Chromatin Dynamics in Living Cells Studied by Tracking in a Two-Photon Microscope

Valeria Levi and Enrico Gratton
Laboratory for Fluorescence Dynamics,
University of Illinois, Urbana, IL 61801

Introduction

Significant advances in the fields of microscopy and molecular biology have in recent years allowed direct visualization of chromatin structure and dynamics in live cells, changing the initial vision of an interphase nucleus containing randomly arranged and static DNA. Several studies in different organisms have described the motion of specific chromatin regions when undergoing apparent Brownian motion limited to a nuclear subregion [1-3].

However, there are certain reports indicating that the motion can be sensitive to ATP and changes in metabolic status [4]. Moreover, specific regions of the DNA show a preferential distribution in the nucleus, e.g. late replicating DNA regions are in the nucleus periphery while early replicating regions are preferentially located in more interior positions. These reports suggest that important nuclear functions such as DNA transcription are accompanied by defined changes in the position of the sequence [5-7]. However, there is little experimental support to date for an active mechanism driving these chromatin movements.

Materials and Methods

Cell Lines

The C6-14 CHO cell line has 10-20 copies of a plasmid containing lac-operator repeats and a DHFR cDNA transgene driven by a viral promoter. These copies are integrated at a single, internal chromosome site. The cell line stably expresses EGFP-lac repressor. Hence, the lac operator repeats region is detected as a bright spot in a fluorescence image (Figure 1).

The DHFR-BAC CHO cell line contains multiple copies (8-10) of a BAC with a 170 kbp mouse genomic DNA insertion including the DHFR locus. A 256-copy, lac-operator direct repeat was inserted 75 kbp upstream in the DHFR gene. This cell line also stably expresses EGFP-lac repressor. Thus, the cells present multiple bright spots instead of a single spot as in the C6-14 cell line due to the multiple copies of the lac-operator repeat.

Microscopy Setup and Tracking Procedure

The tracking experiments were carried out with the Olympus IX70 microscope. The two-photon excitation source was a mode-locked titanium-sapphire laser (Mira 900, Coherent, Palo Alto, CA) pumped by an argon ion laser (Innova 300, Coherent) tuned to 920-nm. The laser power at the sample was in the 1 to 10 mW range. The light is directed into the microscope by two galvomotor-driven scanning mirrors (Cambridge Technologies, Watertown, MA) through a scanning lens. During the tracking procedure, the two scanning mirrors are moved independently by two synchronized voltage sine waves shifted 90° relative to each other and generated in a computer card (three-axis card, ISS, Champaign, IL). As a consequence, the laser moves in a circular path. The frequency of the circular orbit was 250 Hz. The position of the scanning center is determined by the offset values of the sine waves.

The laser light is reflected with a low-pass dichroic mirror (transmission between 370- and 630-nm, Chroma Technology, Brattleboro, VT) and focused on the sample with a 63x (dry) 0.8-NA objective. Fluorescence emission collected by the objective passes through the dichroic and a

short-pass filter, exiting the microscope through the side port. A Hamamatsu H7422P-40 photomultiplier tube was used as a detector with its output amplified, passed through a photon counting discriminator (PX01 Photon Counting Electronics, ISS), and counted with a data acquisition card (ISS). The experiments are controlled by a data acquisition program (SimFCS, Laboratory for Fluorescence Dynamics, Champaign, IL).

The particle-tracking method used for this application is described in detail in the ISS Technical Note: Particle Tracking in a Two-Photon Microscope. The frequency of data acquisition and the circular orbit were 32 kHz and 250 Hz, respectively. Each cycle of the tracking routine consisted of 8 orbits, thus the time resolution was 32 ms.

This method can also be used to track two particles simultaneously. The initial positions for the particles are set by selecting them interactively from the fluorescence image computer display. The tracking routine starts on top of one of the particles; after a given number of cycles, the laser jumps to the position of the second particle where it performs the same routine for tracking. Then the center of scanning is moved to the new position determined previously for the first particle. Thus the positions of the particles are recovered alternately.

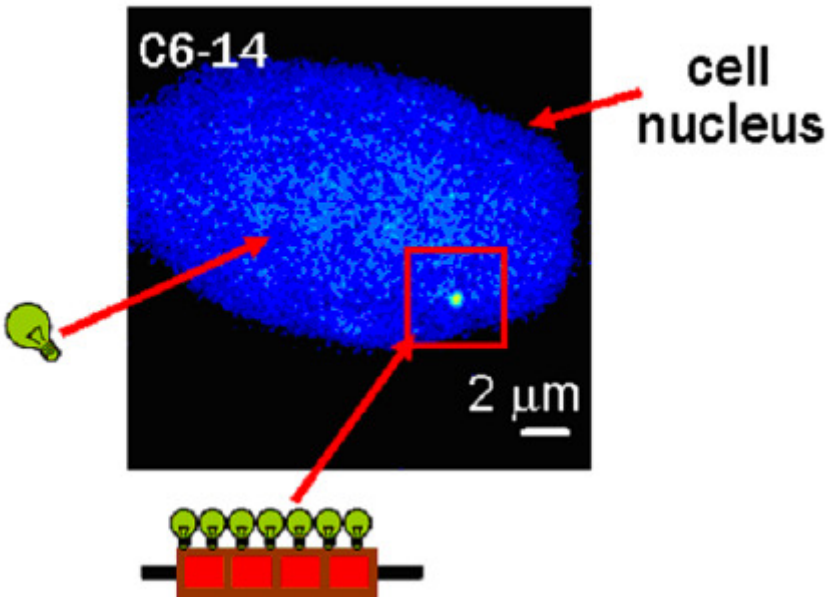

Figure 1. Labeling a chromatin sequence in living cells. The bright spot in the 2-photon fluorescence image corresponds to EGFP-lac repressor molecules (green) bound to the lac operator repeats (orange).

Results

Figure 2A shows a representative trajectory recovered for a C6-14 cell. 77% of the measured trajectories (N = 60) presented larger regions of apparent constrained diffusion connected through curvilinear paths or jumps.

We quantitatively analyzed these trajectories and determined that the characteristic value for the jump size was 150-nm. Also, we calculated the characteristic time that the sequence spends moving within regions of confined motion obtaining a value of 56 s. From the analysis of the number of points in the trajectory during the jumps we could estimate that they occur within 0.3-2 s.

To further distinguish chromatin dynamics from small, submicrometer motions of the nucleus or the cell, we simultaneously tracked two EGFP-tagged sequences within DHFR-BAC nuclei. In

36% of the observed cells (N = 80), similar jumps were observed in the trajectories to those seen for C6-14 cells.

For those DHFR-BAC cells that showed jumps in both trajectories, we analyzed whether they occurred simultaneously and found out that jumps are not correlated in 90% of the cells (Figure 3). This result indicates that most jumps are due to local processes, which are not transmitted long distances through the chromatin chain or nuclear subregion.

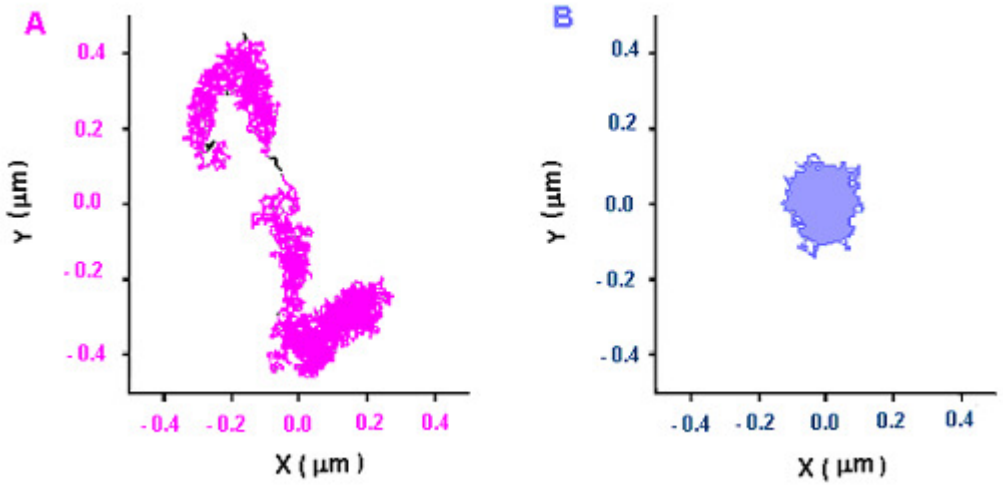

Figure 2. Dynamics of chromatin in living C6-14 cells. Trajectories recovered in a control (A) and an azide-treated (B) cell.

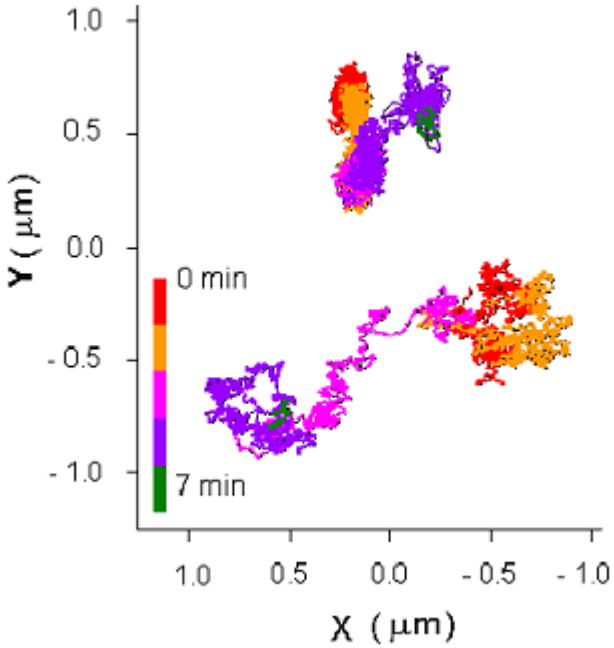

Figure 3. Trajectories registered by simultaneous tracking of two labeled sequences in the DHFR-BAC cell line. The color code represents the time evolution of the trajectories.

To further characterize the physical process responsible for the chromatin position jumps we combined theoretical and experimental analyses. First, we calculated the absolute instantaneous velocity in each trajectory at all the trajectory points. We examined the velocity distribution and observed that the sequence moves faster during the jumps in comparison to the rest of the trajectory. Also the jumps occurred, on average, four times faster than predicted according to a random diffusion model.

We followed the motion of the fluorescent-tagged sequences in cells in which ATP levels had been depleted by previous incubation with sodium azide and 2-deoxyglucose (Figure 2B). After ATP depletion, we did not observe jumps in the trajectories (N = 30), indicating that these curvilinear jump movements are not the consequence of passive diffusion but occur through an energy-dependent process. Also, we measured the chromatin dynamics in C6-14 cells at 21°C after preincubating the cells for 4 h at this temperature. In contrast to the very small changes in motion expected for diffusive processes, the number of trajectories presenting jumps decreased from 77% to 47% (N = 36).

Conclusion

We observed that chromatin in interphase cells undergoes rapid transition between localized motion regimes via jumps in which the locus appears to move in a curvilinear path. Moreover, our results indicate that these jumps reflect energy-dependent chromatin movements rather than diffusive motions driven by thermal fluctuations, supporting a model for active transport of chromatin in the nucleus.

References:

1. W.F. Marshall, A. Straight, J.F. Marko, J. Swedlow, A. Dernburg, A. Belmont, A.W. Murray, D.A. Agard, and J.W. Sedat, Curr. Biol, 7,930, 1997.
2. J.R. Chubb, S. Boyle, P. Perry, and W.A. Bickmore, Curr. Biol., 12, 439, 2002.
3. J. Vazquez, A.S. Belmont, and J.W. Sedat, Curr. Biol., 11, 1227, 2001.
4. P. Heun, T. Laroche, K. Shimada, P. Furrer, and S.M. Gasser, Science, 294, 2181, 2001.
5. J. Ferreira, G. Paolella, C. Ramos, and A.I. Lamond, J. Cell Biol., 139, 1597, 1997.
6. D.L. Spector, Annu. Rev. Biochem, 72, 573, 2003.
7. T. Tumbar and A.S. Belmont, Nat. Cell Biol, 3, 134, 2001.

1602 Newton Drive
Champaign, Illinois 61822 USA
Telephone: (217) 359-8681
Telefax: (217) 359-7879
Email: iss@iss.com