

HUMAN INTERPHASE LYMPHOCYTE DNA CONDENSATION EMPLOYING MITOTIC EXTRACTS

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ABSTRACT

INTRODUCTION: Premature DNA condensation is a powerful cytogenetic tool. Various phosphorylatically regulated cell cycle protein kinases have been characterized, separated and identified in extracts from mitotic and interphase cells. Interphase DNA condensation employing mitotic extracts might be a tool in not only understanding the condensation mechanisms but also will throw light upon the levels of these factors in the various stages of the cell cycle. In this study, we aimed at and have demonstrated DNA condensation capacities of CHO mitotic extracts in human interphase lymphocytes.

MATERIALS & METHODS: CHO cells at Mitotic and Interphase stages were harvested and cytosolic proteins extracted. Protein estimation of the CHO Interphase and Mitotic extracts was done by Bradford method. Protein profile analysis of the extracts was performed by SDS PAGE. Human Lymphocytes were isolated from peripheral blood by Ficoll density gradient method and were subjected to a range of mitotic extract concentrations (10, 20, 30, 40 and 50%). The slides were prepared and cells with varying degrees of condensation were scored and photo documented. A total of 1000 cells per concentration were analyzed for the degree of condensation.

RESULTS: The quantum of interphase human lymphocyte DNA condensation showed a distinct increase from 20% to 50% mitotic extract treatment. It was observed that maximal condensation and also number of cells with such DNA condensation was most prominent in lymphocytes subjected to 50% mitotic extract.

CONCLUSION: Induction of PCC although is achieved to a great degree by somatic cell hybridization techniques, the same if done through isolated proteins might give us a better understanding of finer molecular factors. This also will eliminate the fusion efficiency limitations as is determined by the ratios of fusion partners. Interference of homologous entities when cells of similar phylogenetic lineages are used either for somatic cell hybridizations or for employing specific condensation factors will also be eliminated. Our results show that mitotic cytosolic extracts have potential PCC properties thus paving a path to circumvent cell hybridizations for the same; having wide applications in genotoxicity studies.

KEY WORDS: Mitotic Extracts, DNA condensation cell cycle proteins, Human lymphocytes

INTRODUCTION :

Premature Chromosome Condensation (PCC) has been shown to be a powerful cytogenetic and cytokinetic tool in understanding cell-cycle analysis(1) and also for diagnostic purposes(2,3). Although PCC is a useful cytogenetic tool, it involves somatic cell hybridizations employing a mitotic partner. Understanding of the factors responsible for chromatin condensation and their mechanism of action can throw better light on the cell cycle kinetics and also the protein repertoire of the cells in the various phases of the cell cycle. A number of proteins involved at various levels of cell cycle have been characterized and a variety of protein kinases have been separated and identified in extracts from mitotic and interphase cells. It is also understood that protein phosphorylation / dephosphorylation may be integral to the mechanism of chromosome condensation(4). The induction of PCC in cells using chemicals was well demonstrated and some of the chemical agents employed

are protein phosphatase inhibitors (Okadaic Acid, Calyculin A, Fostreicin, etc.)(5,6).

Protein profiles in the various stages of the cell cycle revealed that in normal cell-cycle, an acidic protein with a molecular weight 35 KDa was specifically associated with chromosome condensation at the mitotic phase(7). It was also found that the condensation factor was cytoplasmic, which is present throughout the cell cycle, but enters the nuclear membrane causing condensation of chromatin during the mitotic stage(8). These factors are collectively known as the "Structural Maintenance of Chromosomes" (SMC). It was shown that mitotic chromosomes are composed of five histones and a large number of non-histone proteins that maintain the chromosomal structural integrity. It has been demonstrated that chromosome "Scaffold Proteins" are important to maintain the condensed structure of the mitotic chromosomes(9).

Many experiments were reported which have employed cytoplasmic extracts from a variety of mitotic cells and their ability to induce condensation of interphase chromatin. It was also shown that these factors responsible for interphase chromatin condensation do not have cross-species barriers or specificity, but rather being able to induce condensation of interphase chromatin across species boundaries and limitations(10).

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Interphase chromatin condensation employing mitotic extracts might be a useful tool in not only understanding the condensation mechanisms but also will throw light upon the levels of these factors in the various stages of the cell cycle. Also, demonstration of cross-species condensation induction might be useful in the relative ease of collection of such factors and employment of the same for human health care apart from a better understanding of such phenomena.

MATERIALS AND METHODS:

CHO cells were cultured by standard procedures. DMEM supplemented with 5% Serum was used as the culture medium and were maintained in 5% CO₂ at 37°C. The same were passaged by routine trypsinization protocols and a continuous culture was maintained. At optimal levels of confluence, CHO cells at Mitotic and Interphase stages were harvested and cytosolic proteins extracted. The mitotic cells were collected by gentle agitation of the flasks and interphase cells collected by trypsinization, were washed once in serum-free medium. The pellet obtained was suspended in cold extraction medium, containing 40 mM Tris base; and spun at 1000 rpm for 10 minutes. The cell pellet was agitated by hand homogenization in a Taronson cell grinding pestle and spun again. The supernatant was collected and left for dialysis in Phosphate Buffered Saline. Further to protein estimation, the two were analyzed for their protein profiles by SDS Page.

Protein estimation of the CHO Interphase and Mitotic extracts was done by Bradford method(11). Bovine Gamma Globulin of 0.225, 0.325, 0.75, 1.125 and 1.50 mg/ml concentrations were used as standards. Protein profile analysis of the extracts was performed by SDS PAGE. A 4% stacking Gel and 10% separating gel was used for optimal resolutions of the separated protein components. Protein molecular marker of medium range (14.3 - 66 KDa) was used. The gel was stained by Coomassie blue and was scanned and analyzed.

Heparinised human peripheral blood was collected by vein-puncture and lymphocytes isolated by Ficoll density. The cell aliquots were subjected to a range of mitotic extract concentrations (the original extract was diluted in PBS to obtain various concentrations) and were incubated for 30 minutes in 4°C followed by 2 hours at 37°C. The concentrations of the Mitotic Extracts employed were 10, 20, 30, 40 and 50%. The same were washed and harvested according to standard methods for cytogenetic analysis(12). The slides were prepared and cells with varying degrees of condensation were scored and photo documented. A total of 1000 cells per concentration were analyzed. According to the levels of the DNA condensation, the cells were positioned in four categories [1. No Condensation (NC), 2. Low Condensation (LC), 3. Intermediate Condensation (IC) and 4. Maximal Condensation (MC)]. The differentiation between the

categories was based on visual microscopic observation as represented in figure 4. The percentage of cells in the four categories and for each of the concentrations were calculated and the results analyzed.

RESULTS:

Protein quantification of CHO interphase and mitotic extracts showed values of 0.54 and 0.6 mg/ml respectively. Further to SDS PAGE analysis, a comparison of protein profiles was made to identify proteins unique to mitotic cell extracts. 4 distinct proteins of higher molecular weights (between 28 KDa and 50 KDa) and a group of low molecular

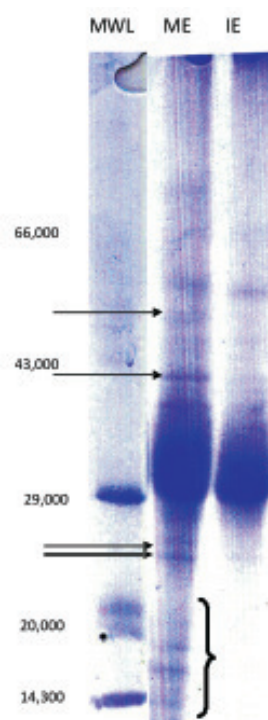


Figure 1: SDS PAGE analysis of Chinese Hamster Ovary Cells (CHO) Mitotic extract (ME) and Interphase extract (IE) with comparison to a medium range molecular weight ladder (MWL). Arrows denote mitotic extract unique higher molecular weight proteins. A group of mitotic unique proteins are also denoted in the range 20,000 and 14,300 Kda.

weight proteins (between 14 – 20 KDa) were observed unique to mitotic extracts (whose corresponding bands were absent in the interphase cell extract). Of these unique mitotic proteins, 2 of molecular weights 38 KDa and 26 KDa were of significant quantities. (Figure 1).

The quantum of interphase human lymphocyte DNA condensation showed a distinct increase from 20% to 50% mitotic extract treatment. A 10 % concentration showed no condensation and values matched control samples. The DNA condensation capacity of the various concentrations of the mitotic extract and levels of condensation are represented in (Figure 2). Maximal condensation was found in lymphocytes treated with 50 % mitotic extract. The

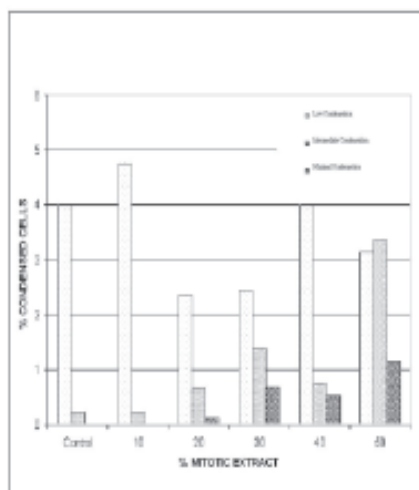


Figure 2: Interphase DNA condensation capacity of various concentrations of the mitotic extracts and levels of condensation

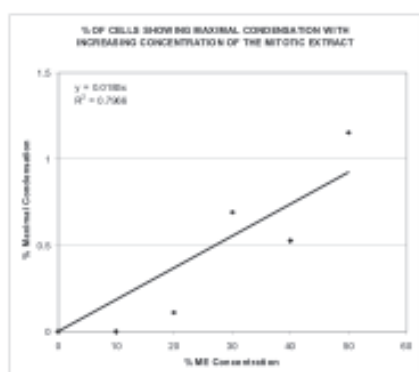


Figure 3: % Interphase human lymphocytes showing maximal condensation with respect to the concentrations of the mitotic extract.

percentage of cells showing maximal condensation are represented in (Figure 3). The lymphocytes showing various levels of chromatin condensation is shown in (Figure 4).

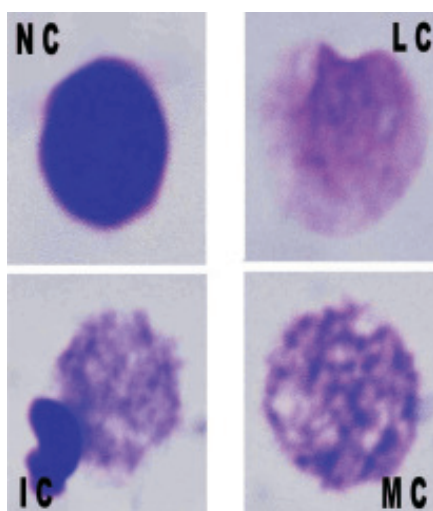


Figure 4: The lymphocytes showing various levels of Interphase DNA condensation: No Condensation (NC), Low Condensation (LC), Intermediate Condensation (IC), and Maximal Condensation (MC).

It was observed that maximal condensation and also number of cells with such DNA condensation was most prominent in lymphocytes subjected to 50% mitotic extract.

DISCUSSION:

Protein profiles during cell cycle are critical for the various cellular mechanisms and proper regulations of the same. Differences in protein repertoire of a cell in its interphase and mitotic phase are important for the various changes necessary for DNA condensation leading to karyokinesis during cell division. This is very clear by the premature chromosome condensation capacity of mitotic extracts in interphase cells and also by the chromosome de-condensation properties of interphase extracts on mitotic cells (13).

Induction of DNA condensation although is achieved to a great degree by somatic cell hybridization techniques, the same if done through isolated proteins will give us a better understanding of the finer molecular factors responsible for the same. This also will eliminate the fusion efficiency limitations as determined by the ratios of the fusion partners (13).

Injection of mitotic extracts into interphase cells and subsequent condensation of interphase DNA has been demonstrated (10). However if large quantities of cells with condensed interphase DNA were required for any study, such an approach would have inherent disadvantages. We therefore have attempted to cause simultaneous DNA condensation of a population of interphase cells by mere subjection of such cells to mitotic extracts with a suitable incubation and processing protocols. Even a maximal condensation index of about 10% of cells would be useful for a variety of reasons. The fact that mitotic extracts of any cell type can induce DNA premature condensation in cells of different phylogenetic lineages becomes an added advantage of this technique. This also eliminates the possible interference of homologous entities when cells of the same phylogenetic lineages are used either for somatic cell hybridizations or for employing specific condensation factors.

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