/loops.

A comprehensive understanding of any complex biological system depends on understanding its structure-function relation-

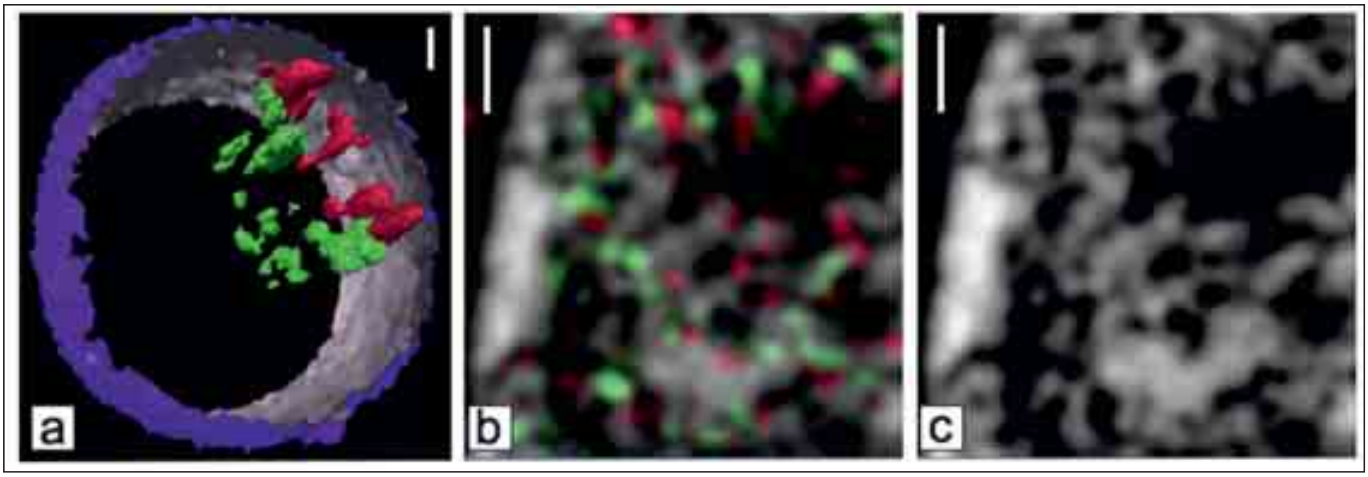
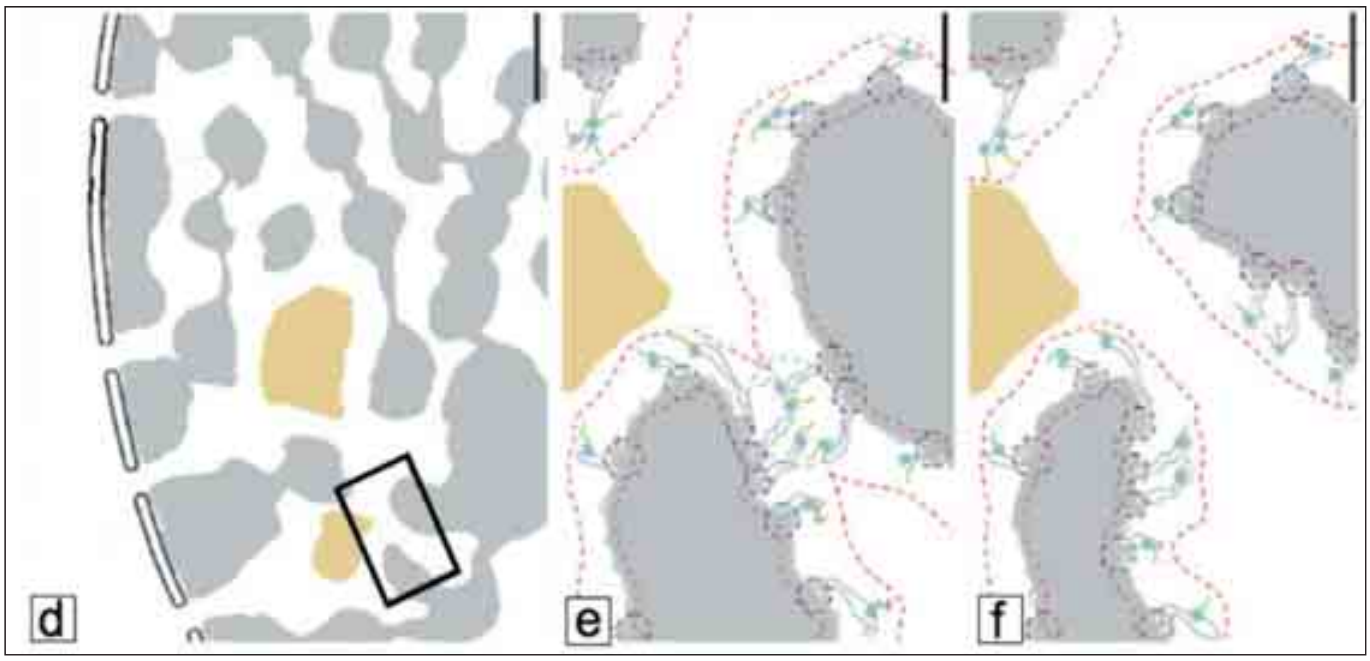


Figure 4. Chromosome territory – interchromatin compartment (CT-IC) model of nuclear architecture.
(a) Partial 3D reconstruction of a human lymphocyte nucleus shows the different radial nuclear distribution of gene rich regions (green signals) and gene poor regions (red signals) located within two HSA 12 CTs. BAC pools for these regions were selected from a HSA 12 BAC (bacterial artificial chromosome) library. After 3D FISH with the two differentially labelled BAC pools, light optical serial sections were obtained with 3D confocal laser scanning microscopy followed by 3D reconstruction. The outside of the reconstructed part of the nuclear border is shown in blue, the inside in silver-grey (Küpper et al. 2007). Bar: 1 µm.
(b, c) These images show a part of a light optical section recorded with 3D structured illumination microscopy (3D SIM) from a nucleus of a mouse mammary tumor cell line (C127) (Markaki et al. 2010). The cell was fixed 15 min after scratch-labeling with both BrUTP (incorporated into nascent RNA and detected by indirect immunoflurescence, red signals) and dUTP conjugated with the fluorophore ATTO 488 (incorporated into nascent DNA, green signals). DAPI-stained DNA is shown in gray color. Bar: 500 nm. Irregular shaped domains of DAPI stained DNA are seen in the nuclear interior, which in 3D reconstructions reveal a network of higher-order chromatin bundles, called the chromatin compartment (CC, compare d). Nascent RNA and nascent DNA is located at the periphery of chromatin domains and bundles. Regions without detectable DAPI stained DNA form a second network, called the interchromatin compartment (IC, compare d)(for electronmicroscopic evidence see Rouquette et al. 2009). The left side of the two images shows a part of the nuclear border with IC channels pervading clusters of heterochromatin located beneath the nuclear lamina. The channels end at nuclear pores (not shown here; see Schermelleh et al. 2008, Markaki et al. 2012).

ships at all levels of organization from molecules to the entire system. The nucleus is a telling example, where a recognizable change of structure may precede or follow a recognizable change of function. The new term 4D nucleome may be useful to describe the whole range of structural and biochemical properties of chromatin, including genetic and epigenetic interactions of chromatin with each other in cis and trans, as well as interactions between chromatin, functional nuclear machineries and bodies. In addition to the massive and well established international efforts of biochemists and molecular biologists to investigate the mechanisms involved in nuclear functions, understanding the 4D nucleome requires quantitative, positional maps of interacting nuclear components in space and time at the nanometer scale. This long term goal necessitates international efforts addressing the following topics:

- 4D topography of the genome and epigenome: from genes, regulatory sequences, non-coding sequences and RNAs with still little explored, functional and/or structural roles, to chromatin domains and chromosome territories;
- 4D topography of nuclear bodies and machineries involved in transcription, co-transcriptional splicing, DNA replication and repair, as well as the assembly and disassembly of macromolecules which form these machineries;
- 4D organization of chromatin domains compatible with functional requirements of 'opening' and 'closure' as requested by different functional states, as well as the structural transformation of chromosome territories into mitotic chromosomes fit for the proper segregation of chromatids to daughter cells. Studies of the 4D organization of well-defined megabase-sized chromatin domains during transcriptional activation and silencing of genes carried by such domains should help to explore for the first time the true extent of 'opening' and 'closure' of chromatin domains or parts of them in correlation with the transcriptional status of their genes;
- 4D nuclear organization of the interchromatin compartment, including its role for the import of macromolecules or macromolecule complexes and their proper allocation, as well as the export of, e.g., ribonucleoprotein particles.

In conclusion, I wish to mention two research areas, which I consider as particularly rewarding:
(I) Comparative studies of the 3D and 4D nucleome. Genome evolution necessitated a whole bunch of structural inventions. Some of them may have persisted in all branches of the phylogenetic tree, other features may have evolved only in certain branches. For example, a lamina built up from lamins serves as



(d) Scheme of a partial nuclear section shows the principle organization of the cell nucleus according to the CT–IC model (Bar: 500 nm). It proposes that the nuclear interior is composed from two interacting, structural networks. The higher order chromatin network consists of interconnected clusters of megabase-sized chromatin domains, which consist of a core of compact chromatin (grey) and a decondensed chromatin periphery (compare e, f). The interchromatin compartment (IC) (white) pervades the space between the chromatin compartment as a second compartment with its own complex structural organization. Splicing speckles and nuclear bodies located within the IC are indicated in caramel.
(e, f) Enlargement of the boxed insert in (d). Bar: 200 nm. A zone of decondensed chromatin, called the perichromatin region (PR), is located at the periphery of chromatin domains and roughly delimited by the red dotted line in this scheme. The PR separates the compact core of chromatin domain clusters from the interior of the IC and represents the nuclear subcompartment, where transcription of genes, co-transcriptional splicing, DNA replication and repair occur. Nascent RNA produced within the PR is symbolized as green threads. Transcription of several genes (see nascent RNA) may preferentially occur in areas (encircled in (e) by a blue dotted line), which are enriched in transcription complexes (symbolized as blue dots). Possibly, transcription factories exist, which carry out the spatially co-regulated transcription of several genes simultaneously. The actual, local width of the IC changes continuously as a result of constrained Brownian chromatin movements. Small chromatin loops from neighboring chromatin domains can directly interact in narrow IC-channels. Such interactions are essential for homologous repair and can give rise for the formation of translocations in cis and trans.

an anchorage site in metazoa, whereas plants, fungi and protists have apprently evolved other proteins for this role (DuBois et al. 2012). The macronucleus of Ciliates with its hugh number of tiny 'gene-sized' chromosomes (Postberg et al. 2008) or nuclei of Dinoflagellates, which lack bulk histones (Talbert and Hennikoff, 2012), may be telling examples to answer the question, whether evolution resulted in profoundly different types of functional nuclear organizations. Comparative studies of the 3D and 4D genome organization in a wide range of both eukaryotes and prokaryotes (as outgroups) will shed light on the prokaryotic history of the evolutionary origin of the cell nucleus (Postberg et al., 2010). One should expect that certain features of nucleomes were already derived from nucleoids of prokaryotes, such as Archae bacteria. Testing the hypothesis that a 'fractal' genome organization already evolved in prokarytes, may provide a case in point. Most important, evidence for the evolutionary preservation of structural features in nuclei and nucleoids demonstrates an important adaptive advantage of such features and thus helps to focus future research on the elucidation of structural features with major functional implications.

(II) Studies exploring the nucleome for the benefit of patients. Chromosomal aberrations can either be changes in chromosome numbers (aneuploidies), translocations between chromosomes or intrachromosomal inversions. FISH with specific chromosome painting probes and subregional probes has made it possible to identify numerical and structural chromosomal aberrations not only in metaphase spreads but also directly in cell nuclei. More than 900 publications listed in the ISI Web of Knowledge signify the importance of this approach, termed interphase cytogenetics (Cremer et al. 1986) or interphase FISH, in research and diagnosis. It has been widely demonstrated that many cancers are defined by recurrent chromosomal aberrations, which deregulate cellular signaling pathways. Their exact diagnosis can be decisive for choosing the appropriate therapy for an individual patient. Exploring the complex ways, in which chromosomal aberrations affect a nucleome's structure and function (Gordon et al., 2012), may help to better understand how aberrations contribute to the deregulation of the tightly controlled transcriptional equilibrium of cells in patients with chromosomal syndromes or cancers.

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The intricacies of apoptotic cell death in *C. elegans*

Nadin Memar and Barbara Conradt

The removal of unwanted cells through programmed cell death is a physiological process that is of fundamental importance for normal development and the maintenance of cellular homeostasis in multi-cellular organisms (Adams 2003, Danial & Korsmeyer 2004). In multi-cellular animals, based on differences at the cellular and molecular level, at least two types of programmed cell death can be distinguished: programmed cell death type I also referred to as 'apoptotic cell death' and programmed cell death type II also referred to as 'autophagic cell death' (Conradt 2009). This report will focus on programmed cell death type I i.e. apoptotic cell death. Together with the identification of the human proto-oncogene *bcl-2* (*bcl*, B-cell lymphoma), genetic studies of the nematode *Caenorhabditis elegans* performed in the 1980's and 1990's form the basis of our current understanding of apoptotic cell death and led to the identification of the conserved molecular machinery that triggers apoptosis in selected cells. These initial findings spawned numerous studies and the founding of the apoptosis field, an inter-disciplinary research area at the interface of cell and developmental biology and medicine. Gratifyingly, research in this field has increased our understanding of common human diseases such as cancer and auto-immune disorders and led to the identification of targets for novel therapeutics (Strasser et al 2011, Thompson 1995). For example, 'BH3 mimetics' are currently in Phase I and Phase II clinical trials as drugs for various types of cancers. While much of the apoptosis research nowadays is performed using mammalian model systems, genetic studies in *C. elegans* continue to uncover new aspects of apoptosis. This is because in *C. elegans*, apoptosis can be dissected genetically as well as cell biologically at single cell resolution. The reason for this is that, due to an essentially invariant somatic cell lineage, the pattern of apoptosis that occurs during the development of, for example, a hermaphrodite is highly reproducible (Sulston & Horvitz 1977, Sulston et al 1983). Specifically, during the development of a hermaphrodite, always the same 131 cells die (Figure 1). Furthermore, *C. elegans* lacks some of the genetic redundancy that has hampered genetic studies of apoptosis in mammals. While the important functions of the central apoptosis machinery in mammals are often performed by several factors with at least partially redundant activities, in *C. elegans* they are performed by one factor with a unique activity (Conradt 2009, Horvitz 2003). Below, we will briefly review the work that led to the discovery of the central apoptosis machinery that is conserved from *C. elegans* to humans. We will then summarize some of the recent research on apoptotic cell death in *C. elegans* that has led to new insight into this process in *C. elegans* and that has revealed aspects of apoptosis that are likely to be conserved in mammals as well.

The conserved, central apoptosis machinery

The cloning of the t(14; 18) (q32; q21) breakpoint, a chromosomal translocation that is found in more than 80% of human follicular lymphomas, led to the identification of a previously unknown gene, *bcl-2*, which when over-expressed can cause tumorigenesis (Tsujimoto et al 1985, Tsujimoto et al 1984). The *bcl-2* gene was subsequently found to be a novel type of proto-oncogene (Vaux et al 1988). The proto-oncogenes described until then were oncogenic due to their ability to enhance cell proliferation. However, the analysis of *bcl-2* revealed that *bcl-2* promotes tumorigenesis by preventing unwanted cells from

Figure 1 The pattern of apoptosis during C. elegans development is highly reproducible. Differential interference contrast (DIC) image of a C. elegans embryo at the 1½-fold stage of embryogenesis. The arrows point to two refractile, button-like structures, which represent two of the 131 cells, ABalpapaapp (left) and ABalpapapapa (right), that reproducibly die during C. elegans development.

dying (Vaux et al 1988). Searches for proteins with homology to the Bcl-2 protein and searches for proteins that interact with the Bcl-2 protein subsequently led to the realization that Bcl-2-like proteins form a super-family with both anti-apoptotic and pro-apoptotic family members, many of which can directly interact with each other (Cory & Adams 2002, Galonek & Hardwick 2006, Gross et al 1999). Within this super-family, three subgroups are distinguished: the anti-apoptotic multi-domain BCL-2-like proteins (such as mammalian Bcl-2, Bcl-XL and Mcl-1), the pro-apoptotic multi-domain Bcl-2-like proteins (such as mammalian Bax and Bak) and the pro-apoptotic BH3-only proteins (such as mammalian Bad, Bid and Puma). Around the same time, genetic screens in *C. elegans* identified four genes, *egl-1* (egl, egg-laying defective), *ced-9* (ced, cell-death defective), *ced-4* and *ced-3*, which when mutated block many of the 131 cell deaths that reproducibly occur during *C. elegans* development (Conradt & Horvitz 1998, Ellis & Horvitz 1986, Hengartner et al 1992). These four genes were found to act in a simple genetic pathway in which *egl-1* acts as an upstream negative regulator of *ced-9*, which, in turn, negatively regulates *ced-4*. The *ced-4* gene then activates *ced-3*, whose activity is sufficient to induce apoptosis (Figure 2). These genetic studies confirmed that apoptosis is controlled by and executed through a specific genetic program. Furthermore, the molecular cloning of the *ced-9* gene revealed that *ced-9* encodes the *C. elegans* homolog of Bcl-2, which demonstrated that this genetic program is conserved from nematodes to humans (Hengartner & Horvitz 1994b) (Figure 2). The molecular cloning of the *egl-1* gene revealed that it encodes a pro-apoptotic BH3-only protein, confirming that the Bcl-2 super-family in *C. elegans* also includes both anti- and pro-apoptotic members (Conradt & Horvitz 1998). Another seminal discovery was the molecular cloning of the *ced-3* gene, which turned out to encode a protease - the founding member of the family of cell death proteases called 'caspases' (caspase, cysteine-dependent aspartate-directed proteases) (Yuan et al 1993). Homologs of *ced-3* were found to play an important role in apoptosis in mammals, confirming evolutionary conservation beyond the Bcl-2 super-family (Miura et al 1993). Subsequent studies demonstrated that the activation of caspases can be considered the 'point of no return' for a cell and that active caspases induce most if not all of the processes that lead to the ordered destruction of the cell and its engulfment and degradation through phagocytes (Crawford et al 2012, Yi & Yuan 2009).

How is the activation of caspases controlled? The activation of caspases is mediated by the 'apoptosome', the assembly of which is directly (*C. elegans*) or indirectly (mammals) controlled by members of the Bcl-2 super-family (Schafer & Kornbluth 2006). The *in vitro* reconstitution of the activation of the mammalian caspase 'caspase 3', the homolog of *C. elegans ced-3*, led to the biochemical purification and identification of two factors that are critical for caspase activation in mammals: cytochrome c and Apaf-1 (Li et al 1997, Zou et al 1997). The identification of cytochrome c uncovered an active role of mitochondria in apoptosis and with the identification of Apaf-1, the target of cytochrome c was discovered. Specifically, the release of cytochrome c from the intermembrane space (IMS) of mitochondria into the cytoplasma induces the assembly of the 'apoptosome', which in mammals is composed of seven Apaf-1 molecules bound to cytochrome c. Several pro-caspases subsequently bind to the apoptosome and, due to a low level of protease activity detected in the pro-enzymes, cause their full activation through inter-molecular cleavage and maturation (Schafer & Kornbluth 2006, Shi 2006). The release of cytochrome c from the IMS is controlled by and mediated through members of the Bcl-2 super-family and occurs through a mechanism that is still not fully understood. However, the activation of the pro-apoptotic multimeric Bcl-2 proteins Bax and Bak is critical for this step, and their activation is triggered by a shift in the balance between the activities of pro- and anti-apoptotic Bcl-2 family members (Basanez et al 2012, Landes & Martinou 2011, Tait & Green 2010). Mammalian Apaf-1 turns out to be the homolog of *C. elegans* CED-4, which forms an apoptosome composed of eight rather than

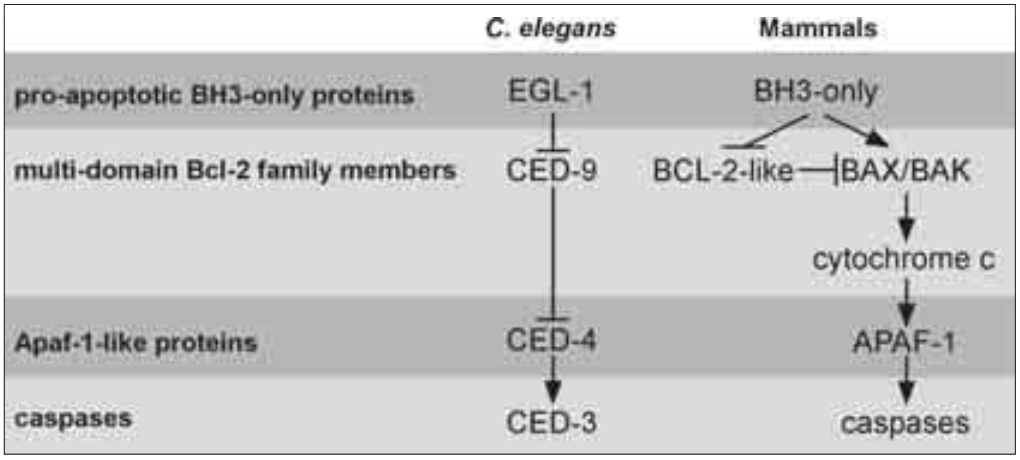

Figure 2 The central apoptosis pathway is conserved from C. elegans to mammals. In both C. elegans and mammals, members of the Bcl-2 super-family, Apaf-1-like proteins and caspases form the central apoptosis machinery. There is currently no evidence for the involvement of cytochrome c in the assembly of the apoptosome in C. elegans, which in C. elegans, is composed of the Apaf-1-like protein CED-4.

seven CED-4 molecules (Qi et al 2010, Yuan & Horvitz 1990, Zou et al 1997). CED-4 assembly appears to be independent of cytochrome c binding. Instead, the ability of CED-4 to oligomerize in healthy cells is blocked by the anti-apoptotic Bcl-2-like CED-9 protein, which directly interacts with a dimer of CED-4 (Chinnaiyan et al 1997, Spector et al 1997, Wu et al 1997, Yan et al 2005). In cells that are programmed to die during *C. elegans* development, this interaction is disrupted by the pro-apoptotic BH3-only protein EGL-1, whose activity is dramatically increased in these cells (Conradt & Horvitz 1999).

'Assisted death'

EGL-1 BH3-only, CED-9 Bcl-2, CED-4 Apaf-1 and CED-3 caspase are thought to act in a 'cell-autonomous' manner i.e. in the cell that is programmed to die to cause that cell's demise (Conradt 2009, Lettre & Hengartner 2006). However, subsequent studies on the engulfment of apoptotic cells revealed that the activity of the central apoptosis pathway can be promoted by 'cell non-autonomous' signals i.e. signals from neighboring cells (Hoeppner et al 2001, Reddien et al 2001). Genetic studies of *C. elegans* have also led to the identification of two partially redundant pathways that act in engulfing cells and that are required for the engulfment of the 131 cells that die during *C. elegans* development: the CED-1 pathway (comprising CED-1 SREC CD91/LRP mEGF10, CED-6 GULP, and the CED-7 ABC transporter) and the CED-2 pathway (comprising CED-2 CrkII, CED-5 Dock180, CED-12 ELMO1 ELMO2/CED-12A, and the CED-10 Rac GTPase) (Lu & Zhou 2012, Reddien & Horvitz 2004). In mammals, these two evolutionary conserved pathways act in professional phagocytes to engulf apoptotic cells. In *C. elegans*, the engulfment of apoptotic cells by neighboring cells (*C. elegans* does not have professional phagocytes) is blocked by mutations in the *ced-3* gene, which demonstrates that the activity of the CED-3 caspase is required to engage and activate the engulfment machinery in the engulfing cells (Ellis & Horvitz 1986). This engagement or activation is, at least in part, mediated through the *ced-3*-dependent exposure of phosphatidylserine ('PS') on the outer leaflet of the plasma-membrane of the apoptotic cell (Lu & Zhou 2012). Therefore, a very surprising finding was that mutations in 'engulfment genes' (*ced-1, -2, -5, -6, -7, -10, -12*) not only block the engulfment of the 131 cells programmed to die during *C. elegans* development but also affect the likelihood of whether these cells actually die. Specifically, mutations that eliminate *ced-3* function result in an average of 12 'extra' cells in the anterior pharynx of larvae; i.e., 12 cells that should have died during embryogenesis failed to do so. Mutations that reduce, but do not eliminate, *ced-3* function result in an average of 2 extra cells in the anterior pharynx (Shaham et al 1999). Surprisingly, if the engulfment pathway(s) are disabled in animals in which *ced-3* function is compromised, the number of extra cells in the anterior pharynx increases significantly (from an average of 2 to an average of 6) (Hoeppner et al 2001, Reddien et al 2001). Blocking engulfment in animals in which CED-3 Caspase activity is compromised therefore increases by three-fold the number of cells that fail to die and therefore the severity of the cell-death defect of these animals. 4D microscopy even revealed that in these double mutants, some of the 131 cell programmed to die start to undergo morphological changes characteristic of apoptotic cells, but then recover and survive (Hoeppner et al 2001). These results demonstrated for the first time that engulfing cells actively participate in the apoptotic process that takes place in the dying cells. How engulfing cells affect the apoptotic machinery (i.e. EGL-1, CED-9, CED-4 and CED-3) in the dying cell is still unclear. However, one might speculate that the CED-1, CED-6, CED-7 and/or CED-2, CED-5, CED-10, CED-12 pathways, which act in the engulfing cell to cause engulfment, are involved in this 'feedback' signaling. Considering the speed with which cells die and are engulfed during *C. elegans* development (cells often have acquired a typical apoptotic morphology and are fully engulfed by neighboring cells within 30 min after the completion of the cell division that generates them), it will be interesting to uncover the molecular mechanisms involved in this signaling event. In mammals, there is mounting evidence for complex signaling between apoptotic cells and their microenvironment and *vice versa* (Conradt 2002, Gregory & Pound 2011). It is therefore feasible that conserved mechanisms and pathways will be uncovered.

Your mother told you so

How do the 131 cells that reproducibly die during *C. elegans* development know that they are 'programmed to die'? Many of these 131 cells appear to be the product of an asymmetric cell division that gives rise to a larger daughter that is programmed to survive and a smaller daughter that is programmed to die (Sulston et al 1983). A number of mutants have been identified, in which some of these asymmetric cell divisions are affected and instead occur in a symmetric manner (Cordes et al 2006, Frank et al 2005, Hatzold & Conradt 2008, Singhvi et al 2011). In these cases, not only the sizes of the daughter cells but also their fates seem to be affected. This suggests that the decision of whether a cell is programmed to survive or die is made in the mother. For example, the NSM (neurosecretory motorneuron) neuroblast (NSMnb) divides to give rise to a larger daughter, the NSM, which survives and differentiates into a serotonergic motorneuron, and a smaller daughter, the NSM sister cell (NSMsc), which is programmed to die (Figure 3). Apoptosis in the smaller NSMsc is triggered through the transcriptional upregulation of the BH3-only gene *egl-1*, the most upstream component of the central apoptosis pathway (Thellmann et al 2003). In the larger NSM, the transcription of the *egl-1* gene is directly repressed through the Snail-related zinc finger transcription factor CES-1, which can be found in the NSM but not the NSMsc (Metzstein & Horvitz 1999, Thellmann et al 2003). Asymmetric *egl-1* transcription in the daughters of the

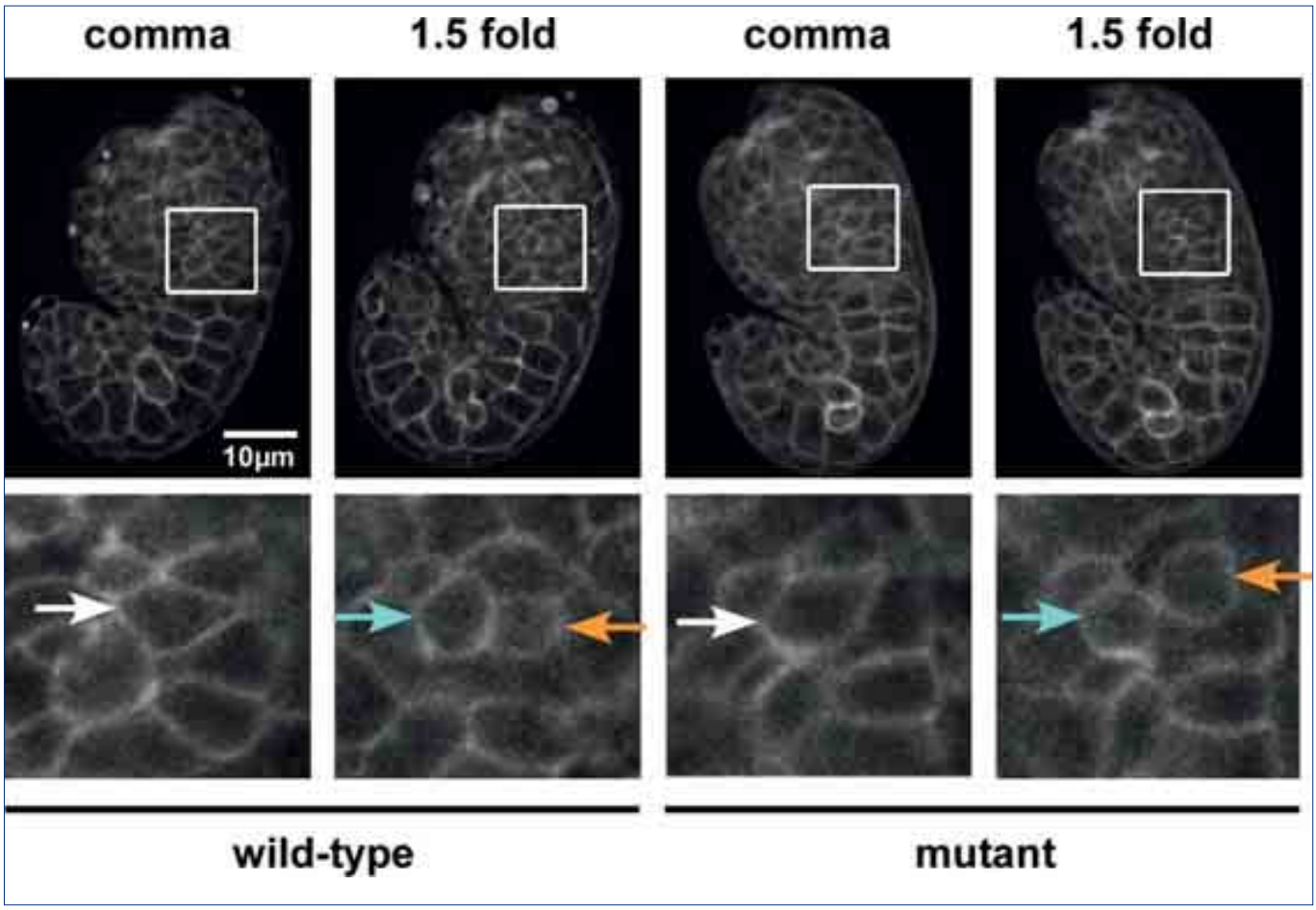

Figure 3 The division of the NSM neuroblast. In wild-type C. elegans embryos (left), the NSM neuroblast (white arrow) divides asymmetrically to give rise to two cells, the larger NSM (green arrow) and the smaller NSM sister cell (orange arrow). In some mutant backgrounds (right), the NSM neuroblast divides symmetrically to give rise to two daughter cells of similar sizes. Shown are embryos at the 'comma' and '1.5 fold' stage of embryonic development. The plasma-membranes of the embryos are labeled with a plasma-membrane targeted red fluorescent protein (RFP::PHPLC1). (Figure courtesy of Bo Yan)

NSMnb is therefore most likely the result of the asymmetric presence of the CES-1 protein in the larger NSM. Interestingly, mutations that result in higher levels of CES-1 in the NSMnb disrupt the polarity of the NSMnb and its ability to divide in an asymmetric manner (Hatzold & Conradt 2008) (Figure 3). Consequently, two daughter cells of similar sizes are generated that both contain CES-1 protein and that both survive. These results demonstrate that CES-1 also plays a role in the NSMnb and that this role is in the establishment and/or maintenance of cell polarity. This suggests that the transcription factor CES-1 normally not only acts in one of the daughter cells to directly block the central apoptosis machinery, but also in the mother to determine which of the daughters should die and which should survive. How CES-1 affects polarity in the NSMnb is currently unknown. Interestingly, the ability of Snail-related transcription factors to directly control the transcription of BH3-only genes is conserved in mammals. Specifically, the mammalian Snail-related protein Slug directly represses the transcription of the BH3-only gene *Puma* in the hematopoietic lineage (Wu et al 2005). Furthermore, Snail-related proteins in mammals have also been implicated in the function and maintenance of stem cells, which divide asymmetrically (Cobaleda et al 2007, Shojaei et al 2005). While a role in asymmetric cell division has not yet been reported for mammalian Snail-related proteins, it has been reported for the Snail-related proteins Snails, Escargot and Worniu of the fruitfly *Drosophila melanogaster* (Ashraf & Ip 2001, Cai et al 2001). For this reason, we speculate that Snail-related transcription factors may play a critical role in stem cells and coordinate asymmetric cell division and the apoptotic fate of their daughter cells.

Mitochondria: yes or no?

In mammals, the release of cytochrome c from the mitochondrial IMS is a critical event that is required for the assembly

of the apoptosome and, hence, the activation of caspases. In *C. elegans*, however, the role of mitochondria in apoptosis is still controversial. *C. elegans* CED-4 lacks the domain that mediates the interaction between Apaf-1, the mammalian homolog of CED-4, and cytochrome c (Yuan & Horvitz 1992, Zou et al 1997). In addition, in *C. elegans*, it is currently not known whether any of the cytochrome c proteins (unlike mammals, *C. elegans* has two cytochrome c genes, *cyc-2.1* and *cyc-2.2* (Vincelli et al 2013)) are released from the IMS during apoptosis. There is, however, evidence that the outer mitochondrial membrane (OMM) in *C. elegans* does become permeable to certain factors during a late stage of the apoptotic process. It has been shown that the *C. elegans* homolog of mammalian AIF, WAH-1 (WAH, worm AIF homolog), can be released from the IMS in an *egl-1*- and *ced-3*-dependent manner (Wang et al 2002). Furthermore, as in mammals, *C. elegans* members of the Bcl-2 superfamily localize predominantly to mitochondria. The anti-apoptotic Bcl-2-like CED-9 protein is associated with the OMM, where it interacts with CED-4, thereby preventing the formation of the apoptosome (Chen et al 2000). In cells that are programmed to die, the BH3-only gene *egl-1* is transcriptionally upregulated (Nehme & Conradt 2008). The resulting EGL-1 protein binds to CED-9 on the OMM (Conradt, B; unpublished), which induces a dramatic conformational change within the CED-9 protein. This conformational change results in the release of CED-4 from CED-9 and the subsequent assembly of the apoptosome (Yan et al 2004). The EGL-1 protein therefore associates with the OMM in a CED-9-dependent manner. Interestingly, the association of EGL-1 with CED-9 in apoptotic cells not only results in the release of CED-4 from CED-9, but also causes mitochondrial fragmentation (Jagasia et al 2005). This fragmentation is induced by *egl-1* and it is dependent on ced-9 as well as the gene *drp-1* (drp, dynamin-related protein). *drp-1* is required for mitochondrial fission and encodes the *C. elegans* homolog of the dynamin-related GTPase Drp1 (Labrousse et al 1999). Reducing *drp-1* activity in *C. elegans* prevents mitochondrial fragmentation and enhances the cell-death defect of weak *ced-3* loss-of-function mutants. Conversely, inducing mitochondrial fragmentation by *drp-1* over-expression causes ectopic cell death, i.e. cells that are programmed to survive now die (Jagasia et al 2005). The mechanism through which DRP-1-mediated mitochondrial fragmentation causes apoptosis is currently unknown. However, *drp-1*-induced ectopic apoptosis can be blocked by mutations in *egl-1*, ced-9, *ced-4* and *ced-3*, which suggests that DRP-1-mediated mitochondrial fragmentation promotes the activity of the central cell death machinery. As mentioned above, mitochondrial fragmentation in cells programmed to die is not only dependent on the pro-apoptotic BH3-only gene *egl-1* and the mitochondrial fission gene *drp-1* but also on the Bcl-2-like gene ced-9, which normally acts to block apoptosis. This suggests that in cells that are programmed to die (i.e. cells in which the transcription of the *egl-1* gene is upregulated and the EGL-1 protein is bound to CED-9), ced-9 acquires a pro-apoptotic function. Indeed, genetic studies support this notion (Hengartner & Horvitz 1994a). Specifically, the loss of ced-9 function increases the number of extra cells in the anterior pharynx of weak *ced-3* loss-of-function mutants from an average of 2 to an average of 6-8 cells. This demonstrates that the loss of ced-9 function in cells that are programmed to die makes these cells less likely to die. We speculate that the CED-9-EGL-1 protein complex directly activates DRP-1-mediated mitochondrial fission, which is supported by the finding that CED-9-EGL-1 can directly interact with the DRP-1 protein and act as a receptor for DRP-1 on the OMM (Lu et al 2011). Mitochondrial fragmentation is also an early event during the apoptotic process in mammalian cells and in *D. melanogaster* (Abdelwahid et al 2007, Frank et al 2001, James & Martinou 2008). Furthermore, in mammals, there is evidence that mitochondrial fragmentation promotes the ability of pro-apoptotic multidomain Bcl-2 proteins (Bax in particular) to mediate cytochrome c release from the IMS (Martinou & Youle 2011, Montessuit et al 2010). How mitochondrial fragmentation promotes the activation of the central apoptosis machinery in *C. elegans* and *D. melanogaster* remains to be elucidated.

Concluding remarks

Research on apoptosis in *C. elegans* continues to teach us about the intricacies of this process. Systems approaches on the one hand and quantitative analysis of single cells on the other hand, represent new opportunities for apoptosis research in *C. elegans* in the future. New challenges are the realization that many of the factors that were thought to specifically function in apoptosis have recently been shown to have non-apoptotic functions as well. Just to give a few examples, EGL-1 and CED-9 function in mitochondrial dynamics not only in apoptotic cells but also non-apoptotic cells, while CED-3 and CED-4 have been implicated in neuronal regeneration (Lu et al 2011, Pinan-Lucarre et al 2012, Rolland et al 2009). Although this makes the analysis of these factors increasingly challenging, it also highlights the degree to which the subcellular components of apoptosis intersect with the everyday life of a typical cell.

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Nadin Memar (right) joined the Conradt lab in 2011 as a postdoctoral fellow. Before moving to the LMU, she studied Biology in Kiel as well as Braunschweig and obtained her PhD from the Technical University in Braunschweig, where she worked with Ralf Schnabel on the role of cell focusing in pattern formation in the C. elegans embryo.

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Back to the Future of Developmental Biology

Joachim Wittbrodt

Developmental biologists have always been trying to delineate the entire fate of indiviual cells, from the early blastomeres to the adult organism. The aim was to understand the origin of organs, tissues and cell types, to identify key steps in their determination and eventually the signals that trigger fate decisions.
In the past, this attempt was facing many technical obstacles that are now gradually overcome. While first fate maps were established by labeling individual cells with ink from the outside, these days individual cells and all of their descendants can be permanently labelled by genetically encoded fluorescent proteins. Since organismal development is a highly dynamic process, not just with respect to the coordination of gene ex-pression, but eventually also with respect to cell motility and behavior it is mandatory to not only label individual cells, but also follow them individually throughout development (and beyond). There are several approaches to this, a steadily growing number of dif-ferent genetically encoded labels that barcode individual cells and allow to trace back identical barcodes to their common origin. An almost science fiction alternative would be to follow all cells by means of microscopy or a combination of genetic and microscopy tracking approaches for the analysis of cells lineages over weeks or months.
This was made possible by a quantum leap in live imaging. Confocal microscopy was initially applied to single cultured cells, often on fixed material. The demand to see and follow cells in their natural environment, drove the development of new technologies. Light sheet microscopy now allows to in vivo image entire transparent embryos and organisms (e. g. flies or fish) with subnuclear resolution over extended periods of time with limited photobleaching and toxicity. For smaller fields of view and shorter time periods modern multiphoton microscopes with highly sensitive detectors facilitate similar approaches in a more conventional setup.
Thus new technology (imaging, genetics, fluorescent labels) allows to now tackle the principal questions of developmental biology, labeling, watching and understanding without interfering with the process per se.

There are questions remaining:
How do we know, we do not interfere by watching? This is almost a philosophical question and obviously we always interfere. It is the degree of interference that could be massively reduced over the last decades. But it is also clear that there will be no analy-sis without minimal interference, even if we could image "label-free".
Can we get mechanistic insights by watching? Yes, of course, if one chooses the proper approach to get the full picture. Seeing and following all cells during organogenesis will present the

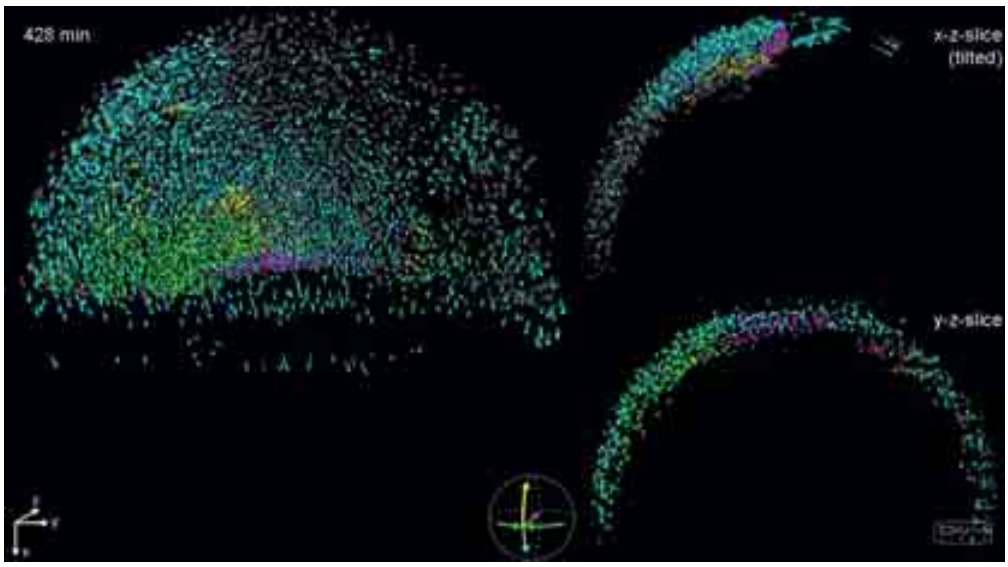

Figure 1: (Cellular choreography of early gastrulation in Zebrafish. Individual cells imaged by DLS Microscopy of have been color coded according to their migratory behavior. Entire dataset was used to visualize onset of gastrulation from different perspectives as indicated. Keller PJ*, Schmidt AD, Wittbrodt J*, Stelzer EH. (2008). Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy. Science 322: 1065-1069.

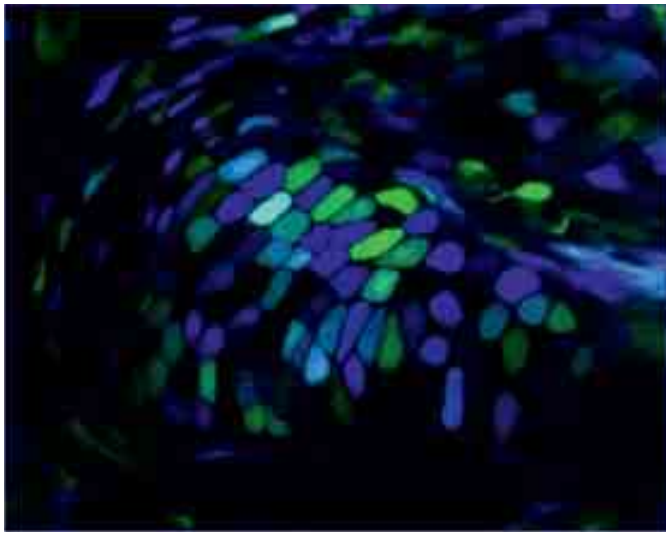

Figure 2: Stochastic barcoding of individual cells with permanent fluorescent labels. Individual cells have been stochastically labeled to generate unambiguous permanent fluorescent barcodes that can be followed throughout life. (Gaudi labelling, Centanin and Wittbrodt, unpublished, courtesy of L. Centanin).

cellular choreography that governs the process, a mechanism that can only be uncovered by watching, just like in the theater (Figure 1)

Other than in the theater, additional attempts can be made to manipulate individual cells while imaging to interfere with the cellular choreography. And once - in the not too far future - we have understood key mechanisms of development - we will be testing our insight by rewriting the choreography of developmental processes.

Behavior of the individual in a population

With the stage set like this we can now engage into a virtual experiment: Let's assume we want to follow the organogenesis of neural crest derived tissues and organs. And to identify the origin of cells that contribute to it. We would have all the nuclei of the embryo labelled with a genetically encoded fluorescent marker and image the entire development up to the completion of embryogenesis. The answer to our questions now resides within in the huge image dataset and "only" requires to be extracted from there. So we have shifted the problem from *in vivo* to *in silico*. And this is one of the new and reoccurring aspects of future biology. It will be multidisciplinary and physics, mathematics and computational sciences will be key to our future progress. Let's assume that image analysis and computational biology have solved our problem and we can now indeed track all cells of the crest derived tissues in silico. With this new information we can develop new hypotheses and these hypotheses will need to be addressed experimentally.

To do so it will be necessary to manipulate the genetic program of individual cells. To activate or inactivate signal transduction pathways, to interfere with direct cell-cell communication or with physical parameters like cell tension and its communication.

Stochastic labeling of individual cells with different colors is achieved by sophisticated genetic approaches (e. g. brainbow, confetti, Gaudí, Figure 2). Upon the activation of Cre recombinase individually lox-P flanked cassettes encoding different fluorescent proteins are randomly flipped or excised to eventually result in the stochastic expression of one particular color. In case cassettes with four different colors are uniquely placed to the genome, the limited activation of Cre recombinase will result in a stochastic distribution of these four colors in individual cells. Assume that color is just a proxy for function (e. g. by T2A fusion of three different genes of interest to three different fluorescent reporters) this approach will deliver a stochastic gain (or loss) of function in individual cells fully controlled by Cre recombinase.

If this approach is now combined with imaging, hypotheses can be precisely tested in individual cells in their natural environment that acutely alter their genetic program. This is clearly one of the ideal scenarios for developmental biology and is highly reminiscent of a natural situation that as such obviously is less appreciated: cancer.

The entire setup established above is fully applicable to follow cells that eventually will turn malignant. Inducible cancer models have been developed and there are examples where the activation of a single gene is a reliable trigger for tumorigenesis. Coming back to our example of the neural crest, one could imagine applying such a system to uncover the "soft spot" in the lineage of cells that leads neural crest derived organs. Are particular stages in the crest lineage particularly receptive and prone to transformation, in vivo, in the adult organism? And since melanomas formed are highly metastatic, clonal labeling and analysis in vivo will eventually allow to address the origin of metastases, just like the origin of tissues and organs in the embryo.

The application of stochastic single cell labeling opens a wide field of potential applications that in the context of metastases will facilitate to trace back their origin. It will ulti-mately pave the way for the analysis of single cell genomes/epigenomes of metastatic cells with the aim to identify genetic and epigenetic hallmarks of metastasis in the individual cell with control over the stages of metastasis. Color markers in the brainbow cassette localized to the nuclear membrane will barcode individual nuclei with a specific and unambiguous color spectrum and will thus facilitate the specific recovery of individual nuclei for downstream analyses.

The example of metastatic cells exiting the primary tumor already touches upon the individuality of cells - and how being different impacts on their decisions. Cancer in that context is an

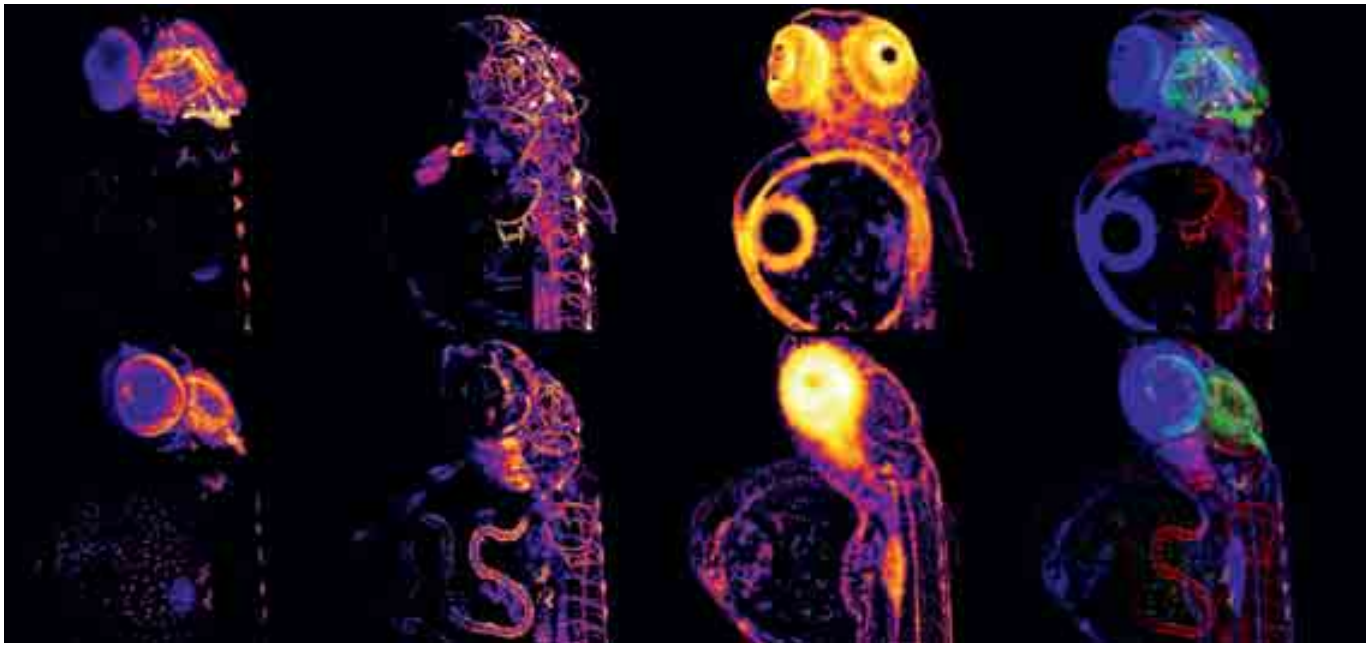

Figure 3: Artistic representation of a multisensor organism. Young fish with parallel sensors for blood vessels, individual axons and specific neuronal domains are represented in false colours (courtesy of L. Centanin).

extreme case of development and the application of the toolbox to other key steps in development will ultimately further our understanding of how a pool of cells develops into tissues, organs and organisms that interact with and respond to their environment. This is the other key aspect and challenge of future developmental biology: to understand the response or an organism across all scales, from the molecule through the cell up to the organism in its complex environment and if you wish beyond that in the interaction with other organisms of the same of different species.

Functional pathway analysis in vivo

To get a closer look at that aspect of development we need to shift our perspective more towards global. Instead of following individual cells of sub-cellular components in the organismal context, rather the decisive processes per se be visualized and correlated with actual morphogenetic decisions in development, growth and regeneration.

Genetically encoded in vivo sensors for most developmentally relevant signaling pathways have been developed and successfully applied in cellular paradigms. Now, in the developmental and organismal context, the correlation of overlapping pathway activity with developmental decisions by in toto imaging will identify new target areas for future research: organizers, stem cell niches, growth and patterning zones that are tagged by overlappingly active signaling pathways. In situ hybridization has pioneered that aspect, but the new tools will not only be relevant for highlighting developmental hot spots but also for studying their response to functional interference. How does the modulation of loss of specific signaling activity impact on the others and ultimately determines the developmental readout? The combination of these sensors with targeted single cell modifications described above will facilitate to zoom in and out depending on the scale of the question to be addressed. To that end the simple ideas presented above will need to be extended by tools that ultimately allow acute knock-down of protein function to minimize the lag time between experimental interference and functional readout to highlight causality rather than coincidence.

Another challenge resides on the level of the organs and there it is obviously the brain that will be most attractive. First attempts have been made to look at the brain while functioning. Genetically encoded calcium sensors combined with in toto microscopy will give a first glimpse on the representation of reality in the most complex of our organs and for sure none of the tools established so far will be sufficient to get a complete picture. However there is a clear perspective and with the rapid advance we see in technology this will be reality in a not to far future. As discussed for early development, stem cells or cancer, the manipulation of individual cells will also here be key to successfully decoding complexity and thus to understanding the basic principles. Optogenetic approaches are used to functionally interfere with neuronal activity and will be applied systematically to identify neuronal networks and query their function. The incredible complexity of the organ massively complicates a decoding of the vertebrate brain, not even by highly parallel approaches. The challenge will be to coordinate and integrate approaches and their results in order to establish a draft of brain function.

Organismal development has evolved and is ideally adapted to the environmental conditions. There are however several degrees of freedom that ensure robustness of the process, compensating and responding to environmental conditions. With a focus on compensatory mechanisms on the level of the individual embryo in response to environmental cues, pathway sensors will address the impact of e. g. oxygen levels, temperature and light/dark cycles that are deeply anchored in the evolution of development.
Nutrition apparently has the most obvious impact on development. Strikingly development will even under starving conditions in most cases still result in a properly proportioned embryo, maybe slightly smaller. This regulation requires complex feedback loops to coordinate overall and specific organ growth and again global pathway sensing and local interference will allow to address the regulatory logic by specifically uncoupling them. An additional class of sensors will be critically contributing to this key aspect of development and growth, namely metabolites. While metabolite analysis is routinely performed for total tissue and organ analysis the development of metabolite sensors is just at the beginning. A number of them have already been shown give an in vivo readout, in particular pH sensors, oxygen sensors or specific sugar sensors. The establishment of multi-sensor organisms (Figure 3) will allow integrating multiple signals and to establish a temporo-spatial correlation to represent functional dependencies. These are testable with the specifically targeted tools described above to eventually present a functionally validated flowchart of development, including the input of the environment.
The implications of this approach directly link developmental analysis with therapeutical applications and pharmacogenetics since the impact of drugs can be immediately monitored and assigned to potentially complex combinations of specific pathways imaged in parallel. That way the multisensor organism represents a universal tool linking development and physiology under conditions of specific drug treatments.

The perspective: in silico biology

Admittedly all this reminds of science fiction but strikingly most of the tools are already in place. Why has this not yet massively impacted? It probably has but we have not realized it since the missing link still is an efficient data integration.
Even in a multi-sensor organism the number of sensors is limited by the available number of non-interfering fluorophores. So for a comprehensive picture clearly data from different experiments need to be integrated. This poses an additional challenge besides the huge amount of imaging data representing the embryo over extended periods of time.
As above, we have once again shifted the problem to the "in silico" side. In my perspective the incorporation of "in silico" biology will be vital for the progress of developmental biology in particular and biology in general. The digital representation of embryos can be efficiently "screened" by computational approaches to establish few hypotheses to be tested in vivo. That way the in silico representation will indeed reduce the number of experiments and present targeted hypotheses. So is the future of developmental biology residing in silico? For sure not, but it will rather be the incorporation of interdisciplinary approaches that will be vital for establishing a comprehensive model of development that can actually be tested on the fly.

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CURRICULUM VITAE

since 2012	full focus on COS Heidelberg
2010	founding and managing director of COS Heidelberg
2007 - 2012	at University of Heidelberg and KIT
2006	Offer for full Professorship at the University of Heidelberg, (accepted)
2006	Offer for Directorship at the Research Center Karlsruhe, (accepted)
2005	Offer for full Professorship at the University of Geneva, Switzerland (declined)
1999 - 2007	Group leader at the European Molecular Biology Laboratory, EMBL Heidelberg, Developmental Biology Unit and Cell Biology and Biophysics Unit
1998	Habilitation in Developmental and Cell Biology, University of Braunschweig
1995 - 1998	Junior Group Leader, SFB 271, MPI for Biophysical Chemistry, Göttingen
1991 - 1994	PostDoc, Biocentre, University of Basle, Basle, Switzerland
1988 - 1990	PhD, Max Planck Institute for Biochemistry, Martinsried
1986 - 1987	Diploma Thesis, Max Planck Institute for Biochemistry, Martinsried
1982 - 1985	Study of Biology, University of Munich

COORDINATING FUNCTIONS

2010 – present	Founding and managing director of the newly established Centre for Organismal Studies, COS, Heidelberg
2010 – present	Member of the executive committee of the SFB 873 "Maintenance and differentiation of stem cells in development and disease"
2007 – 2011	Chairman of the SFB 488 "Molecular and cellular bases of neural Development"
2006 – 2009	Member of the Executive Committee of the Network of Excellence "MAIN"
2002 – 2004	Coordinator of the HFSP grant "Lens-retina interactions"
2001 – present	Founding Member of the Executive Committee of the Medaka Genome Initiative

HONORS

2012	Special price of the University of Heidelberg for exceptional research
2011	ERC advanced grant of the European Commission
2011	Lautenschläger Award at the University of Heidelberg
2009	HMLS award of the Heidelberg Molecular Life Sciences panel
2009	Otto Mangold award of the German Society for Developmental Biology

Fifty Years in Science

The remarkable career of Klaus Weber, a pioneer of the golden age of molecular cell biology

Volker Gerke, Thomas Magin and Manfred Schliwa

Blazing a trail describes the process of exploring new territories in such a way that others can follow, marking the path by leaving blazes on trees. That's how our forebears characterized a person who was bold enough to wander into unexplored ranges. Modern trailblazers are innovators, pioneers, masterminds, originators; they open up a new line of technology or art or, for that matter, research. One of the trailblazers who helped shape the fledgling field of molecular cell biology is Klaus Weber. In his scientific personality he combines a reductionist as well as a holistic reasoner, a technical innovator as well as a meticulous experimentalist, a thinker as well as a down-to-earth practitioner, a traditionalist as well as a visionary with a keen eye for emerging innovations. To many, he has been (and still is) a mentor, advisor, critic, and partner. His research and that of his group cleared the trail that led to modern cell biology, and many of us followed him.

After receiving a PhD from the University of Freiburg, his first personal trail (without blazes) led him to the United States to work with Jim Watson. Important contributions to bacteriophage biochemistry soon earned him a professorship at Harvard University. Eventually, the lure of the Max Planck Society was bigger than a tenured professorship at Harvard, so Klaus and his then already closest collaborator, Mary Osborn, moved to the Max Planck Institute in Göttingen where he remained a director for 30 years to come.

The work on protein biosynthesis mechanisms in bacteriophages marked only the beginning of a remarkable scientific career. Almost *en passant*, so it seems in retrospect, the Weber/Osborn team revolutionized protein biochemistry by establishing the reliability of SDS-PAGE for the determination of the molecular weight of proteins. Then, a stay at the Cold Spring Harbor Laboratory initiated a major shift in Klaus' scientific interest away from straight protein biochemistry. The desire to study not only bacterial but also eukaryotic viruses led to work with cultured cells. Initially a sideline, this work, in combination with the use of specific antibodies, essentially established a whole new field. The 1974 paper with Elias Lazarides on actin immunofluorescence was the snowball that caused an avalanche. Microtubules followed promptly, though the reality of these exceedingly long, often breathtakingly curvy "white lines" seen by immunofluorescence met with considerable resistance by some electron microscopists. The dispute was laid to rest by correlative light- and electron microscopy. In these early days of immunofluorescence microscopy, the phrase "Don't bleach it!" was the one most often heard in the Weber lab when someone was discovering yet another beautiful novel staining pattern in the microscope. Yes, there was no antifading agent at the time, and even the most breathtaking patterns faded into oblivion within seconds.

Klaus Weber in the psychedelic tie era

Actin and tubulin were the first two antigens used in immunofluorescence microscopy to unravel what Klaus at the time called the *molecular anatomy of cells*. A third class of fibers, intermediate filaments (IF), followed on the heels. Many of the fundamental properties of the building blocks of these odd yet seemingly omnipresent fibers, including their sequence, subunit and domain structure, assembly, diversity, evolution and func-

tion, were either entirely unknown or highly controversial at the time. It is not an overstatement to say that Klaus' lab paved the way and settled many issues, some in collaboration with another IF aficionado, Werner Franke. Work in Göttingen established the antiparallel coiled-coil tetramer as the functional building block of IF proteins and, based on rigorous amino acid sequencing of desmin, established the basic structural model of all IF proteins. With Mechthild Hatzfeld, Klaus then solved a long-standing keratin enigma: based on genetically engineered, unique Cys-residues in a type I and type II keratin, they demonstrated the building block of all keratins to be a heterodimer. Further, independently of Inagaki in Nagoya, he provided the first evidence for IF phosphorylation as a regulatory principle.

According to Dobzhanski, nothing in biology makes sense except in the light of evolution, and IFs are captivating and mysterious enough to take a closer look at their natural history. So Klaus embarked on a journey into the oddities of invertebrate IFs. Already in his first paper on nematodes he hypothesized that these proteins may occur in most invertebrates, probably among the lowest metazoa, and possibly even in some unicellular eukaryotes. Recent work provides strong support for this prediction.

Cell biology is a technology-driven field, and novel molecular techniques helped boost the research also on IF function. One of these revolutionary new tools was RNAi. Karabinos and Weber were the first to provide genetic evidence for essential functions of IF proteins in *C. elegans* development using RNAi. A spectacularly successful tool for the study of gene function in invertebrates, RNAi had been utterly unsuccessful in mammalian cells – until Thomas Tuschl appeared on the scene. He teamed up with Klaus and the two of them paved the way for the use of 21mer RNAs, today known as siRNAs, to the study gene function in mammalian cells.

Being a biochemist by training, Klaus never failed to prove that in addition to all that wonderful cell biological work, biochemistry truly was his *métier*. His ability to purify and sequence proteins and analyze their biochemical properties with the cellular background in mind was a key to solving a number of fundamental questions in modern cell biology. This is evident in his prolific work on intermediate filament proteins, but also seen elsewhere. Working together with Joel Vandekerckhove, Klaus established actin fundamentals with regard to its biochemistry, sequence and evolution, not being afraid of "odd" species like *Physarum*. In other groundbreaking and state-of-the-art work Klaus and Anthony Bretscher, his postdoc at that time, identified and characterized proteins regulating actin dynamics, focusing on the intestinal brush border as an ideal starting material for the purification of these proteins. Klaus also applied his exceptional protein chemical knowledge to other elements of the cytoskeleton. Together with Jürgen Wehland he purified the tubulin-tyrosine ligase and characterized its structure and enzymatic properties, and he was also the first to identify the unique repeat structure in annexins by direct protein sequencing. Although only being buoys in a sea of important discoveries, these findings illustrate Klaus' seemingly endless interest in all aspects of the cytoskeleton and his unique approaches combining the art of biochemistry with the beauty of cell biology.

To pay tribute to the man and his science, here are the thoughts and reminiscences of some of his friends, companions, colleagues, and advisees. Jim Watson once said that "biology has at least 50 more interesting years". So, Klaus, 50 more years, please!

Joan A. Steitz

Sometime during my third year as a graduate student at Harvard (1963 – 1967), a dapper young German scientist appeared on the scene in the Watson-Gilbert lab. Klaus Weber was special because he knew about PROTEINS, whereas everyone else in the lab was being trained in the then nascent arts of molecular biology. He inhabited a room around the corner in another wing of the Biolabs that housed a huge instrument, an amino acid analyzer. From that time on, Klaus was to be a very special friend and of critical importance to the course of my science at several points during my career.

In 1966, Klaus was an essential cog in Jim Watson's master plan to decipher the molecular mechanism of protein biosynthesis. At that time, the cellular components involved in initiation and termination of the polypeptide chain, as well as the mechanism of suppression of nonsense codons, were being actively pursued by graduate students Mario Cappechi, Gary Gussin and Jerry Adams in the lab. The messenger RNA used to program *E. coli in vitro* systems was the RNA genome of bacteriophage R17. R17, obtained from Walter Paranchych, was closely related to the f2 phage being worked on in the lab of Norton Zinder, then viewed as our Big Competitor at the Rockefeller Institute. Since the major product of *in vitro* protein synthesis programmed by R17 RNA was the phage coat protein, knowing the sequence of its 139-long polypeptide chain was clearly essential. That was Klaus's assigned mission as a Research Fellow. Through collaboration with Bill Konigsberg, a protein chemist who had recently joined the Biochemistry Department at Yale's Medical School, and with the Zinder lab (competition set aside), Klaus was instrumental in sequencing the tryptic peptides from the f2 bacteriophage coat protein (1966) and later ordered them to complete the f2 coat protein sequence (1966). Klaus then quickly sequenced the tryptic peptides from the RI7 coat protein, allowing deduction of its amino acid sequence (1967). Years later, I would find in the sign-up booklet for the communally-used Beckman ultracentrifuge in the C-Wing of Sterling Hall of Medicine at Yale, Klaus's signature, a relic of his time spent at Yale with Bill Konigsberg during the analysis of the phage coat proteins.

Klaus was also critical to the completion of my thesis. Students in the Watson lab were rarely assigned a specific thesis project. Rather, they "found" one by trying out suggestions of experiments that sounded interesting to various members of the lab. After 5 or 6 failed projects during my second year as a graduate

student, I landed on a successful enterprise – studying the minor protein in the capsid of the R17 phage. I found that lack of the A protein (caused by nonsense mutants in the R17 A gene) resulted in the generation of non-infectious phage dubbed "defective particles". These particles appeared to possess a full complement of the major coat protein and resembled wild-type bacteriophage when viewed in the EM. However, the encapsulated RNA genome was not protected from the action of ribonucleases and the resulting phage were noninfectious. My next goal was to isolate the R17 A protein and characterize its function in phage assembly. Without Klaus's expert advice on protein purification, I never would have succeeded in obtaining semi-pure preparations of the A protein. Subsequently, Jeff Roberts (a Gilbert grad student) and I were able to demonstrate that reconstitution of R17 phage from isolated RNA and coat protein produced infectious phage if A protein were also added (1967). This feat garnered mention in the *New York Times* for Jim Watson as the first test-tube assembly of an infectious bacteriophage.

Shortly before my husband Tom Steitz and I left Cambridge, Massachusetts, for postdoctoral work in Cambridge, England (November, 1967), Jim moved a new postdoc onto the bench opposite mine in the small lab that adjoined his office. She was Mary Osborn – an English undergraduate physicist who had just completed her PhD working on T phage with Stan Person at Penn State. Together, she and Klaus were to publish one of the most highly cited methodology papers in molecular biology, establishing the reliability of determining protein molecular weights by SDS polyacrylamide gel electrophoresis (1969). I recall using tube gels containing SDS (according to a procedure from Jake Maizel's lab, reported in 1967) to examine the purity of the R17 A protein preparations I had isolated. But that was well before Klaus and Mary carried out the systematic analysis of dozens of proteins that validated what was to become – and still is – the method of choice for determining polypeptide molecular weights.

Near the end of our three-year postdoctoral stint at the MRC Laboratory of Molecular Biology in Cambridge, England, Mary joined that lab as a staff scientist. Meanwhile, we had begun playing bridge on evenings and weekends (when we weren't in the lab) with Chris and Jeff Roberts and Mark Bretscher. Mary was a natural fit with this group, her mother being a bridge devotee. During games, we would often be admonished by Mary: "That would not happen in my mother's proper British bridge club." Occasionally, Klaus showed up in Cambridge and whisked Mary away for the weekend. We were not surprised when they married in July, 1972.

Tom and I joined the Yale faculty as assistant professors in the Department of Molecular Biophysics and Biochemistry near the end of 1970. Meanwhile, Klaus had become faculty at Harvard and was involved in multiple seminal discoveries that cast light on the control of gene expression in bacterial cells. Because of the overlap in our interests (I was studying the initiation of protein synthesis, as well as host cofactors for RNA bacteriophage replication, in my new lab at Yale) the projects in Klaus's lab that most impressed and intrigued me were: Alan Weiner's finding of natural readthrough at the single translation termination codon of the Qß coat protein gene (1971), Tom Blumenthal's discovery of the host protein synthesis factors EF Tu and EF Ts as components of the phage Qß replicase (1972), Bill Haseltine's discovery of the production of Magic Spot on the ribosome (1972), and the unanticipated use of restart AUG codons during translation of the lac repressor by Terry Platt and Jeff Miller (1972). Alongside, Klaus continued to pursue his careful dissection of aspartate transcarbamylase, establishing its hexameric nature and the critical role of conformational changes upon ligand binding.

During the early 1970s, Tom and I took joint vacations to warm places with Klaus and Mary. The first was a Christmas trip to the Mexican island of Cozumel, where we stayed in ground-level rooms at the famous El Presidente Hotel (for almost nothing, as it had not yet become a popular destination). After several days on the beach (including snorkeling to see amazing tropical fish on the coral reels), we toured the Yucatan, seeing the famous ruins in Uxmal and spending a day in the capital Merida. Another trip, taken together with Mark Bretscher and his new girlfriend from Cambridge, Barbara Pearse, took us to a tiny Caribbean island called San Andrés – memorable for its panoramic beaches on Johnny Cay and for its many mangy three-legged dogs. Later we visited the rain forests of Costa Rica and toured past the high-fenced estates of various notorious "entrepreneurs", who had taken refuge from US authorities by relocating to Costa Rica.

In 1975, Klaus was offered a Director position at the Max Planck Institute for Biophysical Chemistry in Göttingen and moved there with Mary to establish a new lab focused on the cytoskeleton and its constituent proteins, using not only immunofluorescence but also biochemical and protein chemical techniques. Tom and I were eligible for a sabbatical leave in 1976-77 and decided to spend the first portion of the year (September – May) in Gottingen. While I had focused on the initiation of protein synthesis and other aspects of RNA structure and function in bacteria during my first six faculty years, I decided to jump on the then-growing bandwagon of molecular biologists shifting to the study of RNA in mammalian cells. I was intrigued by the rampant RNA degradation documented for mammalian cell nuclei, with only a tiny fraction (about 10%) surviving to be exported to the cytoplasm as mRNA. (Recall that split genes and splicing were discovered only in 1977.) I thought that perhaps the newly-discovered hnRNP proteins that coat nascent transcripts might be responsible for deciding which parts of the RNA exit to the cytoplasm and which remain behind for turnover. I reasoned that antibodies to hnRNP proteins might be valuable tools for analyzing the situation, and wanted to tap into Klaus's experience in making antibodies to highly conserved proteins. It appeared to be the perfect sabbatical project.

The first step was to isolate hnRNP proteins. Published protocols involved starting with fresh rat liver, making cell extracts and

Robert E. Webster

Klaus and I first met in the mid 60's. He was a postdoctoral fellow with Jim Watson and working on the sequence of the coat protein of the single-stranded RNA phage. I was a postdoctoral fellow with Norton Zinder using the RNA from the same phage to synthesize the coat protein using an in vitro protein synthesis system. It was our common interest in the sequence of the coat protein that got us together. Klaus went on to become a Professor at Harvard and I migrated to Duke University.

We would get together a number of times since then. My favorite was when we met at Cold Spring Harbor. Mary would cook a fantastic meal and the three of us would talk science, politics and have a wonderful time. During one of those visits, Klaus was deciding whether to go back to Germany as a Director at a Max Planck Institute. I said if he did I would do a sabbatical with him. A few months after he went back I got a letter from Klaus saying he was glad I was coming and was I going to bring my big dog. I arrived with the family in June 1978 without the dog and had a wonderful year with Klaus, Mary, and other people in the lab such as Tony Bretscher, Jurgen Wehland and Joel Vandekerckhove. I worked on an analysis of the structure of microtubules in cells and sometimes gave a little help to Tony Bretscher with the isolation of microvilli.

After returning to Duke, Klaus and I kept in contact by telephone. Many times when I got to the laboratory after 8:30 AM, I'd have a message from Klaus saying I was late to work again. In 1997, my wife Beverlee suddenly died. I was devastated and Klaus said it was a good time to come for another sabbatical. I spent 1999 there and remember many evenings with Klaus and Mary eating, drinking wine, talking, laughing and having a great time. My future wife, Gay, visited in July of that year and after meeting Klaus declared that he was absolutely charming. We got married in 2001 and although Klaus was unable to come, Mary did and reported back to Klaus.

We have not been together since then but still keep in touch by telephone or E-mail via Mary. I've always felt it was an honor and privilege to have Klaus as a friend.

Joël Vandekerckhove

My first encounter with Klaus Weber was at the 1972 Cold Spring Harbor RNA-phage meeting. There I presented the amino acid sequence of the A-protein of bacteriophage MS2, a 393 amino acids long and highly insoluble protein, manually sequenced. My previous work was on the coat protein of the same coliphage. This was less successful because all what I discovered was already published by Klaus and his colleagues a few months earlier as the sequence of the coat protein of the bacteriophage R17. Only when my work was at a final stage, it was realized that MS2 and R17 were two different names for the same organism. Although this could be seen as a big step in the mud for my entrance in molecular biology, Klaus was still somehow convinced about my protein-chemical skills and invited me to join his group once he was installed at the Max-Planck Institute (MPI) in Göttingen.

In 1975 I sat down with Klaus and Mary Osborn on the terrace of a pub at what I later learned to be the highest point of Nikolausberg, the Göttingen suburb where the MPI was located. Afterwards I got convinced that this location was selected having some symbolic value for the science quality Klaus was expecting from me and his colleagues. I came up with the proposal for a six-month stay. Unfortunately, my naïve concept of post-doc timing was immediately crashed when Klaus proposed "Entweder zwei Jahre oder nichts". I agreed but, in the back of my mind I kept "die heimliche Idee" that after one year I would have generated so many data that "he would let me go"!

So in the middle of the record hot summer of 1976 my wife and I decided to move to Göttingen. I remember very well our first drive down from Bruges in Belgium to Göttingen (exactly 596 km) following the 51th degree of latitude eastwards. My friends in Belgium warned me I should drive very disciplined in Germany and pay particular attention to the speed limits. To me this meant driving like a snail which on the autobahn provoked the absolute irritation of hundreds of BMW's and Mercedeses. Being tired of the incessant light flashing in my neck, I started driving like a real German, an unrealistic imitation which at destination yielded me two tickets for... exaggerated speed. My wife and I considered this as a highly unfair act against an innocent Belgian couple that came with the intention "to boost" German science at the cost of the Belgian tax payer (because I was still a fellow at the Belgian Research Fund!). Fortunately this was my first and last contact with the German traffic police as I learned very soon that car driving discipline in Germany is written with a capital D.

I started with two projects: the colchicine binding site of tubulin and the molecular nature of the actin isoforms. "You better start with two than with one project" Klaus said, "because when one is crashing, you can still go on with the second". Indeed, after cleaning kilograms of pig brain and centrifuging extracts for hours and days, I decided that the colchicine binding site was taken too high. So I fell back on the second project: the actin heterogeneity. At that time I did not realize this project would fundamentally orient my future research. Experimentally the protein chemistry was also easier and I could fall back on the techniques that were developed earlier at the Ghent University. Taking into account that Belgian science was poorly supported in the late 70ies and as a consequence was done with quite some imagination, Klaus was speechless when I asked him to buy a sewing machine for my work. "Joël, you are at a decent Max-Planck lab and not in a salon de haute couture" Klaus replied. But I could convince him that this would make it easy to pass from one to the other dimension in two-dimensional paper peptide fingerprinting. So he accepted, although I could imagine that this unusual order would have generated somewhat eyebrow frowning at the "Verwaltung". Later I realized that this has been the most efficient machine investment in my entire

career. By using this simple but straightforward technology, we cleaned up the previous mess in the actin literature often originating from the use of complicated chromatographic techniques and we discovered and characterized six actins in warm blooded vertebrates (Vandekerckhove & Weber, 1978). These data have been confirmed by DNA sequencing and even survived the recent genomic tsunamis. The years 76-81 (I stayed all together 5 years instead of the intended two years) turned to be golden years for me and generated several important papers - papers which, in the summer period, were corrected and rewritten by Klaus at the Freibad Nikolausberg. It was frustrating to see my painfully composed manuscripts coming back the next day overloaded with corrections in red ink; frustrating also because that next morning I had to face a look full of compassion. "One day you will be able to write the perfect paper" Klaus said. I could never find out how the secretary was able to put all these upside and sideways written comments in the right order in the final text and this at a record rate of one manuscript version per day. The lab atmosphere was fantastic with many excellent scientist colleagues and visitors. Many of them later reached top positions in academia or industry and with most of them I still keep frequent contacts. In those days we were, so to speak, monopolizing the actin protein chemistry. I had the feeling of being part of a dynamic society making important discoveries and exploring new territories in cell biology. What a sea of difference with my previous status in Ghent, where I had to be satisfied with a pat on the back by my supervisor and where publishing was not the highest priority. One of the most puzzling consequences of our actin data was the question about the nature of these six actin forms. Their nearly identical sequences suggested they should be functionally redundant. But if this would be so, why was their expression so extremely conserved in tissues from mammals and birds? One of the most obvious experiments to perform in those days and being connected with the world leader in immunofluorescence techniques, was to generate antibodies that would be specific for each of the actin isoforms. More easily thought than done, given that the actins were extremely similar with the differentiating sequences predominantly clustered in the 18 first N-terminal residues. In addition actin was not an immunogenic molecule. So I tried the obvious thing: to synthesize the different actin N-termini and to use them as antigens. But it did not work in my hands. At that time I got quite frustrated because another postdoc in Klaus' lab was producing all kinds of highly specific antibodies against the different types of keratins forming the basis of the highly successful tumor typing approach. The Gods of Science were clearly not favorable for me. However other groups were later able to obtain highly specific antibodies; not for all isoactins but for some, enough to get to the first pictures of cellular actin isoform segregation with a special preference for ß-actin localization at the lamellipodia (Skalli, Gimona). The latter was in accordance with findings that ß-actin is synthesized at the leading edge of cells (1994). Although the sky around actins cleared to some extent, the isoactin question remained largely unsolved. In addition, as more papers were further published, more confusion was created.

What started as a straightforward hypothesis, i.e., trying to correlate the diffuse actin network appearance in transformed cells observed by indirect immunofluorescence with the molecular nature of new actin spots noticed in 2D-gels, ended up with the discovery of the actin isoforms. In the particular case, there was no correlation between the apparent lack of stress fibers and potential actin post translational modifications or actin isoform expression. However, later, using the same simple protein chemical tools, we have found actin mutants correlated with the transformed phenotype (1980), while post translational modifications, such as the ADP-ribosylation of Arg-177 in cytoplasmic actins by the *botulinum* C2 toxin were linked with F-actin dysfunction (1988).

Our work in Göttingen, using Whatman paper, a sewing machine, horribly stinking toluene/pyridine tanks to separate peptides that were ultimately analyzed by a daily repaired amino acid analyzer, is still alive and incites the same high interest as more than 30 years ago. Even Klaus would not have predicted that when we sat together at *Zum Klosterkrug* in Nikolausberg in 1975 having a "couple" of Göttinger Edelpils, this work for which he and Mary laid the fundaments, would turn into a project of all seasons.

Anthony Bretscher

I was very fortunate to initially meet Klaus Weber and Mary Osborn through their friendship with my brother Mark. Klaus helped me early on, and then shaped my entire career.

I first met Klaus and Mary in 1971 when they invited Mark to dinner - he asked if he could bring his little brother along, who had just started graduate school in Genetics at the University of Leeds. Since I had a degree in Physics and had never taken a biology course in my life, I was astonishingly naïve. Much of the conversation that night was about my bacterial genetics project, with Klaus offering suggestions and people who I could contact for help. I then met him on a number of other occasions (especially memorable was a party at Mark's house just before Mary and Klaus were married) and he generously invited me to contact him if I ever needed any scientific help. I took him up on this, and joined his lab in Goettingen in 1977 as a postdoc to work on the actin cytoskeleton. It was a very special time as I had the good fortune to overlap with many wonderful scientists, including Tom and Joan Steitz, Bob Webster, Joel Vandekerckhove and Jürgen Wehland. In addition, there was a constant flow of collaborators coming to use the new technique of immunofluorescence microscopy on their favorite samples.

To place our work in context, it was just eight years since Ishikawa et al. (1969) had first identified microfilaments by HMM labeling of cells, six years since Tilney and Mooseker (1971) had isolated actin from intestinal brush borders, and three years since Lazarides and Weber (1974) had first used actin antibody

to visualize microfilaments. Klaus (the protein biochemist) basically said to me (the geneticist): we have no idea what proteins organize actin filaments, so let's isolate a defined structure and determine the function of the new components biochemically. He selected the brush border of intestinal epithelial cells as it could be isolated intact, and highly ordered microfilaments make up the cores of the microvilli. The first year in the lab did not go so well - I was meant to be localizing proteins in the brush border and isolating non-muscle tropomyosin for the generation of antibodies. Klaus took me aside at the Christmas party and asked what my career goals were. I said I wanted a faculty job, and he sighed and lamented 'I thought so'. He was rightfully very frustrated with my progress, and so was I, partly because it was clear that isolated brush borders had too many cytoskeletal components to analyze easily. Then things turned around. First, I saw a micrograph showing the excellent preservation of the intestine of a person who committed suicide by drinking formaldehyde. I had been using isolated brush borders, but now tried filling a mouse intestine with formaldehyde, isolating the cells and doing immunofluorescence. It worked spectacularly well and rapidly led to the localization of muscle-related proteins in epithelial cells (1978). Second, sabbatical visitor Bob Webster from Duke University guided and encouraged me to develop a method for isolating microvilli, and their microfilament core bundles, which had a very simple protein composition. Klaus was very happy with these developments, which lead to the discovery and characterization of villin (1979, 1980) and fimbrin (1980), and has been the foundation of much of my subsequent independent work. By the time I left Göttingen for a faculty position in the US, Klaus and I were enjoying daily scientific discussions together, which I still miss. We have remained friends ever since.

Mathias Gautel

I was a young and green postdoc at EMBL Heidelberg working on the giant protein titin (or connectin). We had just started the biochemical and biophysical characterisation of the C-terminal end of titin, and the question was how this could be tied into a molecular view of M-band ultrastructure and an expanded protein interaction network. Klaus's reputation at EMBL approached something close to a hybrid between Einstein and Captain Flint, and he was spoken of almost in hushed tones. I was duly in trepidation when we first met. My colleague Siegfried Labeit and myself took the fast ICE train to Göttingen, and made our way up to the Olympic heights of the Nikolausberg, where Klaus resides. Klaus met us genially in the department's library, and we got immediately engaged in an in-depth discussion of sequence features, where Klaus revelled in making first-glance assignments of posttranslational modification sites and secondary structure properties. His joy in singing from the song-sheet of single-letter amino acid code was highly infectious, and so was Klaus' genial, witty and relaxed attitude. Our collaboration resulted in two seminal papers on M-band ultrastructure and interactions, but deepened considerably over the next years. When beginning to analyse the Z-disk region of titin, I noted unpredicted PCR products from muscle cDNAs, and sequencing revealed that they differed in discrete numbers of 45-residue segments. Were these just PCR artefacts, as some suggested? I thought not, especially as the number of these "Z-repeats" correlated tightly to the Z-disk thickness of the respective muscle, and seemed in a nutshell to fit the proposed molecular ruler function of titin. Klaus took one look at the sequence and agreed, "Of course it makes sense!" We corroborated the sequences, and mapping some of Dieter Fürst's titin Z-disk antibodies (who at that time worked with Klaus) ultrastructurally and on the primary sequence, it became clear that the Z-repeats had to be localised to the central Z-disk region where actin filaments are crosslinked by alpha-actinin. This became our next key titin paper, this time at the N-terminal end. By this time, my working relationship with Klaus had turned into one of complete trust and enjoyment of his razor-sharp wit- and equal sense of humour! Phone chats with Klaus became a regular highlight, even though I think he raised at least one eyebrow (fortunately invisibly so before the advent of Skype) when I named a new giant muscle protein obscurin. It seemed pretty obscure, binding to Z-disk titin but localising at both Z-disks and M-bands depending on developmental status! But Klaus declared serenely that this wouldn't be surprising if the protein would be peripheral to the sarcomere, as it ultimately turned out to be. Klaus continued to be inspiration and encouragement also after our move to London, and I hope we will share many more inspired discussions of proteins, papers, and politics.

Thomas Tuschl

Klaus Weber is a formidable scientist and mentor. I first met him in his department's library overlooking the forest surrounding the institute and giving a glance at the outlines of the city of Göttingen, of which the 19th century poet Heinrich Heine said, that it only reveals its beauty upon departure from it.

Klaus taught me the spirit of scientific discovery and further reminded me about Heinrich Heine's poetic advice, that we were not given two legs to stand still, or a stump would have sufficed. I arrived in Göttingen in fall 1999 and set up a junior laboratory at the MPI for biophysical chemistry interested in dissecting the biochemical mechanisms of double-stranded RNA (dsRNA) interference (RNAi), a newly discovered gene silencing mechanism. Klaus was already using RNAi in nematodes to silence his favored cytoskeletal genes, but called upon me when he faced a problem silencing a specific isoform that could only be targeted by a short dsRNA and it wasn't yet known if short dsRNAs could work. Though I believe I was of little help in this instance, he kept in touch with me calling me here and then, exchanging his observations regarding attempts to silence mammalian genes using the same approach working well in nematodes but only triggering innate immune responses and cell death in cultured human cells.

CELEBRATION OF A JUBILEE

Several months later and having progressed in biochemical analysis of the RNAi process, we understood that long dsRNA was processed in cells to intermediates of 21-nucleotide (nt) short RNAs, the small interfering RNAs (siRNAs), which were assembled in ribonucleoprotein complexes for targeting complementary mRNAs. These siRNAs, when provided in double-stranded form were very efficient triggers of RNAi in fruitfly embryo lysates and appeared to also work for silencing reporter genes in human cultured cells without triggering unspecific cell death responses. These findings were so exciting that one of my first things to do upon its discovery was to meet Klaus in his library, where he always could be found in the morning, drinking coffee and enclosed by a blue cloud of cigarette smoke, generally in a very good mood and very inquiring. I was hoping to win his support and raise his curiosity about targeting interesting human genes that may reveal interesting cellular phenotypes as one could hope to see by depleting cytoskeletal proteins. Klaus was rather skeptical that this could be so simple, but curious enough so that he recruited his senior postdoc, Jens Harborth, to the project. They selected a gene encoding two isoforms of the nuclear envelope lamin proteins, known as lamin A/C. We chemically synthesized siRNAs matching sequence corresponding to both isoforms, transfected the siRNAs into cultured cells, and 3 days later the lamin A/C gene was off based on immunofluorescence analysis, which Klaus didn't want to believe, until Jens replicated the experiment 7 times and we had been able to also silence 3 other genes. Thereafter he spent a considerable time on the telephone calling some of his science friends and presumably advised them on how to silence their favorite genes in human cells.

Over this important year of intense collaboration I learned from Klaus how to ask and address relevant scientific questions and how to effectively mentor postdocs by having them sit in a cloud of smoke while interrogating them about their experimental progress. Unfortunately, this kind of interrogation technique has now been outlawed at Rockefeller University in New York.

I still draw from these experiences and memory, and just like Klaus had developed necessary tools such as the SDS gel or immunofluorescence to advance his understanding of abundant and ubiquitous cytoskeletal proteins, his example in mind, I have been inspired to develop methods to characterize abundant and ubiquitous non-coding or coding RNA molecules and their localization and function inside cells and tissues.

This article was compiled and edited by VG TM MS

16th Joint Meeting "Signal Transduction – Receptors, Mediators and Genes" together with the DGZ study group

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The 16th Joint Meeting "Signal Transduction – Receptors, Mediators and Genes" took place in Weimar from November 5 to 7, 2012. It was once more jointly organized by the Signal Transduction Society (STS) and four signaling study groups of the German Societies for Cell Biology (DGZ) and for Immunology (DGfI) and the Society for Biochemistry and Molecular Biology (GBM). Other financial and scientific contributions were made by the EU network SYBILLA (Systems biology of T-cell activation in health and disease), the Pancreatic Cancer Consortium (PCC) Kiel and the DFG priority program SPP 1468 'Osteoimmunology - Immunobone'. As in previous years, the conference was organised by Frank Entschladen (Witten), Ottmar Janssen (Kiel), Karlheinz Friedrich (Jena) and Ralf Hass (Hannover) together with the chairpersons of the study groups and members of the STS Advisory Board.

The main focus 2012 was 'Tumor Biology' with keynote lectures given by Stephan Feller (Oxford, UK), Holger Kalthoff (Kiel), Jürgen Ruland (Munich) and Maria Sibilia (Vienna, Austria). In addition, many other facets of signal transduction were addressed in the individual workshops on "Growth Factors, Cytokines, Chemokines" with a keynote lecture by Antonella Viola (Milan, Italy), "Immune Cells and Osteoimmunology", introduced by Gerhard Krönke (Erlangen), and "Pathogens and Disease" with a keynote talk on *Helicobacter*-induced signal transduction by Steffen Backert (Dublin, Ireland). The session on "Stress, Death, Survival" was highlighted by the presentation of Peter Krammer (Heidelberg), Ann Ager (Cardiff, UK) introduced the workshop "Migration and Adhesion" and Yosef Yarden (Rehovot, Israel) set the stage for the session on "Regulation of Signaling and Systems Approaches". All keynote lectures were followed by a number of short talks selected from the submitted abstracts. Here, the mixture of presentations given by group leaders, postdoctoral fellows or PhD students was highly appreciated as a unique feature of the STS meetings.

Since 2010, the STS honors an outstanding researcher in the field of signal transduction research to conclude the workshop program with a "Honorary Medal Lecture". The STS/CCS Honorary Medal was introduced by the STS in cooperation with its open access journal "Cell Communication and Signaling" published by BioMed Central. Following Tony Pawson in 2010 and Tony Hunter in 2011, the 2012 STS/CCS Honorary Medal was awarded to Carl-Henrik Heldin from the Ludwig Institute of Cancer Research in Uppsala, Sweden. He received the medal after a very distinct laudation given by Holger Kalthoff (Kiel). In his award lecture entitled "Signalling via receptors for PDGF and TGF-ß- possible targets in tumor treatment", Professor Heldin highlighted key steps and discoveries during the cloning and functional characterization of platelet-derived and transforming growth factor receptors. These two tyrosine kinase receptors play a pivotal role in mediating growth factor signals that govern normal and cancerous cell growth. Moreover, Professor Heldin pointed to the role of TGF-ß during epithelial-to-mesenchymal transition (EMT) of tumor cells and the potential acquisition of metastatic properties. His enthusiasm and ideas fascinated the audience of the STS conference and he received a long-lasting applause for his outstanding presentation.

Another important aspect of the STS joint meeting has always been the support of young scientists. This year, as many as 18 Bachelor/Master or MD/PhD students received travel grants of 4.500,– € in total to enable them to attend the meeting. Moreover, five poster prizes were selected from the 60 poster presentations and honored with 750,– € of prize money. As a good tradition, all presenting authors had the chance to attract the audience to their posters during the meanwhile well-established 'one minute – one transparency' sessions.

This year's STS Science Award of 1.000,– €, sponsored by BIOMOL GmbH was split between two researchers for their scientific work presented as short oral presentation. One part of the STS Science Award was given to Dr. Sarah Jill de Jong, a young postdoctoral researcher from Erlangen, who presented data on a unique TRAF3-binding motif that confers specificity to a Tio-induced non-canonical NFßB activity. The other part of the prize was awarded to Associate Professor Geert Bultynck from the Katholieke Universiteit Leuven, Belgium, for his work on IP3/Bcl2 complexes in B cell lymphomas to trigger pro-apoptotic Ca^{2+} signaling.

Last but not least, the GBM-Innovation-Award for Young Scientists (500,– €) went to Dr. Stephan Phillip from Kiel for his proteome analysis of cell membranes from human erythrocytes infected with different stages of Plasmodium falciparum.

All together, and also reflected by the on-site evaluation, the 2012 international Joint Meeting 'Signal Transduction' was a great success. Preparations for the 17th Joint meeting of the Signaling Study Groups and the STS have already started. The special focus in 2013 will be "Signaling in Infection and Inflammation". Please mark your calendar: The meeting is scheduled from November 4th to 6th, 2013. The venue will again be the Leonardo Hotel in the historical city of Weimar.

Details and updated information will be available at www.sigtrans.de