Imaging chromatin in embryonic stem cells*

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Abstract

Chromatin recently emerged as one of the governing factors for self-renewal and pluripotency of embryonic stem (ES) cells. Stem cell chromatin is distinct from that of somatic or differentiated cells in several different structural and functional aspects including chromatin modifications, global chromatin arrangement and condensation and compaction of chromosome territories. Live imaging methods further demonstrate that chromatin-binding proteins are more dynamic is ES cells. Here I review recent advances in imaging methods that allow investigations of chromatin and chromatin proteins, mostly in embryonic stem cells, and suggest that the nucleus itself in undifferentiated ES cells is less constrained, giving rise to a 'breathing' chromatin conformation in ES cells.

Introduction

As evident by the different topics covered in this book, chromatin function and epigenetic regulation have lately become the center attractions in the field of stem cell biology. Since chromatin structure, chromatin modifications and epigenetic signatures in stem cells are thoroughly covered by the other chapters in this book, I shall focus on introducing the concepts of live imaging and advanced microscopic techniques to study chromatin protein dynamics

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in living cells. I try to concentrate on ES cells and some early developmental studies, but may occasionally stray into other cell types, where data is absent.

Most of what we know in biology in general and in the chromatin field in particular comes from studies of fixed cells, frozen cells or cell extracts, which are usually used for molecular and biochemical assays. With the advances in fluorescent labeling of molecules, and especially, the usage of green fluorescent protein (GFP) (Prasher et al., 1992) and its color shifted derivatives as markers (Giepmans et al., 2006), we can now study chromatin proteins in living cells.

Chromatin structural changes in stem cell differentiation

Chromatin, as well as other features of nuclear architecture, is distinct in ES cells from differentiating or somatic cells in many respects (Meshorer, 2007; Meshorer and Misteli, 2006). Heterochromatin foci are fewer, larger and dispersed in undifferentiated mouse ES cells (see Figure 1) and the organization of chromatin structure itself is more

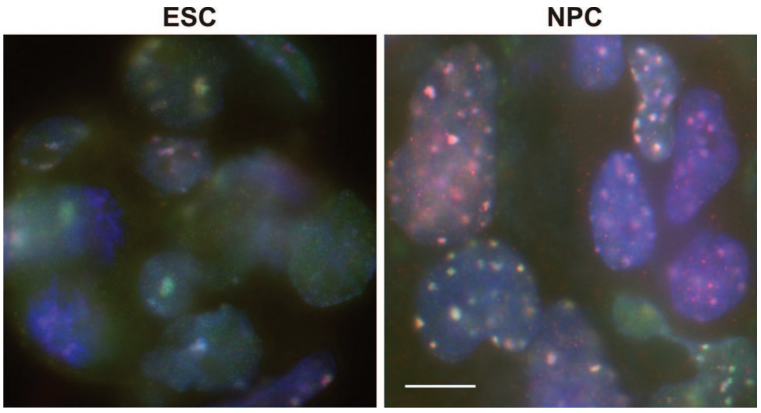


Figure 1. Heterochromatin structure in ES cells. Undifferentiated ES cells (ESC, left) and ES cell-derived neuronal progenitor cells (NPC, right) were immunostained with anti-HP1 antibodies (green), anti-H3K9me3 antibodies (red) and DAPI (blue). In ES cells, heterochromatin appears as larger and fewer foci whereas in neuronal progenitor cells heterochromatin foci are smaller, more condensed and numerous. Bar = 10 μm.

homogeneous (Efroni et al., 2008). Such features were initially observed in fixed murine cells by direct staining of DNA with DNA non-intercalating dyes such as DAPI (Aoto et al., 2006; Meshorer et al., 2006) and by indirect immunofluorescence (IF) using antibodies against heterochromatin-binding proteins such as heterochromatin protein 1 (HP1) or heterochromatin-associated histone modifications such as H3K9me3 (Meshorer et al., 2006). More direct observation of heterochromatin in mouse ES cells was achieved using DNA-fluorescence in situ hybridization (FISH) of the major satellite repeat – another resident of heterochromatin (Meshorer et al., 2006) and by additional microscopic techniques. These include electron microscopy (EM) (Park et al., 2004) – revealing once again, significant increase in condensed heterochromatin following human ES cell differentiation; atomic force microscopy (AFM) (Chen et al., 2005) – demonstrating the paucity and non-uniform organization of heterochromatin in mouse ES cells although a direct comparison with a differentiated cell is lacking in this report, and electron spectroscopic imaging (ESI) – illustrating that chromatin structure in undifferentiated ES cells is more homogenous with less frequent condensed heterochromatic structures (see Figure 2) (Efroni et al., 2008). ESI is an EM-based technique which enables the visualization of phosphate and nitrogen in formaldehyde-fixed cells without the use of heavy metal (or any other) staining methods. In ESI, excitations of the specimen's atoms cause incident electrons to lose specific amounts of energy. An instrument that is both an electron spectrometer and a lens then forms a dark-field energy-filtered image. Even without the use of heavy atom contrast agents, 'endogenous stains' such as phosphorous can be imaged, and thus chromatin serves as one of the best cellular structures readily visualized by ESI (Dehghani et al., 2005).

Additionally, time-lapse visualization of heterochromatin structures in differentiating mouse ES cells was performed using a GFP fusion version of the methyl CpG binding domain protein 1 (MBD1), which resides in chromocenters and renders them fluorescent. The results of these experiments demonstrated elegantly the transition from relatively few (∼7) heterochromatin foci or chromocenters in undifferentiated ES cells to almost thrice as many (∼20)

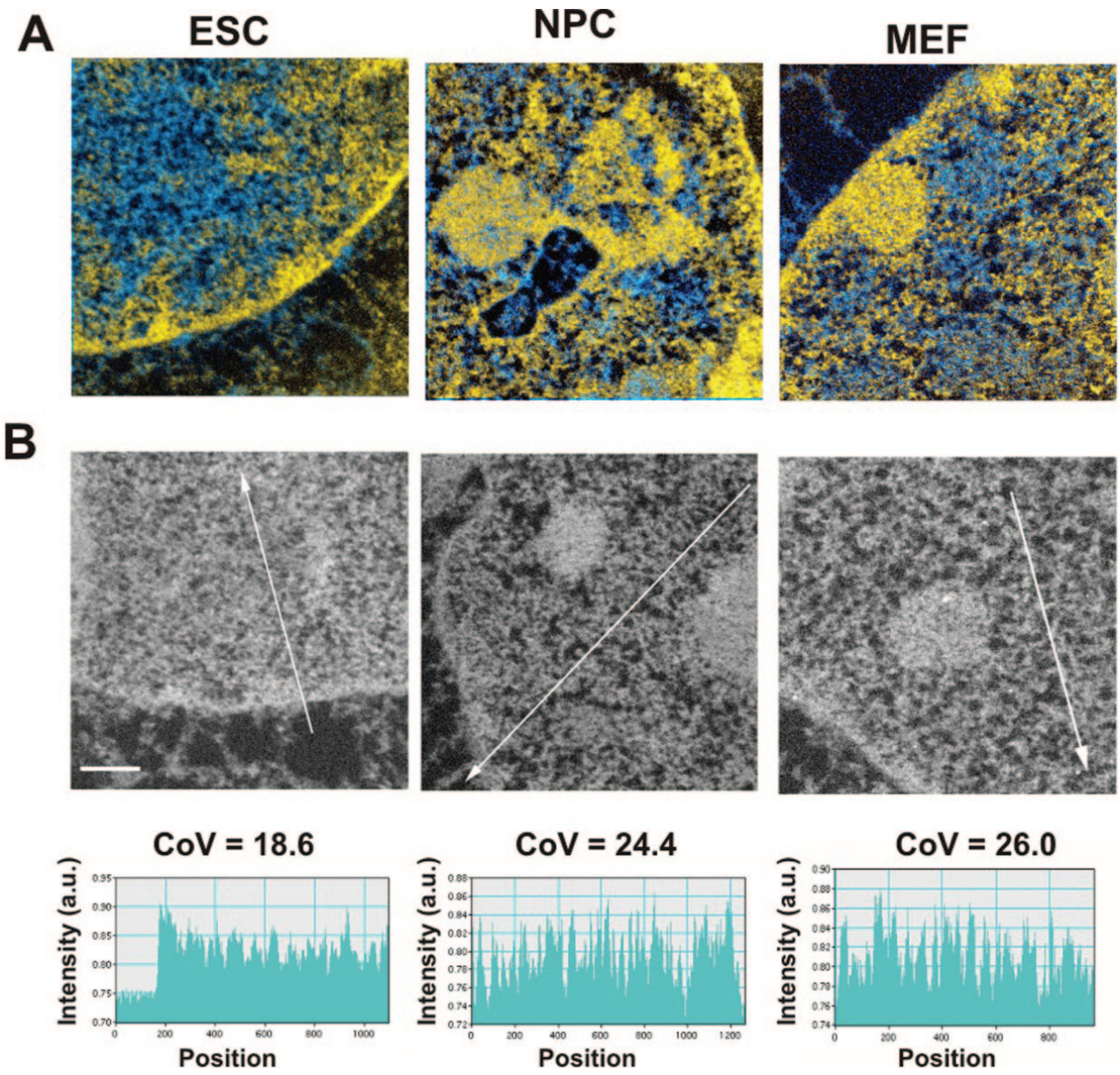


Figure 2. Electrospectroscopic imaging (ESI) in ES cells and ESC-derived neuronal progenitor cells. A. Phosphorus (yellow) and nitrogen-phosphorus (N-P) (blue) maps in undifferentiated ES cells (ESC), ES cells-derived 7 days neuronal progenitor cells (NPC) and mouse embryonic fibroblasts (MEF). ES cells exhibit homogeneous chromatin texture and are devoid of detectable heterochromatin foci. Bar = 500 nm. **B.** Line scans of mass distribution (nitrogen maps) in ES cells (left), NPC (middle) and MEFs (right). CoV = Coefficient of Variation (% standard deviation/mean). Reprinted with permission from Efroni et al., 2008).

in early differentiating cells (Kobayakawa et al., 2007). This shows rapid and highly dynamic changes in chromatin structure in mouse ES cell differentiation.

Although in early differentiating ES cells the number of heterochromatin foci increases, the transition from differentiating stem cells or somatic precursors into terminally differentiated cells usually follows a general decrease in chromocenters, as demonstrated in muscle cells (Brero et al., 2005; Terranova et al., 2005), promyelocytic leukemia cells (Beil et al., 2002) and neuronal cells (Manuelidis, 1984; Takizawa and Meshorer, 2008). It thus seems that the increase in chromocenters is a transient process which might facilitate differentiation, and when cells enter terminal differentiation and exit the cell cycle, clustering of chromocenters takes place (Mayer et al., 2005).

In vivo imaging of chromatin

One way to follow dynamic changes in chromatin structure in vivo is by using transgenic mice expressing a chromatin protein fused with GFP, preferably a histone protein, for which it would be difficult to compete with endogenous protein levels. Transgenic mice expressing H2B-GFP in all of their nucleated cells were generated (Hadjantonakis and Papaioannou, 2004). These mice were fertile, appeared healthy throughout their entire life span and exhibited widespread expression with no morphological abnormalities, suggesting that ectopic expression of histone and fluorescent proteins is not harmful to cells or tissues. These mice were used for a time-lapse study of nuclear dynamics of both preimplantation and postimplantation embryos revealing, for example, dynamic chromatin changes during primitive streak development into mesoderm. In these studies, imaging was done using either a spinning disk confocal microscope (Nakano, 2002) or a 2-photon excitation microscope (White and Errington, 2002). The former allows, through the use of a rotating disk with multiple pinholes (Nipkow disk) aligned with a concentric disk of micro-mirrors (developed by the Japanese company Yokogawa), virtually simultaneous capture of the entire field of view, which enables the use of a charge coupled device (CCD) camera for rapid image collection rather than a photomultiplier, which is used in a conventional confocal laser scanning microscope (CLSM) (Nakano, 2002). For live imaging, the spinning disk is therefore superior to the CLSM in both speed of acquisition and reduced photobleaching and photodamage.

The 2-photon excitation microscope uses, instead of a constant laser beam, a femtosecond pulse laser (usually a solid state mode-locked Ti:Sapphire laser) which first excites an electron to an intermediate state (with the 'first' photon) and then (femtoseconds later) to an excited state (with the 'second' photon). Fluorescence is generated, as always, when the electron reverts back to the ground state. This allows both better light penetration and reduced light scattering (which is proportional to the inverse power of the wavelength) due to the use of infra-red (IR) and near-IR wavelengths, and at the same time less photodamage since energy is lower in the higher wavelengths. In addition, since excitation only occurs where the 2 photons meet, namely in the focal plane, there's almost no photobleaching and photodamage above and below the focal plane, as in a conventional CLSM (White and Errington, 2002). The spinning disk is preferred when rapid image collection is required, usually for capturing rapid processes in living cells, while the 2-photon microscope is chosen when better light penetration is desired, usually for live tissue imaging, but both instruments are extremely advantageous for the study of living cells and tissues.

Live imaging during early embryogenesis in mouse also revealed the dynamic changes of DNA methylation in preimplantation embryos. This was achieved using a synthetic RNA encoding GFP fused to MBD1 microinjected into metaphase-II-arrested and fertilized oocytes (Yamazaki et al., 2007). Injected embryos displayed normal development potential and viability. A dramatic reconfiguration of methylated DNA was observed during the transition from a 2-cell to a 4-cell embryo, from a pro-nuclear like diffuse configuration particularly concentrated around the nucleolus to large-dots configuration, as observed in somatic nuclei. Similar morphological differences was observed for pericentric heterochromatin observed using FISH for the major satellite repeats during early embryogenesis (Probst and Almouzni, 2008).

In vivo imaging of chromatin is still at its infancy but with the advances in spinning disk imaging and especially multi-photon microscopy, together with novel applications of fluorescence tagging, this field will no doubt gain momentum in the next few years.

Photobleaching methods to follow nuclear dynamics

The use of GFP fusion transgenes also opened up the door for monitoring chromatin protein dynamics in living cells using photobleaching methods (Belmont, 2001; Lippincott-Schwartz and Patterson, 2003; Misteli, 2001) such as fluorescence recovery after photobleaching (FRAP). In this method, cells are transfected with GFP fused to a chromatin binding protein, which illuminates its compartment (the chromatin within the nucleus) with green fluorescence. To detect the motion of the tagged protein molecules, a fraction of the nuclear compartment (which can be reduced to the size of a single chromocenter) is bleached using a short pulse of a high energy laser. Since the physical bleaching event is irreversible, recovery of the signal in the bleached zone will occur only if unbleached GFP molecules, making their way from the unbleached territory, will displace the bleached GFP molecules. Fluorescence recovery generates FRAP recovery curves, from which we can extract information regarding the nature of the association between proteins and chromatin, the dynamic fraction, the stably bound fraction, and the unbound fraction, if present (see Figure 3A).

Complementary photobleaching methods include inverse-FRAP (iFRAP), where the entire compartment except a small region of interest is bleached, resulting in a decay, rather than a recovery, curve (see Figure 3B); and fluorescence

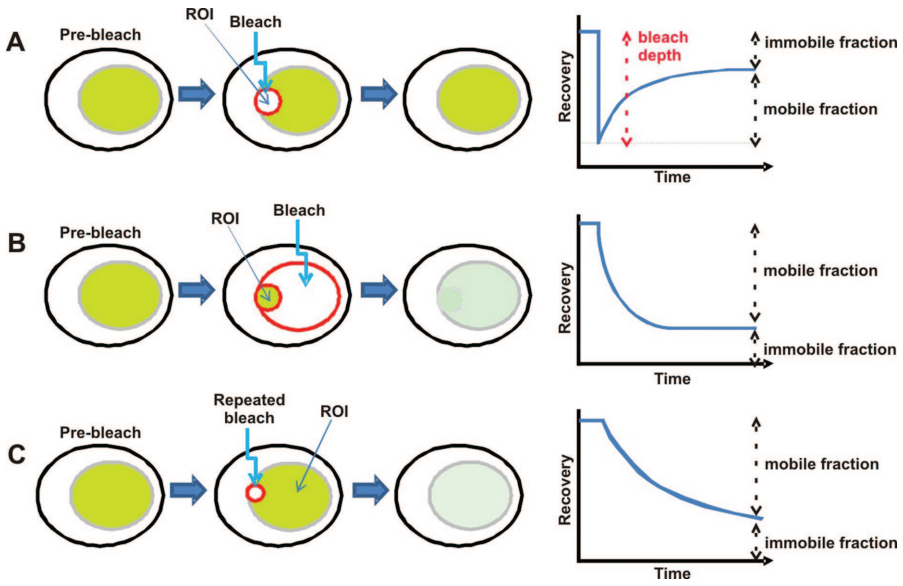


Figure 3. Photobleaching methods used to study chromatin-protein dynamics in living cells. A. Fluorescence recovery after photobleaching (FRAP). Cells expressing a nuclear chromatin-bound GFP-fusion protein are subjected to a short powerful laser beam (bleach, red circle) at a specific region of interest (ROI), and the recovery of the fluorescence signal after the bleach is measured at the ROI. Images are collected before and after the bleach at given intervals, depending on the dynamics of the analyzed protein. A typical FRAP curve is shown on the right. Recovery curves inform on the association between chromatin and the GFP-labeled bound proteins. The immobile fraction can be inferred by the difference between the pre-bleach signal and the maximum recovery while the mobile fraction is the difference between the bleach depth (red) and the recovered signal (right). **B. Inverse FRAP (iFRAP).** Here, the entire nucleus is bleached (red contour) except the ROI (green). Images are collected similarly to FRAP, and fluorescence is measured at the ROI. A usual iFRAP curve is shown on the right. If the molecules are mobile, a rapid displacement usually occurs following the bleach. Immobile proteins will show no decay over time. **C. Fluorescence loss in photobleaching (FLIP).** In FLIP experiments, a repeated bleach is applied at a small specific site within the nucleus (red) and the fluorescence decay is measured elsewhere (green). Mobile molecules will pass, in time, through the bleached region and loss of fluorescence will be recoded. A typical FLIP curve is shown on the right. In FLIP experiments, in order to check continuity of cellular compartments, a different compartment, such as the cytoplasm, can be bleached, while signal loss, if exchange occurs between the two compartments, can be recorded in the nucleus.

loss in photobleaching (FLIP) where a pulse beam photobleaches the same spot repeatedly, again resulting in a slow decay curve when measured elsewhere in the (nuclear) compartment (see Figure 3C) (Dundr and Misteli, 2003). Since the number of the unbleached molecules in iFRAP is significantly larger than the bleached ones, the unbleached molecules do not contribute to new binding events and it is therefore suitable for studying off-rates of proteins residing in relatively small intranuclear compartments. FLIP is best suited for detection of kinetically distinct fractions residing within the same compartment since fluorescence is lost only if the molecules pass through the repeatedly bleached area. It is also appropriate for testing compartment continuity by repeatedly bleaching one compartment and monitoring fluorescence loss in the other.

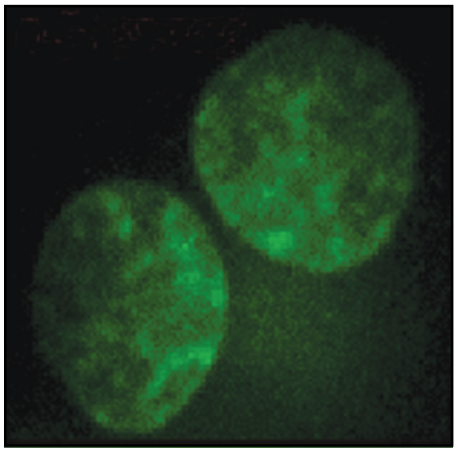
Local protein dynamics can also be studied using fluorescence correlation spectroscopy (FCS). In this method, the fluctuations of the fluorescence intensity are recorded rather than the actual emitted wavelength. When two fluorescent particles (or GFP-labeled proteins) interact, the correlation between their fluctuations increases and when a protein binds chromatin for instance, the diffusion rate of the fluorescent signal decreases. FSC is therefore useful for studying protein-protein interaction as well as diffusion and mobility in living cells, and was applied to determine the concentrations and local mobility of core histones in living cells (Weidemann et al., 2003), and together with FRAP, to monitor H1-GFP increased nuclear mobility following inhibition of histone deacetylation (Rao et al., 2007).

These methods allow studying the mobility, diffusion and exchange rates of fluorescently-labeled proteins in living cells, and specifically, enable us to ask question regarding the nature of the association between chromatin and chromatin-binding proteins.

The hyperdynamic stem cell chromatin

While such photobleaching methods revealed the unexpected dynamic association of nuclear proteins previously perceived to be stably bound to chromatin such as HP1 (Cheutin et al., 2003) and the linker histone H1 (Misteli et al., 2000), the association of chromatin proteins with chromatin in undifferentiated mouse ES cells is hyperdynamic

(Movie 1), revealing a soluble or loosely bound fraction (10–25%) of core and linker histones as well as HP1 in ES



Movie 1. FRAP of H1-GFP in mouse ES cells. In addition to the rapid recovery, note the dynamic 'breathing' chromatin nature.

cells nuclei (Meshorer et al., 2006) (see Figure 4). Hyperdynamic association of chromatin proteins in ES cells likely reflects their global decondensed chromatin structure (see Figure 2). It is also important for maintaining pluripotency and differentiation potential, since mouse ES cells lacking the histone chaperone HirA posses a significantly larger loosely bound fraction of histone H3 and its variant H3.3 than wt ES cells, and the ability of the mutant ES cells to remain fully undifferentiated is impaired (Meshorer et al., 2006).

The dynamics of histone H1-GFP was monitored in mouse ES cells lacking either the DNA methyltransferase Dnmt3 (where both de novo DNA methyltransferases Dnmt3a and Dnmt3b are deleted) or the methyl CpG binding protein 2 (MeCP2) (Gilbert et al., 2007). While lack of Dnmt3 caused reduction in mobility of H1-GFP, the lack of MeCP2 had no effect. These results may suggest that DNA hypomethylation in ES cells is not responsible for their hyperdynamic chromatin. However, it should be noted that when murine ES cells are firstly derived from the inner cell mass (ICM) of the blastocyst, their genomic methylation levels are low. These levels increase during subsequent stages of embryonic development (Oswald et al., 2000; Rougier et al., 1998), and when grown as ES cells, during culturing and passaging (Maitra et al., 2005; Pannetier and Feil, 2007). DNA methylation levels, as well as the effects of perturbing with methylation levels, may therefore vary between different ES cell lines. In any event, additional experiments will be required to delineate the contribution of DNA methylation in ES cells to their hyperdynamic plasticity.

Another possible reason for the chromatin protein hyperdynamics in ES cells is the lack of expression of the nuclear lamina proteins lamins A/C (Constantinescu et al., 2006). Nuclear lamins are known to interact with chromatin

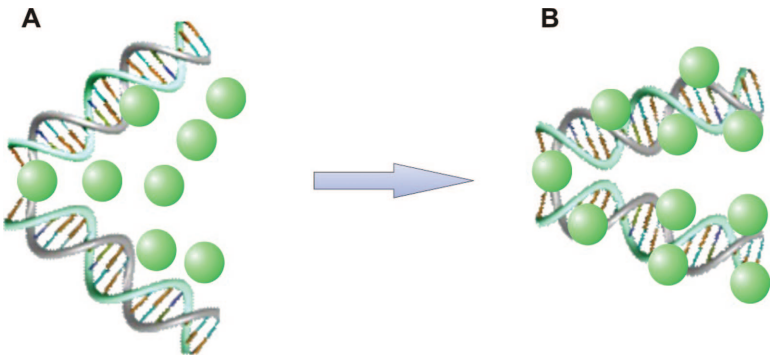


Figure 4. A model for the behavior of chromatin proteins during embryonic stem cell differentiation. A. In undifferentiated ES cells, a fraction of chromatin proteins, including histones and HP1, are loosely bound or unbound, likely contributing to the pluripotent state. **B.** During differentiation, chromatin proteins bind tightly to chromatin, establishing heterochromatin domains during differentiation.

and histone proteins and it is believed that this association contributes to global organization of chromatin within the nucleus and restricts chromatin structural dynamics (Gruenbaum et al., 2005). Supporting this notion, an interesting recent study showed that human ES cells (hESC) display increased nuclear physical plasticity when compared to differentiated cells (Pajerowski et al., 2007). Live cell microaspiration studies of hESC nuclei demonstrated that they deform more readily than differentiated cells, and FRAP of histone H2B-GFP inside the aspirating pipette showed flow of chromatin in undifferentiated cells. Although decondensed chromatin contributes to the noted nuclear plasticity, it appears that lamins A/C have an important role in maintaining nuclear stiffness in differentiated cells as well. Indeed, when lamins A/C levels were reduced in somatic cells, their nuclear deformability was similar to that of hematopoietic stem cells, which possess an intermediate level of stiffness between ES and somatic cells. These data suggest that absence of A-type lamins in ES cells might also contribute to chromatin protein plasticity.

Live imaging studies carried out in mouse preimplantation embryos using an injected RNA coding for HP1β-GFP fusion protein demonstrated a decrease in HP1β mobility during the early stages of development, from an extremely rapid recovery in the fertilized oocyte to a significantly reduced dynamics in the 4-cell stage embryo (Yamazaki et al., 2007), suggesting that chromatin dynamics may play an important role in shaping the organization and function of the early developing nucleus.

Hyperdynamic binding of linker H1 variants was also observed in mouse oocytes and in ES cell-nuclear transfer (Becker et al., 2005). To allow the fusion between the maternal and the paternal genomes following fertilization, global genome reorganization occurs and linker histones are among the major participants in this process. While somatic cells contain several different linker histone subtypes, mammalian oocytes contain one specific linker histone, namely H1FOO. Within 3 hours following ES cell nuclear transfer, somatic linker histones were replaced by H1FOO, which bound more tightly to chromatin than somatic linker histones. Deletion mutants demonstrated that binding specificity is achieved by the N-terminal and globular domains of H1FOO. These results provide insight into the mechanisms of linker histone replacement during fertilization and nuclear transfer, and demonstrate the elegant use of deletion mutants in studying binding determinants in living cells.

These studies collectively demonstrate the important role that chromatin protein dynamics play in ES cell regulation and early development and call for studying the mechanisms that dictate chromatin protein motion in living cells and that distinguishes protein dynamics in pluripotent cells from that of differentiated cells.

Imaging chromatin-protein interactions

As demonstrated above, mutants of GFP-fusion proteins can be combined with photobleaching methods to analyze chromatin-protein interactions. Using this approach, FRAP analysis of GFP fusion mutants of H1(0) demonstrated association of H1(0) with chromatin through the C-terminal domain (Meshorer et al., 2006), and more specifically, via two distinct sites within the globular domain (Brown et al., 2006). Additionally, using FRAP imaged with a 2-photon microscope (2-photon-FRAP), H2A mobility was found to be regulated by both the N- and C-terminal tails (Higashi et al., 2007). Increased dynamics of H2A-GFP was detected following treatment with histone deacetylase (HDAC) inhibitors, as noted above for H1-GFP, supporting a model where histone tails regulate transcription by interacting with nucleosomal DNA via electrostatic interactions.

An additional elegant way to study chromatin-protein interactions in vivo is by fluorescence resonance energy transfer (FRET). FRET occurs when a fluorescing donor molecule excites a fluorescent acceptor dye in the range of the Förster radius of a few nanometers (Jares-Erijman and Jovin, 2006), a signal which decreases exponentially with the sixth power of the distance between the donor and the acceptor dyes. Both donor and acceptor dyes must be chosen carefully so that excitation will occur with minimal wavelength overlap. The most common FRET pair is the cyan fluorescent protein (CFP) as the donor and the yellow fluorescent protein (YFP) as the acceptor, although the GFP-mCherry pair was recently shown to be better suited for FRET imaging by fluorescence lifetime imaging (FLIM) (Tramier et al., 2006). In FLIM, similar to FCS, not the actual emitted light is recorded, but a different physical property of the fluorophore, namely the fluorophore's lifetime or exponential decay duration (Wallrabe and Periasamy, 2005). It is favored over conventional imaging when using different fluorophores emitting light at proximal wavelengths or when using fluorophores with long life-time constants, which translate into better signal-to-noise ratio. FLIM also enjoys reduced photon scattering when recording information from relatively thick specimen.

FRET-FLIM for chromatin-protein interactions was demonstrated using the nucleic acid binding dye Sytox Orange as an acceptor dye and different chromatin proteins as donor fluorophores including GFP-H2B, GFP fused to glucocorticoid receptor (GFP-GR) and GFP-HP1 (Cremazy et al., 2005). Although in this method the cells are

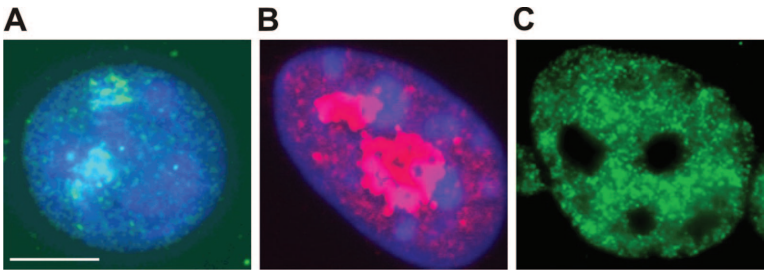


Figure 5. ES cell nuclear architecture. A. Chromosome territories. Chromosome 17 territories in mouse ES cell interphase labeled with a specific chromosome paint probe (green); DAPI staining is in blue. **B. Sites of transcription.** Nascent RNA in interphase nuclei of undifferentiated mouse ES cells is labeled with bromo-uridine (BrU) followed by immunolabeling with anti BrU antibody (red). The nucleoli – the sites of ribosomal RNA transcription – are most conspicuously labeled, shown as two large foci in the center. The remaining red signal marks sites of RNA polymerase II (RNA pol II) transcription. **C. Transcription factories.** Immunostaining of interphase mouse ES cell nuclei with an antibody specific for the phosphorylated form (on serine 2) of the carboxyl terminal domain (CTD) of RNA pol II marks mRNA transcription sites throughout the entire nucleoplasm (green). Bar = 5 μm.

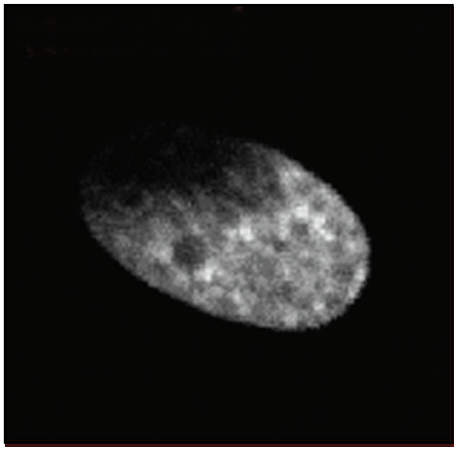
first fixed and then subjected to FRET-FLIM measurements, it enables to distinguish between stably chromatin bound proteins, such as H2B, and more weakly associated proteins, such as GR. In the latter case, FRET signal was shown to be glucocorticoid agonist dependent, attesting to the specificity of this method. In addition, non-specific interactions and random collisions did not contribute to the measured FRET signal. Although not tested specifically in ES cells, this method could be used to compare chromatin-protein interaction between somatic cells and ES cells, or during stem cell differentiation.

Taken together, these emerging methods in imaging and cell biology provide useful means by which we can now study the dynamic interplay between proteins and chromatin in living and fixed cells and ask specific questions about the unique properties of the stem cell chromatin.

Chromatin motion

What was once regarded as an unordered mesh of DNA and proteins filling the cell nucleus un-purposely, eukaryotic chromatin, as well as the housing nucleus, is now recognized to be highly organized (Lamond and Earnshaw, 1998; Misteli, 2005; Swedlow et al., 1993). Chromosomes occupy discrete domains termed ‘chromosome territories’ (Cremer et al., 1982) (see Figure 5A); and numerous proteinacious compartments are present, in which many of the nuclear processes are carried out (Francastel et al., 2000), including nucleoli, where ribosomal RNA (rRNA) synthesis takes place (see Figure 5B), or transcription factories (Faro-Trindade and Cook, 2006), the sites of messenger RNA (mRNA) transcription (see Figure 5C). The nuclear architecture in ES cells has not been studied extensively, but it seems that while the genomic organization and the majority of the nuclear domains are already present in ES cells (Meshorer and Misteli, 2006; Wiblin et al., 2005), some conspicuous differences between ES and somatic cells are also apparent. For example, several nuclear compartments, such as the nuclear lamina, are highly distinct in undifferentiated ES cells (Constantinescu et al., 2006; Meshorer and Misteli, 2006), and a general condensation of chromosome territories takes place during ES cell differentiation (Bartova et al., 2008). However informative, these studies analyzed fixed cells stained with chromosome paints or specific antibodies for nuclear compartments. It is now challenging to monitor direct chromatin movement, as well as the nature of the different nuclear compartments in living ES cells. Indeed, visualization of global chromatin motion in live fertilized mouse eggs was achieved over 15 years ago, demonstrating global paternal chromatin condensation followed by chromatin decondensation in living embryos (Adenot et al., 1991).

Direct visualization of specific chromosomal elements was accomplished using stable chromosomal integration of a plasmid carrying 256 repeats of the Lac operator. Subsequent transfections with a GFP-Lac-repressor protein that binds the Lac repressor site marks the chromosomal location in living cells enabling its visualization (Robinett et al., 1996). Using this method, chromatin was shown to undergo constrained, Brownian motion confined to a subregion of the nucleus (Marshall et al., 1997), a motion that seems to be highly influenced by the association of chromatin with nuclear compartments (Chubb et al., 2002). More recent experiments, using 2-photon microscopy and a particle-tracking method revealed specific dynamics of chromatin motion in living cells (Levi et al., 2005). Constraint diffusion was interrupted by ATP-dependent abrupt leaps of about 150 nm in distance lasting for around 1 second, demonstrating additional, active modes of motion. But despite these advances in imaging techniques with increased spatial and temporal resolutions, no direct chromosomal site imaging was reported in stem cells. Regardless, our own time-lapse observations of GFP-labeled core histones suggest ‘breathing’ chromatin in undifferentiated ES cells (Movie 1), displaying active nuclear dynamics not observed in differentiated or somatic cells (Movie 2).



Movie 2. FRAP of H1-GFP in a mouse 3T3 fibroblast.

Future perspectives

Live imaging of chromatin in stem cells has so far been performed on undifferentiated cells or during the course of differentiation. But chromatin likely plays essential roles during the reverse process of reprogramming from differentiated, or somatic cells, to induced pluripotent (iPS) cells. Future challenges include live imaging of iPS cell differentiation and comparison to ES cells, as well as imaging of chromatin dynamics during the reprogramming process itself. The rapid development of microscopic techniques and markers for live imaging will enable to reach these goals in the foreseeable future. An additional future direction is the use of imaging techniques in living animals. One of challenges would be to monitor chromatin dynamics in early (4, 8, 16... cell stage) embryos as well as in the ICM of the blastocyst in vivo rather than in ES cells taken from the embryo and cultured in vitro or in embryos developing externally.

In summary, we have seen a variety of microscopic imaging techniques that allow visualization of chromatin and chromatin protein dynamics in living (and fixed) cells. Such techniques enable us to study the unique properties of chromatin in stem cells, demonstrating, for example, that undifferentiated ES cells are characterized by hyperdynamic plasticity of chromatin proteins, by fluid nuclei and physical nuclear plasticity, and by global nuclear dynamics, supporting an open conformation model of chromatin in undifferentiated stem cells. Subsequent years will no doubt shed more light on nuclear function and chromatin dynamics in stem cells and during stem cell differentiation and reprogramming.

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