EMBO WORKSHOP REPORT

An eclipse over the cell nucleus Functional organization of the cell nucleus Prague, August 9–12, 1999

I.Raška^{1,2,3}, U.Aebi⁴ and W.C.Earnshaw⁵

¹Department of Cell Biology, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic and ²Laboratory of Gene Expression, 3rd Faculty of Medicine, Charles University in Prague, Albertov 4, CZ-128 00 Prague 2, Czech Republic, ⁴M.E.Mueller Institute for Structural Biology, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland and ⁵Institute of Cell and Molecular Biology, University of Edinburgh, Michael Swan Building, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, UK

³Corresponding author
e-mail: iraska@lfl.cuni.cz

The EMBO workshop 'Functional Organization of the Cell Nucleus' held in Prague at the Hotel of the Postgraduate School of Medicine was attended by 110 participants (49 invited speakers and 61 selected participants) from 22 countries. Such a full range of topics devoted to the cell biology of the nucleus has not been discussed previously in such an intimate meeting in Europe. The workshop not only offered an opportunity for junior scientists to benefit from having an international meeting within Europe and a chance to discuss their work with internationally recognized experts, but it also offered a unique opportunity for interactions among the more established investigators. The fruits of a number of presentations are gathered together in a Special Issue of the *Journal of Structural Biology* which appeared in spring 2000. Last but not least, it is worth mentioning that while stepping through a packed scientific program, the participants did find the time to observe the solar eclipse just before the lunch break of August 11.

Introduction

The nucleus is one of the best studied, yet least understood cellular organelles. Understanding in molecular detail the organizing principles of the nucleus, including how the chromosomal DNA is arranged, how the synthesis, processing, assembly and transport of macromolecules is coordinated and regulated, and how the nucleus is disassembled in mitosis and apoptosis are major goals for cell biology.

The first task of the nucleus is to accommodate the full complement of genomic DNA; not a small task when one considers that the linear DNA length of a mammalian genome may amount to several meters, while the diameter of the nucleus is only 10–15 µm. This problem, which was discussed in one of the sessions, is solved through the interaction of chromosomal DNA with proteins to form chromatin which, in turn, interacts with higher order protein structures so that it becomes folded and compacted to yield eventually condensed chromosomes.

Chromosome condensation, however, gives rise to a second problem, since, within nuclei, specific regions of the genome must be decondensed so that the genes can be transcribed. In addition, the entire genome must be replicated exactly once per cell cycle. The problem of how DNA replication/transcription can be integrated topologically within the nuclear architecture was the subject of another session of the workshop.

For many years, studies on the cell biology of the nucleus were limited by a relative lack of distinctive substructures that can be visualized by microscopy and are amenable to biochemical purification. However, an elaborate picture emerges when antibody or hybridization probes are used to detect specific nuclear factors or genes. Many nuclear proteins are found to localize to distinct regions of the nucleus, including nucleoli and specific nuclear bodies (NBs), which can be recognized by electron microscopy. The role of these nuclear substructures in RNA metabolism and in other processes that take place within the nucleus was the subject of two sessions.

In higher eukaryotes, both the nuclear envelope (NE) and its associated lamina disassemble during mitosis, and reassemble during telophase to achieve spatial segregation of the chromosomes from the cytoplasm. During apoptosis, the nucleus is disassembled by a different mechanism, which facilitates the efficient recycling of cellular components by neighboring cells, and minimizes the harmful effects of cell death for the organism as a whole. Passive diffusion of ions and small molecules as well as active transport of proteins, RNAs and ribonucleoprotein (RNP) particles in and out of the nucleus occur during interphase through dedicated gateways within the NE called nuclear pore complexes (NPCs). Typically, the NE of a mammalian interphase cell nucleus harbors several thousand NPCs, and NPCs can handle bi-directional traffic of $>1 \times 10^6$ cargos per minute per cell. The structure of the NE, nuclear dynamics during mitosis and apoptosis, NPC architecture, together with the signals and factors required for active transport of cargos through the NPC, and recent advances toward a more structure-based functional analysis of nucleocytoplasmic transport were discussed in three sessions.

Chromatin structure, DNA replication and transcription

A.Belmont (Urbana, IL) presented a novel *in vivo* method for selective visualization of specific chromosome regions or segments. Specifically, chromatin fibers with a packing ratio ~20 times that of the 30 nm fibers were observed directly in living cells. These fibers were shown to be stable over several hours in terms of compaction and intranuclear position, although uncoiling of large-scale

chromatin fibers could be induced by targeting of high concentrations of transcriptional activators to particular chromosome regions. T.Cremer (Munich, Germany) talked about the spatial organization of chromosome territories, which he visualized three-dimensionally by combining fluorescence *in situ* hybridization (FISH) and *in vivo* labeling of the replicating chromosomes with fluorescent nucleotides. Chromosome territories were shown to be compartmentalized into mutually exclusive arm domains composed of individual, early and mid-to-late replicating chromatin foci with diameters of 400–800 nm. These experimental findings were complemented by several interesting spatial models emulating chromosome organization. J.Sedat (San Francisco, CA) first reviewed a methodology enabling specific localization and analysis of genetic loci to highly localized three-dimensional positions within *Drosophila* embryonic nuclei. He further demonstrated the possibility (i) of determining homologous chromosome pairing dynamics as a function of development and (ii) of probing dynamic chromosome interactions by four-dimensional studies of live nuclei. Regarding the question of whether nuclear architecture differs for transcriptionally active versus inactive cells for a variegating gene, he analyzed the nuclear architecture in the *Drosophila* eye which exhibits position-effect variegation for the *white* gene. To achieve this, FISH is employed to analyze the nuclear architecture of specific DNA sequences, and the autofluorescence of the eye pigments is used to quantitate *white* gene expression. Thus, three-dimensional nuclear architecture can now be correlated with function on a cell-by-cell basis.

Two talks focused on the composition of origins of DNA replication within the cell nucleus. M.DePamphilis (Bethesda, MD) has documented, through the mapping of the genomic locations of hamster Orc2 protein, a specific binding site adjacent to an origin of bi-directional replication termed ori-b which is located downstream of the hamster *DHFR* gene. Importantly, this binding site appears to facilitate site-specific assembly of a pre-replication complex at ori-b. R.Laskey (Cambridge, UK) discussed the control of DNA replication in normal and neoplastic cells. He has shown that Cdc6 is one of the limiting components for the initiation of replication. Importantly, antibodies against Cdc6 and MCM proteins, which are absent from differentiated G₀ cells, provide exceptional promise for early diagnosis of cancer.

The next two talks discussed the distribution of origins of DNA replication within the cell nucleus. S.Gasser (Lausanne, Switzerland) showed that late-firing origins of replication tend to be distributed toward the nuclear periphery, whereas early origins tested were localized preferentially to the interior. In addition, localization studies demonstrated a high frequency of co-localization for adjacent origins. R.Berezney (Buffalo, NY) described the spatio-temporal dynamics of replication and transcription in the mammalian cell nucleus. He has documented that replication and transcription are separated in non-overlapping zones in early S-phase cell nuclei and that the individual zones are in turn arranged into spatially separate networks that extend throughout the nuclear interior.

The organization of the nucleus with regard to transcription was discussed by R.van Driel (Amsterdam, The Netherlands). He described a striking relationship between

higher order chromatin folding and gene activity, i.e. actively transcribed DNA is located near the surface of compact chromatin domains, whereas only inactive chromatin is found inside such domains. A.Fisher (London, UK) documented that the transcriptionally inactive genes that have been investigated are positioned selectively close to heterochromatic, centromere-containing domains in the nuclei of proliferating lymphocytes. This spatial segregation of inactive and active genes is acquired by resting lymphocytes only as they prepare to enter the cell cycle, and it coincides with the repositioning of an important lymphocyte-associated transcription factor IKAROS. The recruitment of genes to heterochromatic domains is a crucial factor for maintaining the heritable repression and late replication status of these genes. F.Grosveld (Rotterdam, The Netherlands) discussed the dynamics of β -globin gene switching. His data indicated that locus control regions (LCRs) interact with individual globin genes and that LCR–gene interactions are dynamic, with complexes forming and dissociating continually. The levels of expression of each of the genes appear to depend on (i) the frequency of interaction, which is itself dependent on the distance of the gene from the LCR; (ii) the affinity of the LCR for the gene; and (iii) the stability of the LCR–gene complex.

G.Cavalli (Montpellier, France) discussed the regulation of cellular memory by the *Drosophila polycomb* and *trithorax* group of proteins. Using the Fab-7 element, which induces silencing on flanking GAL4-driven *lacZ* reporter and mini-*white* genes, he has shown that a single pulse of GAL4 during embryogenesis is sufficient to release *polycomb*-dependent silencing and allow expression of both reporter genes. Such an activated state is maintained mitotically and thus can be transmitted in a GAL4-independent manner through female meiosis. As mitotic transmission of the activated state involves H4 hyperacetylation as a heritable epigenetic marker, this is the first direct link between *polycomb/trithorax* regulation and chromatin structure. U.Laemmli (Geneva, Switzerland) used a novel chemical approach, exploring cell-permeable pyrroleimidazole polyamides to define the sequence determinants of heterochromatin and to study chromosome dynamics. In the study of a position-effect variegation, he demonstrated that, if fed to *Drosophila* larvae, certain of these cell-permeable compounds binding specific DNA motifs can suppress the whitemottled-4 phenotype.

A model of regulation of gene expression was presented by M.Ptashne (New York, NY). According to this model, cells detect extracellular signals through allostery. Depending on the nature of the allosteric changes, various possibilities of binding are generated so that the cells then give those signals by means of ‘regulated localization’. It was suggested that this formulation, which applies to many biological processes including the action of important kinases, phosphatases, polymerases and proteases, is particularly well illustrated by mechanisms of gene regulation.

News from the nucleolus

Much effort has been devoted to establishing a correlation between the nucleolar structures and nucleolar functions in

the past, the issue remaining controversial. U.Scheer (Würzburg, Germany) found a tight co-localization of newly transcribed rRNA and RNA polymerase I or transcription factor UBF. He concluded that rDNA transcription takes place in nucleolar fibrillar centers. C.Schöfer (Vienna, Austria) studied the structure of rDNA *in situ* using the fiber FISH technique. He described a different anchoring pattern of transcription units and non-transcribing intergenic spacers with respect to cell activity. The nucleolus is assembled and disassembled during each cell cycle. While proteins involved in rDNA transcription are associated with rRNA gene clusters during mitosis, rRNA processing factors are transported to newly formed pre-nucleolar bodies only in late telophase. D.Hernandez-Verdun (Paris, France) described the different kinds of pre-nucleolar bodies, the translocation of which to newly formed nucleoli follows ordered kinetics. This order was confirmed in the *de novo* assembled nucleolus at the mid-blastula transition during *Xenopus laevis* embryogenesis. At this stage, RNA polymerase I-mediated transcription was not activated. Importantly, the nucleolar domain can be assembled without active transcription, but in association with pre-existing pre-rRNAs. M.Schmidt-Zachmann (Heidelberg, Germany) described two novel nucleolar non-ribosomal proteins, NO29 and NOH61. The first is similar to the major nucleolar protein B23 and its unusually high content of negatively charged amino acids suggests its involvement in pre-ribosomal particle assembly. NOH61 belongs to a broad family of DEAD box RNA helicases.

Recent evidence suggests that the nucleolus may fulfil diverse functions in addition to ribosome biogenesis. T.Pederson (Worcester, MA) documented that micro-injected SRP (signal recognition particle) RNA was transiently localized in the nucleolus. In addition, one of the SRP proteins, SRP19, was also found in the nucleolus. Further linkage of different RNA maturation pathways was established in yeast. D.Tollervey (Edinburgh, UK) found that a number of enzymes required for rRNA maturation are also involved mRNA, snRNA and snoRNA biogenesis. These include the 5'-3' exonucleases Rat1p and Xrn1p, endonuclease Rnt1p, 3'-5' exonucleases assembled in the exosome complex, the pseudouridine synthase Cbf5, the putative 2'-O-methylase Nop1p and putative RNA helicases. Finally, A.Tartakoff (Cleveland, OH) presented data to show that rDNA transcription is reduced after mutation of the vesicular traffic machinery. He further documented that this reduction is caused by inhibition of protein import to the nucleus and relocation of multiple nucleolar and nucleoplasmic proteins to the cytoplasm. These observations have revealed a regulatory role for protein shuttling in ribosome maturation.

Nuclear organelles and RNA processing

The cell nucleus contains many non-chromatin structures including SC35 domains and various NBs that accommodate a number of factors involved in RNA biogenesis. It had been claimed that these nuclear structures mostly represent storage sites from which the factors are recruited. The function of several of these structures was discussed during this workshop.

J.Gall (Baltimore, MD) delivered a special lecture on coiled bodies during which he proposed renaming these structures Cajal bodies (CBs), after their discoverer, the Spanish cytologist Ramon y Cajal. He documented that CBs accommodate a number of proteins and snRNAs involved in RNA transcription and processing and proposed that the transcription, splicing and processing machinery is pre-assembled in CBs. A.Lamond (Dundee, UK) studied the dynamic movement of CBs *in vivo* by labeling them with green fluorescent protein (GFP)-tagged coilin, a distinct protein constituent of CBs. Based on their size and movement, Dr Lamond defined two types of CBs. Using GFP fusions to Sm proteins, he documented that upon their import into the nucleus, Sm proteins first accumulate in CBs, suggesting that snRNP assembly occurs in CBs. R.Dirks (Leiden, The Netherlands) showed that GFP-tagged fibrillarin, a protein found in both CBs and the nucleolus, contains different signals for its targeting either to the nucleolus or to CBs. Fibrillarin appeared in CBs and nucleoli at the same time after transfection, suggesting its direct transport to CBs.

One class of nuclear bodies, variously termed PML bodies, ND10 or PODs, represent sites of accumulation of a number of proteins including PML and Sp100 proteins. These bodies fragment under some pathological conditions including acute promyelocytic leukemia and infection with the human simplex virus (HSV) and cytomegalovirus (CMV). Modification of several proteins by the ubiquitin-like protein SUMO-1 is necessary for the maintenance of NB structure. In agreement with these findings, A.Dejean (Paris, France) found that the HSV ICPO and CMV IE1 proteins specifically abrogate SUMO-1 modification of PML and Sp100 proteins. G.Maul (Philadelphia, PA) showed that PML is responsible for recruitment of other ND10-associated proteins. In the case of the Daxx protein, the SUMO-1 modification of PML is required for Daxx targeting to ND10.

The role of SC35 domains (nuclear speckles) in pre-mRNA biogenesis is still a matter of debate. I.Raska (Prague, Czech Republic) documented that Epstein-Barr virus nuclear antigen (EBNA) pre-mRNA accumulated in tracks that extended from the gene to the SC35 domain rather than to the NE. He suggested that bi-directional trafficking of macromolecules must occur. RNAs released from the gene traffick to SC35 domains, whereas splicing factors from SC35 domains traffick to transcription sites. J.Lawrence (Worcester, MA) localized transcripts of both a normal collagen 1A1 allele and an intron retention mutant in osteogenesis imperfecta into tracks that extend through SC35 domains. Most of the 51 introns were spliced at or near the gene, before transiting through the domain and dispersing. In contrast, the intron of the mutant RNA accumulated within tracks. Lawrence suggested that SC35 domains contain both excess factors and specific transcripts in transit from the genes. M.Carmo-Fonseca (Lisbon, Portugal) localized different processing mutants of the β -globin gene by FISH. She found that the ability of RNA to undergo splicing and/or 3' end processing is required for RNA transport from its transcription sites to the cytoplasm. D.Spector (Cold Spring Harbor, NY) reported on a biochemical fractionation procedure which allowed purification of inter-chromatin granule clusters (basically corresponding to

SC35 domains) from mouse liver nuclei and the identification of ~75 enriched proteins. Subsequent mass spectrometry and/or peptide microsequencing of the corresponding protein spots identified 33 known proteins, as well as a number of novel proteins. These included a 137 kDa protein exhibiting striking sequence similarity to a known UV-damaged DNA-binding protein, a protein known to associate with the hereditary disease xeroderma pigmentosum group E, and with the 160 kDa subunit of cleavage polyadenylation specificity factor.

The nucleocytoplasmic transport, nuclear envelope and the NPC

Active transport into and out of the nucleus proceeds through the NPCs and is largely mediated by factors acting as shuttling carriers. Members of the importin- β family of transport receptors represent a prominent class of such factors, which shuttle between the nucleus and the cytoplasm. The interactions between these receptors and their cargos are regulated by the small G-protein Ran. D.Görllich (Heidelberg, Germany) presented an *in vitro* system that was used to follow nuclear import of Ran in complex with NTF2, a small factor binding RanGDP and associating with the NPC via the nucleoporin p62. The transport receptor TAP, which is not a member of the importin- β family, was described by E.Izaurralde (Geneva, Switzerland). Evidently, TAP interacts with hGle2 and E1B-AP5, both of which have been implicated in the export of cellular mRNA, as well as with multiple nucleoporins including Nup98, Nup153, p62 and hCG1, and with three major NPC subcomplexes consisting of Nup93/Nup205, RanBP2/SUMO-RanGAP1 and Nup215-(CAN)/Nup88. Based on her data, Dr Izaurralde suggested that TAP may act as a 'bridge', or an adaptor, between mRNA export cargo and the NPC.

Nuclear export in yeast was discussed by E.Hurt (Heidelberg, Germany). By genetically exploiting mutants of the Nup84p complex, he has found the nuclear import receptor for hnRNP-like proteins (i.e. Mtr10p/Kap11p), and nuclear export factors for mRNA (i.e. Mex67p and Mtr2p). Moreover, he presented functional evidence that the transport receptor TAP (i.e. the human homolog of yeast Mex67p), together with the novel human protein p15, acts as a conserved nuclear mRNA export complex. Using a GFP-tagged version of ribosomal protein L25, he has identified components [rn1-1 (Ran-GAP) and prp20-1 (Ran-GEF), as well as nucleoporins nup49p and nsp1p] involved in the nuclear export of ribosomes. Proteins that interact with transportin 1 (TRN1), the import receptor for shuttling hnRNP proteins harboring an M9 nuclear localization signal (NLS), were searched for by G.Dreyfuss (Philadelphia, PA). A phage display system was used to identify the nucleoporin Nup153, which contains an M9 NLS. It was documented further that Nup153 interacts with TRN1 and other import and export receptors in a RanGTP-regulated fashion. Most interestingly, Dreyfuss provided evidence that Nup153 is a 'mobile' nucleoporin that shuttles between the nuclear and cytoplasmic faces of the NPC. Intranuclear transport of the Balbiani ring (BR) granules (i.e. specific pre-mRNA particles) has been investigated by B.Daneholt (Stockholm, Sweden). He showed that newly synthesized BR particles, upon release

from the BRs, rapidly distribute throughout the nucleoplasm, suggesting their random movement within the entire nucleus prior to their export into the cytoplasm.

Y.Gruenbaum (Jerusalem, Israel) showed that the binding of *Drosophila* lamin Dm0 to chromatin requires two regions in its C-terminal tail domain, and the presence of histones H2A and H2B. It was established that otefin, an inner nuclear membrane protein that is required for the interaction of nuclear vesicles with chromatin in a *Drosophila* nuclear assembly extract, interacts with the lamin Dm0 rod domain. These two proteins appear to be part of the same protein complex *in vivo*. Dr Gruenbaum also presented preliminary results emerging from a functional analysis of the *Caenorhabditis elegans* single B-type lamin, termed CeLam-1. RNA-mediated genetic interference with CeLam-1 activity resulted in early embryonic lethality, thus demonstrating that CeLam-1 is an essential gene. Employing time-lapse fluorescence microscopy of GFP-tagged lamins in living cells, R.Goldman (Chicago, IL) demonstrated that during interphase, lamin foci appear, disappear and are translocated through the nucleoplasm. During S-phase, some of these foci become closely associated with replicons. *Xenopus* interphase extracts were used to show that the function of these replicons could be inhibited reversibly when an N-terminally deleted human lamin A mutant was introduced into nuclei. Interestingly, this mutant lamin did not interfere with nuclear envelope assembly. Dr Goldman also identified a tail deletion mutant of *Xenopus* lamin B, which appeared to disrupt NE assembly by preventing both chromatin decondensation and membrane vesicle fusion around chromatin. R.Foisner (Vienna, Austria) documented that the integral membrane protein LAP2- β may target nuclear membrane vesicles to chromosomes after mitosis and thereby promote assembly of the nuclear lamina. Moreover, the non-membrane-bound LAP2- α was found to dissociate from chromosomes during metaphase upon mitosis-specific phosphorylation and to relocate to chromosomal areas at very early stages of nuclear reassembly, clearly before LAP2- β -containing vesicles and lamins accumulated around chromosomes. A novel C-terminal LAP2- α -specific region was shown to be required for this chromosome association. Furthermore, LAP2- α co-localized transiently with intranuclear and peripheral lamin A during G₁ phase, and it interacted preferentially with A-type lamins *in vitro*. Based on these results, a role for LAP2- α in the post-mitotic establishment of nuclear structure and lamin A assembly was proposed.

S.Georgatos (Heraklion, Greece) talked about the involvement of chromodomain proteins HP1a, M31 and M32 in NE-chromatin interactions. These proteins bound specifically to isolated NEs or nuclear membranes stripped from peripheral components, and specifically decorated the cell nucleus when co-incubated with permeabilized cells. The association of chromodomain proteins with the NE was potently inhibited by cytosolic factors, including a 55 kDa protein that was partially purified and characterized. The 55 kDa polypeptide probably regulates NE-chromosome interactions during mitosis, when cytoplasmically disposed elements gain access to intranuclear structures.

L.Santella's (Napoli, Italy) presentation focused on intranuclear signaling mediated by Ca^{2+} . She showed that functional IP₃ and cADPr receptors reside in the inner nuclear membrane of starfish nuclei. Microinjection of Ca^{2+} channel modulators induced nuclear calcium waves, which emanated from the point of injection and rapidly spread over the entire nucleus. Dr Santella also showed that the Ca^{2+} messenger NAADP⁺ exists in a functional state in starfish nuclei.

The problem of NPC assembly was addressed by E.Kiseleva (Novosibirsk, Russia). Employing *Xenopus* oocytes, *Drosophila* embryos and yeast cells as experimental systems, she documented that NPC assembly *in vitro* and *in vivo* proceeds through a series of intermediates depicted at the cytoplasmic and the nuclear faces of the NE. Moreover, her meticulous morphological analysis identified three modes of NPC assembly in pre-existing NE. Dr Kiseleva presented morphological evidence suggesting that NPC disassembly proceeds in the reverse order to that of assembly. U.Aebi (Basel, Switzerland) presented results on NPC molecular architecture and its functional dynamics. He showed a new tomographic three-dimensional reconstruction of fully native NPCs embedded in a thick amorphous ice layer and visualized using an energy-filtering transmission electron microscope. Toward a structure-based functional analysis of the NPC, Dr Aebi presented time-lapse atomic force microscopy data of fully native NPCs, documenting the reversible opening and closing of the distal ring of an individual NPC's nuclear basket in response to micromolar amounts of calcium in the buffer environment. The distal ring, which acts like an iris diaphragm, might represent a gating device for cargos transported through the NPC into and out of the nucleus.

A.Steven (Bethesda, MD) presented new structural findings on the nucleocytoplasmic exchanges occurring during the course of herpes virus replication. He reviewed current ideas about the course of events governing processing and nuclear import and export of capsid-related structures at successive stages of the virus replication cycle. His morphological data included some striking electron micrographs depicting (i) the initial assembly of a precursor procapsid which, upon maturation, undergoes dramatic changes in structure and composition; and (ii) the capsid-capsid interactions occurring during tegument formation.

Execution of apoptosis

Caspases, core components of the apoptotic machinery, are expressed ubiquitously and activated during apoptosis by various agents and inducers. Beside the death receptor pathway which is activated by the binding of ligands to tumor necrosis factor (TNF) receptor family members, other apoptotic pathways are induced by growth factor deprivation, glucocorticoids, cytotoxic agents and mitogenic oncogenes.

Several of the apoptotic pathways stimulate the release of cytochrome *c* from mitochondria into the cytosol. Cytochrome *c* binds to a cytosolic factor known as apoptotic protease-activating factor-1 (Apaf-1). This binding promotes the formation of a complex with procaspase-9 and its activation. To this end, E.Alnemri

(Philadelphia, PA) documented that full-length Apaf-1 exists as a monomer which is transformed into an oligomeric complex upon binding to cytochrome *c* and hydrolyzing bound dATP. Activation of the complex consisting of caspase-9, Apaf-1 and cytochrome *c* was also investigated by Y.Lazebnik (Cold Spring Harbor, NY) who presented data to support the concept that a complex consisting of caspase-9 and Apaf-1 functions as a holoenzyme, with caspase-9 acting as the catalytic subunit and Apaf-1 as the allosteric regulator. Some oncogene products such as, for example, E1A might facilitate activation of the caspase-9, e.g. by modulating its interaction with Apaf-1. The complexity of the apoptotic machinery was revealed by data demonstrating that the release of cytochrome *c* does not necessarily result in apoptosis. Apoptotic execution can be prevented by caspase inhibitors as shown in nerve growth factor (NGF)-deprived neurons by J.-C.Martinou (Genève, Switzerland). After re-exposure to NGF, mitochondria recovered, with their normal size and cytochrome *c* content being restored.

A cell-free system was employed by W.Earnshaw (Edinburgh, UK) to show that nuclear disassembly can be driven by factors activated downstream of caspases. One such factor, the caspase-activated DNase (CAD) can induce chromatin condensation in the absence of other cytosolic factors. A second factor involved in apoptotic chromatin condensation is the widely studied enzyme topoisomerase II, which appears to interact directly with CAD. CAD is expressed ubiquitously in various tissues, together with two forms of its inhibitory chaperone, ICAD-L and ICAD-S, which are generated by alternative splicing. Activation of caspase-3 leads to cleavage of ICAD which then loses its affinity for CAD, thereby releasing the nuclease to digest the DNA. S.Nagata (Osaka, Japan) reported that nuclei of cells which were transformed with cleavage-resistant ICAD, however, exhibited significant changes in their morphology despite the fact that no signs of DNA fragmentation were observed, either at the level of cleavage of 50–200 kb domain fragments or at the level of producing a nucleosomal ladder. These findings indicate that morphological changes are driven by processes independent of DNA cleavage. The CAD-ICAD complex is also referred to as DNA fragmentation factor (DFF), a heterodimer composed of the two subunits DFF45 (ICAD) and DFF40 (CAD). W.Garrard (Dallas, TX) reported on the sequencing and expression of the human DFF40 subunit. Accordingly, purified DFF40 exhibited an intrinsic DNase activity that was stimulated markedly by the chromatin-associated proteins histone H1 and the high-mobility group proteins. Moreover, DFF40 also triggered chromatin condensation when incubated with nuclei. In many cases, nuclear condensation and fragmentation require the release of cytochrome *c* from mitochondria. S.Orrenius (Stockholm, Sweden) presented data showing that thermotolerant cells with resistance to apoptosis regularly express heat shock proteins that stabilize the mitochondrial transmembrane potential so as to inhibit the release of cytochrome *c* into the cytosol. Hence, bidirectional signaling might occur, in the sense that the nucleus is not only a target but also an inductive/inhibitory signaling center for apoptosis.

Concluding remarks

This EMBO workshop attempted to provide a broad, yet state-of-the-art, coverage of the molecular and structural biology of the cell nucleus, highlighting topics ranging from chromosome territories to apoptic nuclear changes. Ultimately, defects in nuclear components causing disease were discussed in a special session involving shorter talks. In addition to 57 talks, 56 posters were presented, many of them being of remarkable quality, such that *Nature Cell Biology* awarded a special prize to the young scientist chosen for best poster presentation. Admittedly, the scientific program was rather packed, maybe a bit too much for a 4 day workshop. Nevertheless, the workshop received an excellent rating by most participants, who also opted for another within the next 2–3 years.

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