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Structure, function and dynamics of nuclear subcompartments

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The nucleus contains a plethora of different dynamic structures involved in the regulation and catalysis of nucleic acid metabolism and function. Over the past decades countless factors, molecular structures, interactions and posttranslational modifications have been described in this context. On the one side of the size scale X-ray crystallography delivers static snapshots of biomolecules at atomic resolution and on the other side light microscopy allows insights into complex structures of living cells and tissues in real time but poor resolution. Recent advances in light and electron microscopy are starting to close the temporal and spatial resolution gap from the atomic up to the cellular level. Old challenges and new insights are illustrated with examples of DNA replication and nuclear protein dynamics.

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Nuclear structures and protein dynamics

The by far largest molecules in the nucleus are the chromosomes that occupy discrete territories and are anchored at the nuclear envelope [1••,2]. The fact that most nuclear processes occur or at least start at chromosomes makes them the chief organizing factor in the nucleus. Proteins involved in DNA replication, transcription and RNA processing were found enriched in focal structures [3]. The variety of nuclear structures illustrated in Figure 1 raises the question of how these distinct structures are assembled and maintained in the absence of subdividing membranes.

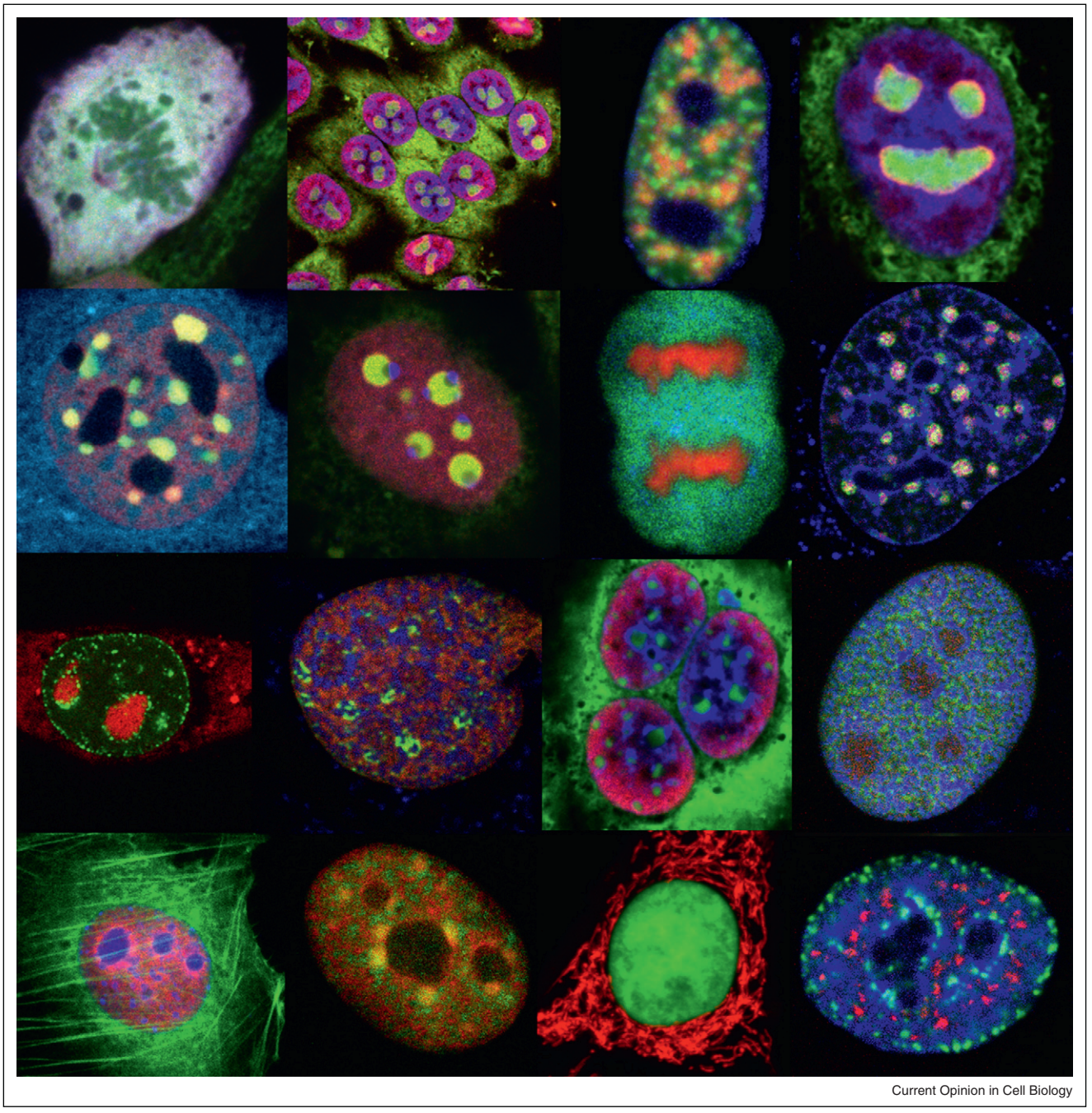
Fluorescence photobleaching experiments demonstrated a surprisingly high mobility of proteins in the nucleus [4••] suggesting that the occurrence of discrete nuclear structures is the net result of association, dissociation and diffusion events. These experiments also indicated that distinct structures could be generated by stable binding as well as rapid exchange of its components [5]. The combination of fluorescence photobleaching experiments with kinetic modeling can in principle provide quantitative data on intracellular binding properties [6]. The occurrence of multiple and variable interactions with unknown numbers of binding sites, however, make it difficult to mathematically dissect out individual mobility classes. Thus, despite the present sophistication of the kinetic modeling [7] the inherent limitations of these ensemble measurements call for complementation by single molecule tracings as discussed below.

Temporal and spatial dimensions of nuclear functions

One of the fundamental nuclear functions and the most prominent and critical event during the cell cycle is the precise duplication of the entire (epi)genetic information. The challenges of DNA replication are easily summarized. Roughly speaking, human cells need to replicate about 6 billion (6×10^9) base pairs in half a day, starting at thousands of sites on 46 chromosomes, precisely copying each and every base pair once and only once. The discovery and biochemical characterization of the DNA double helix and DNA polymerases outlined the basic mechanism but could not explain the efficiency, precision and coordination of cellular DNA replication [8]. Since then an amazing complexity unfolded as countless new factors were identified that contribute to the efficiency and precision of DNA replication.

It is clear that DNA replication *in vivo* is more than just the sum of all participating factors and especially the overall coordination and precision is far from being reproducible *in vitro*. Already early fractionation experiments indicated that newly synthesized DNA and most of the replication activity was associated with higher order structures [9,10]. At the cellular level DNA replication can be visualized by incorporation of modified nucleotides [11], immunofluorescence staining of replication factors [12], expression of fluorescent fusion proteins in living cells [13] and appears highly organized in focal structures (Figure 2). The subnuclear distribution of these foci changes throughout the S-phase [13] in a pattern that roughly corresponds to the underlying chromatin states [14–18] and changes during differentiation [19,20]. Recent studies have addressed how DNA replication is

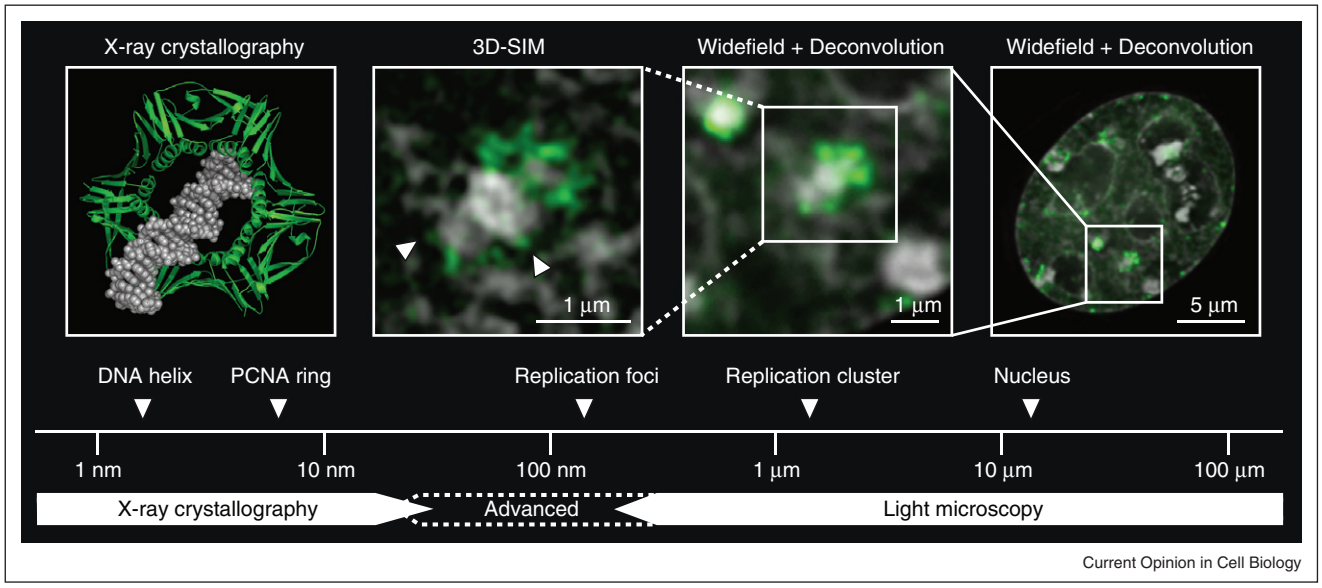
Figure 1



The variety of nuclear structures and subcompartments. This colorful assembly shows a random collection of nuclear structures observed and studied in our lab over the past years. All these distinct structures have in common that they are formed by dynamic assembly and disassembly processes in the absence of organizing membranes.

activated within these foci and how the cell can cope with replicative stress [21,22]. The sizes and numbers of these foci, as obtained from light microscopy analysis, could not be directly linked with numbers of replication units measured *in vitro* in stretched DNA fibers. This and other lines of evidence lead to the hypothesis that the replication foci detected by light microscopy correspond to spatially clustered DNA replication units [23•,24]. Recent studies employing super resolution light microscopy methods, namely STED [25], 3D-SIM and SMI microscopy [26], as well as electron microscopy [27] provided first evidence to the existence of 4000–5000

Figure 2



DNA replication from atomic to cellular scale. This scheme illustrates the orders of magnitude from the level of the well-known crystal structures of key components of DNA replication to the cellular level. The DNA double helix (gray) has a diameter of about two nanometers and the entire diploid genome a combined length of about 2 m, all condensed into a nucleus with a diameter of 5–20 μm. The homotrimeric PCNA ring (green, PDB ID: 1AXC), the central loading platform for DNA replication factors, has a diameter of about 8 nm while the microscopically discernable, cellular replication foci and clusters are in the range from 100 to 2000 nm.

replication units of 125 nm average size at any given time throughout S-phase (Figure 2). These types of studies provide the basis for building and testing qualitative and quantitative models of genome replication.

By way of explanation it is easy to implicate the higher order structures present in living cells but it is hard if not impossible to prove a functional role with the usual methods. In this sense, mutational analysis of nuclear proteins allowed mapping functional domains mediating interactions with other factors involved in particular nuclear functions. In the case of DNA replication, local enrichment of factors involved not only in the DNA synthesis process *per se* but also in cell cycle regulation [28], DNA methylation [29], chromatin assembly [30] and repair of DNA damage [31] was found. Several of these factors were shown to accumulate at replication sites during S-phase via a short peptide sequence mediating binding to the DNA polymerase clamp PCNA [32] and independent from the catalytic domain of the respective enzymes. As PCNA forms homotrimers wrapping around the DNA (Figure 2) with maximally three binding sites for interacting factors sharing the same binding interface, the question arises as to how all these interactions can take place. Furthermore, it was unclear whether such targeting sequences leading to local enrichment of enzymes are required *in vivo* for the function of the enzymes in the particular nuclear process.

These questions were addressed by fluorescence photobleaching/activation experiments in living cells. Several enzymes including DNA ligase I, the flap endonuclease Fen1 and Dnmt1 as well as mutants thereof were photobleached simultaneously with PCNA and their relative kinetics of recovery measured. Whereas PCNA did not exchange over periods of several minutes, the enzymes interacting with it were only transiently associated with replication sites and their entire pools exchanged over periods of a few seconds [33,34,35•]. This analysis suggests that the processivity of DNA replication and the coordination of the different enzymatic activities rely on a stable core component loaded on DNA and transiently interacting factors ensuring the temporal availability of the shared binding site in the stable PCNA ring. This type of kinetics involving the interplay of transient and stable components was also observed in other nuclear processes such as DNA repair [36]. Interestingly, mutating the PCNA interacting domain at least in the DNA methyltransferase Dnmt1 did not prevent DNA methylation but lowered its efficiency [33]. Thus, local enrichment of factors via protein–protein interactions increases the efficiency of enzymatic reactions *in vivo* but does not seem to be an absolute requirement for nuclear function.

Last but not least, also the time scale poses some challenges as the diffusion rate of the participating proteins, the kinetics of their interactions and the incorporation of

single nucleotides are much faster than the imaging rate of regular light microscopy. As discussed below, single molecule tracing offers exciting new insights into the millisecond range but still misses out on contextual information.

Dynamics and interactions of single molecules in the nucleus

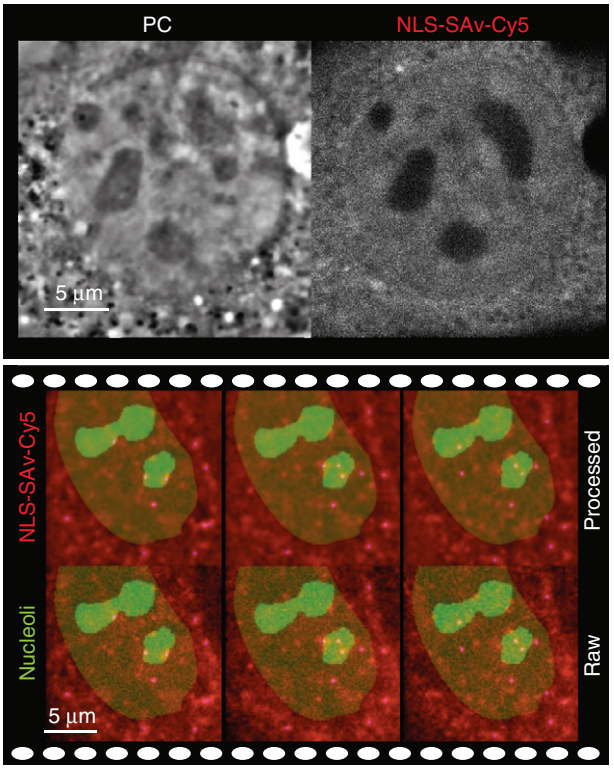
The visualization of cellular components by immunofluorescence staining with antibodies, by *in situ* hybridization for detection of specific nuclei acids and with fluorescently tagged proteins have uncovered a variety of subnuclear compartments [3] as discussed above (Figure 1).

Throughout the last decade systematic analysis of protein dynamics mostly from fluorescence photobleaching/activation experiments [37] has changed our view of static nuclear compartments and revealed new principles of macromolecular complexes [38]. These types of analysis though do not reveal the behavior of individual molecules but rather describe the average behavior of molecular ensembles. In biology important and meaningful information is often lost in the averaging over large numbers of molecules. As an example, the apparent exclusion of multiple nuclear proteins from the nucleolus observed in numerous fixed and living cell studies could be interpreted as lack of accessibility of this major nuclear compartment [39] for these proteins. *In vitro* measurements with nucleoli isolated from Xenopus oocyte nuclei though revealed a low-density structure permeable to molecules such as dextrans [40•]. This apparent contradiction prompted studies of the dynamic behavior of single molecules in living cells.

The tracing of single molecules in cells [41] has been limited by the low signal-to-noise ratio and the small number of photons emitted by the available fluorophores. On the one hand, total internal reflection (TIRF) microscopy [42] gives the best signal-to-noise ratio but is only applicable to structures such as the plasma membrane within about 100 nm distance from the surface. On the other hand, methods based on fluorescence fluctuation such as fluorescence correlation microscopy [43] measure the mobility of single molecules with very high temporal resolution (in the microsecond range) but are restricted to mobile species and to a femtoliter volume within the cell. Direct tracing of single molecules inside cells albeit difficult has been made possible by the development of improved, highly sensitive cameras. This allowed probing to what extent nuclear structures limit the mobility and access of individual proteins by tracing the mobility of fluorescently labeled inert proteins (streptavidin and ovalbumin; [44•,45]). Whereas steady state average distribution (Figure 3, top panel) suggests exclusion of streptavidin protein from the nucleolus, high-speed single molecule tracing microscopy (Figure 3, bottom panel and Movie) clearly indicate that single average size proteins have unrestricted access to the nucleolus and all nuclear subcompartments. Nonetheless, in-depth analysis of the nuclear mobility of proteins uncovered transient trapping on the order of tens of milliseconds. This trapping was mostly pronounced at heterochromatin and least pronounced within the nucleolus. General trapping of factors in specific compartments modulates their local concentration and may thus directly impact on enzymatic reaction velocity and enhance their specificity [46].

Several recent studies use single particle tracking to study the intranuclear dynamics as well as nuclear-cytoplasm

Figure 3



Distribution and dynamics of nuclear proteins. Confocal fluorescence microscopy analysis (top panel) suggests that the probe protein streptavidin (NLS-SAv-Cy5) is, like many other nuclear proteins, excluded from nucleoli which, due to their higher density, appear as dark structures in the phase contrast (PC) image. Single molecule tracing (bottom panel) provide an alternative explanation for this bulk steady-state distribution. Snapshots of a high speed (191 Hz) time lapse microscopy (full sequence in supplementary Movie) of single NLS-SAv-Cy5 protein (red) molecules in and out of nucle(ol)ar (green) subcompartments are shown. The corresponding movie shows streptavidin molecules (NLS-SAv-Cy5) after microinjection into the cytoplasm of C2C12 mouse myoblasts. Raw single molecule data are displayed at the bottom and the same data processed using a 2–20 pixel band pass filter. One pixel corresponds to 96 nm and the whole object field shown is 12.2 μm × 12.2 μm. Further details are described in [44].

transport of, for example, RNP particles [47–49]. The larger size and slower mobility of these particles combined with multiple labels per particle facilitate imaging with good signal-to-noise ratio. Recent improvements in sample illumination strategies [50], increase the signal-to-noise ratio and facilitate direct tracing of single molecules, their interactions and enzymatic reactions in living cells with high spatial and temporal resolution in the range of tens of nanometers and milliseconds.

Outlook

Currently cells are being studied at different size and time scales. The recent development of super resolution light microscopy methods [51] enables the study of cellular structures at ever-increasing resolution far below the classic Abbe diffraction limit approaching the range of electron microscopy and X-ray crystallography (Figure 2). The integration of structural information obtained with different methods at different size scales has been impressively demonstrated by fitting crystal structures into cryo-electron microscopy density maps to assemble a functional architecture of the complete multi-subunit RNA polymerase I [52]. The rapidly increasing capabilities of cryo-electron microscopy to resolve bigger complexes at higher resolution facilitate the combination with advanced light microscopy. Likewise, dramatic advances in electron microscopic tomography recently enabled the direct study of polyribosomes in human cells [53].

Correlative light and electron microscopy in combination with electron microscopic tomography and super resolution light microscopy have recently been used to elucidate intermediate steps of abscission in dividing human cells [54]. In addition to increased spatial resolution, the single molecule light microscopy techniques discussed above expand the time resolution down to the microsecond scale.

Besides the rapid development of advanced electron and light microscopy technologies new reagents like chromobodies that are ten times smaller than conventional antibodies and detectable in living cells [54,55] as well as new genetically encoded tags and contrasting approaches [56••] will further facilitate correlative microscopy and thus the acquisition and integration of structural data over several orders of magnitude.

Last but not least, as imaging data are produced at an ever-increasing rate and volume around the world, at different size and time scales, with different methods and instruments, standardization becomes an essential and urgent prerequisite to compare, integrate, model and thus utilize this accumulating wealth of data [57].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ceb.2011.12.009.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Bolzer A, Kreth G, Solovei I, Koehler D, Saracoglu K, Fauth C, Muller S, Eils R, Cremer C, Speicher MR *et al.*: **Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes**. *PLoS Biol* 2005, **3**:e157.
A major tour de force study visualizing all 46 chromosomes in a human fibroblast and in addition the authors measure and discuss their relative distribution in the nucleus.

2. Cremer T, Cremer C: **Chromosome territories, nuclear architecture and gene regulation in mammalian cells**. *Nat Rev Genet* 2001, **2**:292-301.

3. Spector DL: **Nuclear domains**. *J Cell Sci* 2001, **114**:2891-2893.

4. •• Phair RD, Misteli T: **High mobility of proteins in the mammalian cell nucleus**. *Nature* 2000, **404**:604-609.
The first fluorescence photobleaching recovery measurements demonstrating the surprisingly high mobility of proteins in the nucleus of living mammalian cells.

5. Lamond AI, Spector DL: **Nuclear speckles: a model for nuclear organelles**. *Nat Rev Mol Cell Biol* 2003, **4**:605-612.

6. Phair RD, Scaffidi P, Elbi C, Vecerova J, Dey A, Ozato K, Brown DT, Hager G, Bustin M, Misteli T: **Global nature of dynamic protein–chromatin interactions in vivo: three-dimensional genome scanning and dynamic interaction networks of chromatin proteins**. *Mol Cell Biol* 2004, **24**:6393-6402.

7. Mueller F, Mazza D, Stasevich TJ, McNally JG: **FRAP and kinetic modeling in the analysis of nuclear protein dynamics: what do we really know**. *Curr Opin Cell Biol* 2010, **22**:403-411.

8. Chagin VO, Stear JH, Cardoso MC: **Organization of DNA replication**. In *The Nucleus*. Edited by Misteli T, Spector DL. Cold Spring Harbor Laboratory; 2010:a000737. Cold Spring Harb Perspect Biol, vol 2.

9. Berezney R, Coffey DS: **Nuclear protein matrix: association with newly synthesized DNA**. *Science* 1975, **189**:291-293.

10. Leonhardt H, Cardoso MC: **Targeting and association of proteins with functional domains in the nucleus: the insoluble solution**. *Int Rev Cytol* 1995, **162B**:303-335.

11. Nakamura H, Morita T, Sato C: **Structural organizations of replicon domains during DNA synthetic phase in the mammalian nucleus**. *Exp Cell Res* 1986, **165**:291-297.

12. Bravo R, Macdonald-Bravo H: **Existence of two populations of cyclin/proliferating cell nuclear antigen during the cell cycle: association with DNA replication sites**. *J Cell Biol* 1987, **105**:1549-1554.

13. Leonhardt H, Rahn HP, Weinzierl P, Sporbert A, Cremer T, Zink D, Cardoso MC: **Dynamics of DNA replication factories in living cells**. *J Cell Biol* 2000, **149**:271-280.

14. Casas-Delucchi CS, Brero A, Rahn HP, Solovei I, Wutz A, Cremer T, Leonhardt H, Cardoso MC: **Histone acetylation controls the inactive X chromosome replication dynamics**. *Nat Commun* 2011, **2**:222.

15. Casas-Delucchi CS, van Bemmel JG, Haase S, Herce HD, Nowak D, Meilinger D, Stear JH, Leonhardt H, Cardoso MC:

Histone hypoacetylation is required to maintain late replication timing of constitutive heterochromatin. *Nucleic Acids Res* 2011. doi:10.1093/nar/gkr1723.

16. Lima-de-Faria A, Jaworska H: **Late DNA synthesis in heterochromatin**. *Nature* 1968, **217**:138-142.

17. O'Keefe RT, Henderson SC, Spector DL: **Dynamic organization of DNA replication in mammalian cell nuclei: spatially and temporally defined replication of chromosome-specific alpha-satellite DNA sequences**. *J Cell Biol* 1992, **116**:1095-1110.

18. Wu R, Terry AV, Singh PB, Gilbert DM: **Differential subnuclear localization and replication timing of histone H3 lysine 9 methylation states**. *Mol Biol Cell* 2005, **16**:2872-2881.

19. Hiratani I, Leskovar A, Gilbert DM: **Differentiation-induced replication-timing changes are restricted to AT-rich/long interspersed nuclear element (LINE)-rich isochores**. *Proc Natl Acad Sci USA* 2004, **101**:16861-16866.

20. Hatton KS, Dhar V, Brown EH, Iqbal MA, Stuart S, Didamo VT, Schildkraut CL: **Replication program of active and inactive multigene families in mammalian cells**. *Mol Cell Biol* 1988, **8**:2149-2158.

21. Ge XQ, Blow JJ: **The licensing checkpoint opens up**. *Cell Cycle* 2009, **8**:2320-2322.

22. Ge XQ, Blow JJ: **Chk1 inhibits replication factory activation but allows dormant origin firing in existing factories**. *J Cell Biol* 2010, **191**:1285-1297.

23. • Jackson DA, Pombo A: **Replicon clusters are stable units of chromosome structure: evidence that nuclear organization contributes to the efficient activation and propagation of S phase in human cells**. *J Cell Biol* 1998, **140**:1285-1295.
A thorough quantitative analysis of replication sites *in situ* in mammalian cells and the relationship to replication forks and their progression in stretched DNA fibers.

24. Sadoni N, Cardoso MC, Stelzer EH, Leonhardt H, Zink D: **Stable chromosomal units determine the spatial and temporal organization of DNA replication**. *J Cell Sci* 2004, **117**:5353-5365.

25. Cseresnyes Z, Schwarz U, Green CM: **Analysis of replication factories in human cells by super-resolution light microscopy**. *BMC Cell Biol* 2009, **10**:88.

26. Baddeley D, Chagin VO, Schermelleh L, Martin S, Pombo A, Carlton PM, Gahl A, Domaing P, Birk U, Leonhardt H *et al.*: **Measurement of replication structures at the nanometer scale using super-resolution light microscopy**. *Nucleic Acids Res* 2010, **38**:e8.

27. Koberna K, Ligasova A, Malinsky J, Pliss A, Siegel AJ, Cvackova Z, Fidlerova H, Masata M, Fialova M, Raska I *et al.*: **Electron microscopy of DNA replication in 3-D: evidence for similar-sized replication foci throughout S-phase**. *J Cell Biochem* 2005, **94**:126-138.

28. Cardoso MC, Leonhardt H, Nadal-Ginard B: **Reversal of terminal differentiation and control of DNA replication: cyclin A and Cdk2 specifically localize at subnuclear sites of DNA replication**. *Cell* 1993, **74**:979-992.

29. Leonhardt H, Page AW, Weier HU, Bestor TH: **A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei**. *Cell* 1992, **71**:865-873.

30. Gerard A, Koundrioukoff S, Ramillon V, Sergere JC, Mailand N, Quivy JP, Almouzni G: **The replication kinase Cdc7-Dbf4 promotes the interaction of the p150 subunit of chromatin assembly factor 1 with proliferating cell nuclear antigen**. *EMBO Rep* 2006, **7**:817-823.

31. Otterlei M, Warbrick E, Nagelhus TA, Haug T, Slupphaug G, Akbari M, Aas PA, Steinsbekk K, Bakke O, Krokan HE: **Post-replicative base excision repair in replication foci**. *Embo J* 1999, **18**:3834-3844.

32. Warbrick E: **The puzzle of PCNA's many partners**. *Bioessays* 2000, **22**:997-1006.

33. Schermelleh L, Haemmer A, Spada F, Rosing N, Meilinger D, Rothbauer U, Cardoso MC, Leonhardt H: **Dynamics of Dnmt1 interaction with the replication machinery and its role in postreplicative maintenance of DNA methylation**. *Nucleic Acids Res* 2007, **35**:4301-4312.

34. Sporbert A, Domaing P, Leonhardt H, Cardoso MC: **PCNA acts as a stationary loading platform for transiently interacting Okazaki fragment maturation proteins**. *Nucleic Acids Res* 2005, **33**:3521-3528.

35. • Sporbert A, Gahl A, Ankerhold R, Leonhardt H, Cardoso MC: **DNA polymerase clamp shows little turnover at established replication sites but sequential de novo assembly at adjacent origin clusters**. *Mol Cell* 2002, **10**:1355-1365.
A combined fluorescence photobleaching and spatial recovery analysis of DNA replication demonstrating that replication foci do not move but rather disassemble and reassemble at the DNA to be replicated next.

36. Mortusewicz O, Leonhardt H, Cardoso MC: **Spatiotemporal dynamics of regulatory protein recruitment at DNA damage sites**. *J Cell Biochem* 2008, **104**:1562-1569.

37. Bancaud A, Huet S, Rabut G, Ellenberg J: **Fluorescence perturbation techniques to study mobility and molecular dynamics of proteins in live cells: FRAP, photoactivation, photoconversion, and FLIP**. *Cold Spring Harbor Protocols* 2010. 2010:pdb top90.

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

39. Pederson T: **The nucleolus**. In *The Nucleus,* edn 2010/11/26. Edited by Misteli T, Spector DL.Cold Spring Harbor Laboratory; 2010:a000638. Cold Spring Harb Perspect Biol, vol 3..

40. • Handwerger KE, Cordero JA, Gall JG: **Cajal bodies, nucleoli, and speckles in the Xenopus oocyte nucleus have a low-density, sponge-like structure**. *Mol Biol Cell* 2005, **16**:202-211.
A detailed biophysical analysis of the structural characteristics of nuclear 'organelles', their protein concentration and their permeability to macromolecules.

41. Siebrasse JP, Kubitscheck U: **Single molecule tracking for studying nucleocytoplasmic transport and intranuclear dynamics**. *Methods Mol Biol* 2009, **464**:343-361.

42. Reck-Peterson SL, Derr ND, Stuurman N: **Imaging single molecules using total internal reflection fluorescence microscopy (TIRFM)**. *Cold Spring Harbor Protocols* 2010. 2010:pdb top73.

43. Medina MA, Schwille P: **Fluorescence correlation spectroscopy for the detection and study of single molecules in biology**. *BioEssays* 2002, **24**:758-764.

44. • Grünwald D, Martin RM, Buschmann V, Bazett-Jones DP, Leonhardt H, Kubitscheck U, Cardoso MC: **Probing intranuclear environments at the single-molecule level**. *Biophys J* 2008, **94**:2847-2858.
Using an inert protein the accessibility of subnuclear compartments was measured in living mammalian cells with a very high temporal and spatial resolution.

45. Speil J, Kubitscheck U: **Single ovalbumin molecules exploring nucleoplasm and nucleoli of living cell nuclei**. *Biochem Biophys Acta* 2010, **1803**:396-404.

46. Leonhardt H, Rahn HP, Cardoso MC: **Functional links between nuclear structure, gene expression, DNA replication, and methylation**. *Crit Rev Eukaryot Gene Expr* 1999, **9**:345-351.

47. Grünwald D, Singer RH, Rout M: **Nuclear export dynamics of RNA–protein complexes**. *Nature* 2011, **475**:333-341.

48. Mor A, Suliman S, Ben-Yishay R, Yunger S, Brody Y, Shav-Tal Y: **Dynamics of single mRNP nucleocytoplasmic transport and export through the nuclear pore in living cells**. *Nat Cell Biol* 2010, **12**:543-552.

49. Siebrasse JP, Veith R, Dobay A, Leonhardt H, Daneholt B, Kubitscheck U: **Discontinuous movement of mRNP particles in nucleoplasmic regions devoid of chromatin**. *Proc Natl Acad Sci USA* 2008, **105**:20291-20296.

50. Ritter JG, Veith R, Veenendaal A, Siebrasse JP, Kubitscheck U: **Light sheet microscopy for single molecule tracking in living tissue**. *PloS One* 2010, **5**:e11639.

51. Schermelleh L, Heintzmann R, Leonhardt H: **A guide to super-resolution fluorescence microscopy**. *J Cell Biol* 2010, **190**:165-175.

52. Kuhn CD, Geiger SR, Baumli S, Gartmann M, Gerber J, Jennebach S, Mielke T, Tschochner H, Beckmann R, Cramer P: **Functional architecture of RNA polymerase I**. *Cell* 2007, **131**:1260-1272.

53. Brandt F, Carlson LA, Hartl FU, Baumeister W, Grunewald K: **The three-dimensional organization of polyribosomes in intact human cells**. *Mol Cell* 2010, **39**:560-569.

54. Guizetti J, Schermelleh L, Mantler J, Maar S, Poser I, Leonhardt H, Muller-Reichert T, Gerlich DW: **Cortical constriction during abscission involves helices of ESCRT-III-dependent filaments**. *Science* 2011, **331**:1616-1620.

55. Rothbauer U, Zolghadr K, Tillib S, Nowak D, Schermelleh L, Gahl A, Backmann N, Conrath K, Muyldermans S, Cardoso MC *et al.*: **Targeting and tracing antigens in live cells with fluorescent nanobodies**. *Nat Methods* 2006, **3**:887-889.

56. Shu X, Lev-Ram V, Deerinck TJ, Qi Y, Ramko EB,
•• Davidson MW, Jin Y, Ellisman MH, Tsien RY: **A genetically encoded tag for correlated light and electron microscopy of intact cells, tissues, and organisms**. *PLoS Biol* 2011, **9**:e1001041.

The authors report a new genetically encoded tag for *in situ* visualization of proteins in correlative light and electron microscopy.

57. Swedlow JR, Lewis SE, Goldberg IG: **Modelling data across labs, genomes, space and time**. *Nat Cell Biol* 2006, **8**:1190-1194.