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Nuclear architecture – on higher ground

A report on the Third Elmau Conference on Nuclear Organization: From Basic Science to Application

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The functional architecture of the cell nucleus was poorly understood until about 20 years ago, when the efforts from many laboratories started to yield data on nuclear infrastructure and the link between nuclear structure and function. It has since become clear that dynamic interactions of nuclear components take place in functional sub-compartments within the nucleus, such as chromosome territories, splicing speckles, and a growing number of nuclear 'bodies'. Most, if not all, reactions involved in gene expression, DNA replication and repair occur in spatially separated, dedicated locations. The mosaic-like organization of the nuclear interior plays an important role in the regulation of these processes, which we are now beginning to understand in molecular detail. We can now clearly see how our new-found knowledge of the cell nucleus will be useful in devising ways of fighting cellular dysregulation and designing new therapeutic approaches to tackle human diseases.

From October 7th to October 10th, 2004, more than 50 scientists met at Schloss Elmau on a high plateau in the Bavarian Alps, to delineate the frontiers of our current understanding of nuclear architecture, and discuss ways in which our collective knowledge might be usefully applied. The meeting was organized by Hans Lipps, Susan Gasser and Wolfgang Deppert in the form of an EMBO workshop and was entitled 'Nuclear Organization: From Basic Science to Application'. It was an excellent opportunity for the exchange of ideas between leading scientists in the field of nuclear architecture, and younger participants who are just finding their way into this field of science. This report gives an overview of the four different sessions, which ranged from basic science on telomeres and replication origins, to applied aspects such as the design of episomal vectors for gene therapy.

Telomeres, centromeres and replication origins

The beginning of the conference was all about the end – the physical end of chromosomes, commonly known as telomeres. The ends of linear chromosomes in eukaryotes are problematic for the DNA-replication machinery because of the discontinuous synthesis of the lagging strand. To overcome the problem of shortening chromosomes with each replication event, eukaryotes carry specialized structures at chromosome ends that are replicated by a unique mechanism using the reverse transcriptase telomerase. Aspects of telomerase regulation, which is essential for maintaining genome integrity through the cell cycle, were described in talks presented by **Virginia Zakian** (Princeton, NJ), **Eric Gilson** (Lyon) and **Katrin Paeschke** (Witten). Virginia described how telomerase activity in *S. cerevisiae* is cell-cycle regulated, so that telomere lengthening only occurs during late S/G2 phase. This is surprising, because the catalytic subunit of telomer-

ase, Est2p, is bound to telomeres throughout most of the cell cycle. Thus, telomerase activity seems to be regulated by telomerase-associated proteins such as Pif1p, which reduces telomerase processivity by dissociating telomerase RNA from Est2p.

In a related talk, Eric Gilson discussed the role of chromatin structure in the regulation of telomere length regulation. Eric presented data on a Rap-1 independent pathway, where the subtelomeric proteins Tbf1 and Reb1 affect telomere elongation, most probably via a mechanism in which chromatin remodeling factors are modulated by Tel1p, a PI3 kinase earlier shown to be involved in telomere stability.

Much of our knowledge on telomeres has been gathered in ciliates, elegant model organisms with macronuclei composed of thousands of gene-long linear DNA molecules each with their own two telomeres. Katrin Paeschke showed that telomeres in these organisms are attached to the nuclear matrix via a heterodimeric telomere-binding protein. RNAi knockdown experiments of the two subunits revealed that matrix binding is mediated by the α-subunit of the protein, whereas both subunits are essential to promote G-quadruplex formation. In the course of DNA replication, both the G-quadruplex structure as well as the interaction of the telomere-binding protein with the nuclear matrix is resolved, making telomeres accessible to telomerase action.

Katrin's talk led over to the next main topic of the meeting, the regulation of DNA replication. Many details of this regulation are still unknown, but it is clear that the mechanisms that coordinate DNA replication in temporal and spatial order mainly operate through DNA replication origins and the protein complexes bound to them. Using the LaminB2 origin as a well-characterized model, **Giuseppe Biamonti** (Pavia) described the sequence elements and protein factors essential for the activity of eukaryotic origins of replication. He showed by *in vivo* footprinting that, in addition to the known origin recognition complex (ORC) proteins, other proteins including HoxC13 bind specifically to origin sequences and might be involved in initiation events.

Protein–DNA interactions at eukaryotic origins of replication have also been investigated by **Rolf Knippers** (Konstanz) and his associates. Rolf reported experiments with the well-characterized episomally replicating plasmid pEPI-1 as a model for replication studies both *in vivo* and *in vitro*. He showed that *in vitro* replication of pEPI strongly depends on the presence of ORC and MCM initiator proteins, suggesting that replication start sites are determined by neighboring chromatin structures and pre-bound protein factors rather than the nucleotide sequence of replication origins. Understanding these mechanisms is a prerequisite for the rational design of non-viral vectors for mammalian cells, as discussed in the talk of **Isa Stehle** (Witten). Isa set out to reduce the size of the existing pEPI episomal vector by replacing the genomic S/MAR element with a tetramer of a 155-bp S/MAR-DNA module linked to an upstream transcription unit. She demonstrated that this smaller vector called pMARS still replicates episomally owing to interactions with the nuclear scaffold protein SAF-A, later discussed in detail by Frank Fackelmayer.

Temporal regulation of initiation was addressed by **Olivier Hyrien** (Paris). Olivier used molecular combing of DNA from sperm nuclei replicating in *Xenopus* egg extracts to determine the origin, density and replication timing on single DNA molecules. He demonstrated that the temporal order of origin initiation can be modulated by the checkpoint-abrogating agent caffeine. These data suggest a caffeine-sensitive ATR-dependent checkpoint that adjusts the frequency of initiation to the supply of replication factors, and optimizes fork density for safe and efficient chromosomal replication during normal S phase.

Although replication timing of most genes is invariable throughout development, **David Gilbert** (Syracuse, NY) presented an interesting system in which temporal changes in replication can be examined in homogeneous cell populations. David showed that, in parallel with transcription, several genes residing within AT-rich isochores switch replication timing during the differentiation of mouse embryonic stem cells to neural precursors. It will be interesting to find out how these timing switches are related to changes in chromatin structure or transcriptional activity during the cell cycle.

The talk of **William C. Earnshaw** (Edinburgh) gave interesting insights into the regulation of mitotic events. He outlined the essential roles of chro-

mosomal passenger proteins as regulatory factors at centromeres in mitosis. In particular, Bill showed that borealin, as a novel chromosomal passenger, is required for stability of the bipolar mitotic spindle. Having an even closer look at centromeric chromatin, **Patrick Heun**'s (Berkeley, CA) talk focused on the question of if and how centromer identity is determined epigenetically. He showed that overexpression of CENP-A/CID, a centromere specific histone H3-like protein, produces ectopic kinetochores and leads to serious disarrangements during mitosis possibly causing aneuploidy. Based on these results, he proposed that CENP-A/CID is essential for kinetochore formation and is an epigenetic marker for centromere identity.

Chromatin structure, function and dynamics

A large section of the meeting was dedicated to chromatin structure, function and dynamics. **Peter Becker** (Munich) started the chromatin sessions with his work on chromatin modifications that lead to dosage compensation in *Drosophila*. In contrast to dosage compensation in mammals, which occurs by inactivation of the second X chromosome in females, fruit flies equalize X-linked genes by transcriptional up-regulation of the single X chromosome in males. Peter presented experiments on the 'dosage compensation complex' (DCC). He showed that MOF ('males-absent on the first'), a histone acetylase, is targeted to the X chromosome as part of a dosage compensation complex (DCC) consisting of non-coding roX RNA and at least four other male-specific lethal (MSL) proteins. Peter proposed that activation of MOF's histone acetyltransferase activity upon integration into the DCC restricts the critical histone modification exclusively to the male X chromosome. In addition, Peter discussed fluorescence recovery after photobleaching (FRAP) experiments to determine the dynamics of interaction between MSL2 protein and the X chromosome. As **Roel van Driel** (Amsterdam) pointed out, diffusion constants of cellular components measured by such experiments can be used to establish mathematical models of cellular processes, which will allow a better understanding of complex pathways. On the example of nucleotide excision repair (NER), Roel presented a fascinating model that delineates the general characteristics of this pathway. He showed that sequential assembly rather than random or pre-assembly of the NER complex onto chromatin is advantageous from the standpoint of repair efficiency.

It is clear that higher-order structure of chromatin is an important determinant of gene activity. A number of talks addressed this topic in detail, starting from *in vitro* reconstitution experiments on the 30 nm fiber of chromatin presented by **Phil Robinson** (Cambridge), and determination of their three-dimensional structure by cryo-microscopy. **Ferran Azorin** (Barcelona) presented his work on vigilin, an evolutionarily conserved protein containing multiple tandemly organized K-homology (KH) domains with strong single-strand nucleic acid binding activity. He discussed recent advances in the functional characterization of vigilin in yeast and *Drosophila*, indicating that the protein is involved in heterochromatin formation and chromosome segregation. Of course, chromatin structure is dynamic, and mechanisms are required to locally or globally change chromatin structure in response to intra- or extracellular stimuli. **Peter Verrijzer** (Leiden) showed that single mutations in a component of the SWI/SNF chromatin remodeling complex, humanSNF5, can lead to aberrant chromatin structure. As a consequence, aberrant gene control results in aneuploidy and possibly in cancer.

In her efforts to understand the complex interplay between transcriptional activity and chromatin dynamics, **Susan Gasser** (Geneva) presented mobility measurements in live yeast nuclei. She compared the mobility of active gene loci with transcriptionally inactive genomic regions such as telomeres, centromeres, and the silent mating-type locus. She showed that while active genes in the nuclear interior are subject to rapid random-walk movement, transcriptionally inactive regions are constrained in their mobility, and concluded that chromatin position and mobility are tightly linked to transcriptional state. It will now be interesting to compare these results with chromatin mobility in the nuclei of higher eukaryotes, which are much larger and more complex. In any case, a dependence of chromatin mobility on transcription underlines the different structure of active

chromatin in comparison to inactive, often heterochromatic regions. Chromatin structure of transcriptionally inactive genomic domains was also addressed by **Fabrizio Martino** from Susan Gasser's lab. Fabrizio described experiments to reconstitute subtelomeric silenced heterochromatin *in vitro*, focusing on complex formation between purified components of the Silent Information Regulator (SIR) complex, and their loading onto naked DNA.

The transition of a genomic region from one transcriptional state to another requires chromatin remodeling, and mechanisms to restrict these changes to defined genomic domains. **Haini Cai** (Athens, GA) set out to define these mechanisms in molecular detail and showed that chromatin boundary elements are needed both to modulate enhancer-promoter interactions and block influences from neighboring chromatin. Interestingly, her research on a chromatin boundary element in *Drosophila* shows that these two functions are mediated by different mechanisms and rely on distinct transfactors.

On the lowest level of chromatin organization, transcriptional activity is directly affected by the nucleosomes that make up the basic building blocks of chromatin. **Cleo Bishop** (London) showed that transfected DNA acquires nucleosomes very soon after nuclear entry, which leads to immediate gene silencing. This inhibition can, however, be overcome by inducing replication of the introduced DNA, or by enhancing histone acetylation. In a related talk, **Christoph Lavelle** (Fontenay-aux-Roses) presented evidence that each individual nucleosome has its own characteristics with respect to conformational dynamics and sliding along the DNA, which affect local chromatin structure and dynamics.

Global nuclear organization

Three large sessions of the meeting dealt with our current knowledge of the organization of the nucleus as a whole. In particular, we discussed recent advances in research into well, and not-so-well, established subcompartments of the nucleus, such as the nuclear lamina, chromosome territories, the nuclear scaffold, and the interchromatin compartment. It soon became obvious that our understanding especially of dynamic processes in the nucleus has increased tremendously in recent years. It is now firmly established that nuclear functions can only be understood as a dynamic interplay of a multitude of components in a well-ordered and regulated way. On the level of global nuclear organization, nuclei change their shape and appearance in a variety of diseases, and during normal processes of development. In this context, **Amanda Fisher** (London) described experiments on changes of nuclear structure and gene expression of lymphocyte nuclei in muscle heterokaryons. Her results demonstrate that re-organization of constitutive heterochromatin is an early event in muscle cell differentiation that requires the activity of histone deacetylases (HDAC). Global nuclear architecture was also addressed by **Ana Pombo** (London), who presented a sophisticated chromosome painting procedure with significantly improved painting efficiency. This is an important advance because classical methods of fluorescence *in-situ* hybridization are incapable of resolving whether chromosome territories are mutually exclusive, or intermingle at their borders. Using human lymphocytes, Ana found a low but detectable intermingling that differs for each pair of investigated chromosomes, demonstrating that chromosome territories are not entirely separated *in vivo*. This result does not leave much room for a compartment between chromosome territories, such as a putative interchromatin compartment (IC), in the undisturbed cell. Nevertheless, a novel reversible 'chromatin condensation' protocol described by **Thomas Cremer** (Munich) provides clues that such a compartment may exist, and can be experimentally enlarged by mild hypertonic treatment of living cells. The localization of nascent transcripts in treated cells suggests that transcription may occur mainly at the interface between chromosome territories and the IC. This is compatible with earlier findings that nuclear location and genetic activity are intimately linked. Importantly, **Danielle Zink** (Munich) provided first-time evidence that it is transcriptional activity that dictates nuclear positioning of a gene, and not *vice versa*. It is not yet clear how the spatial organization and regulation of genetic processes is achieved, but work presented by **Frank**

Fackelmayer (Hamburg) provides firm evidence for a functional role of a nuclear scaffold at least for the positioning of DNA replication events. This is in good agreement with results of **Dean Jackson** (Manchester), who also investigated the composition and role of a nuclear scaffold. Dean suggested that nuclear lamins may be major constituents of an intranuclear filament network and showed that RNAi knock down of intranuclear lamins affects gene expression and replication. The nuclear scaffold thus appears to provide a dynamic platform on which genetic processes can occur. In fact, hormone receptors such as the estrogen receptor have recently been shown to be immobilized on the nuclear scaffold in a ligand-dependent way. Other nuclear receptors, such as the peroxisome proliferators-activated receptors (PPAR) investigated by **Laurent Gelman** (Lausanne) are not immobilized, but nevertheless show a reduced mobility due to their interaction with cofactors.

The talks and discussions about the nuclear scaffold and the interchromatin compartments made it clear that more work will be necessary to establish unequivocally whether or not such subcompartments exist *in vivo*, and whether they are important for cellular processes. Research on the nuclear lamina does not suffer from such problems. In fact, as pointed out by **Colin Stewart** (Frederick, MD), the components of the nuclear lamina, the lamins, have clear links to human diseases. Many of these diseases, collectively termed laminopathies, result from mutations in A-type lamins. They can be categorized into three classes, laminopathies affecting striated muscles, lipodystrophies that result in redistribution of white fat and skeletal abnormalities, and a third group including the Hutchison–Gilford Progeria Syndrome (HGPS). It is surprising how many different pathologies arise from point mutations at different sites of the same gene, and we are only beginning to understand why these diseases are so different. Colin's group develops transgenic mouse models for human laminopathies, and characterizes how different defects of lamin A lead to different changes in gene expression and nuclear stability. In a related talk, **Yosef Gruenbaum** (Jerusalem) emphasized that the nuclear lamina is not only important for nuclear morphology, but also seems to play a key role in cell cycle control and germ cell development. He presented several examples in a nematode model system to show how down-regulation or mutations of lamin or lamin-associated proteins like emerin and MAN1 can cause serious abnormalities in the development of *Caenorhabditis elegans*. As mutations in human emerin cause Emery–Dreifuss muscular dystrophy, the results obtained in *C. elegans* may be very helpful to understand the genesis of other laminopathies. In fact, **Josef Gotzmann** (Vienna) demonstrated that a human protein from the same family as emerin, LEM2, requires lamin A for localization in the nuclear envelope. Very much like human and *C. elegans* emerin, LEM2 appears to be involved in cell division and cell-cycle dependent changes in chromatin organization. A role of the lamina for chromatin structure in interphase was also demonstrated by **Roland Foisner** (Vienna). Roland investigated the lamin associated protein LAP2α, which forms nucleoskeletal structures with A-type lamins, and also interacts with chromosomes via its C-terminus in a cell cycle-dependent manner. During mitosis LAP2α translocates from the cytoplasm to telomeres where it is bound at the spindle pole and moves back the cytoplasm in G1 phase, indicating distinct functions of LAP2α in cell cycle progression during interphase and in nuclear reassembly during mitosis.

What is it good for?

The final large session of the meeting was dedicated to applied aspects. Much of the knowledge we have gathered is now at a stage of maturity where application can be envisaged. Naturally, understanding how the activity of genes is regulated and how DNA is replicated in the three-dimensional context of the living cell, has great impact on developing strategies to fight diseases originating in a misregulation of these processes. Typical of such diseases are the various forms of cancer, which are induced by the expression of oncogenes and inactivation of tumor suppressors. **Wolfgang Deppert** and **Andrea Hermannstädter** (both from Hamburg) presented work on the most prominent human tumor suppressor, the p53 protein. Wolfgang described first *in vivo* proof for oncogenic potential of mutant p53, which not only acts as a tumor suppressor

but may, in a mutant form, also lead to advanced tumor progression through 'gain-of-function' effects. Expression of mutant p53 in malignant cells is not well understood, but Andrea suggested that it is regulated at least in part by epigenetic mechanisms. Understanding these mechanisms could open a new way to block the gain-of-function of mutant p53 and lead to more efficient treatment for cancer.

With focus on neuromuscular and cardiovascular diseases, **George Dickson** (Surrey) described a number of approaches to provide genetic therapies by either 'gene addition' or 'gene correction'. Especially the 'gene addition' strategy of therapy demands that gene vectors are both safe and efficient enough to be used in humans. Development of virus-free vectors for gene therapy was discussed in talks of **Aloys Schepers** (Munich) and **Eirini Papapetrou** (Patras), based on different approaches. Aloys described his work on an episomal vector system originally based on the latent origin of replication of Epstein–Barr virus, and his efforts to use a chromatin immunoprecipitation (ChIP) approach to isolate small genomic DNA fragments that support autonomous replication of this episome. Eirini described the evaluation of a chromosome-based episomal vector originally developed in the group of **Hans Lipps** for gene transfer into hematopoietic progenitor cells. She presented evidence that the vector confers long-term expression of a transgene in $CD34^{+}$ cells from umbilical cord blood, and is therefore a very promising candidate for a gene vector for hematopoietic cells. These new type of vectors for gene therapy are based on the activity of 'scaffold attachment regions' (SARs), which tether the plasmid to nuclear substructures and allow the vector to mimic a host chromatin domain. **Jürgen Bode** (Braunschweig) discussed the construction of these 'chromatin minidomains', which led to the development of the pEPI-type vectors, and their properties *in vivo*. As an alternative approach for gene therapy, **Fiorentina Ascenzioni** (Rome) presented the use of artificial minichromosomes. These very long DNA molecules approximate to normal chromosomes, but can be used for cloning vectors with practically unlimited cloning capacity. As exemplified by cloning the entire CFTR gene with its 27 exons, genes on artificial chromosomes are functional and subject to their normal regulation mechanisms. They could thus provide an efficient means for 'gene addition' therapeutic approaches.

The final session of the Elmau meeting put basic research presented in the other sessions into a medical framework. It was this combination of cutting-edge scientific accomplishments in biochemistry, molecular and cellular biology with applied aspects that gave the meeting its unique flavor. Although we may forget many of the details presented in the talks, one thing about the Elmau meeting will certainly stick in our memory: the fascination of seeing how the nuclear architecture field accomplished the transition from the esoteric topic it was a few years ago, into a mature field where our discoveries begin to enable the design of entirely new approaches for the cure of human diseases.