

Preface

All truth passes through three stages.

First, it is ridiculed.

Second, it is violently opposed.

Third, it is accepted as being self-evident.

Arthur Schopenhauer

The organisation of chromatin is non-random and shows a broad diversity across cell types, developmental stages and cell cycle stages. During G_0 and G_1 phases of interphase, chromatin displays a bivalent status. The condensed chromatin (heterochromatin) at the nuclear periphery is mostly associated with low levels of gene expression, while the loosened chromatin (euchromatin) towards the interior of the nucleus is associated with higher gene expression. This quiescent picture of interphase radically changes when the cell cycle progresses towards cell division. Firstly, during S phase, DNA is replicated, and chromatin progressively condenses. This is followed by the G_2 phase that shows a compact heterochromatin recruited towards the centre of the nucleus. At the beginning of mitosis, the chromosomes condense with a significant topological change in their organisation and are segregated during the next stages of the cell division. Meiotic chromosomes are also highly condensed as mitotic chromosomes but show a particular functional structure, which prepares germ cells to exchange DNA sequences between their homologous chromosomes to generate diversity. To summarise, chromatin experiences dramatic organisational changes during mitosis and meiosis. These changes in chromatin organisation during the lifetime of a cell show that chromatin is not a static entity but highly dynamic in nature.

For a variety of reasons, conventional light and electron microscopy have not been able to fully capture the finer details of chromatin organisation and dynamics. For a long time, description of the interphase nucleus was limited to delineate the euchromatin–heterochromatin dichotomy or describe some specific nuclear elements such as the nucleolus. Advancements in molecular biology during the last

thirty years have brought an immense amount of information about how chromatin is organised and genes are regulated. As a classical example, the globin gene has been shown to display a highly constrained shape forced by chromatin looping that brings the regulatory regions to the promoter of the gene. Nowadays, genomic studies can acquire an immense amount of information regarding chromatin organisation and gene regulation, leaving one with the expectation that structure of individual genes could potentially be described visually if sufficient specificity and resolution were reached. With the advent of various super-resolution methods, in particular, single molecule localisation microscopy (SMLM)-based methods and recently developed strategies for labelling DNA, it is now possible to study chromatin organisation and underlying gene regulatory mechanisms at the nanoscale.

During my Ph.D., I have analysed a broad range of nuclear phenotypes using SMLM. My analyses contribute to the description of a periodic and dynamic structure of chromatin. Moreover, I have described several elementary chromatin structures that I call chromatin domains, both in interphase and in meiosis, that are potentially associated with a local function such as gene activation or silencing.

Firstly with colleagues, I established an experimental set-up to study chromatin organisation with single molecule localisation microscopy. I investigated how UV-induced photoconversion of conventional DNA dyes allows increasing sufficiently the labelling density such that it is possible to study various organisational aspects of chromatin in basal interphase. An adequate imaging protocol has been established to bring DNA minor groove binding dyes such as Hoechst 33258, Hoechst 33342 and DAPI (4',6-diamidino-2-phenylindole) into an efficient blinking state necessary to record single molecule locations with high precision. This method was applied to several cell types to investigate the chromatin organisation during different stages of the cell cycle at the highest resolution currently achievable with light microscopy.

The results show that the method can capture several hierarchical levels of chromatin organisation. In reverse hierarchical order, I could describe previously known chromatin territories of 1000 nm, subchromosomal domains of 500 nm, chromatin domains of 100–400 nm (and further subcategories of active or repressed domains) and chromatin fibres below 100 nm, mostly between 30 and 60 nm. Individual nucleosomal domains are also described, which tend to cluster in batches of 10–15 nucleosomes, a number close to one found in genomic studies upstream to promoter regions. Next, with colleagues, I studied the dynamics of chromatin using stress as a model system. It was found that short-term oxygen and nutrient deprivation provokes chromatin to shrink to a hollow, condensed ring and rodlike configuration, which reverses back to the initial structure when the stress conditions cease. The condensed network of rods and rings interspersed with large, chromatin-sparse nuclear voids was 40–700 nm in dimension, capturing another level of chromatin organisation not described before.

Finally, I explored the unique properties of chromatin during meiosis, which has escaped analysis at the single molecule level until now. Single molecule analysis revealed unexpected highly recognisable periodic patterns of chromatin. Firstly, I observed that meiotic chromatin shows unique clusters of 250 nm diameter along

the synaptonemal complex, extended laterally by chromatin fibres forming loops. These clusters show a remarkable periodicity of 500 nm, a pattern possible to spot because of the highly deterministic nature of pachytene chromosomes and the resolution of the experimental set-up. Furthermore, guided by genomic data, I selected histone modifications associated with different chromatin states to dissect the morphology of meiotic chromosomes. I could examine the morphology of these chromosomes into three spatially distinct nanoscale subcompartments. Histone mark H3K4me3 associated with active chromatin was found in a lateral position, potentially located at the places of de novo double-strand breaks. Repressive histone mark H3K27me3 was shown to display a surprising medial symmetrical and periodic pattern, putatively associated with recombination. Finally, centromeric histone mark H3K9me3 locates at one of the meiotic chromosome ends and is potentially associated with repression of repeated regions and pairing of homologous chromosomes at early stages. I summarise these findings in a comprehensive final model.

Overall, I have used new information brought by super-resolution technologies to show the dynamics of chromatin in various processes and novel orders of chromatin compaction, which were not reported previously. Among these new levels of chromatin compaction are the interphase hierarchical chromatin domains, the stress pattern of cells upon oxygen and nutrient deprivation and the novel epigenetic domains found at pachytene stage of meiosis. These architectures show that the organisation of chromatin is more complex than that thought before, is dynamic in nature and shows a high order of periodicity. Further investigation is, therefore, necessary to understand how chromatin transits from a ‘beads-on-string’ model to the intermediary chromatin domains and finally to the commonly observed X-shaped chromosomes.

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Dr. Kirti Prakash

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