FEBS Letters xxx (2015) xxx–xxx

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Chromosome dynamics and folding in eukaryotes: Insights from live cell microscopy

Kerstin Bystricky

[a] University of Toulouse, UPS, Toulouse, France
[b] Laboratoire de Biologie Moléculaire Eucaryote, CNRS, LBME, 31062 Toulouse, France

ARTICLE INFO

Article history:
Received 25 May 2015
Revised 8 July 2015
Accepted 11 July 2015
Available online xxxx

Edited by Wilhelm Just

Keywords:
Live cell fluroescence microscopy
Chromosome conformation
Transcription

ABSTRACT

How chromosomes are folded and how this folding relates to function remain fundamental questions. Answering them is rendered difficult by the stochasticity of chromatin fiber motion which inevitably results in heterogeneity of the populations analyzed. Even if single cell analyses are beginning to yield precious insights, how can we determine whether a snapshot of position is related to function of the probed locus or cell-type? Fluorescence labeling of DNA at single or multiple loci allows determination of their position relative to nuclear landmarks and to each other, enabling us to derive physical parameters of the underlying chromatin fiber. Here I review the contribution of quantitative spatial and temporal analysis of labeled DNA to our understanding of chromosome conformation in different cell types, highlighting live cell imaging techniques and large scale geometrical analysis of multiple loci in 3D.

1. Introduction

Chromosomes take up characteristic positions within the eukaryotic nucleus [1–3], yet the chromatin fiber is in constant motion and its structure is sensitive to biological processes. The spatial organization of the genome can be studied on several scales: composition and density of nucleosome particles, looping of fibers, formation of chromosome domains and positioning relative to landmarks of nuclear architecture. These properties are related both to each other and to chromatin function, making it important to keep a clear distinction between them (Fig. 1).

In the past two decades, fluorescence microscopy approaches to studying chromatin have benefitted from significant improvements in hardware and in computational image analysis, as well as from new tools for labeling DNA in vivo. Fluorescence imaging both of fixed cells, using in situ hybridization and immunofluorescence, and of living cells, using tracking of loci labeled with protein chimaeras, have revealed preferential positioning of specific loci and distribution relative to each other. It has also brought to light correlations between chromatin organization and local or global changes in DNA metabolism such as transcription and repair [4–10].

This review focusses on the contribution of fluorescence imaging to in situ analysis of DNA dynamics and chromosome folding in yeast and in mammalian cells, the most intensively studied models, while acknowledging that studies of worm, drosophila and plant cells have also provided important insights [see for example [11–13]].

Three major aspects will be discussed:

- Intrinsic properties of the chromatin fiber, compaction and flexibility.
- Chromosome conformation and folding.
- Chromatin behavior during transcription activation.

2. Main text

2.1. Visualizing DNA in living eukaryotic cells

Nuclear DNA can be visualized in bulk either directly by incorporating injected or transfected fluorescent nucleotides during replication, or indirectly by expressing fluorescent histones. Visualization of DNA at specific genomic loci requires labeling techniques that create a fluorescent focus detectable above background levels. Several systems useful for live cell imaging have been developed: FROS (fluorescent repressor operator system) [14], which is based on the insertion of numerous bacterial lac, tet or lambda operator sequences to which fluorescent repressor fusion proteins bind; CRiSPR/inactive Cas9 [15,16] or TALE [17,18], which when fused to fluorescent proteins enable visualization of the naturally occurring repetitive sequences they target;

E-mail address: kerstin@biotoul.fr

http://dx.doi.org/10.1016/j.febslet.2015.07.012
0014-5793/

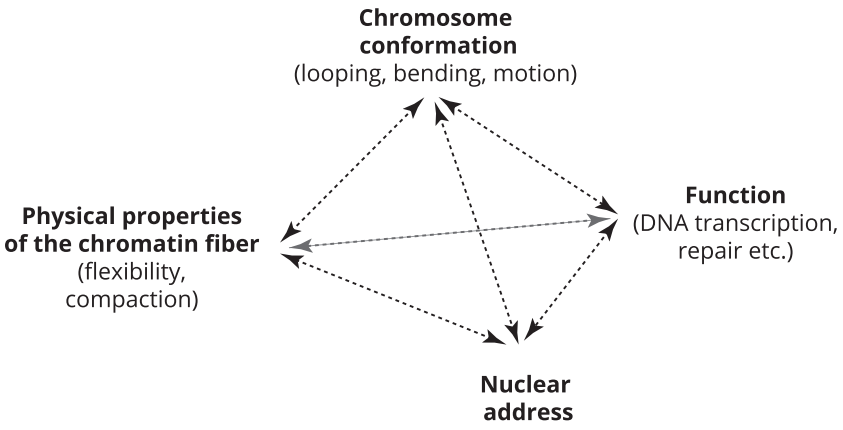

Fig. 1. Cause or consequence? Interdependence of the main parameters governing dynamics of the nucleosome fiber.

suntag [19] which is based on the binding of multiple fluorescent antibodies to a protein bound to a DNA locus; and ANCHOR (ParB/INT), based on amplifying the signal from a small binding site of less than 1 kb (INT) by oligomerization (spreading) of the specific binding protein (ParB) [20]. All these new DNA based tools are derived from the microbial world, a deep reservoir of natural innovation whose potential is no doubt far from exhausted.

Ideally, a labeling system should disrupt neither locus nor neighboring DNA, persist for the duration of the experiment and require no or minimal modification of the genome (Table 1). FROS has proven to be a valuable tool for determining position and dynamics of DNA loci in yeast and to a lesser extent in drosophila and mammalian cells. Three systems of distinct specificity have been developed for use alone or in combination, and a variety of fluorescent proteins of separate emission spectra is available for visualization of each of the three repressor proteins [14,21–23]. In yeast for example, analysis of the motion of FROS-labeled sites led to identification of the pathways involved in positioning telomeres near the nuclear periphery, and to charting changes in mobility associated with replication, with homologous recombination -mediated repair of double-strand breaks, and with regulation of transcription upon change of carbon source [7,14,20,24–27]. The motion of DNA in ectopically inserted operator arrays was shown to be constrained near nucleoli and the nuclear envelope of mammalian cells [28]. Plasticity of chromatin condensation was seen to be correlated with differentiation of ES cells [29], and the dynamics of X chromosome pairing was revealed [30]. In addition, the consequences of DNA cleavage and induced translocations were assessed in living cells [31,32]. Despite these successful applications, use of FROS in higher eukaryotic cells has often proven difficult, owing to the highly repetitive nature of the operator arrays. In particular, the sheer size of the inserted sequences and the tight binding of the repressor proteins can interfere with normal chromatin structure and function, creating fragile sites and perturbing transcription regulation [33,34].

The inactive Cas9 and TALE fluorescent fusion systems avoid this drawback by targeting natural sequences, thus circumventing the need for insertional disruption of the genome. Their use to label non-repetitive sites is however hampered by the need to amplify the signal to visible levels by multimerizing the constructs. Amplification through multiplexing Cas9 involves generating numerous adjacent sites of local DNA unwinding for guide RNA annealing over several kb of sequence, potentially creating DNA damage and nucleosome displacement. Consequently, use of these systems has so far been restricted largely to labeling of naturally repeated sequences e.g. telomeres [35]. Suntag is a non-invasive technique but appears thus far to be reserved for selected proteins that retain their function within the construct [19]. The INT sequence of the ANCHOR system has the advantage of being short, non-repetitive and non-disruptive of chromatin structure [20]. In

Table 1
DNA labeling tools for use in eukaryotic living cells.

System	Origin	Applications, advantages, drawbacks	Genome editing	References
FROS (fluorescent repressor operator system)	*E. coli* chromosome (lactose); Tn10 transposon (tetracycline gene); phage lambda	Yeast, difficult in metazoans; replication blockage (fragile sites); potential chromatin disruption, interference with transcription	Required; insertions 5-10 kb of arrays of repetitive DNA sequences	[14,23,22]
TALE (transcription activator like effector proteins)	*Xanthomonas oryzae* transcription activator	Any cell type; restricted to repetitive sequences	No	[17,18]
CRISPR/Cas9	Streptococcus pyrogenes (Sp), *Neisseria meningitidis* (Nm), and *Streptococcus thermophilus* (St1)	Any cell type; restricted to repetitive sequences or numerous sites (several kb); local DNA unwinding/triple helix formation	No	[15,16]
Suntag	Antibody	Potentially any cell type; very specific, lack of versatility; final complex very large (up to 1400kDa); high probability of instability, aggregation and interference with function of protein studied	Required; insertion of protein binding sites	[19]
ANCHOR (ParB/INT)	Burkholderiaecae chromosome partition systems	Any cell type; versatile; negligible interference with DNA processing, transcription	Required; insertions of 0,4-1 kb unique sequences	[20]
Fluorescent dNTPs		Bulk labeling; whole genome; unspecific; labeling requires single cell injections or 'rubbing'	No	[103]
Histones (H2B-GFP etc.)		Bulk labeling; whole genome; unspecific; stable fluorescence; photoactivatable fusion allows activating individual chromosomes	No	[49,93]

Please cite this article in press as: Bystricky, K. Chromosome dynamics and folding in eukaryotes: Insights from live cell microscopy. FEBS Lett. (2015), http://dx.doi.org/10.1016/j.febslet.2015.07.012