Differentiation (2008) 76:83–90 DOI: 10.1111/j.1432-0436.2007.00234.x

ORIGINAL ARTICLE

Karien Wiesmeijer · Ilke M. Krouwels · Hans J. Tanke · Roeland W. Dirks

Chromatin movement visualized with photoactivable GFP-labeled histone H4

Received May 9, 2007; accepted in revised form August 22, 2007

Abstract The cell nucleus is highly organized with chromosomes occupying discrete, partially overlapping territories, and proteins that localize to specific nuclear compartments. This spatial organization of the nucleus is considered to be dynamic in response to environmental and cellular conditions to support changes in transcriptional programs. Chromatin, however, is relatively immobile when analyzed in living cells and shows a constrained Brownian type of movement. A possible explanation for this relative immobility is that chromatin interacts with a nuclear matrix structure and/or with nuclear compartments. Here, we explore the use of photoactivatable GFP fused to histone H4 as a potential tool to analyze the mobility of chromatin at various nuclear compartments. Selective photoactivation of photoactivatable-GFP at defined nuclear regions was achieved by two-photon excitation with 820 nm light. Nuclear speckles, which are considered storage sites of splicing factors, were visualized by coexpression of a fluorescent protein fused to splicing factor SF2/ASF. The results reveal a constrained chromatin motion, which is not affected by transcriptional inhibition, and suggests an intimate interaction of chromatin with speckles.

Key words chromatin dynamics · fluorescent protein · two-photon microscopy · photoactivation · speckles · live cell imaging

Karien Wiesmeijer · Ilke M. Krouwels · Hans J. Tanke · Roeland W. Dirks (✉)
Department of Molecular Cell Biology
Leiden University Medical Center
Postal zone S1-P
P.O. Box 9600, 2300 RC Leiden, The Netherlands
Tel: +31 715269222
E-mail: r.w.dirks@lumc.nl

Introduction

The mammalian interphase nucleus is highly organized, which is reflected by the organization of chromosomes in discrete chromosome territories during interphase and the localization of many nuclear proteins to discrete nuclear compartments. Chromosome territories vary in size and shape, intermingle to some extent, and their spatial positioning in the nucleus appears to be non-random as gene-poor chromosomes locate toward the nuclear periphery and gene-rich chromosomes locate toward the nuclear interior (Croft et al., 1999; Boyle et al., 2001; Cremer et al., 2001, 2006; Branco and Pombo, 2006). This spatial three-dimensional positioning correlates well with the preferred peripheral localization of transcriptional silent chromatin in the cell nucleus (Andrulis et al., 1998) but also with local gene density (Goetze et al., 2007; Küpper et al., 2007). In addition, specific spatial relationships among individual chromosome territories have been shown. As a consequence, translocations may occur more frequently among certain chromosome pairs than others (Roix et al., 2003). This spatial three-dimensional organization of chromosome territories is not static but dynamic and changes due to dramatic alterations in gene activity as a result of cell proliferation or differentiation (Bridger et al., 2000; Kim et al., 2004). However, other data indicate that chromosomes are predominantly randomly organized (Cornforth et al., 2002), suggesting that a strict order of chromosome neighbors is not required for proper cell functioning.

Within individual chromosome territories, a variety of levels of chromatin organization can be discriminated, ranging from more condensed compact regions to open unfolded structures (van Driel et al., 2003). Some of the unfolded chromatin creates loops that emanate from the chromosome territory in a transcription-dependent way. The functional significance of such loops

is not yet clear but they may provide genes that are positioned at these loops a better access to components of the transcription machinery (Volpi et al., 2000; Mahy et al., 2002). Transcriptional activity, however, is not limited to genes that have a position at the border or even outside the chromosome territory or subchromosomal domain. Active genes are also found distributed throughout chromosome territories. Nevertheless, it is assumed that some transcriptionally active genes are collectively arranged in functional transcription units or factories where their activities are controlled in a coordinated manner (Chakalova et al., 2005). Hence, more important than being at the edge or interior of a chromosome territory is the spatial positioning of genes in transcription-competent environments. For a number of genes, it was shown that their spatial localization in the nucleus changed after altering their transcriptional activity (Zink et al., 2004). Also, the positioning of transcriptionally active genes close to centromeric or peripheral heterochromatin leads to gene silencing (Brown et al., 1999; Francastel et al., 1999; Li et al., 2001). Thus, the spatial positioning of at least a subset genes is most likely dynamic in nature and is possibly directed by cellular activities, including transcription, RNA processing, and DNA repair. Consequently, nuclear positioning is a critical determinant of gene regulation (Kosak and Groudine, 2002; Cremer et al., 2004; Lanctot et al., 2007).

An interesting question, yet to be answered, is whether associations of chromosome loci with nuclear compartments, like Cajal bodies, PML bodies, and speckles, could possibly contribute to the establishment of a nuclear organization and possibly to some nuclear functions as well. Histone genes as well as snRNA and snoRNA loci were shown to associate with Cajal bodies (Smith et al., 1995; Smith and Lawrence, 2000; Frey and Matera, 2001; Shopland et al., 2001) and consistent with these findings, also many active, non-characterized, transcription sites were shown to associate with Cajal bodies (Kießlich et al., 2002), suggesting that these bodies play a more general role in facilitating transcription or RNA processing.

Other chromatin regions were shown to associate preferentially with PML nuclear bodies rather than with Cajal bodies. Like Cajal bodies, however, there seems to be a preference for transcriptionally active chromatin regions to associate with PML bodies (LaMorte et al., 1998; Boisvert et al., 2000; Kießlich et al., 2002; Wang et al., 2004). Interestingly, chromatin seems to establish true physical connections with PML bodies as shown for 10-nm chromatin fibers that make physical connections to the cores of PML bodies (Eskiw et al., 2004). Therefore, it has been proposed that PML bodies may serve as anchoring sites for euchromatin (Eskiw et al., 2004), consistent with the observation that PML bodies are nearly immobile in the nucleus (Wiesmeijer et al., 2002).

Most prevalent associations of gene loci with a nuclear compartment have, however, been found for nuclear speckles. Speckles, or SC-35 domains, are distinct irregular-shaped compartments containing poly(A) RNA, various RNA processing factors as well as many other factors as revealed by proteomic analysis (Saitoh et al., 2004). Many different genes and gene-rich R-bands on chromosomes have been found in association with speckles, which themselves are immobile in the nucleus (Shopland et al., 2003). Interestingly, also gene transcripts were shown to access these speckle domains, suggesting that these speckles play a role in RNA processing (Hattinger et al., 2002; Shopland et al., 2002).

Together, these studies indicate that nuclear bodies function in close association with chromatin by which it may regulate nuclear functions. However, the nature and dynamics of associations between nuclear compartments and chromatin in live cells have thus far been poorly studied, which is also because of technical limitations.

In this study, we show the applicability of photoactivatable GFP (PA-GFP) to study the movement of chromatin in the cell nucleus, also in relation to different nuclear compartments. For this purpose, we expressed histone H4 fused to PA-GFP in combination with a fusion protein that localizes specifically to a certain nuclear compartment in U2OS cells. Activation of PA-GFP is achieved by irradiation with 408 nm light or by two-photon excitation at 820 nm, leading to a threefold increase in fluorescence upon excitation with 488 nm light (Patterson and Lippincott-Schwartz, 2002). Chromatin movement is registered by taking time-lapse images at regular time intervals with 488 nm light. The advantage of the two-photon approach is that the PA-GFP fusion protein can be irradiated selectively within a defined region or spot that is positioned anywhere in the three-dimensional space of a cell and that any phototoxicity in cells associated with single photon activation can be ruled out (Post et al., 2005).

Methods

Generation of constructs

The histone H4 (Z46261) sequence was amplified from a cDNA that was generated from human osteosarcoma cells (U2OS), using the forward primer 5′-GCGCGCGGTACCATGTCTGGTAGAGGCAAAGG-3′containing the KpnI site and the reverse primer 5′-GCGCGCCCCGGGTCAGCCACCAAAGCCGTACA-3′ containing the *Xma*I site. Purified PCR fragments were inserted in-frame into the *Kpn*I-*Xma*I fragment of pPA-GFP-C1 (Patterson and Lippincott-Schwartz, 2002). The coding sequence of SF2/ASF was originally cloned into the pEGFP-C1 vector (Molenaar et al., 2004) and subcloned into DsRed-Express (Clontech, Mountain View, CA). The plasmid EYFP-SUV39H1 has been described before (Krouwels et al., 2005).