Spatial and dynamic organization of molecular structures in the cell nucleus

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CHAPTER 1

General introduction

1. The cell nucleus

1.1 A concise historical perspective

The eukaryotic cell nucleus was first named in 1831 by Robert Brown when he observed an opaque structure in orchid cells. Most likely, he was not the first person who observed the cell nucleus. Already in 1802, it was Franz Bauer who described a structure in plant cells that could reflect the cell nucleus. Also, it is even possible that van Leeuwenhoek was actually the first person who observed the cell nucleus back in 1682 when he studied plant cells. These scientists, however, had no clue about the content and function of this organelle at that time. Knowledge about the constituents and function of the nucleus evolved rapidly after the first isolation of the nucleus in 1869 by Friedrich Miescher (Miescher, 1871). For this isolation he used white blood cells derived from pus, which he treated with a pig-stomach extract and acid. Miescher discovered that the nucleus contains a substance made up of large molecules containing phosphorus and nitrogen which he named 'nuclein'. When the substance was separated into protein and acid molecules it was in 1889 referred to as nucleic acid by a pupil of Miescher, Richard Altmann. It was only since the discovery of the chemical structure of DNA by James Watson and Francis Crick in 1953 that the precise role of this molecule in life became known.

1.2 What is inside the cell nucleus?

1.2.1 Chromatin

The mammalian nucleus is surrounded by a double membrane and contains subcompartments that partition macromolecular machineries to facilitate and coordinate the various nuclear functions, including DNA replication, DNA repair, gene transcription, RNA processing, RNA transport and the transduction of intra- and extracellular signals (Stenoien, 2000; Carmo-Fonseca, 2002; Rippe, 2007). Essentially, there are two main compartments that can be distinguished in the cell nucleus, one is the chromosome territory, and the other is the remaining space, called the interchromatin domain (ICD) (Cremer, 2002). The basis for this assumption is the model (for which substantial evidence has been presented) that each chromosome forms a distinct chromosome territory that shows no or little intermingling with neighbouring chromosomes (Manuelidis, 1985). Within a chromosome territory, DNA is folded around octamers of histone proteins forming nucleosomes separated by linker DNA. The resulting "beads on a string" conformation is a platform for other proteins to bind and is collectively called chromatin. This structure is proposed to fold into 30 nm fibers that form, in turn, DNA loop domains (Cook & Brazell, 1976; Paulson & Laemmli, 1977). These DNA loops vary in size from 20 to 200 kb and contain many genes and clusters of functionally related genes. DNA loops are not only thought to be important for gene regulation, but also for the organization of replicons (a region of DNA that replicates from a single origin of replication in the genome). DNA loop anchorage sites were shown to colocalize with replication origins (van der Velden, 1984; Razin, 1986) and DNA loop sizes were shown to correlate with that of the replicons (Buongiorno-Nardelli, 1982; Marilley & Gassend-Bonnet, 1989). DNA loops are suggested to be attached to the nuclear matrix via Loop Anchorage Regions (LARs).

These regions have a rather complex structure and may include several elements, e.g., topoisomerase II binding sites (for review see Razin, 1996; Vassetzky, 2000b). Together, these studies suggest a strict organization principle for chromatin. The reality is, however, that we know very little about the organization of chromatin in the cell nucleus. Even there is debate whether the 30 nm fibre exists in living cells (Maeshima et al., 2010).

Typically, euchromatin is referred to as a transcriptionally active open chromosome structure having ample access to the transcription and RNA processing machinery, while heterochromatin is referred to as a transcriptionally inactive, compact chromatin structure (John, 1988; Felsenfeld & Groudine, 2003). However, these morphological terms do not provoke a very clear functional distinction, as some genes show transcriptional activity in supposed heterochromatic regions (Bühler & Moazed, 2007) and some are silenced in supposed euchromatic regions. Despite some cell type specific variation, heterochromatin is mainly positioned at the nuclear periphery and around nucleoli. It is probably also for this reason that chromatin at the nuclear periphery shows a relatively low transcriptional activity and a low gene density (Boyle, 2001; Finlan, 2008). Transcriptionally competent regions preferentially localize towards the interior of the cell nucleus and to the periphery of chromosomal territories (Verschure, 1999). The status of chromatin is characterized best by the presence or absence of specific histone and DNA modifications, rather than relying on morphological features. Histone modifications associated with transcriptional repression include methylation of histone H3 on Lysine 9 (Steward, 2005) and Lysine 36 (Strahl, 2002), and deacetylation (leading to hypoacetylation) of histone H3 (Grunstein, 1997; Turner, 2000). Histone H3 lysine 9 (H3-K9) methylation creates a specific binding site for heterochromatin protein 1 (HP1), which is targeted there by the methylating enzyme SUV39H1 (Steward, 2005; Krouwels et al., 2005). However, methylated H3-K9 is also able to suppress transcription in absence of HP1 by a mechanism involving histone deacetylation (Steward, 2005).

Methylation is the most common form of alkylation, and in biochemistry it refers to the replacement of a hydrogen atom with a methyl group (CH3). In biological systems, DNA methylation is mediated by a conserved group of proteins called DNA (cytosine-5) methyltransferases (Goll & Bestor, 2005). In vertebrates DNA base methylation typically occurs at cytosine-phosphate-guanine sites (CpG sites), DNA regions where a cytosine is directly followed by a guanine in the DNA sequence. This methylation results in the conversion of the cytosine to 5-methylcytosine, and the formation of Me-CpG is catalyzed by the enzyme DNA methyltransferase. CpG sites are uncommon in vertebrate genomes but are often found at higher density near vertebrate gene promoters where they are collectively referred to as CpG islands. The methylation state of these CpG sites can have a major impact on gene activity/expression in somatic cells. In eukaryotes, typically 2-7% of cytosines (bases that are part of the nucleotides which constitute DNA) are methylated, and this methylation is often tissue specific (Razin & Cedar, 1991). Cytosine methylation is common to all large-genome eukaryotes and present in only a few small-genome eukaryotes. Not only is there a clear correlation between gene expression and undermethylation, transfection experiments clearly demonstrated that this modification acts as a repressor of transcription (Razin & Cedar 1991). Tissue-specific genes appear to be methylated in almost all cell types and presumably undergo demethylation when expressed in a specific tissue type. In contrast, housekeeping genes contain CpG islands that are

unmethylated in all cells (Bird, 1986). Both histone and DNA methylation can act as epigenetic markers providing heritable mechanisms for gene silencing (Nakayama, 2001; Grewal & Rice, 2004).

1.2.2 The interchromatin domain

The interchromatin domain is inevitably a crowded space since both proteins and RNAs travel through this compartment to reach their destination or exert their function in this compartment. RNA forms together with proteins ribonucleoprotein (RNP) particles, which are thought to form a continuous nuclear network. This structure is the source of the RNP particles that are released from the nucleus by chemical or mechanical extraction (Smetana, 1963). RNA-selective staining procedures have made a complete ultrastructural characterization of the nuclear RNP network possible (Bernhard, 1969; Biggiogera & Fakan, 1998). Making use of EDTA regressive staining to localize RNA, Monneron & Bernhard were able to define, characterize and classify the interconnected nuclear RNP structures and distinguished interchromatin granule clusters, perichromatin fibrils, perichromatin granules and coiled bodies (Monneron & Bernhard, 1969). The discovery of these structures was important for our understanding of nuclear RNA metabolism (Misteli & Spector, 1998; Misteli, 2000). Perichromatin fibrils are sites of RNA transcription (Bachellerie, 1975; Cmarko, 1999), whereas interchromatin granule clusters (or speckles) play a central role in the assembly and/or modification of pre-mRNA splicing factors (Mintz, 1999; Smith, 1999; Spector, 2001).

To ensure unimpeded exchange of molecules between the nucleus and the cytoplasm, the ICD has direct access to nuclear pores. Nuclear pores are multiprotein complexes embedded in the nuclear envelope, which mediate and regulate nucleocytoplasmic transport (Vasu & Forbes, 2001; Fahrenkrog & Aebi, 2003). The ‘basket’ structure at the nucleoplasmic side of the nuclear pore consists of eight filaments, which attach to a distal ‘ring’ structure. Several reports suggest that these ‘rings’ connect to filaments that extend into the nucleus and facilitate nucleocytoplasmic transport (Cordes, 1993; Parfenov, 1995; Cordes, 1997).

1.2.3 Chromatin organization is dynamic.

It has been hypothesized that the spatial arrangement of the genome in the interphase nucleus is an important factor in the regulation of gene activity (Zink, 2004) and possibly also in orchestrating DNA replication and DNA repair. Gene loci positioned megabases apart on the same or even different chromosomes were shown to interact, suggesting that some genes are spatially positioned together in a microenvironment to coordinate their transcription and/or to facilitate the processing of their RNA transcripts (Branco & Pombo, 2006; Lonard & O'Malley, 2008). Although it is a proven fact that the cell nucleus is an ordered and structured compartment, the same structure is highly dynamic to regulate key functions such as transcriptional activity in response to signaling events and differentiation. In particular, fluorescence in situ hybridization and the application of chromosome conformation capture techniques, or a combination of both, provided important insight in the existence and dynamics of long-range

chromatin-chromatin interactions (Dekker, 2006). Also the positioning of specific chromatin regions at particular nuclear bodies are examples supporting the notion that the genome is not randomly organized in the cell nucleus (Smith, 1995). The challenge now is to unravel the underlying mechanisms that establish and maintain this non-random organization of chromatin in the cell nucleus.

Understanding the organization principles of the nucleus is important because rearrangements in nuclear organization have been observed in cells derived from various diseases, including cancer, and in cells with a senescent or apoptotic phenotype (Vijg & Dollé, 2002; Busuttil, 2004; Raz, 2008; Shin et al., 2010). Furthermore, a striking change in nuclear organization has been observed in embryonic stem cells at the onset of differentiation (Butler et al., 2009). Most profound rearrangements in chromatin structure have been observed when a sperm pronucleus and an egg nucleus fuse after fertilization. In many species, the size of DNA loops increases from ca. 50 kbp in early embryogenesis to 200 kbp in cells of the adult organism (Buongiorno-Nardelli, 1982). Notably, the average size of DNA loops was observed to decrease in transformed cells (Linskens, 1987). In several human cancer cell lines the DNA loop size was found to be about 50 kbp, i.e. significantly smaller than in normal cells where it varies between 70-700 kbp (Oberhammer, 1993). It is important to unravel the mechanisms that control these aspects of nuclear organization to understand their impact on the etiology, progression, and possibly treatment of human diseases. Once understood, the hope is that this new knowledge might open possibilities for treatment strategies of human disease.

1.3 Nuclear bodies

In addition to soluble components, the interchromatin domain (ICD) contains different kinds of subcompartments or nuclear bodies that vary in size, composition and function (Figure 3; Tsutsui, 2005). Unlike the organelles present in the cytoplasm, nuclear bodies are not surrounded by a membrane structure. Therefore, it is still an open question how these bodies assemble and maintain their unique protein constellation. It came more or less as a surprise that most if not all proteins that reside in bodies are in a dynamic equilibrium with their surroundings (Misteli, 2001). A few of these proteins have been reported to shuttle between various bodies (Snaar, 2000; Olson, 2004). Thus far, up to twelve different types of bodies have been identified, which are either permanently or temporally present in the cell nucleus depending on the physiological state of the cell (Spector, 2001). The most prominent nuclear bodies are discussed below.

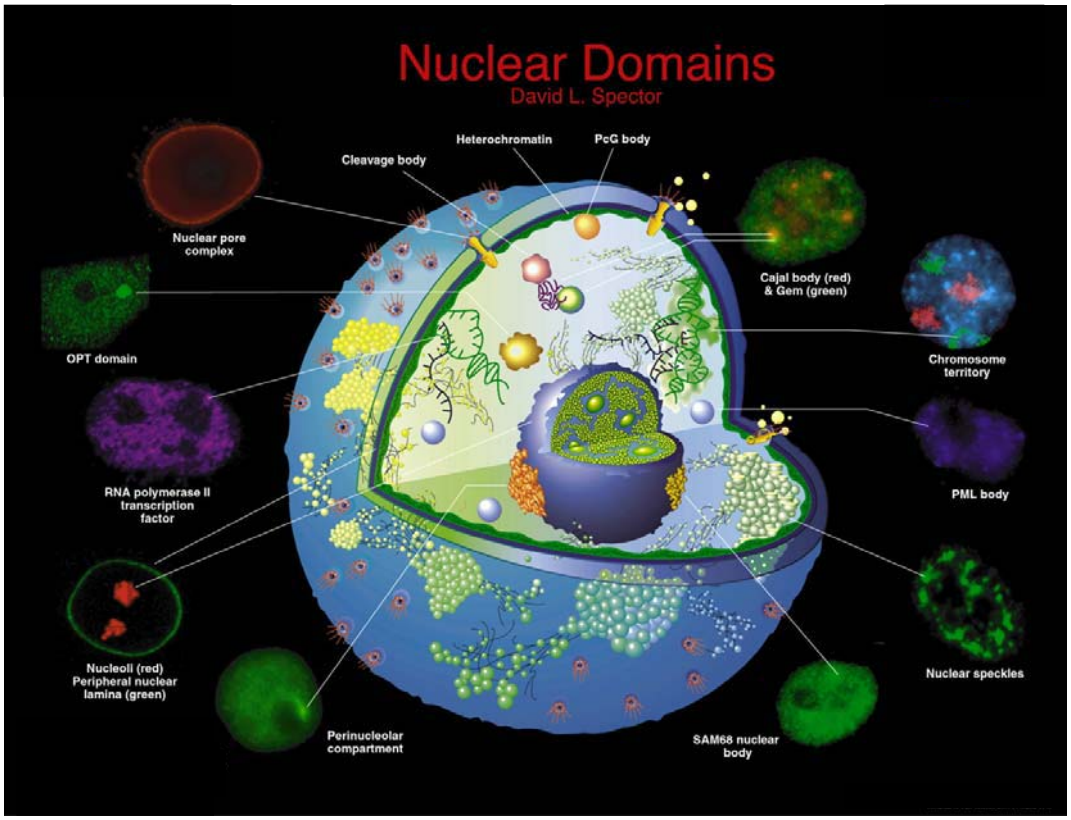

Figure 3. Protein domains present in the mammalian cell nucleus.
OPT domains: transcriptionally active sites that contain a specific set of transcription factors and RNA pol II, appear close to nucleoli in G1. Nuclear pore complex: multiprotein complexes where the inner and outer nuclear membranes are fused and where materials can transit between the cytoplasm and the nucleus. Cleavage body: either overlap or are localized adjacent to Cajal bodies, they consist of factors involved in the cleavage and polyadenylation steps of pre-mRNA processing. Heterochromatin: inactive chromatin. PcG body: have been found to be associated with pericentric heterochromatin (Saurin, 1998) and contain polycomb group proteins (i.e. RING1, BMI1 and hPc2). Gems: Gemini of Cajal bodies, they have been found adjacent to or coinciding with Cajal bodies. Gems are characterized by the presence of the survival of motor neurons gene product (SMN) and an associated factor, Gemin2 (Matera, 1999). SAM68 nuclear bodies/ Perinucleolar compartments (PNC): have been identified as unique structures that are associated with the surface of nucleoli and are thought to play a role in RNA metabolism (Huang, 2000). Both structures are predominantly found in cancer cells and they are rarely observed in primary cells. Other nuclear bodies are discussed in the text. (Adapted from Spector, 2001)

1.3.1 Nucleolus

The nucleolus was one of the first subcellular structures that were identified by early users of the light microscope (Montgomery, 1898). Nucleoli appeared as highly refractive black dots in the nucleus of cells, reflecting its dense protein content. The nucleolus is a dynamic multifunctional nuclear domain where ribosomal RNA is synthesized and the ribosomal subunits are assembled (Olson, 2002). Using mass spectrometry, up to 700 human proteins have been characterized in purified nucleoli

and up to 30% of these proteins are encoded by previously uncharacterized genes (Andersen, 2002; Andersen, 2005). Although it is not expected that all proteins found in nucleoli also have a function in this structure, their diversity is consistent with the idea that the nucleolus performs additional roles beyond generating ribosomal subunits (Pederson, 1998; reviewed by Olson, 2002). For example, many proteins related to cell cycle regulation (about 3.5% of the identified proteome), DNA damage repair (about 1%) and pre-mRNA processing (about 5%) have been identified in isolated nucleoli. Nucleoli have therefore been implicated in processes such as cell cycle regulation (Yamauchi, 2007), virus-replication (Jacob, 1968), regulation of tumor suppressor and oncogene activities (Itahana, 2003), DNA damage repair (van den Boom, 2004), signal recognition particle assembly (Jacobson & Pederson, 1998), RNA modification (Sansam, 2003), tRNA processing (Paushkin, 2004), aging by modulating telomerase function (Kieffer-Kwon, 2004; Zhang, 2004), regulation of protein stability (Mekhail, 2004; Rodway, 2004), senescence (reviewed by Comai, 1999; Rosete, 2007) and apoptosis (Baran, 2003). In addition, nucleoli are thought to play a role in the maturation and transport of mRNAs (Schneiter, 1995).

A possible function of the nucleolus in mRNA export was proposed 25 years ago based on observations in interspecies heterokaryons obtained from fusing chicken erythrocytes with mouse cells. It was observed that in the dormant chicken nucleus gene expression was initiated at precisely the same time when a nucleolus became detectable (Sidebottom & Harris, 1969; Deák, 1972; Harris, 1972). Furthermore, it was observed that UV irradiation of the chicken nucleolus in these heterokaryons greatly suppressed chicken-specific gene expression (Perry, 1961; Deák, 1972). Additional support for a role of nucleoli in mRNA export came by the observation that processed myc and myoD transcripts, unlike actin or lactate dehydrogenase transcripts, are present in the nucleolus of several cell types (Bond & Wold, 1993). Because myc intron 1-containing pre-mRNA was not detected in nucleoli but instead in the nucleoplasm, it was suggested that the nucleolar localization of Pol II transcripts is a general phenomenon for transcripts that have a rapid cytoplasmic turnover only (Bond & Wold, 1993). It should be noted, however, that these observations have thus far not been confirmed by others. In cells derived from species that vary from sea urchins to humans, nuclear poly(A)+ RNA is found present primarily in discrete "transcript domains", which often concentrate around nucleoli (Carter, 1991). Thus, whether nucleoli are involved in some steps of nuclear mRNA export has yet to be confirmed.

1.3.2 Cajal bodies

Cajal bodies are spherical nuclear bodies that are generally present in dividing cells and in cells that show high transcriptional activity. They are prominently present in most tumor cells, which rapidly proliferate, and in neurons that are metabolically active (Cajal, 1903; Ogg & Lamond, 2002). They were first reported in 1903 by the Spanish cytologist Ramón y Cajal who named them “nucleolar accessory bodies”, because of their prominent association with nucleoli in neuronal cells (Cajal, 1903). Cajal bodies were subsequently rediscovered by numerous researchers and given a variety of names in different cell types (Gall, 2000). The name “coiled body” was coined by electron microscopists, referring to their morphology in EM sections. It was not

until 1999 that Joseph Gall suggested to link Cajal's name to the nuclear body that was originally described by him in 1903 (Gall, 1999). The number and size of Cajal bodies varies among cell types (in mammalian cells typically 0 –10 per nucleus, ranging 0.1–2 μm in diameter) and they also show cell cycle variation within cell types. Cajal bodies can be discriminated in the nucleus by the presence of the protein coilin, either by immunocytochemistry or by exogenous expression of coilin-GFP (Snaar, 2000; Ogg & Lamond, 2002).

Recent studies indicated that Cajal bodies play a role in the assembly and/or modification of the transcription and RNA-processing machinery (Gall, 1999; Jády, 2003). Cajal bodies are enriched in snRNPs (small nuclear ribonucleoproteins) and snoRNPs (small nucleolar ribonucleoproteins) spliceosome subunits. Solid evidence has been provided that the final steps in snRNP maturation including snRNA base modification, U4/U6 snRNA annealing, and snRNA-protein assembly of both snoRNAs and snRNAs occur in Cajal bodies (Darzacq, 2002; Verheggen, 2002; Jady, 2003; Stanek, 2008). Despite their role in splicing factor maturation, Cajal bodies do not represent major sites of transcription *per se*, but they were observed frequently in association with a few specific genes coding for small nuclear snRNAs and histone genes in interphase cells. Because Cajal bodies do not contain either DNA (Thiry, 1994) nor non-snRNP protein splicing factors (Raska, 1991; Carmo-Fonseca, 1992) it is unlikely that these bodies are sites of transcription or pre-mRNA splicing. Thus, the current view is that Cajal bodies play a crucial role in the spliceosome cycle in which the production of new snRNPs is promoted by the import and modification of substrates (reviewed by Staněk & Neugebauer, 2006). In addition, Cajal bodies may play a role in the recycling of snRNPs from splicing complexes that are released after finishing pre-mRNA splicing. Interestingly, also the RNA subunit (hTR) of the enzyme telomerase was shown to accumulate in Cajal bodies (Jady, 2004; Zhu, 2004). During the S-phase, when telomerase is likely to act, hTR has been found to associate with a subset of telomeres while Cajal bodies are present at close distance (Jady, 2006; Tomlinson, 2006). Mutant hTR, which fails to accumulate in Cajal bodies, was fully capable of forming catalytically active telomerase in vivo. Telomere extension, however, turned out to be strongly impaired (Cristofari, 2007). This functional deficiency was accompanied by a decreased association of telomerase with telomeres suggesting that Cajal bodies also play an important role in telomere elongation.

1.3.3 Speckles

Speckles, also referred to as SC35 domains or interchromatin granule clusters (IGC), are thought to be storage sites of factors involved in mRNA synthesis, splicing, and RNA export (Dirks, 1999; reviewed by Lamond & Spector, 2003). The prevailing view is that splicing factors are recruited from speckles to sites of active transcription (Dirks, 1997; Misteli, 1997). At the electronmicroscopical level of resolution, IGCs range in size from one to several micrometers in diameter and are composed of 20–25-nm granules that are connected by thin fibrils, resulting in a beaded chain appearance (Thiry, 1995)..Other splicing factor containing structures in the nucleus are perichromatin fibrils, Cajal bodies and interchromatin-granule-associated zones, also referred to as paraspeckles (Visa, 1993). Speckles, however, can be easily discriminated from these structures by their morphology and protein content and are pre-

sent throughout the nucleoplasm in regions that contain little or no DNA (Thiry, 1995). Furthermore, *in situ* hybridization studies revealed that speckles do not contain genes. Instead, active transcription sites were found positioned throughout the nucleoplasm and also next to speckles. Some genes have been reported to localize preferentially close to speckles (Huang, 1991; Xing, 1993; Xing, 1995; Smith, 1999; Johnson, 2000).

These observations indicate that speckles are functionally related to gene expression. Hall and coworkers proposed that speckles are hubs that spatially link the synthesis of specific pre-mRNAs to a rapid recycling of copious RNA metabolic complexes, thereby facilitating expression of many highly active genes (Hall, 2006). In addition to increasing the efficiency of each step, sequential steps in gene expression might be structurally integrated at each speckle, consistent with evidence that the biochemical machineries for transcription, splicing, and mRNA export are coupled (Hall, 2006). The observation that speckles also contain poly(A)$^+$ RNA led to the suggestion that speckles play a role in RNA metabolism and export (Carter, 1991, 1993; Molenaar, 2004). A substantial amount of mature mRNA is found to be retained in nuclear speckles until ATP is added, suggesting that speckles prevent the export of otherwise fully processed mRNAs until an energy-requiring cellular signal releases them (Schmidt, 2006).

1.3.4 PML bodies

The most mysterious of all nuclear bodies is the PML body, also known as ND10 (nuclear domain 10) or Kremer bodies (Kr) (Dyck, 1994; Koken, 1994; Weis, 1994). Promyelocytic leukemia bodies (PML bodies) are nuclear protein bodies, ranging in size from 0.3 µm to 1.0 µm in diameter and are characterized by the presence of the PML protein. Typically there are 10-20 PML nuclear bodies (PML-NB) present in the cell nucleus and they are believed to be tightly associated with nuclear matrix proteins (Stuurman, 1992). Electron microscopy studies have shown that PML-NBs are composed of a ring-like protein structure that does not contain nucleic acids in the centre of the ring (Boisvert, 2000; Dellaire & Bazett-Jones, 2004). At the periphery of the ring, however, PML-NBs are believed to make extensive contacts with chromatin fibers through protein-based threads that extend from the core of the bodies (Eskiw, 2004). These contacts have been proposed to be essential for maintaining the integrity and positional stability of PML-NBs in the nucleus.

PML bodies were originally characterized using human auto-antibodies derived from patients with primary biliary cirrhosis (Bernstein, 1984; Szostecki, 1990; Maul, 2000). Using such antibodies, Bernstein *et al.* described in 1984 the presence of certain typically speckled structures, which later came to be known as nuclear domain 10 (ND10), PML bodies, or PODs. PML bodies, however, were first named after examining cells derived from patients with acute promyelocytic leukemia (APL) (de The, 1991). Most APL patients carry the chromosomal translocation t(15,17), resulting in a fusion protein between the retinoic acid receptor-α (RAR) and the PML protein (de The, 1991; Melnick & Licht, 1999). The PML-RARα fusion protein fails to locate to PML bodies (Melnick& Licht, 1999) and is thought to block differentiation of bone marrow cells (Naeem, 2006). In addition, the leukemic blast cells of APL patients reveal fragmented or dispersed PML bodies. Treatment of APL patients with all-trans-

retinoic acid or arsenic trioxide results in the degradation of the PML-RARα fusion protein, restoration of PML bodies and remission of the disease (Koken, 1994; Weis, 1994). Recently, it has been shown that arsenic-induced degradation of PML or PML-RARα is mediated by the ubiquitin ligase RNF4 (Lallemand-Breitenbach, 2008; Tatham, 2008).

In PML bodies, nearly eighty different proteins have been found present. Among them are Sp100, Sp140, SUMO-1, HAUSP (USP7), CBP and BLM, Daxx, pRB, and p53 (Hodges, 1998; LaMorte, 1998; Alcalay, 1998; Zhong, 1999; Zhang, 1999; Zhong, 2000; for a review see Salomoni & Pandolfi, 2002). Because of this variety of proteins, PML bodies have been implicated in many different functions, such as transcription regulation, protein storage, senescence and interferon-induced antiviral defense (Chelbi-Alix, 1995; Maul, 1998). Concerning transcription regulation, PML bodies have been suggested to be involved in both transcriptional activation (Maul, 1998; Zhong, 2000) and transcriptional repression (Everett, 1999). However, whether PML bodies play indeed an essential role in transcription is not clear since $PML^{-/-}$ mice show a very moderate phenotype. $PML^{-/-}$ mice are morphologically normal and do not have higher rates of spontaneous cancers than littermate controls (Wang, 1998a; Wang, 1998b). Some regions of the human genome that display high transcriptional activity do, however, associate frequently with PML NBs, although RNAi-mediated knockdown of PML did not perturb the expression of these genes (Wang, 2004).

PML bodies have also been implicated in DNA damage repair as several repair factors transit through PML bodies in a temporally regulated manner (Graham & Bazett-Jones, 2004). Furthermore, PML bodies have been shown to recruit single-stranded DNA (ssDNA) molecules in response to exogenous DNA damage (Bøe, 2006). PML bodies are also associated with the sites of initial viral DNA transcription/ replication in virus infected cells (Maul, 1996; Maul, 1998; Guldner, 1992; Stadler, 1995). PML bodies are subsequently disrupted at later stages in the infectious viral cycle (Maul, 1993). Upon treatment of cells with interferon, PML is induced and the number of nuclear bodies increases dramatically (Lavau, 1995; Gaboli, 1998). This suggests a role for PML and the nuclear bodies as part of the anti-viral defense machinery activated by interferons in viral infections. DNA and RNA viruses have a variety of effects on PML body morphology, where arenaviruses and the human immunodeficiency virus (HIV) transport PML to the cytoplasm, and herpesviruses "unwind" PML bodies (Borden, 1998; Melnick & Licht**,** 1999; Maul, 2000; Turelli, 2001). However, findings with HIV infected cells are somewhat controversial, since another group did not see PML NBs translocate during infection (Bell, 2001).

It has been established that PML is the primary essential component of PML NBs, and conjugation of SUMO-1 to PML is suggested to be a prerequisite for PML body formation (Ishov, 1999; Zhong, 2000). PML SUMOylation likely plays a regulatory role in the structure, composition, and function of PML bodies (Sternsdorf, 1997). Elegant studies demonstrate that the RING domain of PML directly interacts with Ubc9, an enzyme which covalently attaches the SUMO1 protein onto distal regions of PML, including one B-box and a region near the nuclear localization signal (Duprez, 1999).

It has been demonstrated that PML contains a SUMO binding motif that is independent of its SUMOylation sites and is required for PML-NB formation. A model for PML-NB formation was proposed in which PML SUMOylation and noncovalent binding of PML to SUMOylated PML through the SUMO binding motif constitutes the nucleation event for subsequent recruitment of SUMOylated proteins and/or proteins containing SUMO binding motifs to the PML NBs (Shen, 2006).

Alternatively, PML bodies may have the ability to self-assemble. Purified RING-domains (small zinc-binding domains) of PML and other proteins have been shown to self-assemble into supramolecular structures *in vitro* that resemble the structures they form in cells (Kentsis, 2002). Over-expression of SUMO-1 prevented the stress-mediated breakdown of PML bodies, indicating that PML body stability is partially dependent on SUMO-1 (Eskiw, 2003). Interestingly, many of the proteins found in the PML NBs have been shown to be SUMOylated (Seeler & Dejean, 2003).

Like PML bodies, also the PML protein has been implicated in different cellular functions including suppressing cell growth and cell transformation (Mu, 1994; Ahn 1995; Koken, 1995; review: Melnick & Licht 1999). Transduction of APL patient derived NB4 cells with a retrovirus harboring the coding sequence for PML suppressed the ability of these cells to form colonies in soft agar. In addition, conditioned medium from these cells suppressed colony formation of wild-type NB4 cells, suggesting the release of negative growth control factors (Mu, 1994). Furthermore, PML-overexpressing NB4 cells, when injected into nude mice, yielded smaller tumors that appeared with a longer latency than vector-expressing cells (Mu, 1994). In various human tumors, PML expression was shown to be decreased (Gurrieri, 2004a) and in some cases it was shown that low levels of PML correlated with poor disease outcome (Chang, 2007). Consistent with a role as tumor suppressor, it has been reported that overexpression of PML suppresses the growth of various cancer cells (Liu, 1995; Mu, 1997; Le, 1998). Also, PML knockout mice revealed an increased susceptibility to chemical-induced carcinogenesis (Wang, 1998a) and spontaneous tumorigenesis (Trotman, 2006).

Probably one of the most important functions of PML is to control apoptosis. The physiological relevance of this is emphasized by *in vivo* studies demonstrating that mice and cells that lack PML are resistant to a vast variety of apoptotic stimuli (Wang, 1998a). Although the molecular mechanism remains largely unknown, PML is thought to be a pivotal factor in γ irradiation-induced apoptosis (Wang, 1998a) and essential for the induction of programmed cell death by Fas, tumor necrosis factor α (TNF), ceramide and type I and II interferons (IFNs) (Wang, 1998a; Quignon, 1998). In support of these thoughts, PML−/− mice and PML−/− cells are resistant to the lethal effects of γ-irradiation (Wang, 1998a; Yang, 2002).

2. The nuclear matrix

2.1 Evidence for a nuclear matrix structure?

For more than 30 years it has been hypothesized that the mammalian cell nucleus contains a filamentous framework, referred to as nuclear matrix or karyoskeleton, which provides structural support to the various nuclear components and a framework for all

nuclear activities. It is the observation that nuclei withstand strong hydrodynamic shear force, compression and friction during cell or tissue homogenization as well as extreme osmotic pressure that prompted scientists to believe in a nuclear matrix structure (Maggio, 1963a; Penman, 1966; Blobel & Potter, 1966; Dounce, 1995; Pederson 1997). The term 'nuclear matrix' was first used in 1974 to describe a filamentous structure that remained present when cell nuclei were salt extracted using 1.0- 2.0 M NaCl (Berezney & Coffey, 1974). Numerous studies followed since, using variations on extraction protocols until proteins, RNA- and DNA-sequences were all shown to be connected to the nuclear matrix (Berezny & Jeon, 1995). Interestingly, Jackson and Cook observed in 1988 an extensively anastomatized nuclear network of filaments after performing nuclear extractions of cells that were encapsulated in agarose spheres (Jackson & Cook, 1988). This network is believed to resemble the filamentous structure that remains present after a high ionic strength extraction of the nucleus (Capco, 1982). Many studies found 3-5 and 10-30 nm ribonucleoprotein elements/filaments remaining present in the nucleus after extraction using RNP-depleted and RNP-containing resinless electron microscopy (resin is an embedding material that scatters electrons in a similar way as the embedded specimen does) or whole mount electron microscopy (Monneron & Bernhard, 1969; Berezney & Coffey, 1974; Comings & Okada, 1976; Capco, 1982; Small, 1985; Fey, 1986; Jackson & Cook, 1988). RNP filament domains are thought to be very important for nuclear matrix organization and for some time it was not possible to remove chromatin from the nucleus without removing the RNP filament domains as well (Fey, 1986).

These ultrastructural studies of sectioned cell nuclei did, however, not confirm the presence of a filament system that was thought to comprise the nuclear matrix in situ. In fact enormous doubt was raised concerning the procedures used to extract cell nuclei could possibly reveal the nuclear matrix structure that may exist *in vivo*. All nuclear matrix preparation procedures used thus far involved harsh treatments, including the removal of nucleic acids, heat (Mirkovitch, 1984; Martelli, 1991), Cu^{2+} (Mirkovitch, 1984; Neri, 1997), sulfhydryl cross-linking (Kaufmann & Shaper, 1984), and highly concentrated monovalent salts such as 2 M NaCl (Berezney & Coffey, 1977). Significantly, it has been noted that such treatments themselves result in protein rearrangements and protein aggregations (Palade & Siekevitz, 1956; Tashiro, 1958; Madison & Dickman, 1963; Lothstein, 1985). Also, protein-protein interactions and van der Waals forces between proteins and water change profoundly when high ionic strength is used (Kauzmann, 1959; Varshavsky & Ilyin, 1974), which is true for most standard nuclear matrix preparation procedures. Consequently, such artificially introduced protein filaments might easily be interpreted as a nuclear matrix structure (Finkelstein, 1997). The existence of a nuclear matrix still needs to be confirmed by other techniques, like for example live cell imaging and RNA interference.

Many who did not believe in the existence of the nuclear matrix became converted by the idea that ribonucleoproteins are functionally integrated elements of the nuclear architecture. Several groups reported pre-mRNA and splicing-intermediates to be retained in RNP-containing nuclear matrix preparations (Ben Ze'ev, 1982; Ciejek, 1982; Mariman, 1982; Ross, 1982; Gallinaro, 1983; Ben Ze'ev & Aloni, 1983). Also, several studies showed that the hyperphosphorylated form of the largest subunit of RNA polymerase II is associated with nuclear sites that are rich in pre-mRNA splicing factors, and importantly, are retained in nuclear matrix preparations (Mortillaro 1996, Vincent 1996). Hyperphosphorylation of RNA polymerase II is functionally linked to the most

active form of this enzyme (Dahmus, 1996). Taking all this evidence into account it can be concluded that it is very likely that there is a nuclear matrix, but still further research is necessary to precisely define its components.

Recent advances in the study of the protein composition of the nuclear matrix allowed the characterization of several proteins that are specifically associated with the nuclear matrix in tumor cells (Konety & Getzenberg, 1999). Some of these proteins are used for the diagnosis of cancer; e.g., NMP22 is specifically present in the nuclear matrix of bladder cancer cells (Ozen, 1999). Hence, detecting changes in the nuclear matrix structure may serve as a valuable tool in cancer diagnostics.

2.2 The nuclear lamina

The nuclear envelope is a double-layered membrane that encloses the contents of the nucleus during most of the cell's lifecycle and forms a boundary between chromosomes and the cytoplasm in eukaryotic cells. The main components of the nuclear envelope are the inner nuclear membrane, the outer nuclear membrane, which is continuous with the endoplasmatic reticulum, and the nuclear pore complexes (Stuurman, 1998; Goldman, 2002). On the inner surface of the nuclear membrane, the nuclear lamins (type-V intermediate filaments) are polymerized to form a thin fibrous structure, 20-50 nm thick. The nuclear lamins form together with the inner nuclear membrane (INM) proteins the 'nuclear lamina', a stable yet dynamic network that maintains extensive interactions with both INM-specific integral membrane proteins and chromatin (Hutchison, 2002). There are two classes of lamins, A-type lamins (lamin A/lamin C, each alternatively spliced from the same gene) and B-type lamins which bind to the lamin B receptor (LBR). Mutations in the lamin A/C and lamin B genes result in diseases ranging from cardiac and skeletal myopathies and partial lipodystrophy to peripheral neuropathy and premature aging (Mounkes, 2003). Specifically, mutations in the genes encoding for A-type lamins and their binding partners have been associated with Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy, Dunnigan-type familial partial lipodystrophy and Hutchinson-Gilford progeria syndrome (Bonne, 1999; Fatkin, 1999; Cao, 2000; Shackleton, 2000; Burke & Stewart, 2002; De Sandre-Giovannoli, 2003; Eriksson, 2003). B-type lamins are constitutively expressed in all somatic cells and contain a stable C-terminal farnesyl modification, which mediates tight association with the INM. Unlike B-type lamins, the A-type lamins are expressed only in differentiated cells (Lebel, 1987; Stuurman, 1998). They are components of the peripheral lamina and of structures in the nuclear interior (Moir, 2000). The lamina may be linked to nuclear pore baskets through Nup153 (Foisner, 2001).

The nuclear lamina is considered to be an important determinant of interphase nuclear architecture (Lenz-Bohme, 1997; Schirmer, 2001) because it plays an essential role in maintaining the integrity of the nuclear envelope and provides anchoring sites for chromatin (Moir, 1995; Gant & Wilson, 1997; Stuurman, 1998; Gant, 1999). In addition to the well-characterized peripheral location of lamins, there is considerable evidence for an intranuclear distribution of lamins. Both, localization in intranuclear spots (Goldman, 1992; Bridger, 1993; Moir, 1994) and a diffuse distribution throughout the nucleus (Hozak, 1995) have been reported. Intranuclear lamins have been shown to localize at sites of DNA replication (Jenkins, 1995; Goldman, 2002; Mar-

tins, 2003; Gruenbaum, 2003) and to support nuclear activities such as DNA replication and RNA synthesis (Nili, 2001; Spann, 2002; Wilkinson, 2003; Haraguchi, 2004). It is not yet clear whether intranuclear lamins form a network and whether such a network would be required for the activities supported by the lamins.

The nuclear lamins bind to several INM proteins, including lamina-associated polypeptides 1 and 2ß (LAP1, LAP2ß), emerin and Man1, which share a common structural motif of about 40 amino acid residues, called the LEM (LAPs, emerin and Man1) domain (Lin, 2000). Although additional LEM domain proteins such as Nesprin, Otefin, and Lem-3 have been identified (Lin, 2000). All LAP1 isoforms and LAP2α interact preferentially with A-type lamins, while the lamin B receptor and LAP2β interact with the B-type lamins and emerin interacts with both types of lamins (Foisner, 2001). Lamins can also bind to chromatin proteins (histone H2A or H2B dimers), as well as ostensibly soluble proteins including lamina-associated polypeptide-2α (LAP2α), Kruppel-like protein (MOK2), actin, retinoblastoma protein (RB), barrier-to-autointegration factor (BAF), sterol-response-element-binding protein (SREBP) and one or more components of RNA-polymerase-II-dependent transcription complexes and DNA-replication complexes (Gruenbaum, 2003; Zastrow, 2004). In cells that lack A-type lamins, many of these proteins are not retained at the NE but instead drift throughout the NE/ER network (Sullivan, 1999; Lee, 2002; Liu, 2003; Muchir, 2003; Wagner, 2004). Lamins and their associated proteins are proposed to have roles in large-scale chromatin organization (Sullivan, 1999; Liu, 2000; Guillemin 2001; Liu, 2003; Raz, 2006; Raz, 2008), the spacing of nuclear pore complexes (Liu, 2000; Schirmer, 2001), the positioning of the nucleus in cells (Starr, 2001; Starr 2002) and the reassembly of the nucleus after mitosis (Lopez-Soler, 2001). Lamins have been shown to interact with chromatin at more than 1,300 sharply defined large domains, 0.1-10 megabases in size (Guelen, 2008). These lamina-associated domains are typified by low gene-expression levels, indicating that they represent a repressive chromatin environment (Guelen, 2008).

The nuclear lamina is linked to the cytoskeleton via the nesprin protein family, which include high molecular weight proteins embedded in the inner and outer nuclear membrane (Zhang, 2001; Mislow, 2002). When nesprins are associated with the outer nuclear membrane, the amino-terminus is exposed towards the cytoplasm and binds to microfilaments (Zhang, 2001; Zhen, 2002) and intermediate filaments (Wilhelmsen, 2005). As such they connect the nucleus to the cytoskeleton (Wang & Suo, 2005). This anchorage of the nuclear membrane to the cytoskeleton is essential for migration and correct localization of the nucleus inside the cell. Nesprins at the inner nuclear membrane (smaller isoforms) bind to lamin A/C and emerin (Mislow, 2002; Padmakumar, 2005) through their spectrin repeats in the carboxy-terminus, and – as such – interact closely with the nuclear lamina. In this way lamins play not only an essential role in the structural integrity of the nucleus but also in the structural integrity of the whole cell, via connections between nuclear lamina, cytoskeleton and extracellular matrix (Lammerding, 2004; Broers, 2004; Broers, 2005). Absence of the A-type lamins or mutations in these structural components of the nuclear lamina leads to an impaired cellular response to mechanical stress (Lammerding, 2004). Laminopathies show clinical phenotypes comparable to those seen for diseases resulting from genetic defects in cytoskeletal components, further indicating that lamins play a central role in maintaining the mechanical properties of the cell.

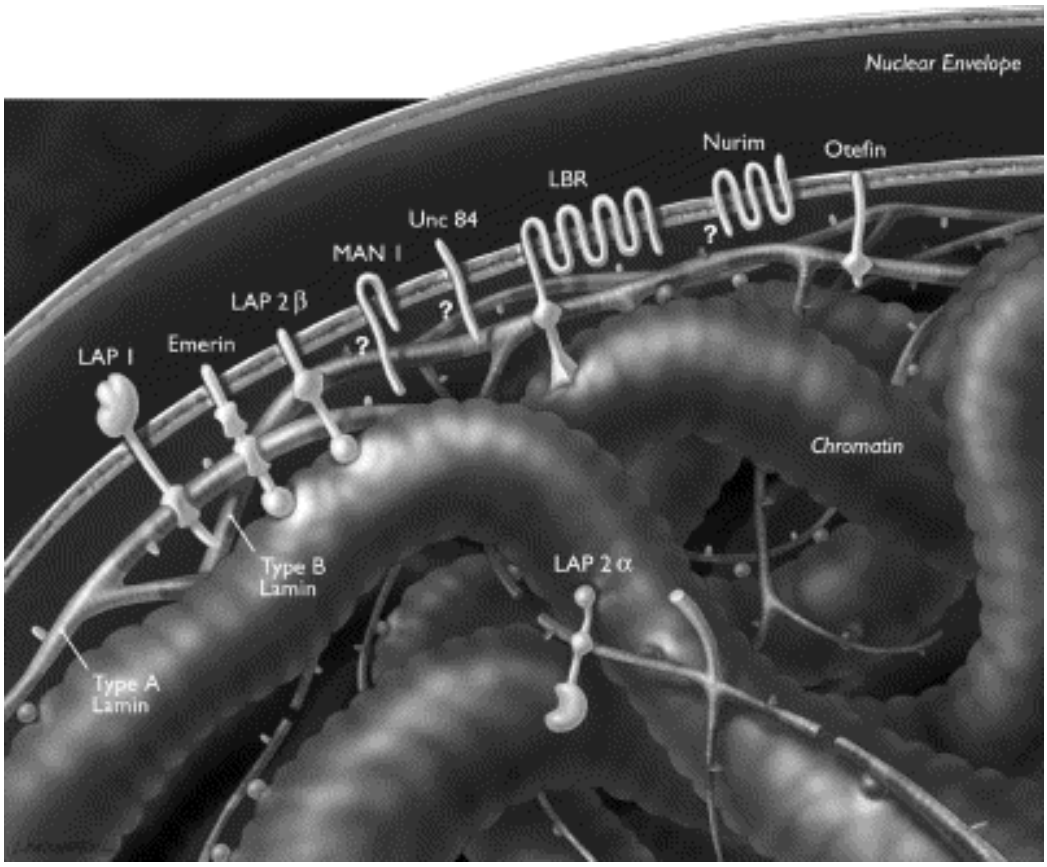

Figure 4. Schematic view of the nuclear envelope, lamina and chromatin. The inner and outer membranes of the nuclear envelope are shown with their enclosed lumen. Also lamin filaments and selected nuclear envelope proteins, including lamina-associated protein 1 (LAP1), emerin, LAP2β, MAN1, UNC-84, lamin B receptor (LBR), nurim and otefin, are shown. (Adapted from Cohen, 2001).

2.3 Are lamins part of the nuclear matrix?

Considering the spatial distribution of lamins in the cell nucleus, the question is raised whether lamins are part of the nuclear matrix. A large fraction of the filaments seen in resinless section images of (RNP-containing) nuclear matrix preparations are 10-11 nm in diameter (Jackson & Cook, 1988; He, 1990; Hozák, 1995; Wan, 1999), which correspond to the size of an intermediate filament. In one report, it has been described that these nuclear filaments react with a lamin A specific antibody (Hozák, 1995). Intruigingly, many different intermediate filament proteins revealed binding affinity for nucleic acids and also share some amino acid sequence homology with transcription factors (Traub & Shoeman, 1994). Other studies have also found lamins as discrete foci present in the nucleoplasm (Goldman, 1992; Bridger, 1993; Moir, 1994), and it has been shown that nucleoplasmic lamins undergo dynamic assembly-disassembly in

vivo (Goldman, 1992; Moir, 1994; Schmidt, 1994). Altogether this evidence is in favour of a role for lamins constituting part of the nuclear matrix, possibly by forming a complex network with other proteins like emerin, protein 4.1, nuclear actin and nuclear myosin (Pestic-Dragovich, 2000; Kiseleva, 2004).

2.4 Nuclear actin

Since one of the presumed functions of the nuclear matrix is to support and to facilitate/regulate intranuclear transport, possible nuclear matrix components may be similar to protein filament systems already characterized in the cytoplasm. Thus far, there is little if no evidence for the presence of tubulin or microtubules in the nucleus. Nuclear actin, however, is present and functional in the cell nucleus of various cell types (Clark & Merriam, 1977; Fukui, 1978; Fukui & Katsumaru, 1979; Clark & Rosenbaum, 1979; Osborn & Weber, 1980; Welch & Suhan, 1985; De Boni, 1994; Yan, 1997; Rando, 2000; Pederson and Aebi, 2002; Bettinger, 2004; Castano et al., 2010). This is also true for nuclear actin binding proteins (Ankenbauer, 1989; Rimm & Pollard, 1989) and nuclear myosin (Hauser, 1975; Berrios & Fisher, 1986; Hagen, 1986; Rimm & Pollard, 1989; Nowak, 1997). Nuclear actin has initially been suggested to play a role in transcription (Scheer, 1984) and later also in mRNA processing (Sahlas, 1993), chromatin remodelling and nuclear export (Machesky & May, 2001; Goodson & Hawse, 2002; Olave, 2002). Nuclear actin is also found present in the nucleolus (Clark & Merriam, 1977; Funaki, 1995) and TEM analysis of *Xenopus* oocyte nuclei suggested that short bundles of actin extend from nucleoli towards the nuclear envelope (Parfenov, 1995). In addition to actin, nuclei also contain a specific isoform of myosin I, nuclear myosin 1 (NM1), which is an actin-dependent motor. Antibodies directed against nuclear myosin I block transcription by RNA polymerase II when injected into mammalian cells and inhibit isolated transcription complexes in vitro (Pestic-Dragovich, 2000).

The most important questions about nuclear actin revolve around its polymeric state(s). Nuclear actin does not form long actin filaments ('F-actin'), it is proposed to assume shorter, potentially novel conformations (Pederson & Aebi, 2002; Bettinger, 2004). Nuclear actin 'rods,' 'bundles,' and 'tubules' have been described by a number of investigators (Fukui & Katsumaru, 1979; Iida, 1986; Iida & Yahara, 1986; Nishida, 1987; Wada, 1998), but their supramolecular organization has remained elusive except for one case (Sameshima, 2001). Sameshima *et al*. have described a new type of actin rods formed both in the nucleus and the cytoplasm of *Dictyostelium discoideum* that have been implicated in the maintenance of dormancy and viability at the spore stage of the developmental cycle. Examination of their ultrastructure has revealed these actin rods as bundles of hexagonally packed actin tubules consisting of three actin filaments each. (Interestingly, cytoplasmic actin is known to form short 'protomer' filaments (e.g. at branched intersections with protein 4.1, tropomyosin and spectrin), as well as tubes, sheets and short branched filaments (Pederson & Aebi, 2002).). Nuclear actin was shown to interact with many structural proteins in the nucleus: the intermediate filament protein lamin A (Sasseville & Langelier, 1998), membrane protein emerin (Holaska, 2004), the nesprin family of filamentous proteins (Zhang, 2002; Zhen, 2002) and nuclear-specific isoforms of protein '4.1', an actin scaffolding pro-

tein (Correas, 1991; Krauss, 1997; Luque & Correas, 2000). An actin network is proposed to exist at the INM and to mechanically reinforce the lamina network (Holaska, 2004). The actin-binding domain of nuclear-specific isoforms of protein 4.1 is found to be essential to reconstruct nuclei after mitosis (Krauss, 2003). In conclusion, there is ample evidence that actin is present in the cell nucleus and is involved in a variety of nuclear processes.

3. Telomeres & Nuclear organization

3.1 Telomere biology

Telomeres are structures at the ends of eukaryotic chromosomes (in greek: telo= end , mere= part). They are protein-DNA complexes that protect chromosome termini from unregulated degradation, recombination and fusion. They also serve to limit the loss of genetic material from chromosome ends that occurs during (incomplete) DNA replication. After about 60-80 cell divisions, telomere repeats are shortened from a typical initial length of 10-15 kb in human cells to ~5 kb and below, which triggers cell senescence or apoptosis (Harley, 1990; Martens, 2000; Blasco, 2007). The number of cell divisions that a normal cell can make before entering in to a state of senescence is also referred to as the 'Hayflick limit'. Leonard Hayflick demonstrated in 1965 that normal human diploid cells in a cell culture divide about 50 +/- 10 times (Hayflick, 1965).

Telomeres consist of tandemly repeated DNA sequences (TTAGGG) bound by various telomeric proteins, such as telomere repeat binding factor 1 (TRF1), telomere repeat binding factor 2 (TRF2) and protection of telomeres 1 (POT-1) (Blackburn, 2001). These proteins bind telomeric DNA directly and are interconnected by three additional proteins, TIN2, TPP1 and Rap1. Together they form a complex called the shelterin complex (figure 6) that allows cells to distinguish telomeres from sites of DNA damage (d'Adda di Fagagna, 2004; Shay & Wright, 2004; de Lange, 2005). The single stranded end-part of the telomere forms a loop structure, called the t-loop (Griffith, 1999), which is lost when TRF2 function is inhibited by expressing a dominant negative allele of TRF2 (van Steensel, 1998). Telomeres have a nonnucleosomal chromatin structure, whereas subtelomeric DNA is assembled into nucleosomes (Wright, 1992). It is possible that t-loops create an organization similar to nucleosomes that conceal the chromosome ends from the DNA damage surveillance, thus preventing telomeres from being degraded. It has been proposed that TRF2 plays an important part in protecting telomeres *in vivo* (Griffith, 1999). Most, if not all TRF2 is in a complex with human (h)Rap1, which has been identified as a direct interacting partner of TRF2 (Li, 2000). Two other telomeric proteins, TIN2 and the DNA repair protein Ku, interact with telomeres via binding to TRF1 (Kim, 1999; Hsu, 2000). TRF1 alone is insufficient to control telomere length in human cells, and the TIN2 protein is thought to be an essential mediator of TRF1 function (Kim, 1999). Moreover, TIN2 is thought to bind TRF1 and TRF2 simultaneously, stabilizing the TRF2 complex on telomeres (Ye, 2004). TPP1 was also found to interact with both TRF1 and TRF2 and to operate as a negative regulator of telomere length (Houghtaling, 2004).

The length of telomeres is well-controlled by several different factors. If this were not the case, the chromosomes would shorten dramatically with every cell division. In most organisms, telomeres are lengthened by the enzyme telomerase (Greider & Blackburn, 1985; Greider, 1996). With the exception of a few cell types, including stem cells, human somatic cells undergo programmed telomere shortening, a process that appears to involve repression of telomerase expression (Cooke & Smith, 1986; de Lange, 1990; Harley, 1990; Hastie, 1990; Counter, 1992; Kim, 1994). This progressive decline of telomere length with each cell division may constitute a tumor suppressor mechanism that limits the replicative potential of transformed cells. In agreement, telomerase is frequently activated in human and mouse tumors and restoration of telomere length is correlated with immortalization of human cells in vitro (Counter, 1992, 1994a, b; Kim, 1994; Blasco, 1996; Broccoli, 1996). During the malignant progression of cancer cells, the maintenance of telomere length is a crucial prerequisite for immortalization (Bacchetti, 1996). Therefore, telomere length has emerged as a promising clinical marker to predict the risk and prognosis of patients with malignant disorders (reviewed by Svenson & Roos, 2009). Most cancer cells activate a telomere maintenance pathway and about 90% of these tumors show telomerase activity (Shay & Bachetti, 1997). A significant minority of tumors use an alternative lengthening of telomeres (ALT) mechanism (Bryan, 1995; Bryan, 1997).

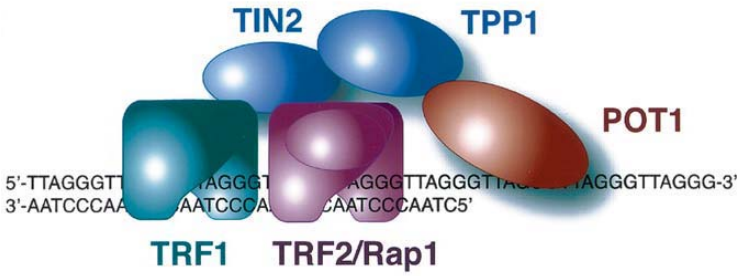

Figure 6. Schematic representation of shelterin on telomeric **DNA.** The shelterin complex consists of six subunits, TRF1,TRF2, POT1, TIN2, TPP1 and Rap1. For simplicity, POT1 is only shown as binding to the site closest to the duplex telomeric DNA although it can also bind to the 3′ end. (Adapted from de Lange, 2005)

3.2 ALTernative lengthening of telomeres

Approximately 10% of all human cancers use instead of the enzyme telomerase an alternative mechanism for telomere elongation, the ALT-mechanism. Although details of the molecular mechanism of ALT are largely unknown, previous studies have shown that the ALT mechanism in human cells likely involves recombination between telomeres (Murnane, 1994; Dunham, 2000). *Saccharomyces cerevisiae* cells that survive in the absence of telomerase require a functional RAD52 gene, a protein

required for DNA recombination (Lundblad, 1993). Also, individual telomeres in human ALT cells undergo steady telomere attrition upon which sudden lengthening and shortening events are superimposed in a manner that is suggestive for recombination (Murnane, 1994). Finally, functional evidence for the involvement of recombination in the ALT mechanism was provided by showing that DNA sequences are copied from telomere to telomere in ALT cells (Dunham, 2000). Telomere lengthening is also possible via intra-telomeric DNA copying (Muntoni, 2009). These observations are all consistent with a recombination-mediated DNA replication mechanism.

The hallmarks of human ALT cells include a large variance in telomere length, with telomeres that range from very short ~5kb, to very long ~50 kb (Bryan, 1995), and the presence of ALT-associated promyelocytic leukemia nuclear bodies (APBs) containing telomeric DNA and telomere binding proteins (Yeager, 1999). ABPs are a subset of PML bodies that are not found in normal cells, or in tumor cells that express telomerase, and contain additional proteins involved in DNA replication, recombination and repair that are not found in normal PML bodies (Yeager, 1999; Yankiwski, 2000; Stavropoulos, 2002; Tarsounas, 2004). APBs are found in a minority of cells, approximately 5% within asynchronously dividing ALT cell populations, from which it may be concluded that their formation is cell cycle-dependent (Yeager, 1999; Grobelny, 2000; Wu, 2000). It has been suggested that APBs may have an integral role in the ALT mechanism (Yeager, 1999; Grobelny, 2000; Wu, 2000, 2003; Molenaar, 2003). Consistent with this suggestion, inhibition of ALT in somatic cell hybrids, formed by fusing ALT and telomerase-positive cells, resulted in a substantial decrease in APBs (Perrem, 2001). It has been shown that inhibition of ALT is accompanied by a reduction of APBs, providing evidence for a direct link between APBs and ALT activity (Jiang, 2005). Furthermore, it has recently been shown that the DNA recombination endonuclease MUS81 is involved in ALT specific telomerase recombination and localizes to APBs (Zeng, 2009).

Observational and clinical studies on ALT positive tumors may help to fill the gaps in our understanding of the ALT mechanism. ALT is most commonly activated in tumors of neuroepithelial origin (astrocytomas) or mesenchymal origin, including osteosarcomas, and in soft tissue sarcomas (Henson, 2005). The reason for this is unknown, but it is possible that some mesenchymal and neuroepithelial cells repress telomerase more tightly than epithelial cells and therefore have a higher probability of activating ALT during tumorigenesis. In sarcomas, ALT is more frequently activated in subtypes that have a complex karyotype, which could be linked to chromosomal instability (Montgomery, 2004; Ulaner, 2004). It could be argued that the ALT mechanism is, in part, the cause of this instability because the critically short telomeres found in ALT cells are prone to end-to-end fusions, anaphase bridge formation, break–fusion–break events and ultimately severe chromosomal rearrangements. However, not all soft tissue sarcomas showing complex karyotypes are ALT-positive (Henson, 2005), indicating that other factors contribute to chromosomal instability as well.

Activation of the ALT pathway has been reported to be a prognostic marker for cancer progression. In case of glioblastoma, ALT correlated with a better patient prognosis, whereas no influence was detected for osteosarcomas. One of 16 non–small cell lung cancer (NSCLC) cell lines (VL-9, SK-LU-1, and VL-7) that lacked telomerase activity and displayed characteristics of an ALT mechanism showed significantly re-

duced tumorigenicity *in vitro* and *in vivo* compared to the telomerase positive NSCLC cell lines (Brachner, 2006)**.** It can be concluded that there is some evidence indicating that the ALT mechanism is indicative for a better patient prognosis, although further research is needed to substantiate this conclusion.

3.3 Telomeres and the nuclear matrix

The positioning of telomeres in the cell nucleus varies among organisms (Dong & Jiang 1998). In yeast, telomeres are positioned at the nuclear periphery while in mammalian cells they seem randomly distributed in the nucleoplasm (Henderson, 1996; Bilaud, 1997; Broccoli, 1997; van Steensel, 1998). Biochemical and ultrastructural data suggest that in mammalian cells telomeric DNA and telomere binding proteins colocalize in individual condensed structures at the nuclear matrix (Ludérus, 1996). The shelterin complex component TIN2 is believed to play a dual role in tethering telomeres to the nuclear matrix (Kaminker, 2009). Consistent with this association to the nuclear matrix, telomeric TTAGGG repeats were found to contain an array of nuclear matrix attachment sites at a frequency of at least one per kb. The nuclear matrix association is supposed to involve large domains of up to 20-30 kb telomeric DNA, encompassing the entire length of most mammalian telomeres (Ludérus, 1996). Because of their association to a nuclear matrix structure, telomeres are thought to play an important role in nuclear organization (de Lange, 2002).

In situ hybridization studies revealed that in yeast telomeres are organized in clusters at the nuclear periphery Gilson, 1993; Gotta, 1996). This organization in clusters may contribute to the repression of transcription of nearby genes, a phenomenon termed telomere position effect (TPE) (Gottschling, 1990). Telomeres in yeast have a nonnucleosomal chromatin structure, whereas subtelomeric DNA is assembled into nucleosomes (Wright, 1992). Subtelomeric chromatin in yeast has therefore many of the hallmarks of heterochromatin as present in mammalian cells: it imposes transcriptional repression (Gottschling, 1990) and late replication of nearby sequences (Ferguson, 1991).

3.4 Telomere and chromatin mobility in the cell nucleus

Considering the high DNA content and the large amounts of RNAs and proteins in the nucleus, one might intuitively think of the nucleus as a viscous, gel-like environment. If this were true, the movement of proteins within the organelle might be severely restricted and specific transport mechanisms would be required to deliver proteins to their destinations. Photobleaching experiments have now shown, however, that most proteins are highly mobile within the nucleus. The difference between the diffusional mobility of nonphysiological solutes in the nucleus as compared to that in an aqueous solution is only about fourfold (Fushimi & Verkman, 1991; Seksek, 1997). Thus, macromolecules such as fluorescently tagged proteins or RNAs move within the nuclear space by simple thermal diffusion at an unexpectedly high speed (Huang, 1998; Rademakers, 1999; Phair & Misteli, 2000**;** Pederson, 2000; Shopland & Lawrence, 2000; Snaar, 2000; Misteli, 2001,). The time required for travelling from the centre to the periphery of the nucleus is in the order of several seconds for an average sized

monomeric protein, and only several minutes for a large complex such as a spliceosome or ribosome.

If the subnuclear positioning of any particular chromosomal locus reflects a state of transcriptional activity, then genes must be able to move from a transcriptional repressive subenvironment to a transcriptional competent environment and to obtain a tissue-specific and/or developmental-stage-specific spatial organization. In the past few years, various studies addressed the dynamic properties of chromatin in general or specific sequences in particular. Using time-lapse imaging of GFP-tagged chromosomal loci, Sedat and coworkers showed in yeast and later in flies that chromatin is engaged in a continuous random-walk-like motion (Marshall, 1997). Later, slightly less constrained random movements were described for multiple yeast loci by monitoring the movements of specific chromosomal sites fused to lac repressor binding sites that were tagged with GFP-lac repressor proteins (Heun, 2001a; Heun, 2001b). Telomeres and transcriptionally active non-telomeric loci showed clear differences in movement. Active chromosomal loci displayed a random walk movement within a radius of 0.5–0.7 μm (Heun, 2001a; Gartenberg, 2004; Sage, 2005). This represents more than one-quarter of the nuclear diameter in yeast, but less than one-tenth of the nuclear diameter in mammalian cells. Because 50% of the yeast nuclear volume is contained within a peripheral shell that is <0.4 μm thick, most yeast genes have a high probability to encounter the nuclear membrane. Silent telomeres, however, moved in a highly constrained manner along the inner surface of the nuclear envelope and only rarely occupy the nuclear core (Heun, 2001a; Hediger, 2002; Gartenberg, 2004; Sage, 2005). The movement of a typical yeast telomere is restricted to an area at the inner-nuclear-envelope surface occupying ~12% of the total nuclear volume (Rosa, 2006). Interestingly, a similar constraint movement was observed for a subset of active genes. Notably, galactose-induced loci were shown to associate with nuclear pores upon induction. In addition, a subtelomeric gene was shown to shift from a telomeric focus to a nuclear pore upon induction by low glucose (Cabal, 2006; Taddei, 2006). Given their lateral dynamics and striking radial confinement, it was suggested that a subset of active genes move from pore to pore (Cabal, 2006).

Outline of this thesis

The aim of this thesis is to provide a better understanding of the principles that underlie the spatial dynamic organization of the cell nucleus. Chapter 1 reviews the current status of knowledge about the structural and functional organization of the cell nucleus. In **chapter 2**, the development of a computer program is described that has been designed to track the 2D and 3D motion of objects in the nucleus of living cells. The functionality of the program is demonstrated by tracking the movements of GFP-tagged telomeres in the nuclei of tumor cells (U2OS) and normal mouse embryonic fibroblasts (W8 MEFS). GFP-tagged proliferating cell nuclear antigen (PCNA) is used as a nuclear counterstain to correct for cell movements, and as a cell cycle marker. In **chapter 3**, evidence is provided for the existence of a nuclear matrix structure that is composed of lamin proteins, emerin and actin. By analyzing the dynamics of telomeres in nuclei of cells showing reduced levels of lamin expression, it is investigated whether telomeres anchor to an inner nuclear lamina structure. In **chapter 4** the de novo formation of PML nuclear bodies is described. Using live cell imaging and immunocytochemistry it is demonstrated that telomeres play a role in the de novo formation of PML bodies. In **chapter 5** it is investigated whether nuclear bodies are associated with chromatin in the cell nucleus. After treating cells with DNA alkylating agent MMS, the dynamics of PML bodies, Cajal bodies and speckles has been analyzed relative to chromatin in the 3D space of the cell nucleus. In chaper 6 the results of our studies and future implications are discussed.

References

Miescher F. 1871. Ueber die chemische Zusammensetzung der Eiterzellen. *Hoppe-Seyler's Med. Chem.Unters*. **4**, 441-460

Stenoien D.L., Simeoni S., Sharp Z.D., Mancini M.A. 2000. Subnuclear dynamics and transcription factor function. *J Cell Biochem.* **3,** 99 –106

Carmo-Fonseca M. 2002. The contribution of nuclear compartmentalization to gene regulation. *Cell* **108,** 513–521

Rippe K. 2007. Dynamic organization of the cell nucleus. *Current Opinion in Genetics & Development* **17,** 373-380

Cremer T., Kreth G., Koester H., Fink R.H., Heintzmann R., Cremer M., Solovei I., Zink D. & Cremer C. 2002. Chromosome territories, interchromatin domain compartment, and nuclear matrix: An integrated view of the functional nuclear architecture. *Crit Rev Eukaryot Gene Expr.* **10,** 179-212

Manuelidis L. 1985. Individual interphase chromosome domains revealed by in situ hybridization. *Hum. Genet.* **71**, 288-293

Cook P.R. & Brazell I.A. 1976. Conformational constraints in nuclear DNA. *J Cell Sci.* **22,** 287-302

Paulson J.R. & Laemmli U.K. 1977. The structure of histonedepleted metaphase chromosomes. *Cell* **12,** 817-825

van der Velden H.M., van Willigen G., Wetzels R.H. & Wanka F.1984. Attachment of Origins of Replication to the Nuclear Matrix and the Chromosomal Scaffold. *FEBS Lett*. **171,** 13–16

Razin S.V., Kekelidze M.G., Lukanidin E.M. 1986. Replication origins are attached to the nuclear skeleton. *Nucl. Acids Res*. **14,** 8189–8207

Buongiorno-Nardelli M., Micheli G., Carri M.T. & Marilley M. 1982. A relationship between replicon size and supercoiled loop domains in the eukaryotic genome. *Nature* **298,** 100–102

Marilley M. & Gassend-Bonnet G. 1989. Supercoiled loop organization of genomic DNA: A close relationship between loop domains, expression units, and replicon organization in rDNA from *Xenopus laevis*. *Exp. Cell Res.* **180,** 475–489

Razin S.V. 1996. Functional architecture of chromosomal DNA domains. *Crit. Rev. Eukaryot. Gene Expr.* **6,** 247–269

Vassetzky Y., Lemaitre J.M. & Mechali M. 2000b. Specification of chromatin domains and regulation of replication and transcription during development. *Crit. Rev. Eukaryot. Gene Expr.* **10,** 31–38

John B. 1988. The Biology of Heterochromatin. (ed.Verma R.S.) (Cambridge Univ. Press, Cambridge).

Felsenfeld G. & Groudine M. 2003. Controlling the double helix. *Nature* **421,** 448–453

Bühler M. & Moazed D. 2007. Transcription and RNAi in heterochromatic gene silencing. *Nature Structural & Molecular Biology* **14,** 1041-1048

Boyle S., Gilchrist S., Bridger J.M., Mahy N.L., Ellis J.A. & Bickmore W.A. 2001. The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells. *Hum. Mol. Genet.* **10,** 211–219

Finlan L.E., Sproul D., Thomson I., Boyle S., Kerr E., Perry P., Ylstra B., Chubb J.R., Bickmore W.A. 2008. Recruitment to the Nuclear Periphery Can Alter Expression of Genes in Human Cells. *PLoS Genet.* **4**, e1000039

Verschure P.J., van Der Kraan I., Manders E.M. & van Driel R. 1999. Spatial relationship between transcription sites and chromosome territories. *J. Cell Biol.* **147**, 13– 24

Stewart M.D., Li J. & Wong J. 2005. Relationship between Histone H3 Lysine 9 Methylation, Transcription Repression, and Heterochromatin Protein 1 Recruitment. *Molecular and cellular biology* **25,** 2525–2538

Strahl B.D., Grant P.A., Briggs S.D., Sun Z.W., Bone J.R., Caldwell J.A., Mollah S., Cook R.G., Shabanowitz J., Hunt D.F.& Allis CD. 2002. Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. *Mol. Cell. Biol.* **22**, 1298 -1306

Grunstein M. 1997. Histone acetylation in chromatin structure and transcription. *Nature* **389,** 349-352

Turner B.M. 2000. Histone acetylation and an epigenetic code. *BioEssays* **450,** 1-10

Krouwels I.M., Wiesmeijer K., Abraham T.E., Molenaar C., Verwoerd N.P., Tanke H.J., and Dirks R.W. 2005. A glue for heterochromatin maintenance: stable SUV39H1 binding to heterochromatin is reinforced by the SET domain *J. Cell Biol*. **170,** 537-549

Goll M.G. & Bestor T.H. 2005. Eukaryotic cytosine methyltransferases. *Annual Review of Biochemistry* **74,** 481-514

Razin A. & Cedar H. 1991. DNA methylation and gene expression. *Microbiol. Rev.* **55,** 451-458

Bird A.P. 1986. CpG-rich islands and the function of DNA methylation. *Nature* **321,** 209-213

Nakayama J., Rice J.C., Strahl B.D., Allis C.D., Grewal S.I. 2001. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science* **292,** 110–113

Grewal S.I. & Rice J.C. 2004. "Regulation of heterochromatin by histone methylation and small RNAs". *Curr. Opin. Cell Biol.* **16**, 230–238

Maeshima K., Hihara, S., and Eltsov, M. 2010. Chromatin structure: does the 30-nm fibre exist in vivo? Curr. Opin. Cell Biol. In press

Smetana K., Steele W. J. & Busch H. 1963. A nuclear ribonucleoprotein network. *Exp. Cell Res.* **31**, 198-201

Bernhard W. 1969. A new staining procedure for electron microscopical cytology. *J. Ultrastruct. Res.* **27**, 250-265

Biggiogera M. & Fakan S. 1998. Fine structural specific visualization of RNA on ultrathin sections. *J. Histochem. Cytochem.* **46**, 389-395

Monneron A. & Bernhard W**.** 1969. Fine structural organization of the interphase nucleus in some mammalian cells. *J. Ultrastruct. Res.* **27**, 266- 288

Misteli T. & Spector D.L. 1998. The cellular organization of gene expression. *Curr. Opin. Cell Biol.* **10**, 323-331

Misteli T. 2000. Cell biology of transcription and pre-mRNA splicing: nuclear architecture meets nuclear function. *J. Cell Sci.* **113**, 1841-1849

Bachellerie J.P., Puvion E. & Zalta J.P. 1975. Ultrastructural organization and biochemical characterization of chromatin - RNA – protein complexes isolated from mammalian cell nuclei. *Eur. J. Biochem.* **58**, 327- 337

Cmarko D., Verschure P.J., Martin T.E., Dahmus M.E., Krause S., Fu X.D., van Driel R. & Fakan S. 1999. Ultrastructural analysis of transcription and splicing in the cell nucleus after bromo-UTP microinjection. *Mol. Biol. Cell* **10**, 211-223

Mintz P.J., Patterson S.D., Neuwald A.F., Spahr C.S. & Spector D.L. 1999. Purification and biochemical characterization of interchromatin granule clusters. *EMBO J.* **18**, 4308-4320

Smith K.P., Moen P.T., Wydner K.L., Coleman J.R. & Lawrence J.B. 1999. Processing of endogenous pre-mRNAs in association with SC-35 domains is gene specific. *J. Cell Biol.* **144**, 617-629

Spector D.L. 2001. Nuclear domains. *J. Cell Sci.* **114,** 2891–2893

Vasu S. & Forbes D.J. 2001. Nuclear pores and nuclear assembly. *Curr. Opin. Cell Biol.* **13**, 363-375

Fahrenkrog B. & Aebi U. 2003. The nuclear pore complex: nucleocytoplasmic transport and beyond. *Nat. Rev. Mol. Cell Biol.* **4**, 757- 766

Cordes V.C., Reidenbach S., Kohler A., Stuurman N., van Driel R. & Franke W.W. 1993. Intranuclear filaments containing a nuclear pore complex protein. *J. Cell Biol.* **123**, 1333-1344

Cordes V., Reidenbach S., Rackwitz H.R. & Franke W.W. 1997. Identification of protein 270/Tpr as a constitutive component of the nuclear pore complex-attached intranuclear filaments. *J. Cell Biol.* **136**, 515-529

Parfenov V.N., Davis D.S., Pochukalina G.N., Sample C.E., Bugaeva E.A. & Murti, K.G. 1995. Nuclear actin and their topological changes in frog oocytes. *Exp. Cell Res.* **217**, 385-394

Zink D., Amaral M.D., Englmann A., Lang S., L.A. Clarke L.A., Rudolph C., Alt F., Luther K., Braz C., Sadoni N., Rosenecker J. & Schindelhauer D. 2004. Transcription-dependent spatial arrangements of CFTR and adjacent genes in human cell nuclei. *J. Cell Biol*. **166,** 815–825

Branco M.R. & Pombo A. 2006. Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. *PLoS Biol.* **4**, 780-788

Lonard D.M. & O'Malley B.W. 2008. Gene transcription: Two worlds merged. *Nature* **452**, 946-947

Dekker J. 2006. The three 'C' s of chromosome conformation capture: controls, controls, controls. *Nature Methods* **3**, 17-21

Smith K.P., Carter K.C., Johnson C.V., Lawrence J.B. 1995. U2 and U1 snRNA gene loci associate with coiled bodies. *J. Cell. Biochem.* **59,** 473-85

Vijg J. & Dollé M.E.T. 2002. Large genome rearrangements as a primary cause of aging. *Mechanisms of ageing and development* **123,** 907-915

Busuttil R.A., Dollé M.E.T., Campisi J., Vijg J. 2004. Genomic Instability, Aging, and Cellular Senescence. *Ann. N. Y. Acad. Sci*. **1019,** 245–255

Raz V., Vermolen B.J., Garini Y., Onderwater J.J.M., Mommaas-Kienhuis M.A., Koster A.J., Young I.T., Tanke H.J. & Dirks R.W. 2008. The nuclear lamina promotes telomere aggregation and centromere peripheral localization during senescence of human mesenchymal stem cells. *Journal of Cell Science* **121,** 4018-4028

Shin D-M., Kucia M., Ratajczak M.Z. 2010. Nuclear and chromatin reorganization during cell senescence and aging – a mini-review. Gerontology, in press

Butler J.T., Hall L.L., Smith K.P. & Lawrence J.B. 2009. Changing nuclear landscape and unique PML structures during early epigenetic transitions of human embryonic stem cells. *J. Cell Biochem.* **107**, 609-621

Buongiorno-Nardelli M., Micheli G., Carri M.T., Marilley M. 1982. A relationship between replicon size and supercoiled loop domains in the eukaryotic genome. *Nature* **298**, 100-102

Linskens M.H., Eijsermans A., Dijkwel P.A. 1987. Comparative analysis of DNA loop length in nontransformed and transformed hamster cells. *Mutat. Res* **178**, 245-256

Oberhammer F., Wilson J.W., Dive C., Morris I.D., Hickman J.A., Wakeling A.E., Walker P.R., Sikorska M. 1993. Apoptotic death in epithelial cells: cleavage of DNA to 300 and/or 50 kb fragments prior to or in the absence of internucleosomal fragmentation. *EMBO J* **12**, 3679-3684

Tsutsui K.M., Sano K. & Tsutsui K. 2005. Dynamic View of the Nuclear Matrix. *Acta Med. Okayama* **59,** 113-120

Misteli T. 2001. Protein dynamics: implications for nuclear architecture and gene expression. *Science* **291**, 843 -847

Snaar S., Wiesmeijer K., Jochemsen A.G., Tanke H.J., Dirks R.W. 2000. Mutational analysis of fibrillarin and its mobility in living human cells. *J. Cell. Biol.* **151,** 653-662

Olson M.O.J. 2004. Nucleolus. BookISBN-10: 0306478730 ISBN-13: 978-0306478734

Spector D.L. 2001. Nuclear domains. *J. Cell Sci.* **114,** 2891–2893

Saurin A.J., Shiels C., Williamson J., Satijn D.P., Otte A.P., Sheer D. & Freemont P.S. 1998. The human polycomb group complex associates with pericentromeric heterochromatin to form a novel nuclear domain. *J. Cell Biol.* **142**, 887 -898

Matera A.G. 1999. Nuclear bodies: multifaceted subdomains of the interchromatin space. *Trends Cell Biol.* **9**, 302 -309

Huang, S. 2000. Review: perinucleolar structures. *J. Struct. Biol.* **129**, 233 -240

Montgomery T.H. 1898. Comparative cytological studies, with especial regard to the morphology of the nucleolus. *J. Morphol.* **15,** 265-565

Olson M.O., Hingorani K. & Szebeni A. 2002. Conventional and nonconventional roles of the nucleolus. *Int. Rev. Cytol.* **219**, 199-266

Andersen J.S., Lyon C.E., Fox A.H., Leung A.K., Lam Y.W., Steen H., Mann M. & Lamond A.I. 2002. Directed proteomic analysis of the human nucleolus. *Curr. Biol.* **12**, 1-11

Andersen J.S., Lam Y.W., Leung A.K.L., Ong S.E., Lyon C.E., Lamond A.I. & Mann M. 2005. Nucleolar proteome dynamics. *Nature* **433**, 77-78

Pederson T. 1998. The plurifunctional nucleolus. *Nucleic Acids Res.* **26**, 3871-3876

Yamauchi K., Yang M., Hayashi K., Jiang P., Yamamoto N., Tsuchiya H., Tomita K., Moossa A.R., Bouvet M., Hoffman R.M. Imaging of nucleolar dynamics during the cell cycle of cancer cells in live mice. 2007. *Cell cycle* **6,** 2706-2708

Jacob J. 1968. Involvement of the nucleolus in viral synthesis in the cells of primary renal tumors of leopard frogs. *Cancer Research* **28,** 2126-2136

Itahana K., Krishna P., Jin B.A., Itahana Y., Hawke D., Kobayashi R. & Zhang Y. 2003. Tumor suppressor ARF degrades B23, a nucleolar protein involved in ribosome biogenesis and cell proliferation. *Molecular Cell* **12**, 1151–1164

van den Boom, V., Citterio, E., Hoogstraten, D., Zotter, A., Egly, J.M., van Cappellen, W.A., Hoeijmakers, J.H., Houtsmuller, A.B. and Vermeulen, W. 2004. DNA damage stabilizes interaction of CSB with the transcription elongation machinery. *J. Cell Biol.* **166**, 27-36

Jacobson M.R. & Pederson T. 1998. Localization of signal recognition particle RNA in the nucleolus of mammalian cells. *PNAS* **95,** 7981-7986

Sansam C.L., Wells K S. & Emeson R.B. 2003. Modulation of RNA editing by functional nucleolar sequestration of ADAR2. *Proc. Natl. Acad. Sci. USA* **100**, 14018-14023

Paushkin S.V., Patel M., Furia B.S., Peltz S.W. & Trotta C.R. 2004. Identification of a human endonuclease complex reveals a link between tRNA splicing and pre-mRNA 3' end formation. *Cell* **117**, 311-321

Kieffer-Kwon P., Martianov I. & Davidson I. 2004. Cell-specific nucleolar localization of TBP-related factor 2. *Mol. Biol. Cell* **15**, 4356-4368

Zhang S., Hemmerich P. & Grosse F. 2004. Nucleolar localization of the human telomeric repeat binding factor 2 (TRF2). *J. Cell Sci.* **117**, 3935-3945

Mekhail K., Gunaratnam L., Bonicalzi M.E. & Lee S. 2004. HIF activation by pH-dependent nucleolar sequestration of VHL. *Nat. Cell Biol.* **6**, 642-647

Rodway H., Llanos S., Rowe J. & Peters G. 2004. Stability of nucleolar versus non-nucleolar forms of human p14(ARF). *Oncogene* **23**, 6186-6192

Comai L. 1999. The nucleolus: a paradigm for cell proliferation and aging. *Brazilian Journal of Medical and Biological Research* **32,** 1473-1478

Rosete M., Padros M.R., Vindrola O. 2007. The nucleolus as a regulator of cellular senescence. **67,** 183-194

Baran V., Fabian D., Rehak P., Koppel J. 2003. Nucleolus in apoptosis-induced mouse preimplantation embryos. *Zygote* **11,** 271-283

Schneiter R., Kadowaki T. & Tartakoff A.M. 1995. mRNA transport in yeast: time to reinvestigate the functions of the nucleus. *MCB* **6,** 357-370

Sidebottom E. & Harris H. 1969. The role of the nucleolus in the transfer of RNA from nucleus to cytoplasm. *J. Cell Sci*. **5,** 351-364

Deák I., Sidebottom E. & Harris H. 1972. Further experiments on the role of the nucleolus in the expression of structural genes. *J. Cell Sci*. **11,** 379-391

Harris H. 1972. A new function for the nucleolus. *Aust. J. Exp. Biol. Med. Sci*. **50,** 827-832

Perry R.P., Hell A. & Errera M. 1961. The role of the nucleolus in ribonucleic acid and protein synthesis. *Biochem. Biophys. Acta* **49**, 47-57

Bond V.C. & Wold B. 1993. Nucleolar localization of myc transcripts. *Mol. Cell. Biol.* **13,** 3221-3230

Carter K.C., Taneja K.L. & Lawrence J.B. 1991. Discrete nuclear domains of poly(A) RNA and their relationship to the functional organization of the nucleus. *J. Cell Biol*. **115,** 1191-1202

Cajal S.R. 1903. Un sencillo método de coloración selectiva del retículo protoplásmico y sus efectos en los diversos órganos nerviosos de vertebrados e invertebrados. *Tra. Lab. Invest. Biol.* **2,** 129–221

Ogg S.C. & Lamond A.I. 2002. Cajal bodies and coilin - moving towards function. *J Cell Biol.* **159,** 17–21

Gall J.G. 2000. Cajal bodies: the first 100 years. *Annu. Rev. Cell Dev. Biol.* **16,** 273-300

Gall J.G., Bellini M., Wu Z. & Murphy C. 1999. Assembly of the nuclear transcription and processing machinery: Cajal bodies (coiled bodies) and transcriptosomes. *Mol. Biol. Cell* **10**, 4385–4402

Snaar S. Wiesmeijer K., Jochemsen A.G., Tanke H.J., Dirks R.W. 2000. Mutational analysis of fibrillarin and its mobility in living human cells. *J.Cell Biol.* **151,** 653-662

Jády B.E., Darzacq X., Tucker K.E., Matera, A.G., Bertrand E. & Kiss T. 2003. Modification of Sm small nuclear RNAs occurs in the nucleoplasmic Cajal body following import from the cytoplasm. *EMBO J.* **22,** 1878–1888

Darzacq X., Jady B.E., Verheggen C., Kiss A.M, Bertrand E. & Kiss T. 2002. Cajal body-specific small nuclear RNAs: a novel class of 2'-O-methylation and pseudouridylation guide RNAs. *EMBO J.* **21,** 2746–2756

Verheggen C., Lafontaine D.L., Samarsky D., Mouaikel J., Blanchard J.M., Bordonne R., & Bertrand E. 2002. Mammalian and yeast U3 snoRNPs are matured in specific and related nuclear compartments. *EMBO J.* **21,** 2736–2745

Staněk D., Přidalová-Hnilicová J., Novotny I., Huranová M., Blažíková M., Wen X., Sapra A.K. & Neugebauer K.M. 2008. Spliceosomal snRNPs repeatedly cycle through Cajal Bodies. *Mol Biol Cell* **19,** 2534-2543

Thiry M. 1994. Cytochemical and immunocytochemical study of coiled bodies in different cultured cell lines. *Chromosoma* **103,** 268–276

Raska I., Andrade L.E., Ochs R.L., Chan E.K., Chang C.M., Roos G. & Tan E.M. 1991. Immunological and ultrastructural studies of the nuclear coiled body with autoimmune antibodies. *Exp. Cell Res.* **195,** 27–37

Carmo-Fonseca M., Pepperkok R., Carvalho M.T. & Lamond A.I. 1992.
Transcription-dependent colocalization of the U1, U2, U4/U6, and U5 sn-RNPs in coiled bodies. *J. Cell Biol.* **117,** 1–14

Staněk D. & Neugebauer K.M. 2006. The Cajal body: a meeting place for spliceosomal snRNPs in the nuclear maze. *Chromosoma* **115,** 343–354

Jady B.E., Bertrand E. & Kiss T. 2004. Human telomerase RNA and box H/ACA scaRNAs share a common Cajal body-specific localization signal, *J. Cell Biol.* **164**, 647–652

Zhu Y., Tomlinson R.L., Lukowiak A.A., Terns R.M. & Terns M.P. 2004. Telomerase RNA accumulates in Cajal bodies in human cancer cells. *Mol. Biol. Cell* **15**, 81–90

Jady E., Richard P., Bertrand E. & Kiss T. 2006. Cell cycle-dependent recruitment of telomerase RNA and Cajal bodies to human telomeres. *Mol. Biol. Cell* **17,** 944–954

Tomlinson R.L., Ziegler T.D., Supakorndej T., Terns R.M. & Terns M.P. 2006. Cell cycle-regulated trafficking of human telomerase to telomeres, *Mol. Biol. Cell* **17**, 955–965

Christofari G., Adolf E., Reichenbach P., Sikora K., Terns R.M., Terns M.P. & Lingner J. 2007. Human telomerase RNA accumulation in Cajal bodies facilitates telomerase recruitment to telomeres and telomere elongation. *Molecular Cell* **27,** 882–889

Dirks R.W., Hattinger C.M., Molenaar C. & Snaar S.P. 1999. Synthesis, processing, and transport of RNA within the three-dimensional context of the cell nucleus. *Critical reviews in eukaryotic gene expression*. **9,** 191-201

Lamond A.I. & Spector D.L. 2003. Nuclear speckles: a model for nuclear organelles. *Nat. Rev. Mol. Cell. Biol*. **4,** 605-612

Dirks R.W., de Pauw E.S. & Raap A.K. 1997. Splicing factors associate with nuclear HCMV-IE transcripts after transcriptional activation of the gene, but dissociate upon transcription inhibition: evidence for a dynamic organization of splicing factors. *J. Cell Sci.* **110,** 515–522.

Misteli T., Caceres J.F. & Spector D.L. 1997. The dynamics of a pre-mRNA splicing factor in living cells. Nature **387,** 523-527

Thiry M. 1995. The interchromatin granules. *Histol. Histopathol.* **10**, 1035–1045

Visa N., Puvion-Dutilleul F., Bachellerie J. P. & Puvion E. 1993. Intranuclear distribution of U1 and U2 snRNAs visualized by high resolution *in situ* hybridization: revelation of a novel compartment containing U1 but not U2 snRNA in HeLa cells. *Eur. J. Cell Biol.* **60**, 308–321

Huang S. & Spector D.L. 1991. Nascent pre-mRNA transcripts are associated with nuclear regions enriched in splicing factors. *Genes Dev.* **5**, 2288–2302

Xing Y., Johnson C.V., Dobner P.R. & Lawrence J.B. 1993. Higher level organization of individual gene transcription and RNA splicing. *Science* **259**, 1326–1330

Xing, Y., Johnson, C. V., Moen, P. T., McNeil, J. A. & Lawrence, J. B. 1995. Nonrandom gene organization: structural arrangements of specific pre-mRNA transcription and splicing with SC35 domains. *J. Cell Biol.* **131**, 1635–1647

Smith K.P., Moen P.T., Wydner K.L., Coleman J.R. & Lawrence J.B. 1999. Processing of endogenous pre-mRNAs in association with SC-35 domains is gene specific. *J. Cell Biol.* **144**, 617–629

Johnson C., D. Primorac, M. McKinstry, J. McNeil, D. Rowe & J. Bentley Lawrence. 2000. Tracking *COL1A1* RNA in osteogenesis imperfecta. Splice-defective transcripts initiate transport from the gene but are retained within the SC35 domain. *J. Cell Biol.* **150**, 417–432

Hall L.L., Smith K.P., Byron M., Lawrence J.B. 2006. Molecular anatomy of a speckle. *The Anatomical record part A* **288A**, 664–675

Carter K.C., Taneja K.L., Lawrence J.B. 1991. Discrete nuclear domains of poly(A) RNA and their relationship to the functional organization of the nucleus. *J.Cell Biol*. **115,** 1191-1202

Carter K.C., Bowman D., Carrington W, Fogarty K., McNeil J.A., Fay F.S., Lawrence J.B. 1993. A three-dimensional view of precursor messenger RNA metabolism within the mammalian nucleus. *Science* **259**, 1330–1335

Molenaar C., Abdulle A., Gena A., Tanke H.J. & Dirks R.W. 2004. Poly(A)$^+$ RNAs roam the cell nucleus and pass through speckle domains in transcriptionally active and inactive cells. *J. Cell Biol.* **165,** 191-202

Schmidt U., Richter K., Berger A. B. & Lichter P. 2006. In vivo BiFC analysis of Y14 and NXF1 mRNA export complexes: preferential localization within and around SC35 domains. *J. Cell Biol.* **172**, 373-381

Dyck, J.A., Maul, G.G., Miller, W.H., Jr., Chen, J.D., Kakizuka, A. & Evans R.M. 1994. A novel macromolecular structure is a target of the promyelocyte- retinoic acid receptor oncoprotein. *Cell* **76**, 333-343

Koken M.H.M., Puvion-Dutilleul F., Guillemin M. C., Viron A., Linares- Cruz G., Stuurman N., de Jong L., Szostecki C., Calvo F., Chomienne C. 1994. The t(15;17) translocation alters a nuclear body in a retinoic acid- reversible fashion. *EMBO J.* **13**, 1073-1083

Weis K., Rambaud S., Lavau C., Jansen J., Carvalho T., Carmo-Fonseca M., Lamond A. & Dejean A. 1994. Retinoic acid regulates aberrant nuclear localization of PML-RAR alpha in acute promyelocytic leukemia cells. *Cell* **76**, 345-356

Stuurman N., De Graaf A., Floore A., Josso A., Humbel B., De Jong L., Van Driel R 1992. A monoclonal antibody recognizing nuclear matrix-associated nuclear bodies. *J Cell Sci* **101,** 773-784

Boisvert F.M., Hendzel M.J. & Bazett-Jones D.P. 2000. Promyelocytic leukemia (PML) nuclear bodies are protein structures that do not accumulate RNA. *J. Cell Biol.* **148**, 283–292

Dellaire G. & Bazett-Jones D.P. 2004. PML nuclear bodies: dynamic sensors of DNA damage and cellular stress. *Bioessays* **26,** 963–977

Eskiw C.H., Dellaire G. & Bazett-Jones D.P. 2004. Chromatin contributes to structural integrity of promyelocytic leukemia bodies through a SUMO-1-independent mechanism. *J. Biol. Chem.* **279**, 9577–9585

Bernstein R.M., Neuberger J.M., Bunn C.C., Callender M.E., Hughes G.R., Williams R. 1984. Diversity of autoantibodies in primary biliary cirrhosis and chronic active hepatitis. *Clin. Exp. Immunol.* **55**, 553-560

Szostecki C., Guldner H.H., Netter H.J., Will H. 1990. Isolation and characterization of cDNA encoding a human nuclear antigen predominantly recognized by autoantibodies from patients with primary biliary cirrhosis. *J. Immunol.* **145**, 4338-4347

Maul G.G., Negorev D., Bell P., Ishov A.M. 2000. Review: properties and assembly mechanisms of ND10, PML bodies, or PODs. *J. Struct. Biol.* **129**, 278-287

de The H., Lavau C., Marchio A., Chomienne C., Degos L & Dejean A.1991. The PML-RARα fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell* **66,** 675-684

Melnick A. & Licht J.D. 1999. Deconstructing a disease: RARα, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. *Blood* **93,** 3167-3215

Naeem M., Harrison K., Barton K., Nand S. & Alkan S. 2006. A unique case of acute promyelocytic leukemia showing monocytic differentiation after ATRA (all-trans retinoic acid) therapy. *Eur. J. Heamatol*. **76,** 164-166

Weis K., Rambaud S., Lavau C., Jansen J., Carvalho T., Carmo-Fonseca M., Lamond A., Dejean A. 1994. Retinoic acid regulates aberrant nuclear localization of PML-RARα in acute promyelocytic leukemia. *Cell* **75,** 347-386

Lallemand-Breitenbach V., Jeanne M., Benhenda S., Nasr R., Lei M., Peres L., Zhou J., Zhu J., Raught B. & de Thé H. 2008. Arsenic degrades PML or PML—RARα through a SUMO-triggered RNF4/ubiquitin-mediated pathway. *Nature Cell Biol.* **10,** 547-555

Tatham M.H., Geoffroy M.-C., Shen L., Plechanovova A., Hattersley N., Jaffray E.G., Palvimo J.J. & Hay R.T. 2008. RNF4 is a poly-SUMO-specific E3 ubiquitin ligase required for arsenic-induced PML degradation. *Nature Cell Biol.* **10,** 538-546

Hodges M., Tissot C., Howe K., Grimwade D. & Freemont P.S. 1998. Structure, organization, and dynamics of promyelocytic leukemia protein nuclear bodies, *Proc. Natl. Acad. Sci. U.S.A.* **59**, 94–101

LaMorte V.J., Dyck J.A., Ochs R.L. & Evans R.M. 1998. Localization of nascent RNA and CREB binding protein with the PML-containing nuclear body. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 4991–4996

Alcalay M., Tomassoni L., Colombo E., Stoldt S., Grignani F., Fagioli M., Szekely L., Helin K. & Pelicci P.G. 1998. The promyelocytic leukemia gene product (PML) forms stable complexes with the Retinoblastoma protein. *Mol. Cell Biol.* **18**, 1084–1093

Zhong S., Hu P., Ye T.Z., Stan R., Ellis N.A. & Pandolfi P.P. 1999. A role for PML and the nuclear body in genomic stability. *Oncogene* **18**, 7941–7947

Zhang T., Xiong H., Kan L.-X., Zhang C.-K., Jiao X.-F., Fu G., Zhang Q.-H., Lu L., Tong J.-H., Gu B.-W., Yu M., Liu J.-X., Licht J., Waxman S., Zelent A., Chen E., Chen S.-J. 1999. Genomic sequence, structural organization, molecular evolution, and aberrant rearrangement of promyelocytic leukemia zinc finger gene. *Proc. Natl. Acad. Sci. USA* **96,** 11422-11427

Zhong S., Muller S., Ronchetti S., Freemont P.S., Dejean A. & Pandolfi P.P. 2000. Role of SUMO-1-modified PML in nuclear body formation. *Blood* **95,** 2748–2752

Salomoni P. & Pandolfi P.P. 2002. The role of PML in tumor suppression. *Cell* **108,** 165-170

Chelbi-Alix M.K., Pelicano L., Quignon F., Koken M.H., Venturini L., Stadler M., Pavlovic J., Degos L., de Thé H. 1995. Induction of the PML protein by interferons in normal and APL cells. *Leukemia* **9,** 2027–2033

Maul G.G. 1998. Nuclear domain 10, the site of DNA virus transcription and replication. *Bioessays* **20,** 660–667

Everett R.D., Earnshaw W.C., Pluta A.F., Sternsdorf T., Ainsztein A.M., Carmena M., Ruchaud S., Hsu W.L. & Orr A. 1999. A dynamic connection between centromeres and ND10 proteins. *J. Cell Sci.* **112,** 3443–3454

Wang Z.G., Ruggero D., Ronchetti S., Zhong S., Gaboli M., Rivi R. & Pandolfi P.P. 1998a. PML is essential for multiple apoptotic pathways. *Nature Genet.* **20,** 266–272

Wang Z.G., Delva L., Gaboli M., Rivi R., Giorgio M., Cordon-Cardo C., Grosveld F. & Pandolfi P.P. 1998b. Role of PML in cell growth and the retinoic acid pathway. *Science* **279,** 1547–1551

Wang J., Shiels C., Sasieni P., Wu P.J., Islam S.A., Freemont P.S., Sheer D. 2004. Promyelocytic leukemia nuclear bodies associate with transcriptionally active genomic regions. *J. Cell Biol*. **164,** 515-526

Graham D. & Bazett-Jones D.P. 2004. PML nuclear bodies: dynamic sensors of DNA damage and cellular stress. Bioessays. **26,** 963-977

Bøe S.O., Haave M., Jul-Larsen Å., Grudic A., Bjerkvig R. & Lønning P.E. 2006. Promyelocytic leukemia nuclear bodies are predetermined processing sites for damaged DNA. *J Cell Sci* **119,** 3284-3295

Maul G.G., Ishov A.M. & Everett R.D. 1996. Nuclear Domain 10 as Preexisting Potential Replication Start Sites of Herpes Simplex Virus Type-1. Virology **217,** 67–75

Maul G.G. 1998. Nuclear domain 10, the site of DNA virus transcription and replication. *Bioessays* **20,** 660–667

Guldner H.H., Szostecki C., Grotzinger T. & Will H. 1992. IFN enhance expression of Sp100, an autoantigen in primary biliary cirrhosis. *J. Immunol.* **149,** 4067–4073

Stadler M., Chelbi-Alix M.K., Koken M.H., Venturini L., Lee C., Saïb A., Quignon F., Pelicano L., Guillemin M.C. & Schindler C. 1995. Transcriptional induction of the PML growth suppressor gene by interferons is mediated through an ISRE and a GAS element. *Oncogene* **11,** 2565–2573

Maul G.G., Guldner H.H. & Spivack J.G. Modification of discrete nuclear domains induced by herpes simplex virus type 1 immediate early gene 1 product (ICP0). *J. Gen. Virol.* **74,** 2679-2690

Lavau C., Marchio A., Fagioli M., Jansen J., Falini B., Lebon P., Grosveld F., Pandolfi P.P., Pelicci P.G., Dejean A. 1995. The acute promyelocytic leukaemia-associated PML gene is induced by interferon. *Oncogene* **11,** 871–876

Gaboli M., Gandini D., Delva L., Wang Z.G. & Pandolfi P.P. 1998. Acute promyelocytic leukaemia as a model for cross-talk between interferon and retinoic acid pathways: from molecular biology to clinical applications. *Leuk. Lymph.* **30,** 11–22

Borden K.L.B., Campbell Dwyer E.J. & Salvato M.S. 1998. An arenavirus RING (zinc-binding) protein binds the oncoprotein promyelocyte leukemia protein (PML) and relocates PML nuclear bodies to the cytoplasm. *J. Virol.* **72,** 758-766

Melnick A. & Licht J.D. 1999. Deconstructing a disease: RARα, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. *Blood* **93,** 3167-3215

Maul G.G., Negorev D., Bell P. & Ishov A.M. 2000. Review: properties and assembly mechanisms of ND10, PML bodies, or PODs. *J. Struct. Biol.* **129,** 278-287

Turelli P., Doucas V., Craig E., Mangeat B., Klages N., Evans R., Kalpana G. & Trono D. 2001. Cytoplasmic recruitment of INI1 and PML on incoming HIV preintegration complexes: interference with early steps of viral replication. *Mol. Cell* **7,** 1245-1254

Bell P., Montaner L.J. & Maul G.G. 2001. Accumulation and intranuclear distribution of unintegrated human immunodeficiency virus type 1 DNA. *J. Virol*. **75,** 7683-7691

Ishov A.M., Sotnikov A.G., Negorev D., Vladimirova O.V., Neff N., Kamitani T., Yeh E.T., Strauss J.F. 3rd & G.G. Maul. 1999. PML is critical for ND10 formation and recruits the PML-interacting protein daxx to this nuclear structure when modified by SUMO-1. *J. Cell Biol.* **147,** 221–234

Zhong S., Müller S., Ronchetti S., Freemont P.S., Dejean A. & Pandolfi P.P. 2000. Role of SUMO-1-modified PML in nuclear body formation. Blood **95,** 2748-2752

Sternsdorf T., Jensen K. & Will H. 1997. Evidence for Covalent Modification of the Nuclear Dot–associated Proteins PML and Sp100 by PIC1/SUMO-1. *J. Cell Biol.* **139,** 1621–1634

Duprez E., Saurin A.J., Desterro J.M., Lallemand-Breitenbach V., Howe K., Boddy M.N., Solomon E., de The H., Hay R.T. & Freemont P.S. 1999. SUMO-1 modification of the acute promyelocytic leukaemia protein PML: implications for nuclear localization. *J. Cell Sci.* **112,** 381-393

Shen T.H., Lin H.-K., Scaglioni P.P., Yung T.M. & Pandolfi P.P. 2006. The Mechanisms of PML-Nuclear Body Formation. *Mol Cell.* **24,** 331-339

Kentsis A., Gordon R.E. & Borden K.L.B. 2002. Control of biochemical reactions through supramolecular RING domain self-assembly. PNAS **99,** 15404–15409

Eskiw C.H., Dellaire G., Mymryk J.S. & Bazett-Jones D.P. 2003. Size, position and dynamic behavior of PML nuclear bodies following cell stress as a paradigm for supramolecular trafficking and assembly *J. Cell Sci.* **116**, 4455−4466

Seeler J.S. & Dejean A. 2003. Nuclear and unclear functions of SUMO. *Nat Rev Mol Cell Biol.* **4,** 690-699

Mu Z.M., Chin K.V., Liu J.H., Lozano G. & Chang K.S. 1994. PML, a growth suppressor disrupted in acute promyelocytic leukemia. *Mol Cell Biol* **14,** 6858-6867

Ahn M.J., Nason-Burchenal K., Moasser M.M., Dmitrovsky E. 1995. Growth suppression of acute promyelocytic leukemia cells having increased expression of the non-rearranged alleles: RAR alpha or PML. *Oncogene* **10,** 2307-2314

Koken M.H.M., Linares-Cruz G., Quignon F., Viron A., Chelbi-Alix M.K., Sobczak-Thepot J., Juhlin L., Degos L., Calvo F. & de The H. 1995. The PML growth-suppressor has an altered expression in human oncogenesis. *Oncogene* **10**, 1315–1324

Melnick A. & Licht J.D. 1999. Deconstructing a disease: RARα, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. *Blood* **93,** 3167-3215

Gurrieri C., Capodieci P., Bernardi R., Scaglioni P.P., Nafa K., Rush L.J., Verbel D.A., Cordon-Cardo C., Pandolfi P.P. 2004a. Loss of the tumor suppressor PML in human cancers of multiple histologic origins. *J Natl Cancer Inst* **96**, 269–279

Chang H.J., Yoo B.C., Kim S.W., Lee B.L., Kim W.H. 2007. Significance of PML and p53 protein as molecular prognostic markers of gallbladder carcinomas. *Pathol Oncol Res* **13**, 326–335

Ferbeyre G., de Stanchina E., Querido E., Baptiste N., Prives C. & Lowe S.W. 2000. PML is induced by oncogenic *ras* and promotes premature senescence. *Genes Dev.* **14,** 2015–2027

Liu J.H., Mu Z.M., Chang K.S. 1995. PML suppresses oncogenic transformation of NIH/3T3 cells by activated neu. *J Exp Med* **181**, 1965–1973

Mu Z.M., Le X.F., Vallian S., Glassman A.B., Chang K.S. 1997. Stable overexpression of PML alters regulation of cell cycle progression in HeLa cells. *Carcinogenesis* **18**, 2063–2069

Le X.F., Vallian S., Mu Z.M., Hung M.C., Chang K.S. 1998. Recombinant PML adenovirus suppresses growth and tumorigenicity of human breast cancer cells by inducing G1 cell cycle arrest and apoptosis. *Oncogene* **16**, 1839–1849

Wang Z.G., Ruggero D., Ronchetti S., Zhong S., Gaboli M., Rivi R. & Pandolfi P.P. 1998a. PML is essential for multiple apoptotic pathways. *Nat Genet* **20**, 266–272

Trotman L.C., Alimonti A., Scaglioni P.P., Koutcher J.A., Cordon-Cardo C., Pandolfi P.P. 2006. Identification of a tumour suppressor network opposing nuclear Akt function. *Nature* **441**, 523–527

Quignon F., De Bels F., Koken M., Feunteun J., Ameisen J.C., de The H. 1998. PML induces a novel caspase-independent death process. *Nature Genet.* **20,** 259–265

Yang S., Kuo C., Bisi J.E. & Kim M.K. 2002. PML-dependent apoptosis after DNA damage is regulated by the checkpoint kinase hCds1/Chk2. *Nature Cell Biol.* **4**, 865 -870

Tsukamoto T., Hashiguchi N., Janicki S.M., Tumbar T., Belmont A.S., Spector D.L. 2000. Visualization of gene activity in living cells. *Nature Cell Biol.* **2,** 871–878

Maggio R., Siekevitz P. & Palade G.E. 1963a. Studies on isolated nuclei. I. Isolation and chemical composition of a nuclear fraction from guinea pig liver. *J. Cell Biol*. **18**, 267-291

Penman S. 1966. RNA metabolism in the HeLa cell nucleus. *J. Mol. Biol*. **17,** 117-130

Blobel G. & Potter V.R. 1966. Nuclei from rat liver: isolation method that combines purity with high yield. *Science* **154,** 1662-1665

Dounce A.L. 1955. Isolation and composition of nuclei and nucleoli. *In The Nucleic Acids* (Chargaff E. & Davidson J.N. eds), pp. 93-153, Academic Press, New York

Pederson T. 1997. Preparation of nuclei from tissue or suspension culture. In Cells: A Laboratory Manual (Spector, D. L., Goldman, R. D. & Leinwand, L. A., eds), vol. 1, *Cold Spring Harbor Laboratory Press*, pp. 43.7-43.11, Plainview, NY

Berezney R. & Coffey D.S. 1974. Identification of a nuclear protein matrix. *Biochem. Biophys. Res. Comm.* **60**, 1410-1417

Berezny R. & Jeon K. 1995. Nuclear matrix: structural and functional organization. Book.

Jackson D.A. & Cook P.R. 1988. Visualization of a filamentous nucleoskeleton with a 23 nm axial repeat. *EMBO J.* **7,** 3667–3677

Capco D.G., Wan K.M., Penman S. 1982. The nuclear matrix: three-dimensional architecture and protein composition**.** *Cell* **29,** 847-858

Monneron A. & Bernhard W. 1969. Fine structural organization of the interphase nucleus in some mammalian cells. *J Ultrastruct Res.* **27,** 266-288

Comings D.E. & Okada T.A. 1976. Nuclear proteins. III. The fibrillar nature of the nuclear matrix. *Exp. Cell Res*. **103,** 341-60

Small D., Nelkin B. & Vogelstein B. 1985. The association of transcribed genes with the nuclear matrix of *Drosophila* cells during heat shock. *Nucleic Acids Research* **13,** 2413-2431

Fey E.G., Krochmalnic G. & Penman S. 1986. The nonchromatin substructures of the nucleus: The ribonucleoprotein (RNP)-containing and RNP-depleted matrices analyzed by sequential fractionation and resinless section electron microscopy. *The J. Cell Biol.* **102,** 1654-1665

Jackson D.A. & Cook P.R. 1988. Visualization of a filamentous nucleoskeleton with a 23 nm axial repeat. *EMBO J.* **7**, 3667-3677

Mirkovitch J., Mirault M.E. and Laemmli U.K. 1984. Organization of the higher-order chromatin loop: Specific DNA attachment sites on nuclear scaffold. *Cell* **39**, 223-232.

Martelli A.M., Falcieri E., Gobbi P., Manzoli L., Gilmour R.S. & Cocco L. 1991. Heat-induced stabilization of the nuclear matrix: A morphological and biochemical analysis in murine erythroleukemia cells. *Exp. Cell Res.* **19,** 216-225

Neri L.M., Fackelmayer F.O., Zweyer M., Kohwi-Shigematsu T. & Martelli A.M. 1997. Subnuclear localization of s/mar-binding proteins is differently affected by in vitro stabilization with heat or Cu^{2+}. *Chromosoma* **106**, 81-93

Kaufmann S.H. & Shaper J.H. 1984. A subset of non-histone nuclear proteins reversibly stabilized by the sulfhydryl cross-linking reagent tetrathionate. Polypeptides of the internal nuclear matrix. *Exp. Cell Res*. **155**, 477-495

Berezney R. & Coffey D.S. 1977. Nuclear matrix. Isolation and characterization of a framework structure from rat liver nuclei. *J. Cell Bio*l. **73**, 616-637

Palade G.E. & Siekevitz P.1956. Liver microsomes. An integrated morphological and biochemical study. *J. Biophys. Biochem. Cytol.* **2**, 171–200

Tashiro Y. 1958. Studies on the ribonucleoprotein particles. III. Some physicochemical characteristics and the degradation with ribonuclease and trypsin of the microsomal ribonucleoprotein particles. *J. Biochem.* **45,** 803–813

Madison J.T. & Dickman S.R. 1963. Beef pancreas ribosomes: stability. *Biochem.* **2,** 325–332

Lothstein L., Arenstorf H.P., Chung S.-Y., Walker B.W., Wooley J.C. & LeStourgeon W.M. 1985. General organization of protein in HeLa 40 S nuclear ribonucleoprotein particles. *J. Cell Biol.* **100,** 1 1570–1581

Kauzmann W. Some factors in the interpretation of protein denaturation. 1959. In: C.B. Anfinsen, Jr, M.L. Anson, K. Bailey & J.T. Edsall editors. *Advances in Protein Chemistry* **14**, 1–63

Varshavsky A.J. & Ilyin Y.V. 1974. Salt treatment of chromatin induces redistribution of histones. *Biochim. Biophys. Acta* **340,** 207–217

Finkelstein A.V., Protein structure: what is it possible to predict now? 1997. *Curr. Opin. Struct. Biol.* **7**, 60–71

Ben-Ze'ev A., Abulafia R. & Aloni Y. SV40 virions and viral RNA metabolism are associated with subcellular structures. 1982. *EMBO J.* **1,** 1225–1231

Ciejek E.M., Nordstrom J.L., Tsai M.-J. & O'Malley B.W. 1982. Ribonucleic acid precursors are associated with the chick oviduct nuclear matrix. *Biochemistry* **21**, 4945–4953

Mariman E.C.M., van Eckelen C.A.G., Reinders R.J., Berns A.J.M. & van Venrooij W.J. 1982. Adenoviral heterogeneous nuclear RNA is associated with the host nuclear matrix during splicing. *J. Mol. Biol.* **154**, 103–119

Ross D.A., Yen R.-W. & Chae C.B. 1982. Association of globin ribonucleic acid and its precursors with the chicken erythroblast nuclear matrix. *Biochemistry* **21**, 754–771

Gallinaro H., Puvion E., Kister L. & Jacob M. 1983. Nuclear matrix and hnRNP share a common structural constituent associated with premessenger RNA. *EMBO J.* **2**, 953–960

Ben-Ze'ev A. & Aloni Y. 1983. Processing of SV40 RNA is associated with the nuclear matrix and is not followed by the accumulation of low-molecular-weight RNA products. *Virol.* **125**, 475–479

Mortillaro M.J., Blencowe B.J., Wei X., Nakayasu H., Du H., Warren S.L., Sharp P.A. & Berezney R. 1996. A hyperphosphorylated form of the large subunit of RNA polymerase II is associated with splicing complexes and the nuclear matrix. *Proc. Natl Acad. Sci. USA* **93**, 8253–8257

Vincent M., Lauriault P., Dubois M.-F., Lavoie S., Bensaude O. & Chabot B. 1996. The nuclear matrix protein p225 is a highly phosphorylated form of RNA polymerase II largest subunit which associates with spliceosomes. *Nucl. Acids Res.* **24,** 4649–4652

Dahmus M.E. 1996. Reversible phosphorylation in the C-terminal domain of RNA polymerase II. *J. Biol. Chem.* **271**, 19009–19012

Konety B.R., Getzenberg R.H. 1999. Nuclear structural proteins as biomarkers of cancer. *J. Cell Biochem.* **32/33,** 183-191

Ozen H. 1999. Bladder cancer. *Curr. Opin. Oncol.* **11**, 207-212

Stuurman N., Heins S., Aebi U. 1998. Nuclear lamins: their structure, assembly, and interactions. *J. Struct Biol.* **122**, 42–66

Goldman R.D., Gruenbaum Y., Moir R.D., Shumaker D.K., Spann T.P. 2002. Nuclear lamins: building blocks of nuclear architecture. *Genes Dev*. **16,** 533–547

Hutchison C.J. 2002. Lamins: building blocks or regulators of gene expression? *Nat.Rev. Mol. Cell Biol.* **3**, 848–858

Mounkes L., Kozlov S., Burke B., Stewart C.L. 2003. The laminopathies: nuclear structure meets disease. *Curr. Opin. Genet. Dev.* **13**, 223–230

Bonne G., Di Barletta M.R., Varnous S., Bécane H.M., Hammouda E.H., Merlini L., Muntoni F., Greenberg C.R., Gary F., Urtizberea J.A., Duboc D., Fardeau M., Toniolo D., Schwartz K. 1999. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat. Genet.* **21,** 285-288

Fatkin D., MacRae C., Sasaki T., Wolff M.R., Porcu M., Frenneaux M., Atherton J., Vidaillet H.J. Jr, Spudich S., De Girolami U., Seidman J.G., Seidman C., Muntoni F., Müehle G., Johnson W., McDonough B. 1999. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *N. Engl. J. Med.* **341,** 1715-1724

Cao H. & Hegele R.A. 2000. Nuclear lamin A/C R482Q mutation in canadian kindreds with Dunnigan-type familial partial lipodystrophy. *Hum. Mol. Genet.* 2000. **9,** 109-112

Shackleton S., Lloyd D.J., Jackson S.N., Evans R., Niermeijer M.F., Singh B.M., Schmidt H., Brabant G., Kumar S., Durrington P.N., Gregory S., O'Rahilly S., Trembath R.C. 2000. LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. *Nat. Genet.* **24,** 153-156

Burke B. & Stewart C.L. 2002. Life at the edge: the nuclear envelope and human disease. *Nat. Rev. Mol. Cell Biol.* **3,** 575-585

De Sandre-Giovannoli A., Bernard R., Cau P., Navarro C., Amiel J., Boccaccio I., Lyonnet S., Stewart C.L., Munnich A., Le Merrer M., Lévy N. 2003. Lamin A truncation in Hutchinson-Gilford progeria. *Science* **300,** 2055

Eriksson M., Brown W.T., Gordon L.B., Glynn M.W., Singer J., Scott L., Erdos M.R., Robbins C.M., Moses T.Y., Berglund P., Dutra A., Pak E., Durkin S., Csoka A.B., Boehnke M., Glover T.W. & Collins F.S. 2003. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* **423,** 293-298

Lebel S., Lampron C., Royal A., & Raymond Y. 1987. Lamins A and C Appear during Retinoic Acid-induced Differentiation of Mouse Embryonal Carcinoma Cells. *The Journal of Cell Biology* **105,** 1099-1104

Stuurman N., Heins S., Aebi U. 1998. Nuclear lamins: their structure, assembly, and interactions. *J. Struct. Biol*. **122**, 42–66

Moir R. D., Miri Yoon, Satya Khuon & Robert D. Goldman. 2000. Nuclear Lamins A and B1: Different Pathways of Assembly during Nuclear Envelope Formation in Living Cells. *J. Cell Biol.* **151,** 1155-1168

Foisner R. 2001. Inner nuclear membrane proteins and the nuclear lamina. *J. Cell Sci.* **114,** 3791-3792

Lenz-Bohme B., Wismar J., Fuchs S., Reifegerste R., Buchner E., Betz H., Schmitt B. 1997. Insertional mutation of the *Drosophila* nuclear lamin Dm0 gene results in defective nuclear envelopes, clustering of nuclear pore complexes, and accumulation of annulate lamellae. *J. Cell Biol.* **137,** 1001–1016

Schirmer E.C., Guan T., Gerace L. 2001. Involvement of the lamin rod domain in heterotypic lamin interactions important for nuclear organization. *J. Cell Biol.* **153,** 479–489

Moir R.D., Spann T.P. & Goldman R.D. 1995. The dynamic properties and possible functions of nuclear lamins. *Int. Rev. Cytol*. **162B**, 141-182

Gant T.M. & Wilson K.L. 1997. Nuclear assembly. *Annu. Rev. Cell Dev. Biol*. **13**, 669-695

Stuurman N., Heins S. & Aebi U. 1998. Nuclear lamins: Their structure, assembly and interactions. *J. Struct. Biol.* **122**, 42-66

Gant T.M., Harris C.A., Wilson K.L. 1999. Roles of LAP2 proteins in nuclear assembly and DNA replication: truncated LAP2beta proteins alter lamina assembly, envelope formation, nuclear size, and DNA replication efficiency in *Xenopus laevis* extracts. *J. Cell Biol.* **144,** 1083–1096

Goldman A.E., Moir R.D., Montag-Lowy M., Stewert M. & Goldman R.D. 1992. Pathway of incorporation of microinjected lamin A into the nuclear envelope. *J. Cell Biol.* **119**, 725-735

Bridger J.M., Kill I.R., O'Farrell M. & Hutchison C.J. 1993. Internal lamin structures within G1 nuclei of human dermal fibroblasts. *J. Cell Sci*. **104**, 297-306

Moir R.D., Montaglowy M. & Goldman R.D. 1994. Dynamic properties of nuclear lamins: Lamin B is associated with sites of DNA replication. *J. Cell Biol.* **125,** 1201-1212

Hozak P., Sasseville A.M-J., Raymond Y. & Cook P.R. 1995. Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. *J. Cell Sci.* **108**, 635-644

Jenkins H., Whitfield W.G., Goldberg M.W., Allen T.D., Hutchison C.J. 1995. Evidence for the direct involvement of lamins in the assembly of a replication competent nucleus. *Acta. Biochim. Pol.* **42,** 133–143

Goldman R.D., Gruenbaum Y., Moir R.D., Shumaker D.K. & Spann T.P. 2002. Nuclear lamins: building blocks of nuclear architecture. *Genes Dev*. **16,** 533-547

Martins S., Eikvar S., Furukawa K., Collas P. 2003. HA95 and LAP2 beta mediate a novel chromatin-nuclear envelope interaction implicated in initiation of DNA replication. *J. Cell Biol.* **160,** 177–188

Gruenbaum Y., Goldman R.D., Meyuhas R., Mills E., Margalit A., Fridkin A., Dayani Y., Prokocimer M., Enosh A. 2003. The nuclear lamina and its functions in the nucleus. *Int. Rev. Cyt.* **226**, 1–62

Nili E., Cojocaru G.S., Kalma Y., Ginsberg D., Copeland N.G., Gilbert D.J., Jenkins N.A., Berger R., Shaklai S., Amariglio N., Brok-Simoni F., Simon A.J., Rechavi G. 2001. Nuclear membrane protein LAP2 mediates transcriptional repression alone and together with its binding partner GCL (germ-cell-less). *J. Cell Sci.* **114,** 3297–3307

Spann T.P., Goldman A.E., Wang C., Huang S., Goldman R.D. 2002. Alteration of nuclear lamin organization inhibits RNA polymerase II-dependent transcription. *J. Cell Biol.* **156,** 603–608

Wilkinson F.L., Holaska J.M., Zhang Z., Sharma A., Manilal S., Holt I., Stamm S., Wilson K.L., Morris G.E. 2003. Emerin interacts *in vitro* with the splicing-associated factor, YT521-B. *Eur. J. Biochem.* **270,** 459–2466

Haraguchi T., Holaska J.M., Yamane M., Koujin T., Hashiguchi N., Mori C., Wilson K.L., Hiraoka Y. 2004. Emerin binding to Btf, a death-promoting transcriptional repressor, is disrupted by a missense mutation that causes Emery–Dreifuss muscular dystrophy. *Eur. J. Biochem.* **271,** 1035–1045

Lin F., Blake D.L., Callebaut I., Skerjanc I.S., Holmer L., McBurney M.W., Paulin-Levasseur M., Worman H.J. 2000. MAN1, an inner nuclear membrane protein that shares the LEM domain with lamina-associated polypeptide 2 and emerin. *J. Biol. Chem.* **275,** 4840–4847

Foisner R. 2001. Inner nuclear membrane proteins and the nuclear lamina. *J. Cell Sci.* **114,** 3791-3792

Gruenbaum Y., Goldman R.D., Meyuhas R., Mills E., Margalit A., Fridkin A., Dayani Y., Prokocimer M., Enosh A. 2003. The nuclear lamina and its functions in the nucleus. *Int. Rev. Cyt.* **226**, 1–62

Zastrow, M.S., Vlcek, S. & Wilson, K.L. 2004. Proteins that bind A-type lamins: integrating isolated clues. *J. Cell Sci.* **117**, 979–987

Sullivan T., Escalante-Alcaldea D., Bhattb H., Anverc M., Bhatc N., Nagashimac K., Stewarta C. L.& Burked B. 1999. Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. *J. Cell Biol.* **147**, 913–920

Lee K.K., Starr D., Cohen M., Liu J., Han M., Wilson K.L. & Gruenbaum Y. 2002. Lamin-dependent localization of UNC-84, a protein required for nuclear migration in C. elegans. *Mol. Biol. Cell* **13,** 892–901

Liu J., Lee K.K., Segura-Totten M., Neufeld E., Wilson K.L. & Gruenbaum Y. 2003. MAN1 and emerin have overlapping function(s) essential for chromosome segregation and cell division in *C. elegans*. *Proc. Natl Acad. Sci. USA* **100**, 4598–4603

Muchir A., van Engelen B.G., Lammens M., Mislow J.M., McNally E., Schwartz K. & Bonne G. 2003. Nuclear envelope alterations in fibroblasts from LGMD1B patients carrying nonsense Y259X heterozygous or homozygous mutation in lamin A/C gene. *Exp. Cell Res.* **291**, 352–362

Wagner N., Schmitt J. & Krohne G. 2004. Two novel LEM-domain proteins are splice products of the annotated *Drosophila melanogaster* gene CG9424 (*Bocksbeutel*). *Euro. J. Cell Biol.* **82**, 605–616

Guillemin K., Williams T. & Krasnow M.A.A. 2001. Nuclear lamin is required for cytoplasmic organization and egg polarity in *Drosophila*. *Nature Cell Biol.* **3**, 848–851

Raz V., Carlotti F., Vermolen B.J., van der Poel E., Sloos W.C.R., Knaän-Shanzer S., de Vries A.A.F., Hoeben R.C., Young I.T., Tanke H.J., Garini Y. & Dirks R.W. 2006. Changes in lamina structure are followed by spatial reorganization of heterochromatic regions in caspase-8-activated human mesenchymal stem cells. *J. Cell Sci.* **119,** 4247-4256

Raz V., Vermolen B.J., Garini Y., Onderwater J.J.M., Mommaas-Kienhuis M.A., Koster A.J., Young I.T., Tanke H.J. & Dirks R.W. 2008. The nuclear lamina promotes telomere aggregation and centromere peripheral localization during senescence of human mesenchymal stem cells. *J. Cell Sci.* **121,** 4018-4028

Liu J., Ben-Shahar T.R., Riemer D., Treinin M., Spann P., Weber K., Fire A. & Y.Gruenbaum. 2000. Essential roles for *Caenorhabditis elegans* lamin gene in nuclear organization, cell cycle progression, and spatial organization of nuclear pore complexes. *Mol. Biol. Cell* **11**, 3937–3947

Schirmer E.C., Guan T. & Gerace L. 2001. Involvement of the lamin rod domain in heterotypic lamin interactions important for nuclear organization. *J. Cell Biol.* **153,** 479–490

Starr D.A., Hermann G.J., Malone C.J., Fixsen W., Priess J.R., Horvitz H.R. & Han M. 2001. *unc-83* encodes a novel component of the nuclear envelope and is essential for proper nuclear migration. *Development* **128**, 5039–5050

Starr D.A. & Han M. 2002. Role of ANC-1 in tethering nuclei to the actin cytoskeleton. *Science* **11**, 406–409

Lopez-Soler R.I., Moir R.D., Spann T.P., Stick R. & Goldman R.D. 2001. A role for nuclear lamins in nuclear envelope assembly. *J. Cell Biol.* **154**, 61–70

Guelen L., Pagie L., Brasset E., Meuleman W., Faza M.B., Talhout W., Eussen B.H., de Klein A., Wessels L., de Laat W., van Steensel B. 2008. Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. *Nature* **453**, 948-951

Zhang Q., Skepper J.N., Yang F., Davies J.D., Hegyi L., Roberts R.G., Weissberg P.L., Ellis J.A. & Shanahan C.M. 2001. Nesprins: a novel family of spectrin-repeat-containing proteins that localize to the nuclear membrane in multiple tissues. *J. Cell Sci.* **114**, 4485–4498

Mislow J.M., Kim M.S., Davis D.B. & McNally E.M. 2002. Myne-1, a spectrin repeat transmembrane protein of the myocyte inner nuclear membrane, interacts with lamin A/C. *J. Cell Sci.* **115**, 61–70

Zhen Y.Y., Libotte T., Munck M., Noegel A.A. & Korenbaum E. 2002. NUANCE, a giant protein connecting the nucleus and actin cytoskeleton, *J. Cell Sci.* **115**, 3207–3222

Wilhelmsen K., Litjens S.H., Kuikman I., Tshimbalanga N., Janssen H., van den Bout I., Raymond K. & Sonnenberg A. 2005. Nesprin-3, a novel outer nuclear membrane protein, associates with the cytoskeletal linker protein plectin. *J. Cell Biol.* **171**, 799–810

Wang N. & Suo Z. 2005. Long-distance propagation of forces in a cell. *Biochem. Biophys. Res. Commun.* **328,** 1133–1138

Mislow J.M., Holaska J.M. Kim M.S., Lee K.K., Segura-Totten M., Wilson K.L. & McNally E.M. 2002. Nesprin-1alpha self-associates and binds directly to emerin and lamin A in vitro. *FEBS Lett.* **525**, 135–140

Padmakumar V.C., Libotte T., Lu W., Zaim H., Abraham S., Noegel A.A., Gotzmann J., Foisner R. & Karakesisoglou I. 2005. The inner nuclear membrane protein Sun1 mediates the anchorage of Nesprin-2 to the nuclear envelope. *J. Cell Sci.* **118,** 3419–3430

Lammerding J., Schulze P.C., Takahashi T., Kozlov S., Sullivan T., Kamm R.D., Stewart C.L. & Lee R.T. 2004. Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. *J. Clin. Invest.* **113**, 370–378

Broers J.L., Peeters E.A., Kuijpers H.J., Endert J., Bouten C.V., Oomens C.W., Baaijens F.P. & Ramaekers F.C. 2004. Decreased mechanical stiffness in LMNA−/− cells is caused by defective nucleo-cytoskeletal integrity: implications for the development of laminopathies. *Hum. Mol. Genet.* **13**, 2567–2580

Broers J.L., Kuijpers H.J., Ostlund C., Worman H.J., Endert J. & Ramaekers F.C. 2005. Both lamin A and lamin C mutations cause lamina instability as well as loss of internal nuclear lamin organization. *Exp. Cell Res.* **304**, 582–592

Cohen M., Lee K.K., Wilson K.L., Gruenbaum Y. 2001.Transcriptional repression, apoptosis, human disease and the functional evolution of the nuclear lamina. *Trends Biochem Sci.* **26,** 41-47

Jackson D.A. & Cook P.R. 1988. Visualization of a filamentous nucleoskeleton with a 23 nm axial repeat. *EMBO J.* **7**, 3667-3677

He D., Nickerson J.A. & Penman S. 1990. Core filaments of the nuclear matrix. *J. Cell Biol.* **110**, 569-580

Hozák P., Sasseville A.M.-J., Raymond Y. & Cook P.R. 1995. Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. *J. Cell Sci.* **108**, 635-644

Wan K.M., Nickerson J.A., Krockmalnic G. & Penman S. 1999. The nuclear matrix prepared by amine modification. *Proc. Natl. Acad. Sci. USA* **96,** 933–938

Traub P. & Shoeman R.L. 1994. Intermediate filament proteins: cytoskeletal elements with gene-regulatory functions? *Int. Cytol. Rev*. **154**, 1-103

Goldman A.E., Moir R.D., Montag-Lowy M., Stewart M. & Goldman R.D. 1992. Pathway of incorporation of microinjected lamin A into the nuclear envelope. *J. Cell Biol.* **119**, 725-735

Bridger J.M., Kill I.R., O'Farrell M. & Hutchison C.J. 1993. Internal lamin structures within G1 nuclei of human dermal fibroblasts. *J. Cell Sci.* **104**, 297-306

Moir R.D., Montag-Lowy M. & Goldman R.D. 1994. Dynamic properties of nuclear lamins: lamin B is associated with sites of DNA replication. *J. Cell Biol*. **125**, 1201-1212

Schmidt M., Tschödrich-Rotter M.M., Peters R. & Krohne G. 1994. Properties of fluorescently labeled Xenopus lamin A in vivo. *Eur. J. Cell Biol*. **65**, 70-81

Kiseleva E., Drummond S.P., Goldberg M.W., Rutherford S.A., Allen T.D. & Wilson K.L. 2004. Actin- and protein-4.1-containing filaments link nuclear pore complexes to subnuclear organelles in Xenopus oocyte nuclei. *Journal of Cell Science* **117,** 2481-2490

Pestic-Dragovich L., Stojiljkovic L., Philimonenko A.A., Nowak G., Ke Y., Settlage R.E., Shabanowitz J., Hunt D.F., Hozak P., de Lanerolle P. 2000. A Myosin I Isoform in the Nucleus. *Science* **290,** 337-341

Clark T.G. & Merriam R.W. 1977. Diffusible and bound actin in nuclei of *Xenopus laevis* oocytes. *Cell.* **12,** 883–891

Fukui Y. 1978. Intranuclear actin bundles induced by dimethyl sulfoxide in interphase nucleus of Dictyostelium. *J. Cell Biol.* **76,** 146–157

Fukui Y., Katsumaru H. 1979. Nuclear actin bundles in Amoeba, Dictyostelium and human HeLa cells induced by dimethyl sulfoxide. *Exp. Cell Res.* **120,** 451–455

Clark T.G. & Rosenbaum J.L. 1979. An actin filament matrix in hand-isolated nuclei of *X. laevis* oocytes. *Cell* **18,** 1101–1108

Osborn M.& Weber K. 1980. Dimethylsulfoxide and the ionophore A23187 affect the arrangement of actin and induce nuclear actin paracrystals in PtK2 cells. *Exp. Cell Res.* **129**, 103–114

Welch W.J. & Suhan J.P. 1985. Morphological study of the mammalian stress response. characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. *J. Cell Biol.* **101**, 1198-1211

DeBoni U. 1994. The interphase nucleus as a dynamic structure. *Int. Rev. Cytol.* **150**, 149-171

Yan C., Leibowitz N., Mélèse T. 1997. A role for the divergent actin gene, ACT2 in nuclear pore structure and function. *EMBO J.* **16,** 3572–3586

Rando O. J., Zhao K. & Crabtree G.R. 2000. Searching for a function for nuclear actin. *Trends Cell Biol.* **10**, 92-97

Pederson T. & Aebi U. 2002. Actin in the nucleus: what form and what for? *J. Struct. Biol*. **140**, 3-9

Bettinger B.T., Gilbert D.M. & Amberg D.C. 2004. Actin up in the nucleus. *Nature Reviews Molecular Cell Biology* **5,** 410-415

Ankenbauer T., Kleinschmidt J.A., Walsh M.J., Weiner O.H. & Franke W.W. 1989. Identification of a widespread nuclear actin binding protein. Nature, **342**, 822-825

Rimm D.L. & Pollard T.D. 1989. Purification and characterization of an Acanthamoeba nuclear actinbinding protein. *J. Cell Biol.* **109**, 585-591

Hauser M., Beinbrech G., Groschel-Stewart U. & Jockusch B.M. 1975. Localisation by immunological techniques of myosin in nuclei of lower eukaryotes. *Expt. Cell Res.* **95** 127–135.

Berrios M. & Fisher P.A. 1986. A myosin heavy-chain-like polypeptide is associated with the nuclear envelope in higher eukaryotic cells. *J. Cell Biol.* **103,** 711-724

Hagen S.J., Kiehart D.P., Kaiser D.A. & Pollard T.D. 1986. Characterization of monoclonal antibodies to *Acanthamoeba* myosin-I that cross react with both myosin-II and low molecular mass nuclear proteins. *J. Cell Biol.* **103,** 2121–2128

Rimm D.L. & Pollard T.D. 1989. Purification and characterization of an *Acanthamoeba* nuclear actin-binding protein. *J. Cell Biol.* **109**, 585–591

Nowak G., Pestic-Dragovich L., Hozak P., Philomenokov A., Simerly C., Schatten G. & de Lanerolle P. 1997. Evidence for the presence of myosin I in the nucleus. *J. Biol. Chem.* **272**, 17176–17181

Scheer U., Hinssen H., Franke W.W. & Jockusch B.M. 1984. Microinjection of actin-binding proteins and actin antibodies demonstrates involvement of nuclear actin in transcription of lampbrush chromosomes. *Cell* **39**, 111-122

Sahlas D.J., Milankov K., Park P.C. & De Boni U. 1993. Distribution of snRNPs, splicing factor SC-35 and actin in interphase nuclei: immunocytochemical evidence for differential distribution during changes in functional states. *J. Cell Sci.* **105**, 347-357

Machesky L.M. & May R.C. 2001. Arps: actin-related proteins. *Results Probl. Cell Diff.* **32**, 213-229

Goodson H.V. & Hawse W.F. 2002. Molecular evolution of the actin family. *J. Cell Sci.* **115**, 2619-2622

Olave I., Wang W., Xue Y., Kuo A., Crabtree G.R. 2002. Identification of a polymorphic, neuron-specific chromatin remodeling complex. *Genes Dev.* **16,** 2509–2517

Clark T.G. & Merriam R.W. 1977. Diffusible and bound actin in nuclei of *Xenopus laevis* oocytes. *Cell* **12**, 883-891

Funaki K., Katsumoto T. & Iino A. 1995. Immunocytochemical localization of actin in the nucleolus of rat oocytes. *Biol. Cell* **84**, 139-146

Parfenov V.N., Davis D.S., Pochukalina G.N., Sample C.E., Bugaeva E.A. & Murti K.G. 1995. Nuclear actin and their topological changes in frog oocytes. *Exp. Cell Res.* **217**, 385-394

Pestic-Dragovich L., Stojiljkovic L., Philimonenko A.A., Nowak G., Ke Y., Settlage R. E., Shabanowitz J., Hunt D.F., Hozak P. & de Lanerolle P. 2000. A myosin I isoform in the nucleus. *Science* **290**, 337- 341

Pederson T. & Aebi U. 2002. Actin in the nucleus: what form and what for? *J. Struct. Biol*. **140**, 3-9

Bettinger B.T., Gilbert D.M. & Amberg D.C. 2004. Actin up in the nucleus. *Nature Reviews Molecular Cell Biology* **5,** 410-415

Fukui Y. & Katsumaru H. 1979. Nuclear actin bundles in Amoeba, Dictyostelium and human HeLa cells induced by dimethyl sulfoxide. *Exp. Cell Res.* **120**, 451–455

Iida K., Iida H. & Yahara I. 1986. Heat shock induction of intranuclear actin rods in cultured mammalian cells. *Exp. Cell Res.* **165,** 207–215

Iida K. & Yahara I. 1986. Reversible induction of actin rods in mouse C3H–2K cells by incubation in salt buffers and by treatment with non-ionic detergents. *Exp. Cell Res.* **164,** 492–506

Nishida E., Iida K., Yonezawa N., Koyasu S., Yahara, I. & Sakai H. 1987. Cofilin is a component of intranuclear and cytoplasmic actin rods induced in cultured cells. *Proc. Natl. Acad. Sci. USA* **84,** 5262–5266

Wada A., Fukuda M., Mishima M. & Nishida E. 1998. Nuclear export of actin: a novel mechanism regulating the subcellular localization of a major cytoskeletal protein. *EMBO J.* **17,** 1635–1641

Sameshima M., Kishi Y., Osumi M., Minamikawa-Tachino R., Mahadeo D. & Cotter D. A. 2001. The formation of actin rods composed of actin tubules in *Dictyostelium discoideum* spores. *J. Struct. Biol.* **136,** 7–19

Sasseville A.M-J. & Langelier Y. 1998. *In vitro* interaction of the carboxy-terminal domain of lamin A with actin. *FEBS Lett*. **425**, 485-489

Holaska J.M., Kowalski A.K. & Wilson K.L. 2004. Emerin caps the pointed end of actin filaments: evidence for an actin cortical network at the nuclear inner membrane. *PLoS Biol.* **2**, E231

Zhang Q., Ragnauth C., Greener M J., Shanahan C.M. & Roberts R.G. 2002. The nesprins are giant actin-binding proteins, orthologous to *Drosophila melanogaster* muscle protein MSP-300. *Genomics* **80**, 473-481

Zhen Y.Y., Libotte Y., Munck M., Noegel A.A. & Korenbaum E. 2002. NUANCE, a giant protein connecting the nucleus and actin cytoskeleton. *J. Cell Sci.* **115**, 3207-3222

Correas I. 1991. Characterization of isoforms of protein 4.1 present in the nucleus. *Biochem. J.* **279**, 581-585

Krauss S.W., Larabell C.A., Lockett S., Gascard P., Penman S., Mohandas N. & Chasis J.A. 1997. Structural protein 4.1 in the nucleus of human cells: dynamic rearrangements during cell division. *J. Cell Biol.* **137**, 275-289

Luque C.M. & Correas I. 2000. A constitutive region is responsible for nuclear targeting of 4.1R: modulation by alternative sequences results in differential intracellular localization. *J. Cell Sci.* **113**, 2485-2495

Holaska J.M., Kowalski A.K. & Wilson K.L. 2004. Emerin caps the pointed end of actin filaments: evidence for an actin cortical network at the nuclear inner membrane. *PLoS Biol.* **2**, E231

Krauss S.W., Chen C., Penman S. & Heald R. 2003. Nuclear actin and protein 4.1: essential interactions during nuclear assembly *in vitro*. *Proc. Natl Acad. Sci. USA* **100**, 10752–10757

Castano E., Philimonenko V.V., Kahle M., Fukalova J., Kalendova A., Yildirim S., Dzijak R., Dingova-Krasna H., Hozak P. 2010. Actin complexes in the cell nucleus: new stones in an old field. Histochem *Cell Biol.* **133,** 607-626

Harley C.B., Futcher A.B. & Greider C.W. 1990. Telomeres shorten during ageing of human fibroblasts. *Nature* **345,** 458-460

Martens U.M., Chavez E.A., Poon S.S., Schmoor C. & Lansdorp P.M. 2000. Accumulation of short telomeres in human fibroblasts prior to replicative senescence. *Exp. Cell Res*. **256,** 291-299

Blasco M.A. 2007. Telomere length, stem cells and aging. *Nat Chem Biol* **3,** 640-649

Hayflick L. 1965. The limited in vitro lifetime of human diploid cell strains. *Experimental Cell Research* **37**, 614–636

Blackburn E.H. 2001. Switching and signaling at the telomere. *Cell* **106**, 661-73

de Lange T. 2005. Shelterin: The protein complex that shapes and safeguards human telomeres. *Genes Dev.* **19,** 2100–2110

Shay J.W. & Wright W.E. 2004. Telomeres are double-strand DNA breaks hidden from DNA damage responses. *Mol. Cell* **14,** 420–421

d'Adda di Fagagna F., Teo S.H. & Jackson S.P. 2004. Functional links between telomeres and proteins of the DNA-damage response. *Genes Dev.* **18,** 1781–1799

Griffith J.D., Comeau L., Rosenfield S., Stansel R.M., Bianchi A., Moss H., de Lange T., Karlseder J., Broccoli D., Dai Y. & Hardy S. 1999. Mammalian telomeres end in a large duplex loop. *Cell* **97,** 503-514

van Steensel B., Smogorzewska A. & de Lange T. 1998. TRF2 Protects Human Telomeres from End-to-End Fusions. *Cell* **92,** 401-413

Wright J.H., Gottschling D.E. & Zakian V.A. 1992. Saccharomyces telomeres assume a non-nucleosomal chromatin structure. *Genes Dev.* **6,** 197–210

Li B., Oestreich S. & de Lange T. 2000. Identification of human Rap1: implications for telomere evolution. *Cell* **101,** 471–483

Hsu H.L., Gilley D., Galande S.A., Hande M.P., Allen B., Kim S.H., Li G.C., Campisi J., Kohwi-Shigematsu T., Chen D.J. 2000. Ku acts in a unique way at the mammalian telomere to prevent end joining. *Genes Dev* **14**, 2807-2812

Kim S.H., Kaminker P., Campisi J. 1999. TIN2, a new regulator of telomere length in human cells. *Nat Genet* **23**, 405-412

Ye J.Z., Donigian J.R., van Overbeek M., Loayza D., Luo Y., Krutchinsky A.N., Chait B.T., de Lange T. 2004. TIN2 binds TRF1 and TRF2 simultaneously and stabilizes the TRF2 complex on telomeres. *J. Biol. Chem.* **279,** 47264-47271

Houghtaling B.R. Cuttonaro L., Chang W. & Smith S. 2004. A dynamic molecular link between the telomere length regulator TRF1 and the chromosome end protector TRF2. *Curr. Biol*. **14,** 1621-1631

Greider C.W. & Blackburn E.H. 1985. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. *Cell* **43,** 405-413

Greider C.W. 1996. Telomere Length Regulation. *Annu. Rev. Biochem.* **65,** 337-365

Cooke H.J. & Smith B.A. 1986. Variability at the telomeres of the human X/Y pseudoautosomal region. *Cold Spring Harbor Symp. Quant. Biol.* **51,** 213-219

de Lange T., Shiue L., Myers R.M., Cox D.R., Naylor S.L., Killery A.M. & Varmus H.E. 1990. Structure and variability of human chromosome ends. *Mol. Cell. Biol.* **10,** 518-527

Harley C.B., Futcher A.B.& Greider C.W. 1990. Telomeres shorten during ageing of human fibroblasts. *Nature* **345,** 458-460

Hastie N.D., Dempster M., Dunlop M.G., Thompson A.M., Green D.K. & Allshire R.C. 1990. Telomere reduction in human colorectal carcinoma and with ageing. *Nature* **346,** 866-868

Counter C.M., Avilion A.A., LeFeuvre C.E., Stewart N.G., Greider C.W., Harley C.B. & Bacchetti S. 1992. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *EMBO* **11,** 1921-1929

Kim N.W., Piatyszek M.A., Prowse K.R., Harley C.B., West M.D., Ho P.L.C., Coviello G.M., Wright W.E., Weinrich S.L. & Shay J.W. 1994. Specific association of human telomerase activity with immortal ceils and cancer. *Science* **266,** 2011-2015

Counter C.M., Botelho F.M., Wang P., Harley C.B. & Bacchetti S. 1994a. Stabilization of short telomeres and telomerase activity accompany immortalization of Epstein-Barr virus-transformed human B lymphocytes. Z *Virol.* **68,** 3410-3414

Counter C.M., Hirte H.W., Bacchetti S. & Harley C. 1994b. Telomerase activity in human ovarian carcinoma. *Proc. Natl. Acad. Sci. USA*. **91,** 2900-2904

Blasco M.A., Rizen M., Greider C.W. & Hanahan D. 1996. Differential regulation of telomerase activity and telomerase RNA during multi-stage tumorigenesis. *Nature Genet.* **12,** 200-204

Broccoli D., Godley L.A., Donehower L.A., Varmus H.E. & de Lange T. 1996. Telomerase activation in mouse mammary tumors: lack of detectable telomere shortening and evidence for regulation of telomerase RNA with cell proliferation. *Mol. Cell. Biol.* **16,** 3765-3772

Bacchetti S. 1996. Telomere maintenance in tumour cells. *Cancer Surv.* **28,** 197-216

Svenson U. & Roos G. 2009.Telomere length as a biological marker in malignancy. *Biochim Biophys Acta*. **1792,** 317-323

Shay J.W. & Bacchetti S. 1997. A survey of telomerase activity in human cancer. *Eur. J. Cancer* **33**, 787–791

Bryan T.M., Englezou A., Gupta J., Bacchetti S., Reddel R.R. 1995. Telomere elongation in immortal human cells without detectable telomerase activity. *EMBO J.* **14**, 4240–4248

Bryan T.M., Englezou A, Dalla-Pozza L., Dunham M.A., Reddel R.R. 1997. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. *Nat. Med.* **3**, 1271–1274

Murnane J.P., Sabatier L., Marder B.A. & Morgan W.F. 1994. Telomere dynamics in an immortal human cell line. *EMBO J.* **13**, 4953–4962

Dunham M.A., Neumann A.A., Fasching C.L., Reddel R.R. 2000. Telomere maintenance by recombination in human cells. *Nat. Genet.* **26,** 447–450

Lundblad V. & Blackburn E.H. 1993. An alternative pathway for yeast telomere maintenance rescues *est1*$^{-}$ senescence. *Cell* **73**, 347–360

Muntoni A., Neumann A.A., Hills M. & Reddel R.R. 2009. Telomere elongation involves intra-molecular DNA replication in cells utilizing alternative lengthening of telomeres. *Hum Mol Genet.* **18,** 1017-1027

Bryan T.M., Englezou A., Gupta J., Bacchetti S., Reddel R.R. 1995. Telomere elongation in immortal human cells without detectable telomerase activity. *EMBO J* **14**, 4240–4248

Yeager T.R., Neumann A.A., Englezou A., Huschtscha L.I., Noble J.R., Reddel R.R. 1999. Telomerase-negative immortalized human cells contain a novel type of promyelocytic leukemia (PML) body. *Cancer Res.* **59,** 4175–4179

Tarsounas M., Munoz P., Claas A., Smiraldo P.G., Pittman D.L., Blasco M.A. & West S.C. 2004. Telomere maintenance requires the RAD51D recombination/repair protein. *Cell* **117,** 337–347

Yankiwski V., Marciniak R.A., Guarente L., Neff N.F. 2000. Nuclear structure in normal and Bloom syndrome cells. *Proc. Natl. Acad. Sci. USA* **97**, 5214–5219

Stavropoulos D.J., Bradshaw P.S., Li X., Pasic I., Truong K., Ikura M., Ungrin M., Meyn M.S. 2002. The Bloom syndrome helicase BLM interacts with TRF2 in ALT cells and promotes telomeric DNA synthesis. *Hum. Mol. Genet.* **11,** 3135–3144

Grobelny J.V., Godwin A.K., Broccoli D. 2000. ALT-associated PML bodies are present in viable cells and are Screen for ALT genes enriched in cells in the G2/M phase of the cell cycle. *J. Cell Sci.* **113**, 4577–4585

Wu G., Lee W.H., Chen P.L. 2000. NBS1 and TRF1 colocalize at promyelocytic leukemia bodies during late S/G2 phrases in immortalized telomerase-negative cells. Implication of NBS1 in alternative lengthening of telomeres. *J. Biol. Chem*. **275,** 30618–30622

Wu G., Jiang X., Lee W.H., Chen P.L. 2003. Assembly of functional ALT-associated promyelocytic leukemia bodies requires Nijmegen breakage syndrome 1. *Cancer Res.* **63,** 2589–2595

Molenaar C., Wiesmeijer K., Verwoerd N.P., Khazen S., Eils R., Tanke H.J. & R.W. Dirks 2003. Visualizing telomere dynamics in living mammalian cells using PNA probes. *EMBO J.* **22,** 6631–6641

Perrem K., Colgin L.M., Neumann A.A., Yeager T.R., Reddel R.R. 2001. Coexistence of alternative lengthening of telomeres and telomerase in hTERT-transfected GM847 cells. *Mol. Cell Biol.* **21,** 3862–3875

Jiang W.Q., Zhong Z.H., Henson J.D., Neumann A.A., Chang A.C., Reddel R.R. 2005. Suppression of alternative lengthening of telomeres by Sp100-mediated sequestration of MRE11/ RAD50/NBS1complex. *Mol. Cell Biol.* **25,** 2708–2721

Zeng S., Xiang T., Pandita T.K., Gonzalez-Suarez I., Gonzalo S., Harris C.C. & Yang Q. 2009. Telomere recombination requires the MUS81 endonuclease. *Nat Cell Biol.* **11**, 616-623

Jeyapalan J.N., Mendez-Bermudez A., Zaffaroni N., Dubrova Y.E., Royle N.J. 2008. Evidence for alternative lengthening of telomeres in liposarcomas in the absence of ALT-associated PML bodies. *Int. J. Cancer* **122,** 2414-2421

Henson J.D., Hannay J.A., McCarthy S.W., Royds J.A., Yeager T.R., Robinson R.A., Wharton S.B., Jellinek D.A., Arbuckle S.M., Yoo J., Robinson B.G., Learoyd D.L., Stalley P.D., Bonar S.F., Yu D., Pollock R.E., Reddel R.R. 2005. A robust assay for alternative lengthening of telomeres (ALT) in tumors demonstrates the significance of ALT in sarcomas and astrocytomas. *Clin. Cancer Res.* **11**, 217–225

Ulaner G.A., Hoffman A.R., Otero J., Huang H.Y., Zhao Z., Mazumdar M., Gorlick R., Meyers P., Healey J.H. & Ladanyi M. 2004. Divergent patterns of telomere maintenance mechanisms among human sarcomas: sharply contrasting prevalence of the alternative lengthening of telomeres mechanism in Ewing's sarcomas and osteosarcomas. *Genes Chromosomes Cancer* **41**, 155–162

Montgomery E., Argani P., Hicks J.L., DeMarzo A.M. & Meeker A.K. 2004. Telomere lengths of translocation-associated and nontranslocation-associated sarcomas differ dramatically. *Am. J. Pathol.* **164**, 1523–1529

Scheel C., Schaefer K.L., Jauch A., Keller M., Wai D., Brinkschmidt C., van Valen F., Boecker W., Dockhorn-Dworniczak B. & Poremba C. 2001. Alternative lengthening of telomeres is associated with chromosomal instability in osteosarcomas. *Oncogene* **20,** 3835–3844

Hakin-Smith V., Jellinek D.A., Levy D., Carroll T., Teo M., Timperley W.R., McKay M.J., Reddel R.R., Royds J.A. 2003. Alternative lengthening of telomeres and survival in patients with glioblastoma multiforme. *Lancet* **361,** 836–838

Brachner A., Sasgary S., Pirker C., Rodgarkia C., Mikula M., Mikulits W., Bergmeister H., Setinek U., Wieser M., Chin S.-F., Caldas C., Micksche M., Cerni C. & Berger W. 2006. Telomerase- and alternative telomere lengthening–independent telomere stabilization in a metastasis-derived human non–small cell lung cancer cell line: effect of ectopic hTERT. *Cancer Res*. **66,** 3584-3592

de Lange T. 2002. Protection of mammalian telomeres. *Oncogene* **21**, 532–540

Dong F. & Jiang J. 1998. Non-Rabl patterns of centromere and telomere distribution in the interphase nuclei of plant cells. *Chromosome Res*. **6,** 551–558

Gilson 1993; Gilson E., Laroche T. & Gasser. S.M. 1993. Telomeres and the functional architecture of the nucleus. *Trends Cell Biol.* **3,** 128–134

Gotta M., Laroche T., Formenton A., Maillet L., Shertan H. & Gasser S.M. 1996. Cytological evidence for the clustering of telomeres and their colocalization with Rap1, Sir3 and Sir4 proteins in wild-type S. cerevisiae. *J. Cell Biol.* (in press)

Gottschling D.E., Aparicio O.M., Billington B.L. & Zakian V.A. 1990. Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. *Cell* **63,** 751–762

Conrad M.N., Wright J.H., Wolf A.J. & Zakian V.A. 1990. RAP1 protein interacts with yeast telomeres in vivo: overproduction alters telomere structure and decreases chromosome stability. *Cell* **63,** 739–750

Wright J.H., Gottschling D.E. & Zakian V.A. 1992. Saccharomyces telomeres assume a non-nucleosomal chromatin structure. *Genes Dev.* **6,** 197–210

Ferguson B.M., Brewer B.J., Reynolds A.E. & Fangman W.L. 1991. A yeast origin of replication is activated late in S phase. *Cell* **65,** 507–515

de Bruin D., Kantrow S., Liberatore R. & Zakian V.A. 2000. Telomere folding is required for the stable maintenance of telomere position effects in yeast. *Mol. Cell. Biol.* **20**, 7991–8000

Csink A.K. & Henikoff S. 1996. Genetic modification of heterochromatic association and nuclear organization in Drosophila. *Nature* **381**, 529–531

Dernburg A.F., Broman K.W., Fung J.C., Marshall W.F., Philips J., Agard D.A., Sedat J.W. 1996. Perturbation of nuclear architecture by long-distance chromosome interactions. *Cell* **85**, 745–759

Henderson S., Allsopp R., Spector D.L., Wang S.S. & Harley C. 1996. *In situ* analysis of changes in telomere size during replicative aging and cell transformation. *J. Cell Biol.* **134,** 1–12

Bilaud T., Brun C., Ancelin K., Koering C.E., Laroche T. & Gilson E. 1997. Telomeric localization of TRF2, a novel human telobox protein. *Nat. Genet.* **17,** 236–239

Broccoli D., Smogorzewska A., Chong L. & de Lange T. 1997. Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2. *Nat. Genet.* **17,** 231–235

van Steensel B., Smogorzewska A. & de Lange T. 1998. TRF2 protects human telomeres from end-to-end fusions. *Cell* **92,** 401–413

Ludérus M.E., van Steensel B., Chong L., Sibon O.C.M., Cremers F.F.M. & de Lange T. 1996. Structure, Subnuclear Distribution, and Nuclear Matrix Association of the Mammalian Telomeric Complex.. *J. Cell Biol.* **135,** 867-881

Kaminker P.G., Kim S.-H., Desprez P.-Y. & Campisi J. 2009. A novel form of the telomere-associated protein TIN2 localizes to the nuclear matrix. *Cell Cycle* **8,** 931-939

Seksek O., Biwersi J., Verkman A. S. 1997. Translational Diffusion of Macromolecule-sized Solutes in Cytoplasm and Nucleus *J. Cell Biol.* **138**, 131-142

Fushimi K. and Verkman A. S. 1991. Low viscosity in the aqueous domain of cell cytoplasm measured by picosecond polarization microfluorimetry. *J. Cell Biol.* **112**, 719-725

Rademakers S., Nigg A. L., Hoogstraten D., Hoeijmakers J. H. J., Vermeulen W., Houtsmuller A.B. 1999. Action of DNA Repair Endonuclease ERCC1/XPF in Living Cells *Science* **284**, 958-961

Phair R. D. & Misteli T. 2000**.** High mobility of proteins in the mammalian cell nucleus. *Nature* **404**, 604-609

Huang S., Deerinck T. J., Ellisman M. H., Spector D. L. 1998. The Perinucleolar Compartment and Transcription. *J. Cell Biol.* **143**, 35-47

Snaar S., Wiesmeijer K., Jochemsen A. G., Tanke H. J., Dirks R. W. 2000. Mutational Analysis of Fibrillarin and Its Mobility in Living Human Cells. *J. Cell Biol.* **151**, 653-662

Pederson T. 2000. Diffusional protein transport within the nucleus: a message in the medium. *Nature Cell Biol.* **2**, E73-E74

Shopland L. S. & Lawrence J. B. 2000. Seeking Common Ground in Nuclear Complexity. *J. Cell Biol.* **150**, F1-F4

Misteli T. Protein dynamics: Implications for nuclear architecture and gene expression. 2001. Science **291**, 843-847

Marshall W.F., Straight A., Marko J.F., Swedlow J., Dernburg A., Belmont A., Murray A.W., Agard D.A., Sedat J.W**.** 1997. Interphase chromosomes undergo constrained diffusional motion in living cells. *Curr. Biol.* **7**, 930–939

Heun P., Laroche T., Shimada K., Furrer P. & Gasser S.M. 2001a. Chromosome dynamics in the yeast interphase nucleus. *Science* **294**, 2181–2186

Bystricky K., Heun P., Gehlen L., Langowski J. & Gasser S.M. 2004. Long-range compaction and flexibility of interphase chromatin in budding yeast analyzed by high-resolution imaging techniques. *Proc. Natl Acad. Sci. USA* **101,** 16495–16500

Heun P., Laroche T., Raghuraman M.K. & Gasser S.M. 2001b. The positioning and dynamics of origins of replication in the budding yeast nucleus. *J. Cell Biol.* **152**, 385–400

Jin Q., Trelles-Sticken E., Scherthan H. & Loidl J. 1998. Yeast nuclei display prominent centromere clustering that is reduced in nondividing cells and in meiotic prophase. *J. Cell Biol.* **141**, 21–29

Gartenberg M.R., Neumann F.R., Laroche T., Blaszczyk M., Gasser S.M. 2004. Sir-mediated repression can occur independently of chromosomal and subnuclear contexts. *Cell.* **119**, 955–967

Sage D., Neumann F.R., Hediger F., Gasser S.M. & Unser M. 2005. Automatic tracking of individual fluorescence particles: application to the study of chromosome dynamics. *IEEE Trans. Image Process.* **14**, 1372–1383

Hediger F., Neumann F.R., Van Houwe G., Dubrana K. & Gasser S.M. 2002. Live imaging of telomeres: yKu and Sir proteins define redundant telomere-anchoring pathways in yeast. *Curr. Biol.* **12**, 2076–2089

Rosa A., Maddocks J.H., Neumann F.R., Gasser S.M. & Stasiak A. 2006. Measuring limits of telomere movement on nuclear envelope. *Biophys. J.* **90**, L24–L26

Taddei A., Van Houwe G., Hediger F., Kalck V., Cubizolles F., Schober H., Gasser S.M. 2006. Nuclear pore association confers optimal expression levels for an inducible yeast gene. *Nature* **441**, 774–778

Cabal G.G., Genovesio A., Rodriguez-Navarro S., Zimmer C., Gadal O., Lesne A., Buc H., Feuerbach-Fournier F., Olivo-Marin J.C., Hurt E.C., Nehrbass U. 2006. SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope. *Nature* **441**, 770–773

Coué M., Brenner S.L., Spector I. & Korn E.D. 1987. Inhibition of actin polymerization by latrunculin A. *FEBS Letters* **213,** 316-318