Toward a High-Resolution View of Nuclear Dynamics

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REVIEW

Toward a High-Resolution View of Nuclear Dynamics

Laura Trinkle-Mulcahy* and Angus I. Lamond

The nucleus is the defining feature of eukaryotic cells. It is a highly dynamic, membrane-bound organelle that encloses chromatin and thereby partitions gene transcription from sites of protein translation in the cytoplasm. Major cellular events, including DNA replication, messenger RNA synthesis and processing, and ribosome subunit biogenesis, take place within the nucleus, resulting in a continuous flux of macromolecules into and out of the nucleus through dedicated nuclear pore complexes in the nuclear envelope. Here, we review the impact of new technologies, especially in areas of fluorescence microscopy and proteomics, which are providing major insights into dynamic processes affecting both structure and function within the nucleus.

All eukaryotic cells enclose their genome within a dedicated organelle termed the nucleus. Nuclear chromatin is the template for two of the cell's major metabolic activities, DNA replication and transcription, necessitating a huge flux of macromolecules into and out of the nucleus. For example, import of histones is required during S phase of the cell cycle to package the newly replicated DNA, and both ribosome subunits and mRNA transcripts must be regularly exported from the nucleus for ribosome assembly and protein translation in the cytoplasm. Nuclear functions can vary at different stages of interphase, while during mitosis, in most eukaryotes, the entire nucleus is disassembled (Fig. 1). This involves chromosome condensation, disassembly of the nuclear lamina and envelope, and complete or partial disassembly of subnuclear bodies such as nucleoli and Cajal bodies.

Despite the importance of nuclear functions and extensive research into many aspects of mechanisms affecting nuclear processes, our understanding of the structure and function of the nucleus is still surprisingly limited. In part, this has reflected technological limitations, which have made it difficult to resolve dynamic nuclear events at the cell biological level.

Methodological advances have provided new opportunities to investigate how complex nuclear mechanisms, which have largely been studied in vitro, take place within the context of a living cell. Live cell fluorescence imaging techniques, especially those involving the use of fluorescent protein (FP)-tagged reporters, now allow the localization, dynamic properties, and interactions of proteins and ribonuclear protein (RNP) complexes in the nucleus to be studied quantitatively. Concurrent with this, advances in the sensitivity and time resolution of global approaches, particularly mass spectrometry (MS)-based proteomics, now permit quantitative analyses of complex proteomes and their changes in response to cell growth and perturbations. Importantly, this includes the direct analysis of endogenous proteins and their complex posttranslation modification states.

Here, we highlight recent examples of the application of both imaging and proteomics approaches to uncover mechanisms involved in the dynamic organization of nuclear structure and function throughout the cell cycle. We focus on transcription, replication, and condensation of chromatin and on the regulated assembly and disassembly of nuclear structures during mitosis.

Emerging Technologies: Imaging and Proteomics

Green fluorescent protein (GFP) and its many variants have been major driving forces in enabling the visualization of nuclear structure and function in live cells. There is now an entire "toolbox" of fluorescent proteins with different chromatic and structural properties [reviewed in (*1*)]. Combinations of chromatic variants can be visualized in the same cell, and specific pairs permit protein-protein interactions to be measured in vivo by fluorescence resonance energy transfer (FRET). In fluorescence complementation, the direct interaction of two proteins, each fused to a nonfluorescent fragment of a fluorophore, is reflected by formation of the intact fluorescent complex (*2*). Other interesting variants are photoactivatable GFP (paGFP) and similar fluorophores (*3*). Both GFP and photoactivatable tags, when combined with either laser photobleaching or photoactivation, respectively, allow rates of protein movement within cells to be measured under a wide range of conditions.

A major advantage of fluorescent proteins is that events can be analyzed at the level of a single cell and in the same cell throughout the cell cycle. By fusing FP tags to DNA or RNA binding domains, the movement of specific DNA loci and RNP complexes can be visualized, facilitating the in vivo analysis of gene expression [reviewed in (*4*, *5*)]. A caveat with all FP-tagging techniques, however, is that the presence of the tag can alter the properties and function of the molecule being visualized. Therefore, it is important to control for the effect of the tag in the experiment design and, where possible, validate the results obtained using other methods. An interesting parallel approach developed recently involves "chromobodies," i.e., epitope-recognition fragments of antibody heavy chains fused to fluorophores that can be expressed in living cells and allow live-cell imaging of endogenous proteins (*6*).

Proteomics. An important goal in characterizing nuclear organization is to identify proteins concentrated on chromatin and in each of the different subnuclear domains, which can guide future functional experiments. The method of choice for such analyses is MS-based proteomics, although non–MS-based techniques have also proved useful.

In the case of the nucleus, proteomic analyses have been performed on purified nucleoli, enriched preparations of interchromatin granule clusters, nuclear envelope and pore complexes, and various purified chromatin and chromosome fractions (*7*–*10*). A major limitation, however, is that the resulting "static" proteome provides an averaged view, which does not take into account dynamic changes in the protein composition of an organelle or structure, either at different cell cycle stages or in response to specific stimuli. Furthermore, this first-generation proteome does not distinguish between proteins that are predominantly located within a specific structure, and other components, which are only present in low amounts and/or transiently under specific conditions.

Detailed information concerning the dynamic properties of nuclear structures can be obtained with quantitative techniques such as stable isotope labeling of amino acids in cell culture (SILAC) and isotope tagging for relative and absolute protein quantitation (iTRAQ) [reviewed in (*11*)]. These techniques are based on differential isotope labeling of proteins from cells under two or more different experimental conditions, which allows quantitation of the relative amounts of protein found in each condition. This provides for a "second generation" proteomics approach, which couples protein identification with a quantitative annotation of protein properties, including dynamics, turnover, and interaction partners. For example, isotope labeling in combination with MS has been used to determine the flux of proteins through nucleoli and to measure the turnover rates of many nucleolar proteins (*7*, *12*), while a combination of SILAC-based quantitative proteomics with immunoprecipitation revealed differences in binding partners for two different isotopes of the same nuclear protein phosphatase, PP1 (*13*). In addition, these methods permit dynamic assessment of changes in the

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posttranslational modification state of proteins under a wide range of conditions (*14*).

The sensitivity of proteomics has not yet reached the point where measurements can be performed at the single-cell level. However, it is already possible to combine the advantages of high-throughput proteomics in studying endogenous, untagged proteins in cell populations with the advantages of studying FP-tagged proteins at the single-cell level using fluorescence microscopy. These complementary techniques can be applied in parallel, providing a "dual strategy" with which to analyze the same biological process (Fig. 2). This allows a direct comparison of the behavior of FP-tagged and endogenous forms of the same protein in stable cell lines, which determines if the tagged protein is a valid reporter for the behavior of the endogenous form.

An alternative to proteomics is to adopt a function-based approach, where gene expression is knocked down using RNA interference (RNAi) libraries and cells screened for phenotypes affecting either a specific process or structure of interest. For example, large-scale RNAi screens have been performed to identify mammalian proteins involved in chromosome condensation and apoptosis (*15*). There is considerable scope in the future for applying a combination of RNAi and proteomics to identify proteins required for a range of nuclear activities.

Functional Studies on the Nucleus

Within the nucleus, DNA is highly packaged through its association with histone proteins to form nucleosomes, which in turn are further compacted into higher-order structures [reviewed in (*16*)]. This chromatin complex is important both for controlling the compaction of chromosomes and for regulating access to specific DNA sequences during gene transcription and DNA replication. Both global and localized changes in structure are driven, at least in part, by posttranslational modifications, including methylation and acetylation of specific histone residues. Modification of the DNA itself, such as base methylation of cytosine residues, is also important and is involved in the epigenetic marking of gene loci.

The condensation state of chromatin, which can vary regionally in chromosomes during interphase, changes dramatically at the onset of mitosis when all chromosomes become highly condensed to facilitate accurate segregation to daughter cells. There is still debate over what mechanisms are primarily responsible for the condensation of chromatin, because detailed results from in vitro studies have not correlated completely with in vivo results [reviewed in (*16*)]. For example, although topoisomerase II and the condensin complexes are absolutely required for mitotic chromosome condensation in vitro, pronounced condensation occurs in vivo even after knockdown or genetic knockout of these proteins, although segregation defects are observed. It has been suggested that their primary effect may be on chromosome stability or architecture, or that redundant mechanisms exist. The debate has also raised the important issue of how to measure chromatin condensation accurately. To improve upon subjective measurements based on visual inspection of microscopy images, new approaches have been developed, including measurement of changes in the volume and density of chromatin with fluorescent probes (*17*).

Dynamic studies of transcription and replication have been enabled by the development of a method for visualizing specific DNA sequences, involving a tandem array of lac operator binding sites integrated into the genome and detected by the binding of FP-tagged lac repressor fusion proteins (*18*). This system has been used successfully

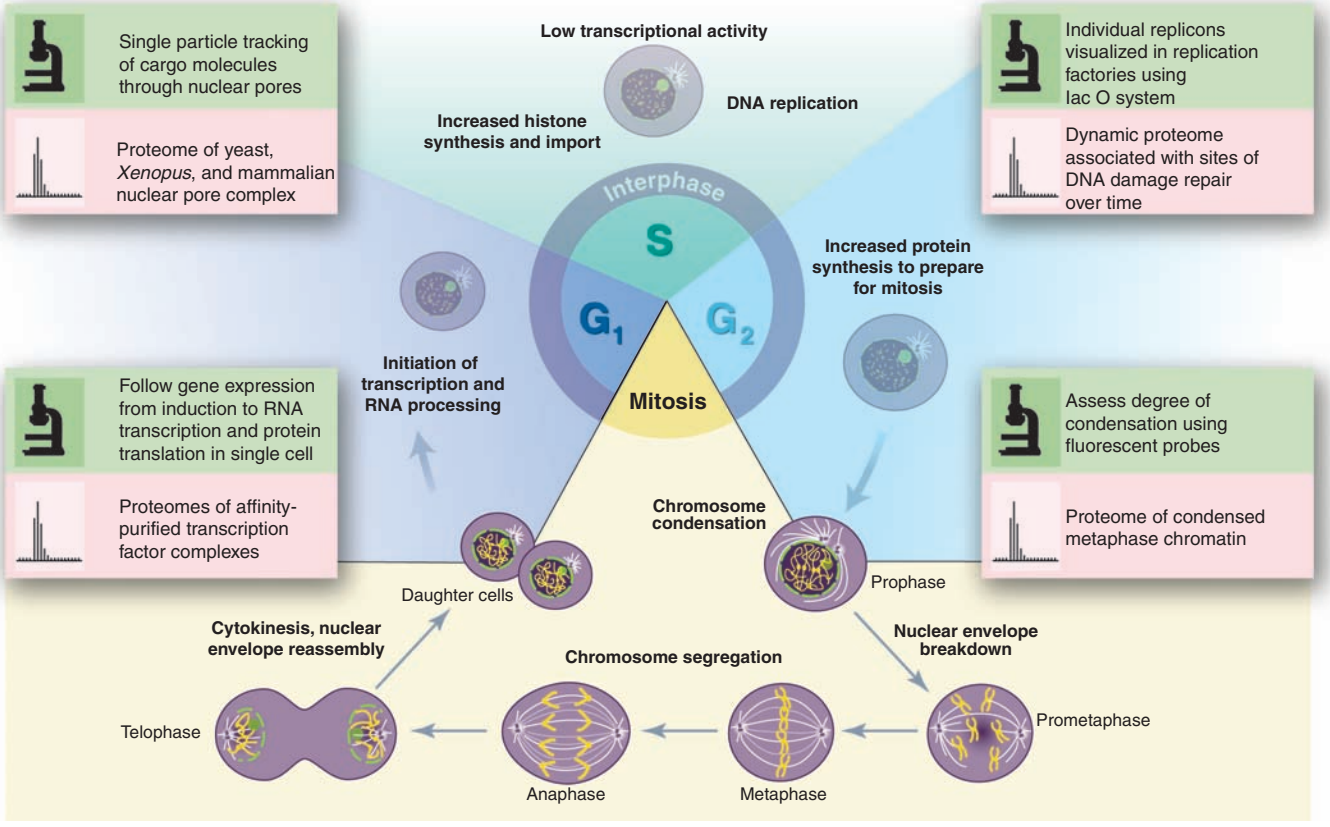

Fig. 1. Dynamic nuclear events. The cell cycle is divided broadly into interphase and mitosis, with cell growth and duplication of chromosomes occurring during interphase and segregation of chromosomes into two new daughter cells occurring during mitosis. Interphase is further divided into three distinct stages—G_1, S, and G_2—with specific functions carried out at each stage and advancement to the next stage dependent on proper progression and completion of the previous one. Recent uses of imaging (green) and proteomics (pink) techniques to study specific nuclear events are highlighted.

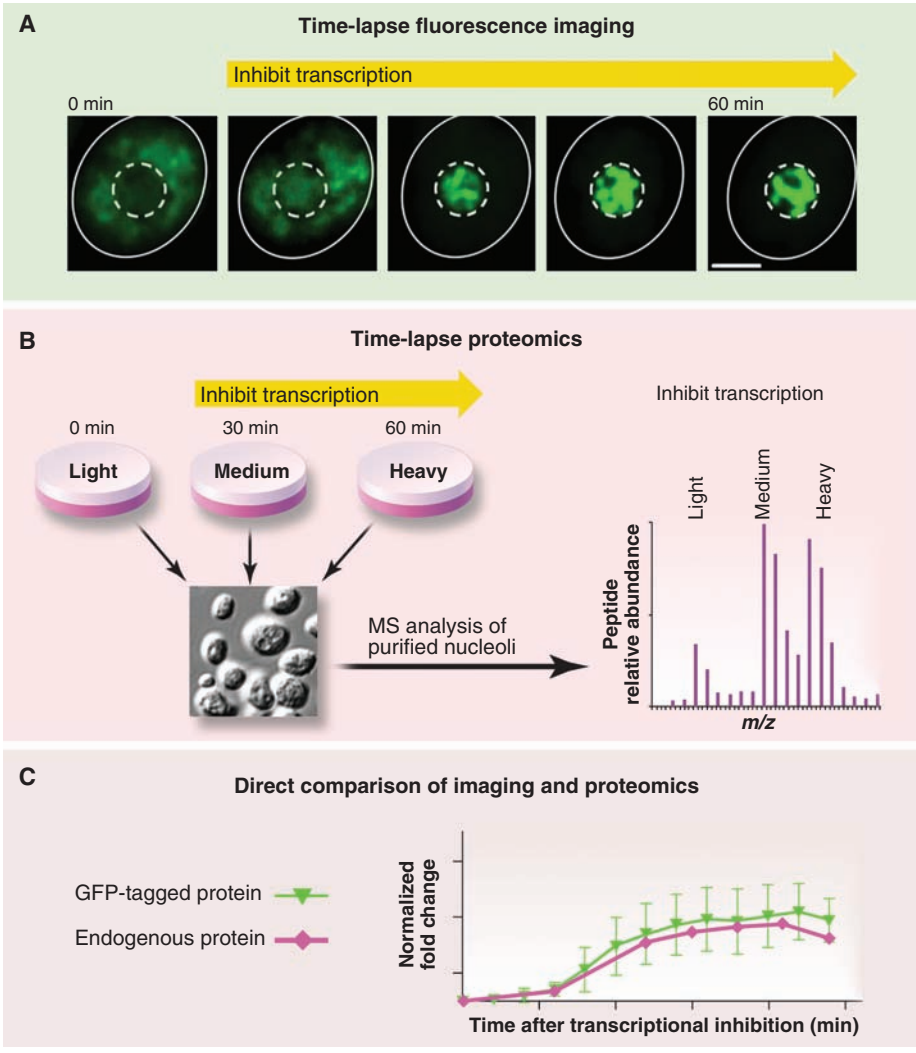

Fig. 2. Dual-strategy imaging and proteomics approach. Fluorescence imaging and MS-based proteomics are complementary methods, which can be used together in a "dual strategy." Time-lapse fluorescent imaging of an FP-tagged protein in a single cell nucleus (**A**) (ellipse) is used here to quantitate the fluorescence intensity within the nucleolus (hashed circle) over time, following inhibition of transcription. The results correlate with time-lapse proteomics data (**B**), in which the relative abundance of the endogenous protein within purified nucleoli was determined by metabolic labeling of cell populations at different time points following transcriptional inhibition. Cells were labeled either with light (^{12}C), medium (^{13}C; ^{4}D), or heavy (^{13}C/^{15}N) isotopes of arginine and lysine, respectively, which cause a measurable shift in the mass of peptides containing these amino acids. When combined for purification of nucleoli and MS analysis, the changing proteome of the nucleolus following transcriptional inhibition is determined by calculating isotopic arginine and lysine ratios for each protein identified. Thus, the increase in fluorescence intensity of the FP-tagged protein within the nucleolus upon inhibition of transcription matches the increase in relative abundance of the endogenous protein within purified nucleoli [plotted together in (**C**)].

in a range of organisms from *Escherichia coli* to eukaryotes and was adapted to analyze quantitatively the kinetics of gene transcription by RNA polymerase II (Pol II) in live mammalian cells (*19*) (Fig. 3A). Using a combination of photobleaching and photoactivation coupled with mathematical modeling, single-cell kinetic measurements were made of promoter binding, initiation, and elongation events. This shows that single-cell analysis of gene expression is already feasible. Further improvement in the sensitivity of detection of fluorescent signals should facilitate characterization of smaller arrays, or even single-copy genes, allowing more gene templates to be analyzed at the single-cell level.

The study of individual DNA replicons in *Saccharomyces cerevisiae* replication factories was also made possible with the lac operon system (*20*). By combining quantitative fluorescence imaging of lac operon– and tet operon–tagged gene loci with an independent label for replication sites, i.e., DNA polymerase, the increase in fluorescence that accompanies duplication of the inserted arrays could be monitored relative to the site of replication. Time-lapse imaging of these two separate foci supports the idea that sister replication forks generated from the same origin remain associated within a replication factory while the DNA moves through it. A related concept of a "transcription factory," in which RNA Pol II remains tethered while the transcribed genes move through, has also been proposed [reviewed in (*21*)].

Chromatin also shows dynamic, localized changes in response to different forms of DNA damage, as factors are recruited to identify and repair the resulting lesions. The dynamic association of repair proteins with chromatin following DNA damage events has been analyzed in vivo with time-lapse fluorescence imaging and photobleaching techniques (*22*). Using a combination of lac and tet operator arrays detected by the binding of their respective FP-tagged repressors, the movement of individual double-strand chromosome breaks was analyzed in live mammalian cells after DNA damage (*23*). The resulting broken ends do not move extensively within the nucleus, and this immobilization requires the DNA-end binding protein Ku80. In addition, ends resulting from double-strand breaks preferentially undergo translocations with other chromosomes in their local vicinity. The DNA repair process has also been analyzed by measuring the increased mobility of chromatin marked by paGFP-tagged histone H2B at sites undergoing DNA damage repair (*24*). The results were consistent with a local change in the condensation state of chromatin, facilitating access of repair factors to sites of damage.

A quantitative proteomics approach based on stable-isotope labeling and affinity purification was used to characterize protein complexes associating with chromatin containing a tagged form of the histone variant H2AX, which is phosphorylated at sites of double-strand DNA breaks (*25*). Through comparison of irradiated and nonirradiated cells, specific proteins were found to leave damaged regions of chromatin, while known repair factors transiently accumulated at H2AX sites, indicating that the H2AX chromatin complex undergoes dynamic changes after DNA damage and resulting DNA repair.

In most cases, the large-scale proteomic analysis of specific DNA-associated protein complexes has proven to be a technically difficult task, albeit an essential one for characterizing active genes and the mechanisms of their activation or repression. Chromatin immunoprecipitation techniques have helped to localize proteins to specific genes and can be combined with MS-based proteomics to identify both the DNA binding site(s) for a tagged protein and its associated protein binding partners [reviewed in (*26*)].

It has proven difficult to isolate transcription factories and to extract engaged RNA Pol II

complexes in native form. However, purification and MS analysis of tandem affinity protein–tagged RNA Pol II holoenzyme complexes identified a minimum stable complex of both known and novel proteins (*27*). The absence of certain expected proteins, such as elongation factors, likely highlights the technical difficulties in isolating intact protein complexes from relatively insoluble chromatin fractions. Recently, a method based on in vivo biotinylation tagging was used to identify transcription and chromatin remodeling factors associated with the essential hematopoietic transcription factor GATA-1 (*28*). A proteomics approach has also been used to identify the protein components of the Mediator complex, which links transcriptional activators with the general transcriptional machinery. It was shown that a common core of proteins is shared by alternate forms of the complex that differentially associate with components of the transcription machinery (*29*).

Major changes are expected in the composition of chromatin-associated proteins during mitosis, and thus mitosis-specific chromatin proteins may have roles in either maintaining or regulating the unique condensed state that occurs during chromosome segregation. Proteomics methods have been used to identify proteins associated with mitotic chromatin and to map protein interactions and posttranslational modifications in mitotic kinases, anaphase-promoting complex, centrosomes, and kinetochores [reviewed in (*10*)]. MS analysis of histone-depleted mitotic chromosomes identified several novel chromosome-associated proteins, including borealin, a component of the chromosomal passenger protein complex. Borealin helps target this essential regulatory complex, which includes aurora B kinase, INCENP, and survivin, to centromeres and the central spindle during mitosis (*30*).

Nucleoli and nuclear bodies. Many nuclear factors involved in events such as DNA replication, transcription, RNA processing, and ribosome subunit biogenesis are organized in spatially distinct nuclear domains. These domains include chromosome territories, interchromatin granule clusters, nucleoli, and assorted nuclear bodies. Although many of the mechanisms controlling the formation, organization, and movement of these domains remain unclear, live-cell experiments with FP-tagged fusion proteins suggest that the interaction of many factors with these domains is highly dynamic (*31*).

FP-tagging and time-lapse imaging strategies have identified protein-trafficking pathways within the nucleus. The use of heterokaryon formation to distinguish between newly assembled and mature components showed that two major classes of nuclear RNPs, i.e., small nucleolar RNAs (snoRNAs) and small nuclear RNAs (snRNAs) complexed with specific proteins, initially concentrate in nuclear domains distinct from their final sites of function. Maturation of both splicing snRNPs and nucleolar snoRNPs and movement to their respective sites of function in nucleoplasmic splicing and ribosomal RNA (rRNA) modification was shown to involve a transient localization in Cajal bodies [reviewed in (*32*)]. More recently, this has been extended to include a role for the nuclear export factor CRM1 in the directed nuclear movement of both snRNPs and snoRNPs (*33*, *34*).

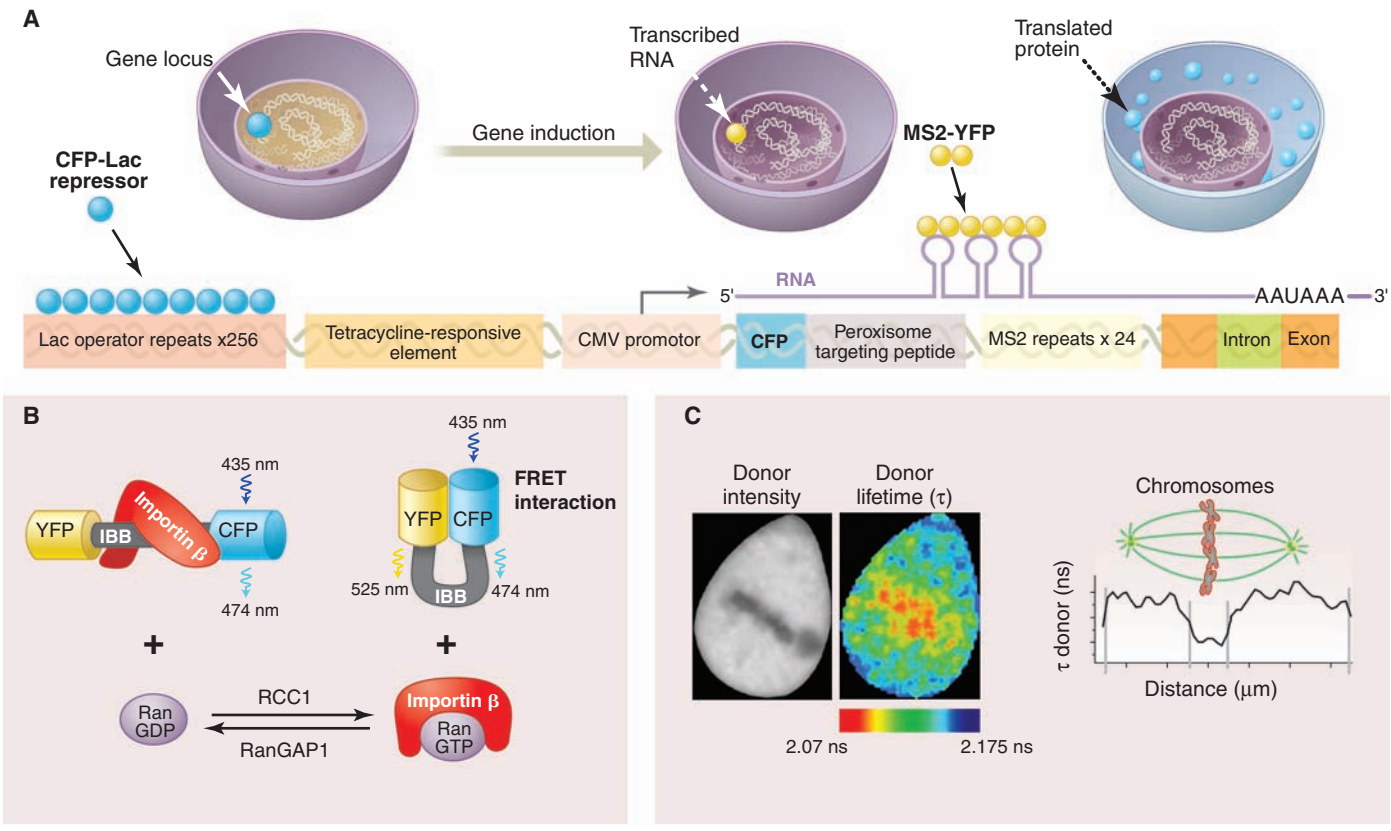

Fig. 3. Fluorescent probes for cellular mechanisms. (**A**) Plasmid used to visualize and quantitatively assess the kinetics of gene expression. The gene locus is marked by cotransfected CFP-lac repressor binding to multiple lac operon repeats, whereas cotransfected MS2-YFP binds transcribed RNA. The translated CFP fusion protein targets to cytoplasmic peroxisomes. (**B**) The biosensor Rango, used to monitor changes in RanGTP/GDP gradients in cells, consists of an importin β binding domain (IBB) flanked by CFP and YFP. Binding of importin β to Rango sterically hinders the FRET interaction between CFP and YFP. Where levels of RanGTP are high, release of importin β relieves this steric hindrance, and FRET can occur. This is observed as a decrease in the fluorescence lifetime of CFP. (**C**) A gradient of fluorescence lifetime is observed for the Rango probe in a metaphase mammalian cell, with shorter lifetimes (red) around chromosomes indicating FRET/free Rango and hence a high level of RanGTP, which is maintained by chromatin-bound RCC1.

Upon entry into mitosis, most nuclear bodies partially or fully disassemble, and nuclear functions such as transcription and pre-mRNA processing shut down. These processes, and reassembly of nuclear complexes and domains at the end of mitosis, have been studied by time-lapse imaging of FP-tagged proteins, including analysis of the sequential entry of components of the gene expression machinery, i.e., RNA Pol II subunits and pre-mRNA processing factors, into daughter nuclei after nuclear envelope (NE) formation (*35*). The initiation-competent form of RNA Pol II and general transcription factors are imported first, with pre-mRNA splicing factors recruited only after transcription is initiated. This suggests that a mechanism, most likely at the level of nuclear import, exists to coordinate transcription and pre-mRNA splicing during the transition from telophase to the establishment of the interphase nucleus.

The best-studied nuclear body is the nucleolus, which forms around the tandemly repeated clusters of ribosomal DNA and is the site of rRNA transcription, processing, and ribosome subunit assembly [reviewed in (*36*)]. Quantitative kinetic analysis of the breakdown and reassembly of the nucleolus with FP-tagged proteins demonstrated that disassembly starts before the onset of NE breakdown with the loss of RNA Pol I subunits from the fibrillar centers (*37*). These subunits, originally believed to remain associated with nucleolar organizing regions (NORs) throughout mitosis, were shown to transiently leave NORs during metaphase. Dissociation of proteins from the remaining nucleolar subcompartments occurred at a faster rate and was coincident with NE breakdown. Reassembly of nucleoli also followed a defined temporal sequence, although not necessarily in the same order, as the proteins were imported into newly formed nuclei. Time-lapse FRET analysis between FP-tagged nucleolar proteins has extended such live-cell imaging experiments to include information about protein-protein interactions during nucleolar reformation (*38*). Both early and late rRNA processing factors pass through the same prenucleolar bodies, and during translocation of these factors, interactions between protein partners from the same rRNA processing machinery can occur and may even induce formation of prenucleolar bodies.

Although proteomics studies have been carried out on various nuclear protein complexes and enriched fractions of interchromatin granule clusters (*8*), nucleoli have proven the most amenable of the nuclear bodies to biochemical purification and detailed proteomic analysis to define their protein composition in both mammalian and plant cells. Nucleoli have also been used to develop the "time-lapse proteomics" strategy, in which, using the SILAC isotope-labeling procedure, changes in the relative levels of nucleolar proteins were systematically evaluated by repeated proteomic analysis at multiple time points after treatment of cells with inhibitors affecting either transcription, proteasome activity, or specific kinases (*7*). Pulse labeling with heavy isotopes has also been used to characterize the different turnover rates of nucleolar proteins (*12*). These studies in the nucleolus have provided the first global characterization of the flux of proteins through a cellular organelle. It will clearly be important in the future to apply similar approaches to study other nuclear bodies and defined complexes.

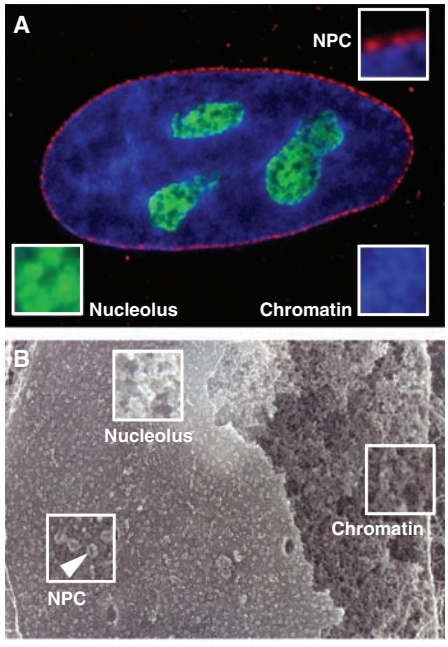

Fig. 4. Visualization of nuclear structures. (**A**) Conventional wide-field fluorescence micrograph of a paraformaldehyde-fixed HeLa cell stained with markers for the NPC (red), nucleolus (green), and chromatin (blue). A resolution limit of 200 nm is imposed by the wavelength of visible light. (**B**) Field-emission scanning electron micrograph of a dry fractured HeLa cell, which permits spatial resolution of structures within 3 to 5 nm. The fracture exposed NPCs (arrowhead), which are clearly visible on the surface of the nucleus, while a fracture across the surface exposes the chromatin within the nucleus. The paler, brighter circular area at the top center is a result of the increased backscatter electron signal from the dense nucleolar region.

Nuclear envelope and transport. The NE and associated nuclear pore complexes (NPCs) provide a barrier between the genome and the cytoplasm and play a major role in controlling entry and exit of the wide range of molecules that must continually move between these cellular compartments [reviewed in (*39*)]. In addition to regulating nucleocytoplasmic exchange, the NE has also been linked to control of both signaling and mitotic progression [reviewed in (*40*)].

Recent advances in imaging and single particle tracking have been applied to study cargo transport, permitting visualization of single molecules interacting with NPCs. Cargo molecules were shown to randomly diffuse within a NPC, exiting as a result of a single rate-limiting step (*41*). The small guanosine triphosphatase (GTPase) Ran plays a central role in the transport mechanism and is also required during mitosis when the NE is no longer present. The common feature is that Ran signals the location of chromatin within the cell through establishment of a gradient in the relative levels of RanGTP/GDP, with a higher RanGTP/GDP ratio occurring in the vicinity of chromatin as Ran interacts with the chromatin-bound GTP exchange factor RCC1. Away from chromatin, hydrolysis of GTP bound to Ran is stimulated by a Ran GTPase activating protein (RanGAP). This gradient defines the directionality of nucleocytoplasmic transport during interphase, is required for the correct formation of bipolar mitotic spindles during mitosis (*42*), and defines the sites where NE and nuclear re-formation will occur at the end of telophase. FRET probes have been used to visualize the RanGTP/GDP gradient in both cell-free systems and in live mammalian cells (*42*, *43*). As shown in Fig. 3, B and C, an importin β binding domain flanked by cyan (CFP) and yellow fluorescent protein (YFP) on opposite ends provides an elegant visual readout that indirectly reflects the relative level of RanGTP by monitoring the RanGTP-dependent cargo release from importin β at different sites within the cell. This convenient assay for monitoring changes in the Ran GTP/GDP gradient upon specific perturbations has been used to probe the contribution of Ran to transport and chromosome-segregation mechanisms (*42*).

In most eukaryotes, chromosome segregation during mitosis occurs in the absence of a defined nuclear structure. Thus, an open mitosis is characterized by the breakdown of the NE and associated structures before association of condensed chromosomes with the spindle and their subsequent segregation during anaphase. NE breakdown is a complex process involving disassembly of the underlying nuclear lamina, tearing apart of the membraneous NE, and disassembly of the NPCs (*44*). Advanced fluorescence and electron microscopy techniques have been applied to visualize NPC disassembly and membrane fenestration. In starfish embryos, NPCs were seen to expand and likely contribute to tearing of the membrane (*45*), while in *Xenopus* the NPCs were dismantled and the pore closed before membrane rupturing (*46*). These apparently conflicting results could be attributable either to differences between species or to technical limitations. Many details of the mechanisms involved in NPC assembly also remain to be characterized. NPCs were recently shown to form de novo from both sides of the NE in pre-assembled *Xenopus* nuclei (*47*), supporting the view that once assembled, an NPC remains stable throughout interphase, with

little or no exchange of nucleoporins (Nups) between separate NPCs.

Considerable work has also been aimed at identifying the detailed protein composition of NPCs in yeast, *Xenopus*, and mammals. Combinations of biochemical fractionation and subtractive proteomics have produced the current inventory of Nups, which includes ~30 proteins, a subset of which share phenylalanine-glycine (FG) repeat domains that bind transport factors and coordinate active transport through the NPC [reviewed in (*9*)]. The proteomics data on NPCs have in turn facilitated large-scale imaging studies aimed at localizing Nups within the NPC by both fluorescence and immunoelectron microscopy, examining FRET interactions between Nup pairs to build a network map of Nup-Nup interactions (*48*), and assessing the effects of RNAi knockdown of various Nups on NPC formation and integrity [reviewed in (*49*)]. Through the use of photobleaching analysis of GFP-tagged Nups to compare residence times, the dynamic organization of the NPC was also mapped (*44*). Nups known to localize to central parts of the NPC were more stable, whereas peripheral components were more dynamic, consistent with their having primarily structural and regulatory roles, respectively. Taken together, these studies demonstrate the complementary contributions of proteomics and imaging approaches to the study of the NE and NPCs.

Conclusion

Both proteomics and imaging technologies have contributed to the recent progress in analyzing dynamic events connected with nuclear structure and function. Proteomics, for example, is rapidly moving away from the simple generation of lists of proteins toward providing quantitative annotation of protein properties, including intracellular distributions, concentrations, turnover dynamics, interaction partners, and posttranslational modifications. As the sensitivity continues to improve, it may yet be possible to reach the goal of performing proteomics studies on material from a single cell. With the major emphasis shifting toward quantitation and the analysis of protein dynamics, proteomics is rapidly evolving into a mainstream research tool for cell biology.

Fluorescence imaging technology also continues to improve, both in sensitivity and resolution. Improvements in instrumentation have brought us closer to bridging the gap between the 1- to 5-nm resolution of electron microscopy and the 200-nm resolution of light microscopy (Fig. 4). With techniques such as stimulated emission depletion (STED) and photoactivated localization microscopy/subdiffraction-limit imaging by stochastic optical reconstruction microscopy (PALM/STORM) that exploit reversibly saturable or photoswitchable transitions of fluorescent probes, a resolution of ~20 nm has been achieved with light microscopy (*50*). Higher-resolution imaging will also drive the further development of techniques exploiting the unique properties of GFP and related fluorophores.

Currently, a major roadblock for the proteomic study of nuclear bodies and complexes is the difficulty in isolating enough pure and intact material for analysis. Progress in this area is likely to come both from improvements in fractionation methods and also from further advances in MS technology that will allow proteomic analysis of complexes isolated from smaller numbers of cells. One promising approach is the development of protein correlation profiling (*51*), which does not rely on absolute purity of the structure or organelle of interest. In this technique, cell lysates are fractionated by density gradient centrifugation and analyzed by liquid chromatography–based MS. Organelle localization is assigned by comparing the ion intensity profiles (based on peptide mass and elution time from the column) of peptides from an unknown protein to those of known marker proteins for particular organelles across the separated fractions.

Another important future goal for proteomics studies is detailed mapping of posttranslational modifications. A large-scale nuclear phosphoproteome has already been mapped (*45*), and SILAC can be used to quantify, in parallel, thousands of changes in phosphorylation sites in response to a cellular perturbation (*46*). Other global proteomics studies are addressing the significance and mechanism of complex posttranslational modifications that occur on DNA-associated proteins in addition to phosphorylation, including methylation, ADP-ribosylation, ubiquitination, and acetylation [reviewed in (*52*)].

A major goal of proteomics now is to quantitate protein abundance, dynamics, modifications, and interactions at the single-cell level. A parallel goal of imaging is time-lapse fluorescence microscopy of living cells with a sensitivity sufficient to detect single molecules and a resolution approximating that of electron microscopy. Improvements in mathematical modeling may also allow a detailed quantitative understanding of specific steps in the mechanism of nuclear events to be derived and analyzed at the single-cell level.

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