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Review

Chromatin condensation dynamics and implications of induced premature chromosome condensation

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ABSTRACT

Somatic cell cycle is a dynamic process with sequential events that culminate in cell division. Several physiological activities occur in the cytoplasm and nucleus during each of the cell cycle phases which help in doubling of genetic content, organized arrangement of the duplicated genetic material and perfect mechanism for its equal distribution to the two daughter cells formed. Also, the cell cycle checkpoints ensure that the genetic material is devoid of damages thus ensuring unaltered transmission of genetic information. Two important phenomena occurring during the cell cycle are the DNA condensation and decondensation cycles in the nucleus along with the cyclic expression and functioning of certain specific proteins that help in the same. Several protein families including Cyclins, cyclin dependent kinases, condensins, cohesins and surivins ensure error free, stage specific DNA condensation and decondensation by their highly specific, controlled orchestrated presence and action. Understanding the molecular mechanisms of chromatin compaction towards formation of the structural units, the chromosomes, give us valuable insights into the cellular physiology and also direct us to techniques such as premature chromosome condensation. The techniques of inducing 'prophasing' of interphase cells are undergoing rapid advances which have multidimensional applications for basic research and direct applications.

1. Introduction

Proliferating cells go through a cycle of events in an ordered progression, the mitotic cell-cycle. This results in the duplication of each chromosome to form closely adjacent sister chromatids, which separate from each other to become daughter chromosomes. The molecular mechanisms underlying the cell cycle are highly conserved in all organisms with nucleated cells. Many of the genes and proteins involved in the mitotic cell cycle have been identified because of their high degree of nucleotide and amino acid sequence similarity to homologous genes and proteins in the more extensively studied and understood budding yeast, *Saccharomyces cerevisiae*. All phases of the cell cycle are marked by an orderly progression of metabolic processes and its progression is regulated at several checkpoints which are necessary to prevent cells with damaged or missing chromosomes from proliferating.

The interphase chromatin is condensed to form compact chromosomes during mitosis. Several protein–DNA associations occur in the presence of highly regulated protein mediators for obtaining 46 chromosomes. The extent of compaction is very significant considering that 46 chromosomes whose combined length is approximately 200 μm are derived from the entire diploid cell DNA which spans about 2 m [1,2]. According to the "chromosome condensation cycle" originally proposed by Mazia et al., chromosomes begin a decondensation process during telophase that continues throughout G_1, maximum decondensation levels are associated with DNA replication during S phase [3]. After replication, chromosomes begin to recondense, and this process continues until the maximum level of compaction is again achieved during metaphase. Many other investigators, using a variety of experimental methods, have provided evidence to support the idea of a chromatin condensation cycle [3–5].

It is possible to view the interphase chromatin in 'as-near-to-chromosome' clarity by the induction of "premature chromosome condensation" (PCC) [6]. By the phenomenon known as 'prophasing' [7], it is possible to induce interphase DNA condensation and compaction in any nucleated cell by a variety of means. These include fusions of target cells with mitotic partners, injection of mitotic extracts and also a few chemical inducers of chromatin condensation. The morphology of prematurely condensed interphase chromatin gives us valuable insights into the mechanisms involved in chromatin condensation dynamics and the molecular

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mechanisms. The final length of PCC is largely independent of the concentration of the inducer molecules or the numerical relations between the fused metaphase and interphase cells. Even when the prematurely condensed chromosomes are held together with metaphase chromosomes for several hours, there is no change in the final length [8]. In general, the final result of PCC depends on the phase of the target cell at the time of fusion, and accordingly, G_1 PCC are very long, single chromatids, those of G_2 are elongated and slender double chromatids, and those of S phase are characterized by their pulverized, fragmented appearance [3,6,8].

The technique of premature chromosome condensation has multifaceted applications in areas including all aspects of cell cycle analysis, cancer biology, mutagenesis and clinical cytogenetics, biodosimetry, prenatal diagnosis, fertility and reproductive medicine. The use of the method of PCC to solve certain applied questions i.e. its relevance to applied research, can be attributed to the advantageous use of three substantial differences between prematurely condensed chromosomes and normal mitotic chromosomes: their increased length, their representation in specialized, non-dividing tissues and their very nature as "interphase" chromosomes. Chemically induced PCC technique allows simple, rapid and precise analysis of chromosomal aberrations and DNA repair kinetics. This seems useful for the study of biological effects of ionizing radiation, particularly in cancer radiotherapy and biodosimetry [9]. Calyculin A induced PCC for biodosimetry overcomes problems such as low mitotic index as faced in conventional metaphase analysis and to a certain extent, the low fusion rate in conventional PCC.

An in-depth understanding of the cyclic events during the 'making and breaking' of chromosomes, as interpretable by PCC induction, may be subverted to advantageous use as a rapid cytogenetic tool to track chromatin dynamics in terms of the physical states of condensation, replication, damage and repair; also indicating the possible link between such cellular phenomena. The phenomenon bears relevance in basic research to human cytogeneticists, in shedding light on complex cytological phenomena and in applied research, to clinical cytogeneticists, in areas including cancer studies, diagnosis and status prediction, prenatal diagnosis, mutagenesis, biodosimetry etc. In the present review, we highlight the molecular mechanisms of chromatin condensation and the implications of induced premature chromosome condensation.

2. Cell cycle

The average number of cells in the body is maintained at any given point of time by cell proliferation or cell division. The mitotic cell cycle is the main cycle responsible for maintaining the requisite number of cells (somatic) in the body and is tightly regulated by a variety of proteins that have been extensively studied. Two major functions must occur during the cell cycle: first, the replication of DNA and second, the proper segregation and packaging of DNA into daughter cells. The entire process of DNA replication and cell division is divided into many stages which contribute collectively to the name: the cell cycle.

The cell cycle comprises of interphase and mitotic phase of which the interphase is the longest part and is further divided into three stages, the Gap1/G_1 phase, the Synthesis/S phase and the Gap 2/G_2 phase [10]. The mitotic phase is further divided into: prophase, prometaphase, metaphase, anaphase, telophase which is followed by cytokinesis [10]. The interphase considered being the resting stage has now proven to be the most active part of the cell cycle. G_1 phase is the first phase in interphase stage where the parent cell begins to grow and organelle synthesis takes place. DNA replication occurs in the S phase, doubling the DNA content and in the G_2 phase, the DNA is checked for errors before the cell divides. During the process of cell cycle, various functions such as replication of DNA, RNA transcription and translation, synthesis of new organelles for each of the daughter cells, etc occur simultaneously. All these various processes occur at the different stages of the cell cycle, with the most dramatic process being the DNA condensation and decondensation phenomena.

3. DNA condensation and decondensation cycle

In diploid human cells, the amount of DNA that must be packaged into the 46 chromosomes as 23 pairs is roughly 6 billion base pairs. Each cell contains DNA that is almost 2 m in the 46 chromosomes whose combined length is only 200 μm [1,2]. The morphology of the chromatin differs during various stages of the cell cycle, depending on the DNA amount and its activity. During the G_1 phase, cells are just beginning to synthesize proteins necessary for DNA replication, and thus the DNA at this stage is usually seen in the form of a network of fibres called as chromatin network. During the S phase there is doubling of DNA without much alteration in the morphology and then it begins condensing during the G_2 phase. During the G_2 phase the morphology alters as it nears the M phase and chromosome condensation begins but the arms of the chromosome are not very clearly discernible. Further condensation and compaction of the chromatin occurs during mitosis. Chromosomes are almost completely condensed during the prophase and complete condensation occurs at the metaphase, the most preferred stage for cytogenetic analysis. The maximally condensed chromosomes split into two equal parts at their centromere/kinetochore at anaphase and no major morphological changes occur during this phase in the chromosome structure. Chromosomes slowly begin reverting back to the uncondensed state at telophase as the cell completes its division. These events occur in a cyclic fashion synchronous with the cell cycle phases and constitute the DNA condensation and decondensation cycle.

4. Factors involved in chromatin condensation

The discovery of proteins belonging to the SMC (Structural Maintenance of Chromosomes) family paved the way for understanding the intricacies of chromatin condensation. Three major proteins have been found to play a crucial role in condensation of chromatin and in the mechanistic shaping of chromosomes. They are the condensin I, condensin II and cohesins. A variety of molecular players have been identified and their roles described in chromatin condensation (Table 1, Fig. 1).

4.1. Structural Maintenance of Chromosomes (SMC) and its role in chromatin condensation

SMCs are the family of chromatin condensation proteins with specific roles which aid in the higher order of chromosome structural organization. The initial identification and characterization of SMC proteins were done in the yeast, *S. cerevisiae* [11]. The SMC proteins have molecular weights ranging from 110 kDa to 170 kDa with 5 distinct domains that make up the protein. The structure consists of amino and carboxy terminal globular regions with ATPases and two self folded anti-parallel coiled domains held together by a flexible hinge in the centre. This causes the formation of an ABC like head domain at one end and a hinge region at the other end. A dimer is then formed between two SMCs by association of their hinge regions. This forms a "V" shaped structure [12]. The best characterized SMC molecule to date is the SMC3, which is made up of 31 exons and spans approximately 45 kb of DNA. Genbank sequences for human SMC3 and SMC4 predict the genes to have 27 and 23 exons respectively [13].