Spatial and dynamic organization of molecular structures in the cell nucleus

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CHAPTER 5

Nuclear body movement is constrained by associations with chromatin

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Summary

The cell nucleus contains distinct nuclear bodies which are involved in key cellular processes. Factors that determine the integrity and spatial positioning of these bodies in the cell nucleus are unknown. Here we investigate the dynamic behavior of three prominent nuclear bodies, PML bodies, Cajal bodies and speckles relative to that of chromatin. All three bodies seem to be associated with chromatin and this association is lost in response to methylmethane sulfonate treatment. Once dissociated from chromatin, the nuclear bodies have more freedom to move in the three dimensional nuclear space. Furthermore, PML bodies will eventually disassemble but not Cajal bodies and speckles, suggesting that PML body structural integrity is dependent on its association with chromatin.

Introduction

The cell nucleus contains various distinct nuclear compartments or bodies which differ in size, morphology, composition and function. The diversity in nuclear bodies has been explained by the various activities that are supported by the cell nucleus including transcription, RNA processing, RNA transport and DNA repair. Each of these activities requires the recruitment of multiple factors and an ordered assembly of multi-component machineries. Among the most prominent and best studied nuclear bodies are nucleoli, PML bodies, Cajal bodies and speckles. Nucleoli are sites of rRNA synthesis, rRNA processing and ribosome subunit assembly. Each of these steps is performed by different protein assemblies and takes place in three different subcompartments of the nucleolus. The functions of the other nuclear compartments are still not fully understood. Speckles have been suggested to function as storage sites of RNA processing and RNA transport factors, from which these factors are recruited to transcriptionally active genes (Misteli et al., 1997; Dirks et al., 1997; Snaar et al., 1999). Observations of active genes that are preferentially associated with speckles suggested that these domains may also function as hubs, facilitating and possibly coordinating the synthesis of mRNAs (Hall et al., 2006). In addition, speckles have also been implicated in steps of mRNA splicing control and nuclear export (Hattinger et al., 2002; Molenaar et al., 2004; Schmidt et al., 2006).

Functionally related to speckles are Cajal bodies, which have been identified as assembly sites of spliceosomal small nuclear ribonucleoproteins (snRNPs) and have been found present mainly in proliferating cell types (Stanek & Neugebauer, 2006).

PML bodies have been implicated in many different cellular processes such as transcription regulation, apoptosis, senescence, DNA repair, proteosomal degradation and genome stability (Bernardi & Pandolfi, 2007). Furthermore, they have been suggested to function as nuclear depots in which proteins are temporally stored (Negorev & Maul, 2001). Recent evidence suggests that PML bodies are sites at which post-translational modification of proteins by both ubiquitin and SUMO is integrated to regulate proteosomal degradation (Bailey & O'Hare, 2005; Sharma et al., 2010).

Although nuclear bodies are distinct, stable structures during interphase, their protein content is in a continuous flux with the surrounding nuclear space, allowing rapid changes in body composition. This protein flux indicates that proteins do not necessarily exert their function within or at the surface of a nuclear body but possibly elsewhere in the nucleus. Nevertheless, there is ample evidence that nuclear bodies have the ability to transiently associate with chromosomal sites. This has not only been reported for nucleoli, which associate with ribosomal DNA genes but also for PML bodies (Ching et al., 2005; Kieβlich et al., 2002; Gialitakis et al., 2010), Cajal bodies (Platani et al., 2002) and speckles (Brown et al., 2008).

Live cell studies revealed that nuclear bodies generally show little movement inside the cell nucleus, which could reflect a rather stable interaction with chromatin. Alternatively, it has been suggested that nuclear body mobility is mainly determined by the poor accessibility and the slow dynamics of chromatin (Görisch et al., 2004). Also, nuclear bodies have been suggested to be connected to a nuclear matrix structure, limiting their movement. Physical associations of chromatin with immobile nuclear bodies may then restrict the movement of chromatin or even support the spatial organization of chromatin in the nucleus (Chubb et al., 2002).

A key question is whether the association of nuclear bodies with chromatin or with activities that take place at chromatin is an essential factor to ensure the stability/integrity of the bodies. Recently, we observed that the mobility of PML nuclear bodies significantly increase in cells that were treated with the DNA-methylating agent methyl methanesulfonate (MMS), before the PML bodies dispersed as a result of this treatment (Brouwer et al., 2009). Whether the increase in PML body dynamics reflects an increase in chromatin dynamics has not yet been investigated. Photoactivatable GFP (PA-GFP) Histone H4 has been used as a marker for chromatin to examine their dynamic properties in living cells (Post et al., 2005; Wiesmeijer et al, 2008). In this study, we used this marker, together with fluorescent markers for PML bodies, speckles and Cajal bodies to investigate their dynamic spatial arrangements in cells exposed to MMS treatment by fluorescence time-lapse imaging.

Materials and Methods

Cell culture and transfection

Human Osteosarcoma cells (U2OS) were cultured in 3.5 cm glass bottom petri dishes (MatTek Corporation, Ashland, MA) in Dulbecco's modified Eagle's medium without phenol red, containing 1.0 mg/ml glucose, 10% fetal bovine serum (FBS), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Invitrogen, Carlsbad, CA). Cells at 70%–80% confluency were transiently transfected with PA-GFP histone H4 (Wiesmeijer et al., 2008) together with either coilin-DsRed, SF2/ASF-DsRed or PML-DsRed by using lipofectamine 2000 (Invitrogen). Coilin, SF2/ASF and PML I were originally cloned into a GFP vector (Molenaar et al., 2004; Wiesmeijer et al., 2002) and subcloned into a DsRed vector (ClonTech, Mountain View, CA). Cells were incubated with 0.01% methyl methanesulfonate (MMS; Sigma-Aldrich, St. Louis, MO) for time periods ranging from 45 minutes to 2 hours. Cells were allowed to recover from MMS-treatment by incubating them in fresh medium containing 20% FBS for 2 to 3 hours.

Photoactivation and microscopy

Photoactivation and subsequent time-lapse imaging were both performed using a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems, Mannheim, Germany) equipped with a climate control box. During the course of the experiments, the temperature of the cells was kept constant at 37°C and the CO_2 concentration at 5%. PA-GFP was activated using the 405 nm laser line at 12% laser power during a 300 ms pulse. Following photoactivation, subsequent time-lapse images were taken with a 63× NA 1.4 PlanApo oil objective lens and 488 nm and 561 nm laser lines of respectively an Argon (multi-line) and a DPSS (561nm) laser. An additional 6-fold scanning zoom was applied to collect high-resolution Z-stacks. At each time-point a two-channel Z-stack of 15-25 Z-slices (thickness 0.4 μm) was acquired using 2-5% laser power, 400 Hz scanning frequency and 512 x 512 pixels per frame. 4D image stacks were collected every 30 seconds for 10 minutes. Image acquisition and analysis were performed using the Leica SP5 software. All images and movies shown are representative of 6-10 repeats of the same experiment.

Results

MMS treatment causes PML bodies to detach from chromatin

On average, PML bodies show little movement in the cell nucleus (Wiesmeijer et al., 2002; Eskiw et al., 2003). Only a small subset of PML bodies display high mobility (Muratani et al., 2002), while directional movements of PML bodies are observed only when cells enter mitosis (Chen et al., 2008). When U2OS cells are treated with the DNA methylating agent MMS, the majority of PML bodies display a dynamic movement until they disperse (Brouwer et al., 2009). There are two possible explanations for this increase in PML body mobility. Either chromatin domains become more dynamic and accessible as a result of the DNA methylation or, alternatively, PML bodies dissociate from the chromatin to which they are bound and become dynamic.

To analyze whether the observed increase in PML body mobility after MMS treatment is related to an increase in chromatin mobility, U2OS cells were transfected with EYFP-PML-I together with DsRed-TRF2, treated with MMS, and 3D image stacks were collected at 30 second time intervals for 10 minutes. At 2 hours following MMS treatment, the majority of PML bodies moved rapidly through cell nucleus as compared to telomeres, which showed little mobility (Fig. 1 and Movie 1). Thereby, the movement of PML bodies was not limited to a confined area in the nucleus but PML bodies were shown to move over large distances. Ultimately, as indicated by arrows, PML bodies fuse with each other and disperse. As telomere movement may not be indicative for the dynamics of other chromatin regions, in particular those surrounding PML bodies, we employed a histone H4 fused at its carboxy terminus with a photoactivatable (PA) GFP, as a marker for chromatin. Incorporation of histone H4-PAGFP into chromatin allows photoactivation of selected chromatin regions in the cell nucleus using 405 nm laser light and there after their visualization using 488 nm laser light. Fluorescence 3D time lapse recordings of PML bodies containing DsRed-PML-I together with photoactivated histone H4-PAGFP surrounding these bodies showed a similar slow mobility for both compartments (Fig. 2A, Movie 2). PML bodies remained enclosed by photoactivated histone H4-PAGFP-labeled chromatin for at least 10 minutes, the maximum time-period in which we imaged cells. A time course of 10 minutes appeared optimal to obtain 3D stacks at a high temporal resolution without photobleaching of the photoactivated H4-PAGFP. A side-view of the same nucleus that is shown in Fig. 2A shows that PML bodies are evenly distributed throughout the cell nucleus (Fig. 2B). Next, U2OS cells expressing DsRed-PML-I and H4-PAGFP were incubated with MMS for 2 hours and chromatin regions directly surrounding PML bodies were photoactivated. DsRed-labeled PML bodies were observed losing contact with the fluorescently labeled chromatin and moving rapidly throughout the nucleus (Fig. 3A, B, Movie 3). The number of PML bodies that lost contact with the surrounding photoactivated chromatin varied among cells but seemed to increase when exposed to MMS for a longer time period. In some cells all PML bodies lost contact with the surrounding chromatin while in others only a few PML bodies did. Notably, during the time course in which PML bodies lose contact with chromatin, the photoactivated chromatin seemed to remain positionally stable and did not change its state of compaction in the cell nucleus. This indicates that the increase in PML body motion is not a result of an increase in chromatin mobility or a change in chromatin compaction as far it can be judged at the light microscopical level of resolution. By looking at the side-view

of cells, it appeared that the spatial distribution of PML bodies changed from being positioned throughout the 3D nuclear volume in untreated cells to a more confined localization in a Z-plane located at the centre in the 3D space of the nucleus following MMS treatment (Fig. 4). These side views in the x-z plane of cells support the conclusion that PML bodies dissociate from chromatin in response to MMS treatment.

Cajal bodies and speckles dissociate from chromatin in response to MMS treatment
Next, we investigated whether the spatial positioning and dynamics of speckles and Cajal bodies in U2OS cells is also altered by MMS treatment. U2OS cells were transiently transfected with the speckle marker construct DsRed-ASF together with construct histone H4-PAGFP. Chromatin regions surrounding speckles were selected and photoactivated and cells were imaged at regular time intervals for 10 minutes. Both speckles and photoactivated chromatin showed a constrained Brownian type of movement (Fig. 5A, Movie 4). Consistent with previous data, chromatin remained associated with speckles during the 10 minutes period in which the cells were imaged (Wiesmeijer et al., 2008). In response to MMS treatment, however, speckles became more dynamic as compared to the chromatin surrounding the speckles (Fig. 5B, Movie 5). This can also be concluded from a side view of the cell (Fig. 4). Frequently, speckles were observed losing contact with the surrounding photoactivated chromatin and remaining detached from it. Probably because speckles are relatively large nuclear structures with an irregular shape, their movements are still confined to a small nuclear region as if restricted by a physical barrier. Different from what we observed for PML bodies, speckles did not disperse after they lost their association with chromatin, even after imaging the cells for another hour. In addition, we did not observe speckles to merge in larger structures. The effect of MMS on speckle behavior proved reversible as cells that were first incubated with MMS and then washed and incubated in fresh medium revealed a speckle distribution and dynamics similar to that of untreated control cells.

To analyze the movement of Cajal bodies together with that of the surrounding chromatin, U2OS cells were cotransfected with DsRed-coilin and histone H4-PAGFP. Time lapse recordings of untreated cells showed that both the Cajal bodies and the surrounding chromatin display similar slow kinetics (Fig. 6A, Movie 6). After the cells were treated with MMS a response similar to that of PML bodies and speckles was observed. The Cajal bodies moved away from the surrounding photoactivated chromatin and became more dynamic (Fig. 6B, Movie 7). Like speckles, Cajal bodies did not become dispersed when they lost contact with chromatin in response to MMS treatment.

Discussion

Recently, it has been questioned whether nuclear bodies physically interact with chromatin foci. The interpretation of time-lapse imaging data of nuclear body and chromatin movement has lead to the proposition that nuclear bodies move by diffusion in a chromatin environment. The mobility of the bodies would then be reflected by the dynamics and accessibility of the chromatin environment (Görisch et al., 2004). The time-lapse imaging data presented in this study show that PML bodies, Cajal bodies and speckles are associated with chromatin. Treatment of cells with the DNA methylating agent MMS result in the dissociation of these nuclear bodies from chromatin. Consequently, both Cajal bodies and PML bodies move through a larger area in the cell nucleus. Consistent with data showing that PML bodies, Cajal bodies and speckles associate with specific chromatin loci, our results suggest that nuclear bodies are relatively immobile in the cell nucleus because of their association with chromatin.

PML bodies have been found associated with specific, mostly transcriptionally active, chromatin loci while a subset of PML bodies have been found transiently associated with telomeric DNA in telomerase negative cells which maintain telomere length by the alternative lengthening of telomeres (ALT) mechanism (Yeager et al., 1999; Molenaar et al., 2003; Jegou et al., 2009). Furthermore, indirect evidence suggests that PML plays a role in higher-order chromatin organization (Kumar et al., 2007). Consistent with these observations, PML has been shown to physically and functionally interact with the matrix attachment region (MAR)-binding protein, a special AT-rich sequence binding protein 1 (SATB1), to organize the major histocompatibility complex (MHC) class I locus into distinct higher-order chromatin-loop structures (Kumar et al., 2007). In this and in our previous work we have shown that treatment of cells with MMS leads to an increase in PML body dynamics before they disperse (Brouwer et al., 2009). By photoactivating chromatin regions that surround PML bodies, using histone H4 fused with a photoactivatable GFP as a marker for chromatin, and subsequent fluorescence time lapse confocal imaging, we show that the increase in PML body dynamics is not accompanied by an increase in chromatin dynamics at PML bodies. This observation is consistent with our previous work showing that MMS treatment has no major impact on general chromatin dynamics (Wiesmeijer et al., 2008). Therefore, the increase in PML body movement might be explained by a retraction of chromatin from the surface of the PML bodies. At present, it is not known to what extent PML bodies interact with chromatin. Also, it is not known which proteins are involved in this interaction. Electron spectroscopic images of human neuroblastoma cells suggest that PML bodies are connected to chromatin at multiple sites (Eskiw et al., 2004).

Since MMS induces alkylating DNA damage and activates a DNA repair mechanism it is conceivable that the increase in PML body dynamics is a direct response to the DNA damage. Recently, it has been shown that following DNA damage, several repair factors transit through PML NBs in a temporally regulated manner implicating these bodies in DNA repair (Dellaire & Bazett-Jones, 2004). It is possible that PML bodies dissociate from chromatin as a result of DNA methylation and finally disassemble into large supramolecular complexes, dispersing associated repair factors to sites of damage.

In addition to PML bodies, Cajal bodies have also been reported to be associated with chromatin sites, including histone and snRNA genes. Although it is suggested that these bodies alternate between an association state with chromatin and a diffusion state in which the bodies move within the interchromatin space (Platani et al., 2002), the association state may continue as long genes are transcribed and ATP is available (Platani et al., 2002).
Compared to PML and Cajal bodies, speckles do not show a dramatic increase in mobility following MMS treatment, although various genes have been observed positioned at the periphery of speckles. Speckles have been implicated in mediating gene associations and to function as hubs (Brown et al., 2008). Most likely, it is the size of this body and its irregular shape that limits its mobility in the chromatin environment.

Methylmethane sulfonate (MMS) is a compound that methylates biologically important nucleophilic sites in DNA, through an S_N2 reaction, and interacts with amino acids in proteins (Paik et al., 1984). When cells are treated with MMS, PML bodies lose their integrity and become dispersed (Conlan et al., 2004; Brouwer et al., 2009). The mechanism of their dispersal is not clear but could be a result of the applied DNA damage. However, there are other examples of cellular stress that cause PML bodies to become dispersed. Environmental stresses such as heat shock, heavy metal shock, or viral protein expression, cause the dissociation of PML bodies into many smaller punctate domains (Maul et al., 1995). MMS has been found to induce hyperacetylation of both cytoplasmic and nuclear proteins as well (Lee et al., 2007). Notably, the acetylation level upon MMS treatment was strongly correlated with the susceptibility of cancer cells, and the enhancement of MMS-induced acetylation by histone deacetylase (HDAC) inhibitors was shown to increase the cellular susceptibility to cancer.

Since PML bodies become dispersed after applying DNA damage, and Cajal bodies and speckles do not, this could implicate that regulated dispersal is an important and functional property of PML bodies. The regulated dispersal of PML bodies may facilitate the enhanced release of DNA repair proteins from NB depots, in order to respond adequately to extensive DNA damage (Conlan et al., 2004). Both speckles (Mintz et al., 1999) and Cajal bodies (Lam et al., 2002) have been isolated and this has not been achieved for PML bodies, further implying structural differences between PML bodies and other nuclear bodies. It is possible that PML body integrity depends on their interaction with chromatin, and that as soon as the interaction with chromatin is lost, PML bodies become dispersed. Further studies are necessary to investigate the effect of the association between chromatin and PML bodies on PML body stability. Also, alkylation or acetylation of the PML protein or other proteins present inside PML bodies may affect the integrity of PML bodies.

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Figures

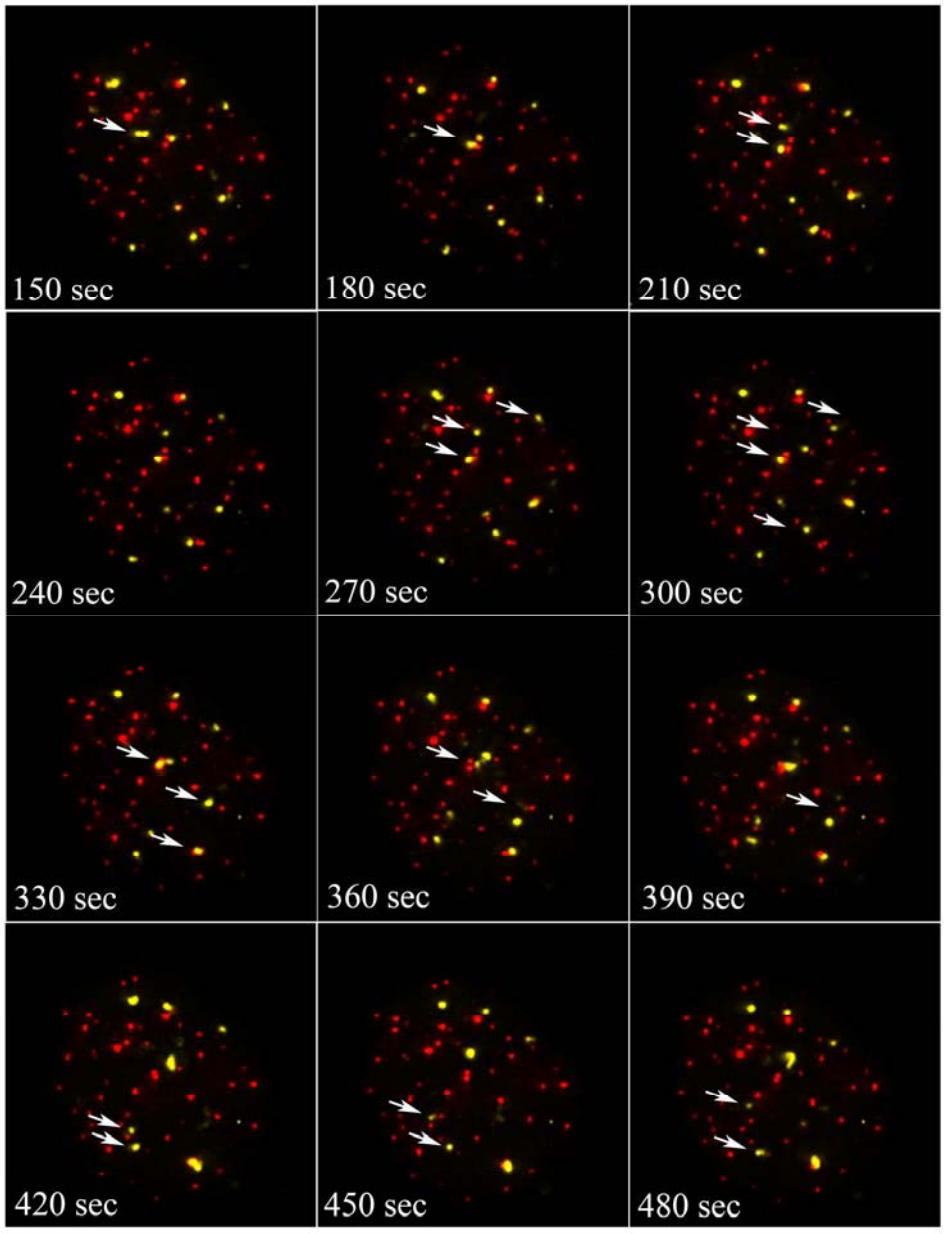

Figure 1. PML/TRF1 dynamics are shown in U2OS cells transfected with EYFPPML and DsRedTRF1 and subsequently treated with MMS for 2 hours. PML bodies are more dynamic than telomeres and fuse with other PML bodies and with telomeres. Dynamic or fusing PML bodies are indicated by arrows.

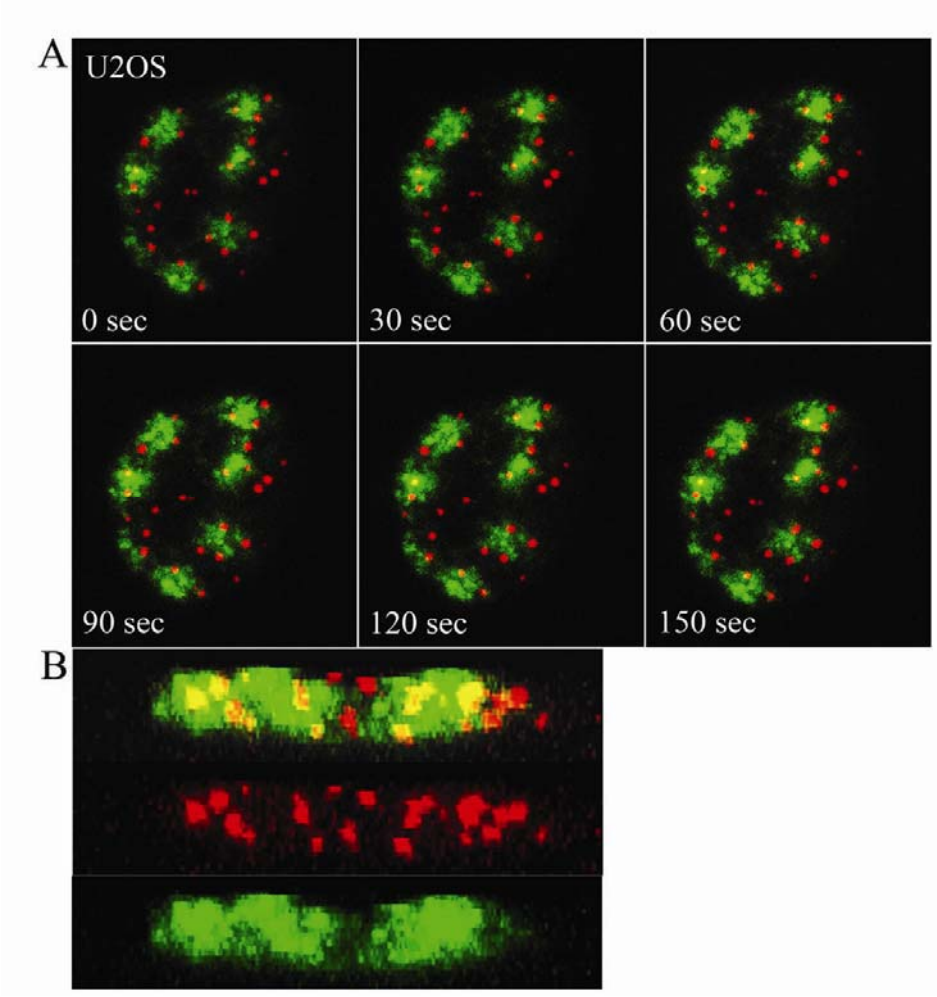

Figure 2 A) Dynamics of PML bodies are shown in untreated U2OS cells. Photoactivatable Histon4 (H4-PAGFP) was used as a marker for chromatin and DsRed-PML-I was used as a marker for PML bodies. H4-PAGFP was photoactivated around PML bodies using the 405 nm laser on a SP5 confocal microscope. DsRed-PML-I together with photoactivated histone H4-PAGFP surrounding these bodies show a similar slow mobility. Images were acquired with 2-4% laser power, 400 Hz scanning frequency and 512 * 512 pixels per frame. B) A side-view of the 15-25 Z-stack slices (thickness 0.4 μm) of the same cell is shown, time step t=0 seconds.

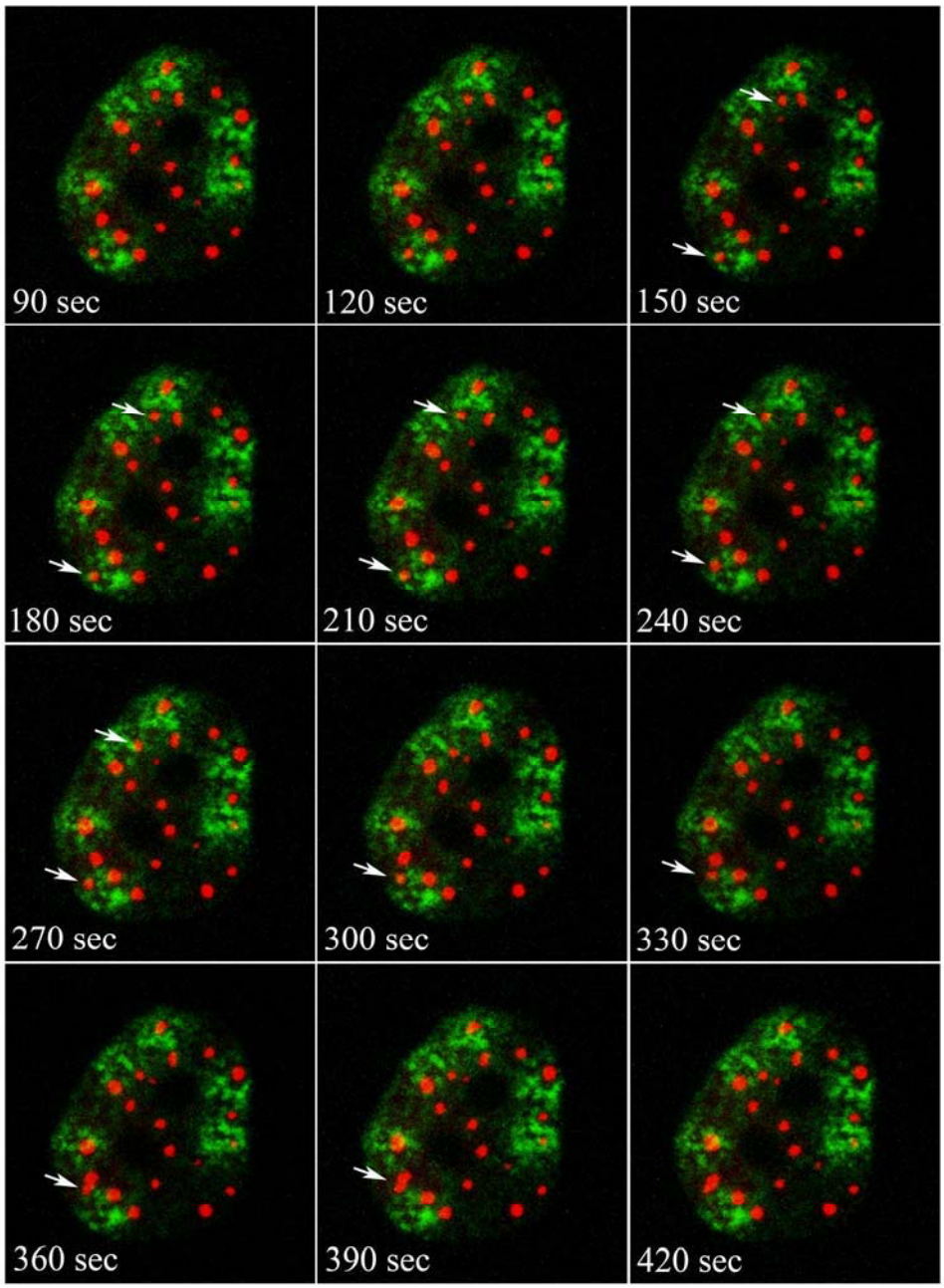

Figure 3A. H4-PAGFP surrounding PML bodies was photoactivated using the 405 nm laser on a SP5 confocal microscope after 2 hours of MMS treatment. Arrows indicate PML bodies that lose contact with the surrounding chromatin and move away from their original positions, displaying increased dynamics compared to the photoactivated H4-PAGFP.

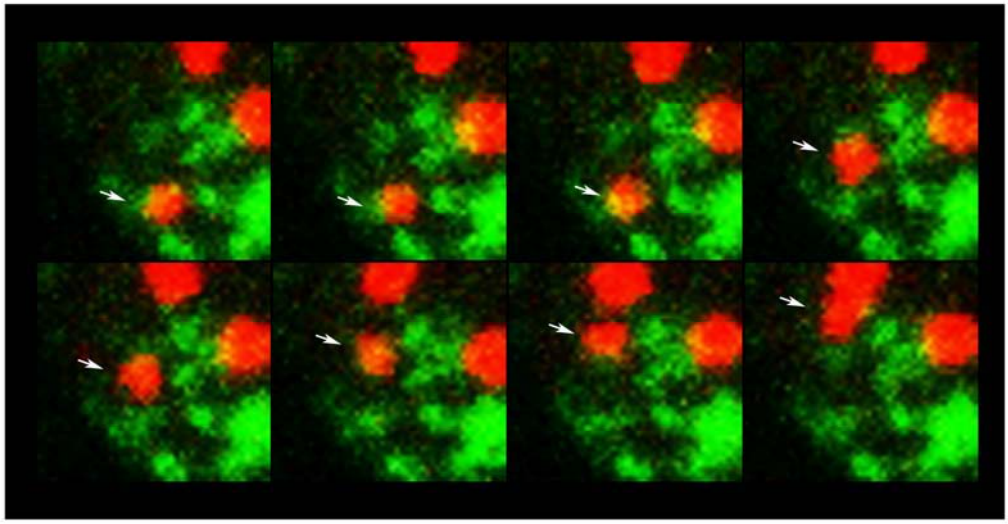

Figure 3B. PML bodies are dynamic after MMS treatment and lose contact with the surrounding chromatin. A section of the nucleus from figure 3A is shown at higher magnification and a PML body dissociates from the surrounding chromatin and moves away from its original position, as a result of MMS induced DNA damage.

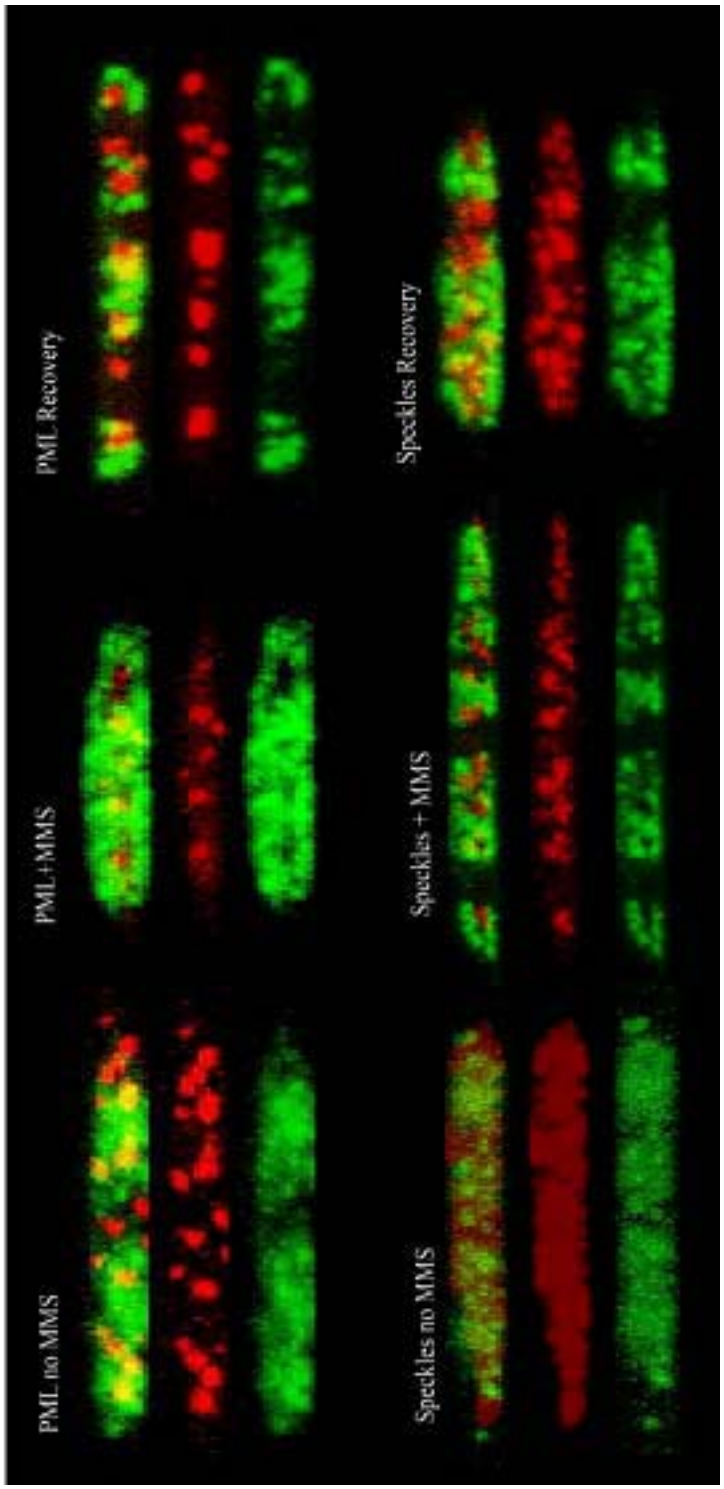

Figure 4. A side-view of photoactivated H4-PAGFP surrounding PML bodies, speckles and Cajal bodies using the 405 nm laser on the SP5 confocal microscope. The spatial distribution of PML bodies appears changed from being positioned throughout the 3D nuclear volume in untreated cells to a more confined localization in a Z-plane located at the centre in the 3D space of the nucleus following MMS treatment.

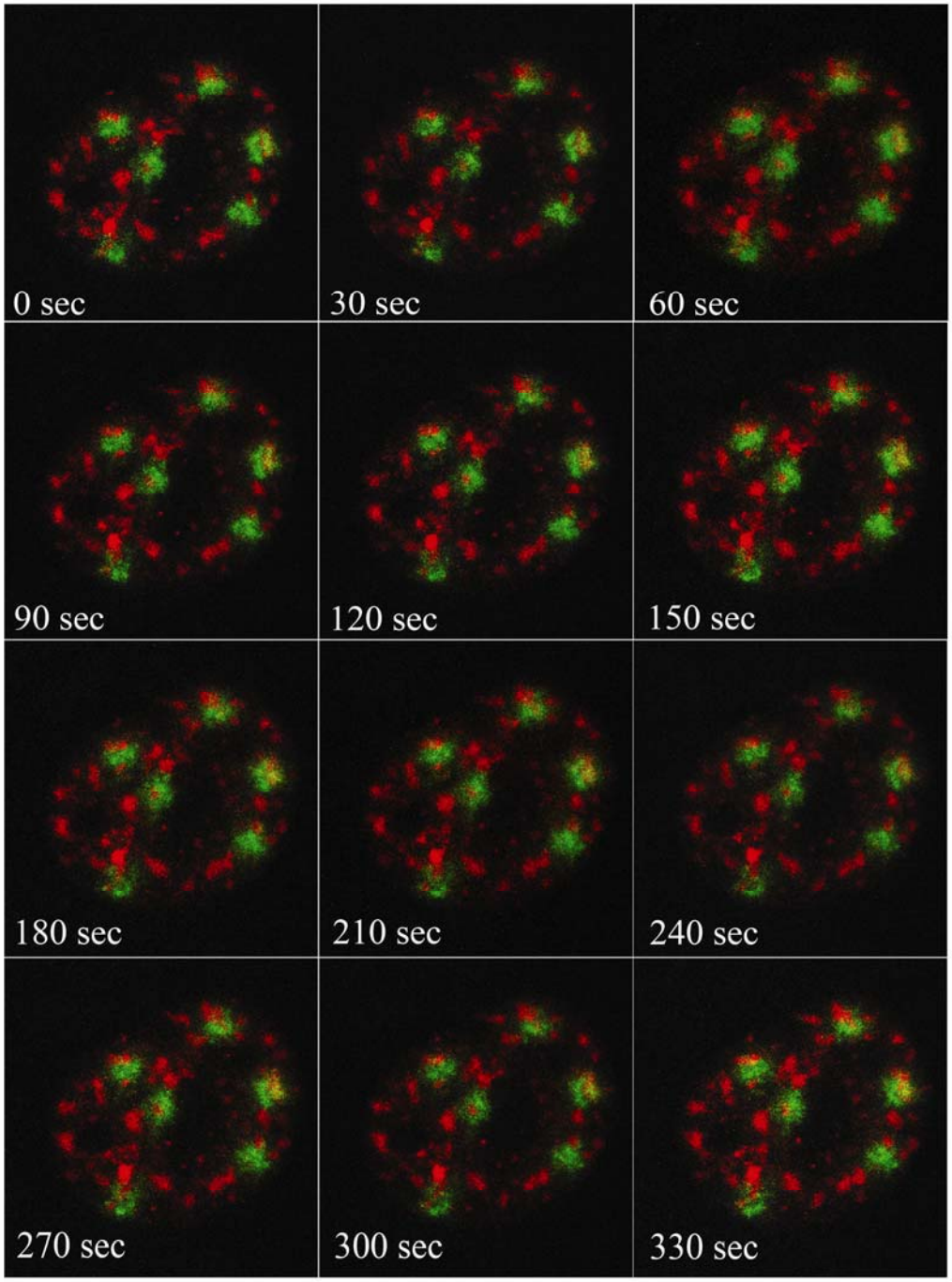

Figure 5A. H4-PAGFP surrounding speckles was photoactivated in U2OS cells transfected with DsRed-ASF as a marker for speckles. Chromatin regions surrounding speckles were selected and photoactivated and cells were imaged at regular time intervals for 10 minutes. Similar to PML bodies, speckles are rather immobile relative to the surrounding chromatin, suggesting that there is an interaction between speckles and the surrounding chromatin that keeps them in a fixed position.

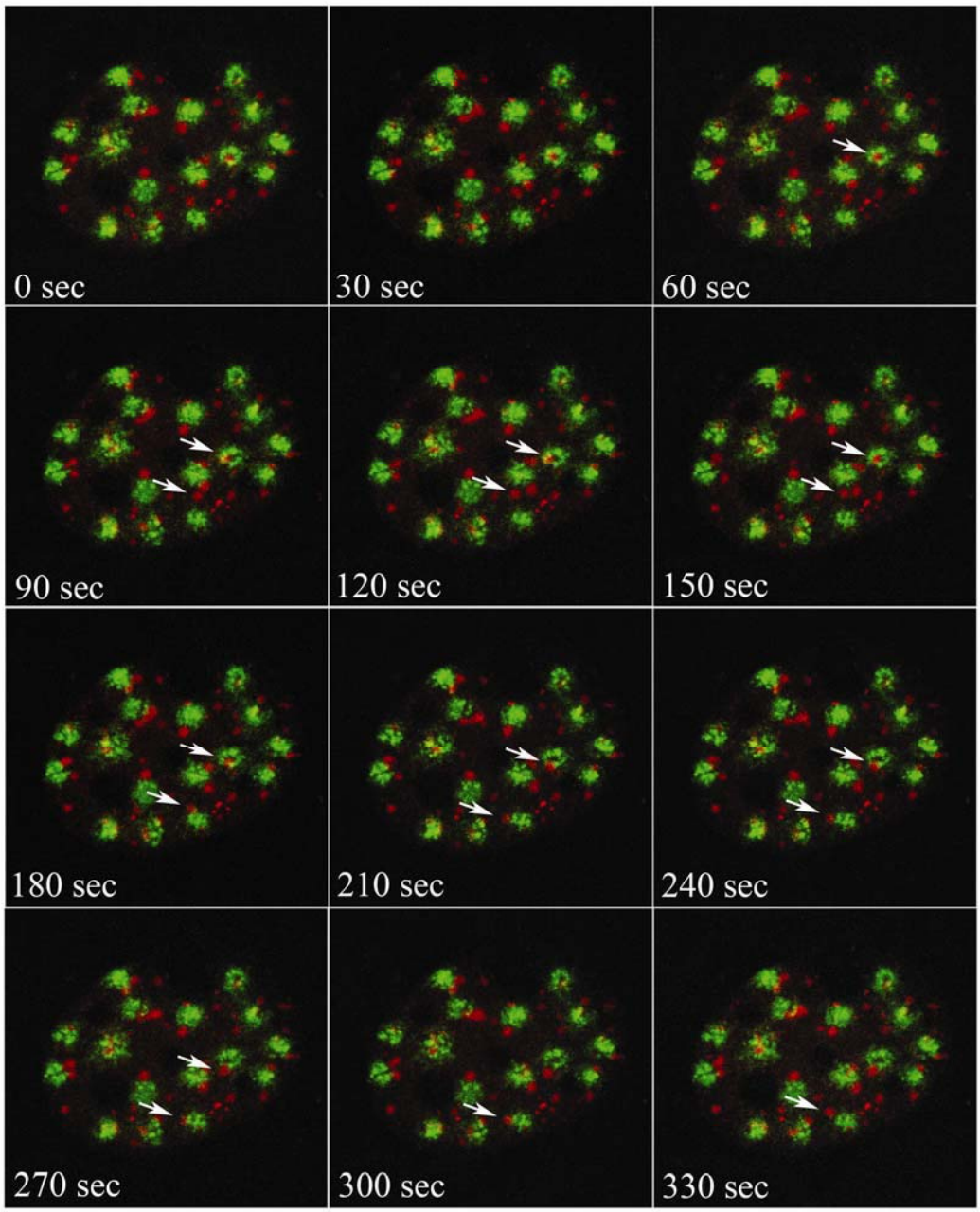

Figure 5B. In response to 2 hours of MMS treatment U2OS cells transfected with H4-PAGFP and DsRed-ASF show a strong increase in dynamics of speckles compared to the chromatin surrounding the speckles. Dynamic speckles that move away from their original position are indicated by arrows.

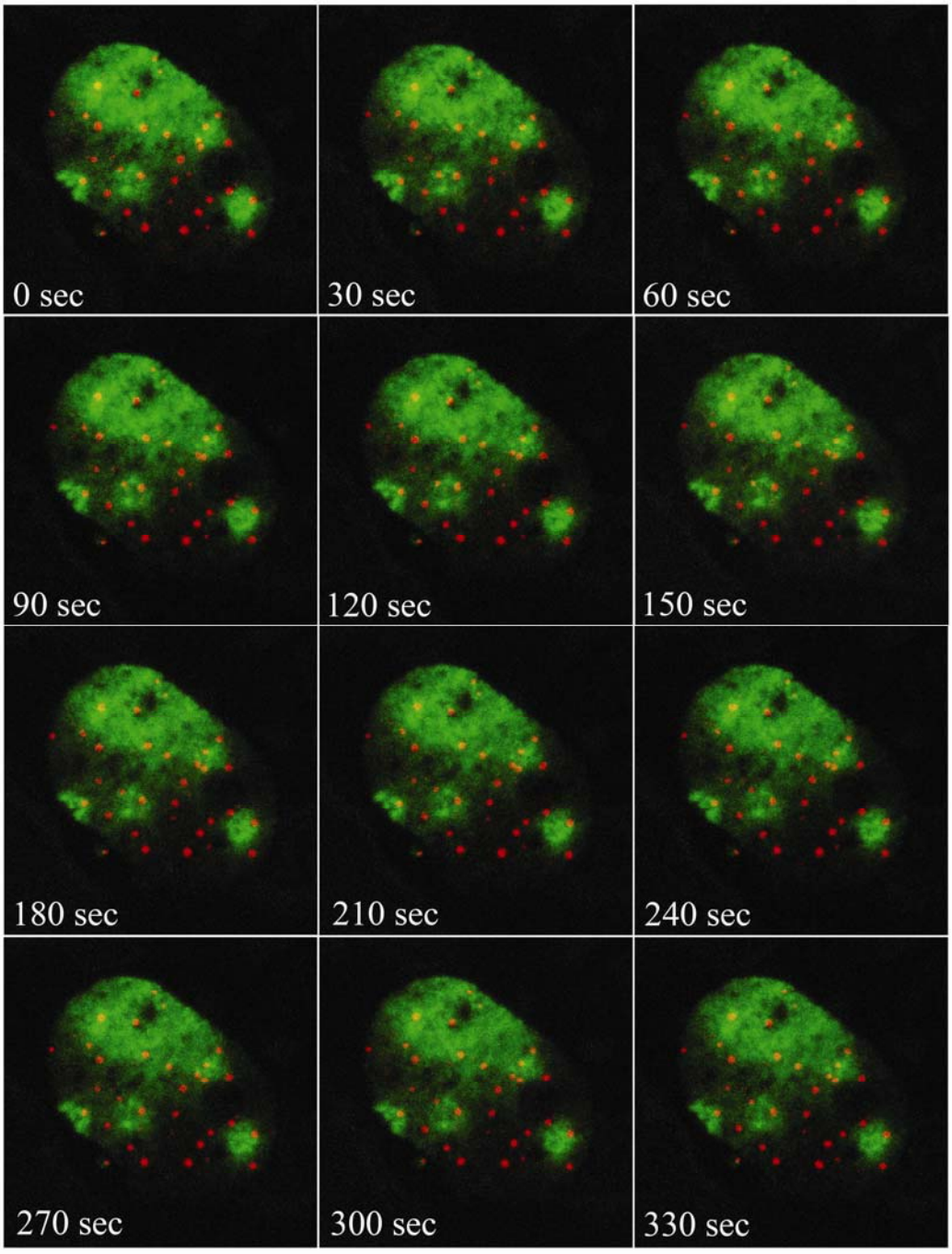

Figure 6A. Histon4-PA was photoactivated around Cajal bodies to study their dynamics compared to the surrounding chromatin. Similar to PML bodies and speckles, Cajal bodies are nearly immobile relative to the chromatin, suggesting that there is an interaction between the Cajal bodies and the surrounding chromatin that keeps the Cajal bodies in a fixed position.

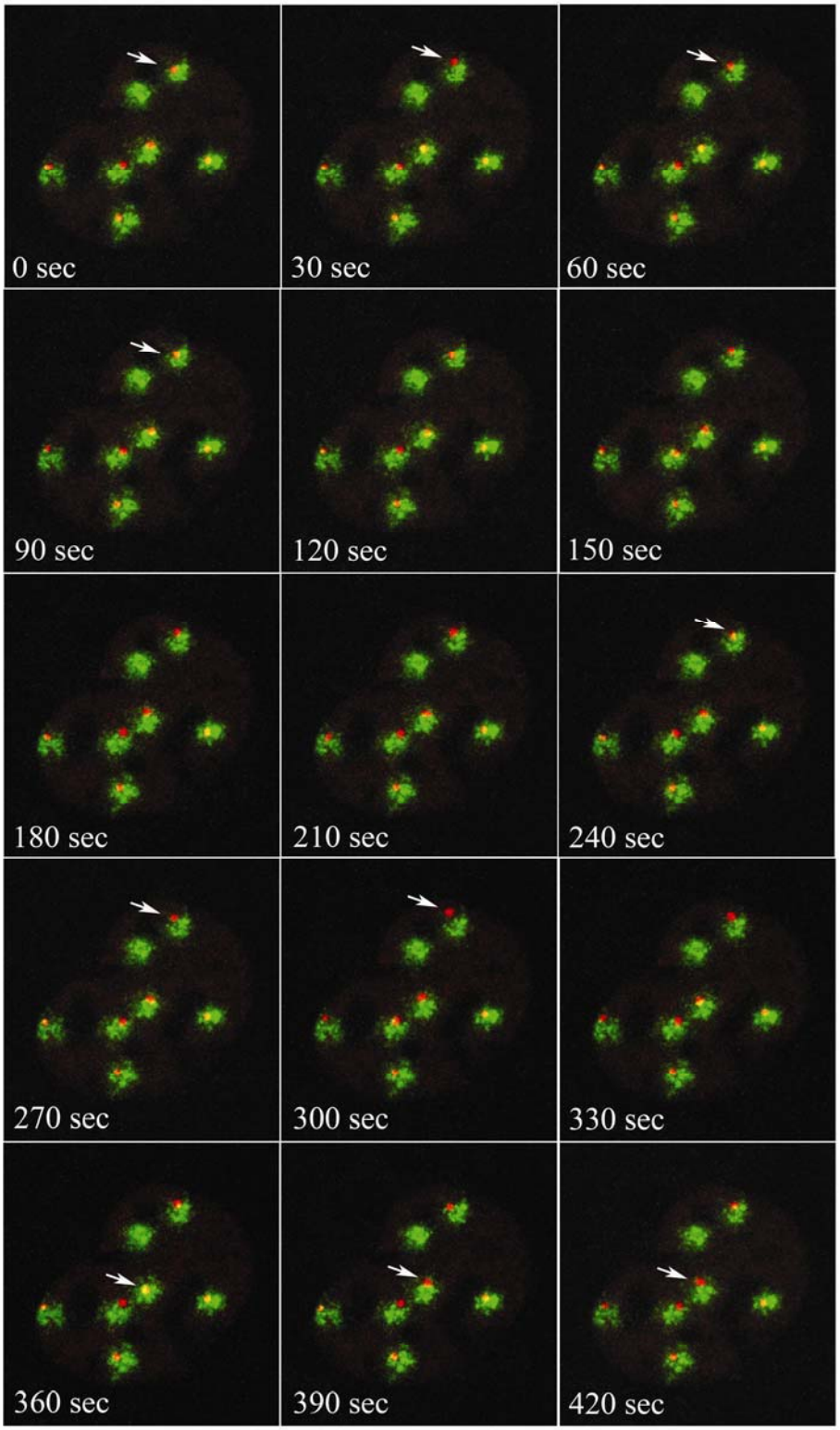

Figure 6B. As a response to MMS treatment Cajal bodies display increased dynamics similar to PML bodies and speckles and likewise Cajal bodies appear to lose contact with the surrounding chromatin. Dynamic Cajal bodies are indicated by arrows.