PRINT: ISSN 0972-3757 ONLINE: 2456-6360
Int J Hum Genet, 7(4): 319-323 (2007)
DOI: 10.31901/24566330.2007/07.04.08

Optimizing Premature Chromosome Condensation (PCC) of Human Lymphocytes by Somatic Cell Hybridization to Study Primary DNA Damages

Maddaly Ravi*, Preetha B, Govind Pai M, Deepa Parvathi V, Sulogna Ghosh and Solomon FD Paul

Department of Human Genetics, Sri Ramachandra Medical College and Research Institute (Deemed University), Porur, Chennai 600 116, Tamil Nadu, India

KEYWORDS Premature chromosome condensation; human lymphocytes; mitotic cells; DNA damages; optimization

ABSTRACT Cytogenetic tools are important in understanding the health effects of various chemical and physical agents implicated in human health. Several biodosimetric and cytogenetic techniques help in the assessment of the genotoxic effects of therapeutic drugs and these become essential for human health care management. There exist methods by which genotoxicity can be identified and quantified employing interphase condensed DNA. Premature Chromosome Condensation (PCC) can be achieved by Somatic Cell Fusions and can result in obtaining condensed DNA into "near chromosome like clarity" and the same can be utilized to check for common end-points such as DNA "breaks" and "gaps", as can be induced by genotoxic drugs. PCC in conjunction with FISH can also be an added advantage for the same. We present here an efficient method to obtain high quality PCC spreads of human lymphocytes, which can be analyzed with relative simplicity for genotoxic monitoring. Mitotic CHO cells were used as the fusion partners followed by incubation and the processing of cells for analysis. Image capturing and analysis was done after fixing and casting cells. The key factors such as the ratio of the fusion partners, fusion and processing protocols are optimized and presented. Although PCC cannot replace conventional lymphocyte culture techniques completely, it can be efficiently utilized for genotoxic monitoring with advantages such as the considerable reduction in time required for processing and analysis of samples. This will also eliminate the chances of genotoxicity underestimation due to the inherent DNA damage repair capacity of human lymphocytes.

INTRODUCTION

Cytogenetic tools continue to be important for understanding and monitoring the health effects of various chemical and physical agents that are used in human diagnosis, therapy or in accidental exposures. An assessment of the genotoxic effects of therapeutic drugs and monitoring the same becomes crucial for human health care management. Several cytogenetic tools are currently available for monitoring genotoxicity including radiation-induced damages (Paul et al. 1996). Alternative methods of cytogenetic analysis are required for detection of primary DNA damages.

Somatic cell hybridization with a fusogen has been reported and has proved a useful technique for a variety of studies (Pontecorvo et al. 1975). Fusion between a mitotic and an interphase cell frequently results in disintegration of interphase nucleus, particularly interphase nuclear membrane and induction of mitotic state in which, the chromatin material condense to visible, discrete "chromosome like structures". This is possible under the influence of certain factors, the "chromosome condensation factors" present in the mitotic fusion partner. Premature Chromosome Condensation (PCC) can be achieved by Somatic Cell Fusions and can result in obtaining condensed DNA into "near chromosome like clarity" (Yoshitaka et al. 1974). The morphology of prematurely condensed chromosomes varies according to the stage of the cells in the cell cycle at time of fusion. Thus, the PCC of G1 phase cells are very elongated and slender single chromatids, and those of the S phase are characterized by their fragmented appearance and G2 type consists of double chromatids, greatly extended and in general much longer than prometaphase elements (Johnson et al. 1970). In certain cases PCC has greater sensitivity in the detection of chromosomal damage induced during interphase than the standard method of scoring metaphase chromosomes. It was shown that after X-ray treatment the incidence of chromatid damage was significantly higher in PCC than in mitotic chromosomes; it was assumed that repair accounts for most of these differences (Hittleman and Rao 1974). Premature chromosome condensation was

Corresponding Author: Maddaly Ravi
Telephone: +91-44- 24768027
Fax: +91-44- 24767008
E-mail: maddalyravi@hotmail.com

used to analyse the interphase chromosome after X- ray and Ultraviolet irradiation (Charles et al. 1974). An important advantage of G0/G1 PCC is that it does not underestimate primary DNA damage (Durante et al. 1998). Detection of primary DNA damage by premature chromosome condensation has also been reported as an useful tool for the assessment of genotoxicity (Garcia et al. 2001).

For the present study, mitotic Chinese hamster ovary (CHO) cells were used as the fusion partners followed by incubation and the processing of cells for analysis, which followed conventional cytogenetic procedures. Image capturing and analysis was done after fixing and casting cells. The process of condensation from initiation to maximal condensation was observed and images analyzed so as to arrive at the optimal fusion and processing parameters. Also, the ideal phase of the cells for PCC was arrived at for visualization of gross genetic damages as can be used in situations such as therapeutic drug monitoring for genetic toxicity.

The efficiency of condensation of chromosome of interphase nuclei depends on various factors like the concentration of inducer molecules, the cell cycle phase of the interphase cells, genomic ratio, concentration of the colcemid and fusogen used, and duration of incubation .In this study, we have optimized various factors like the volume of Polyethylene Glycol and the exposure duration apart from the post fusion processing and staining for analysis.

MATERIALS AND METHODS

Chinese Hamster Ovary (CHO) cells were utilized as the mitotic partner. These cells were routinely cultured at 37^0C in a 5% CO2 humidified incubator in Dulbecco's modified eagle's medium supplemented with 10% Fetal Bovine Serum and mitotic stage arrest was done by spiking the medium with colcemid. The unattached, spherical mitotic cells were harvested by gentle agitation of the culture flasks. Also, cultures exposed to colchicine were used for obtaining sufficient quantities of CHO mitotic cells as fusion partners.

Venous blood was drawn in heparinised tubes from healthy volunteers and Human Peripheral Blood Lymphocytes (HPBL) were separated by the ficoll density gradient method. For the G0/G1PCC isolated lymphocyte pellet washed in the Roosevelt Park memorial institute (RPMI) medium was mixed with the mitotic pellet and taken in a sterile centrifuge tube. For the S and G2 phase, a deviation from the normal HBPL culture set-up was followed. Instead of the whole-blood cultures, lymphocytes that were isolated by density gradient were used and pure lymphocyte cultures were obtained. Methotextrate block was employed on the pure lymphocyte cultures and S and G2 synchronized cells were obtained, washed with RPMI medium and the final cell pellet was used for fusion.

The lymphocytes in the various stages of the cell cycle thus obtained (G0, G1, S and G2 stages) were washed with RPMI medium without serum and mixed with CHO mitotic cells in the ratio 5:1. The mixed cell pellet was subjected to 1 ml of 45% Polyethylene Glycol (PEG). After 1 minute of exposure to PEG, the pellet was diluted in about 30 ml of RPMI medium without serum and washed immediately. The final pellet was re-dispensed to a volume of 1 ml in medium and was incubated at 37^0C for 1 to 1 ½ hours with the addition of colcemid. The pellet was subjected to hypotonic treatment for 10 minutes followed by fixative treatment and casting. After two days of aging in room temperature, the slides were subjected to 3% solid Giemsa staining. The PCC spreads were then scored, analyzed and photo-documented.

RESULTS

Pure lymphocyte cultures (for the S and G2 blocked cells), which were obtained by employing isolated lymphocytes when used for Premature Chromosome Condensation showed better ease of handling for fusion and post-fusion processing when compared to the conventional whole blood culture.

Differentially stained mitotic and interphase spreads were obtained by staining the aged slides with 3% Giemsa to discriminate chromosomes of the mitotic cell partner and the prematurely condensed chromosomes of the interphase cell partner (Fig. 1-A).

The pattern of PCC showed varied degree of compactness of the DNA. The G0/G1 PCC showed characteristic morphology of single chromatids. These showed distinct thread-like pattern of condensation of the interphase DNA and single chromatid-like structures were obtained (Fig. 1-B). The S phase PCC showed

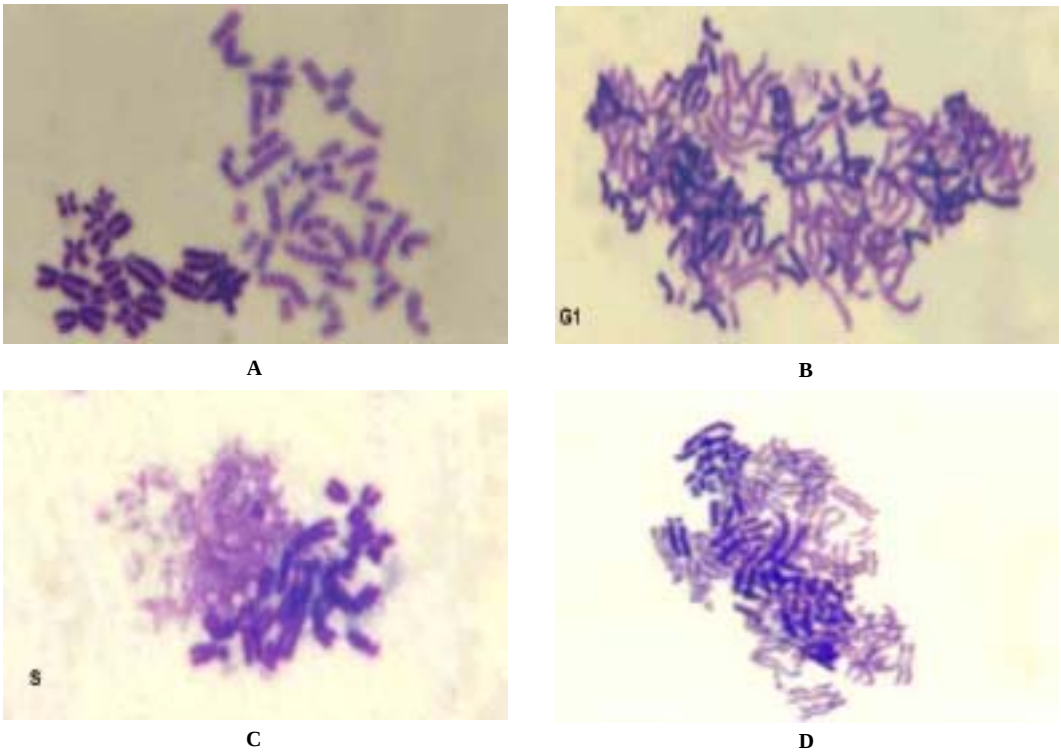
highly pulverized appearance of the interphase DNA (Fig. 1-C). The G2 phase PCC showed characteristic double chromatid structure with condensation resulting in near-chromosome-like-structures (Fig. 1-D).

The various optimized factors have been summarized in the Table 1.

DISCUSSION

The present study was conducted to arrive at the most optimal conditions required for premature chromosome condensation by somatic cell hybridization. There is no restriction as to the source of the mitotic cell partners, as it has been observed that no cross-species barriers exist in terms of the mitotic factors to induce chromosome condensation prematurely (Schmiesing et al. 2000; Sunkara et al. 1979). Therefore, any available cell line maintained *in vitro* can be blocked at the mitotic phase and such cells can be readily used for PCC of practically any cell type at the interphase stage.

However, the properties of the CHO cells make them best choice for mitotic partner for somatic cell hybridization. The ability to distinguish prematurely condensed chromosomes from mitotic inducer chromosomes becomes critical especially when PCC is followed by other techniques such as Fluorescent *in situ*



A **B** **C** **D**

Fig. 1: A: 3% Giemsa solid staining of PCC demonstrating the discrimination of compact chromosomes of the mitotic cell partner and the prematurely condensed chromosomes of the interphase cell partner; B. G1 PCC showing distinct thread-like pattern of condensation of the interphase DNA. Single chromatid-like structures are visible and can be applied for assessment of Primary DNA damages in terms of breaks and gaps; C. S PCC showing highly pulverized appearance of the interphase DNA that can be applied more towards studies such as cell-cycle analysis and in combination of Fluorescent *in situ* Hybridization; D: G2 PCC showing double chromatid structure with condensation resulting in near-chromosome-like-structures. The applications include biodosimetry, genotoxic studies and primary DNA damage analysis that need to assess gaps, breaks which are of single strand or double stranded damages too.

Table 1: The various optimized parameters for arriving at Permaturely Condensed Chromosomes through Somatic Cell Hybridization which can be applied to a host of applications ranging from Cell-Cycle analysis to assessing primary DNA damages and for genotoxic studies.

S.No	*Factors*	*Optimum Parameters*		*Remarks*
1.	Choice of mitotic partner	Chinese hamster ovary (CHO) cells		The ease of maintenance, low chromosome number and low doubling time makes it the best choice for mitotic partner.
2.	Choice of interphase partner	Peripheral blood lymphocytes (HPBL)		It is the most commonly used sample for postnatal chromosome investigations due to its ease of handling and collection.
3.	Choice of medium for fusion and post-fusion incubation	RPMI		RPMI medium is found to support growth of hybrid cells and is commonly used for fusion protocols and it supports the growth of peripheral lymphocytes.
4.	Genomic ratio	Cell number of mitotic partner should not be too low		Though a ratio of 1:5 has been used for PCC protocols it should be noted that excess of interphase partner and very low mitotic partner will not yield good results.
5.	Concentration of PEG	45% W/V		Pure PEG has no fusogenic properties but a proportion with water results in efficient fusion.
6.	Concentration of colchicine	10 µl/ 5ml of medium		Increased concentration of colchicine is found to be toxic to fused cells.
7.	Duration of incubation	45 minutes – 1hour		Maximum condensation is reached 1 hour after incubation. Prolonging the duration of incubation does not increase the efficiency of condensation.
8.	Processing conditions	Hypotonic treatment	5-10 minutes KCL (0.075M)	Since fused cells are very fragile a mild hypotonic treatment is given.
		Casting height	more than 20 cm	To avoid the overlap.
		Staining	3% Giemsa	To discriminate between the mitotic and prematurely condensed chromosomes

Hybridization. The CHO cells have an advantage that their chromosome number is relatively low (2n = 22) apart from being phylogenetically distant from humans. The doubling time of these cells is eleven hours thus attaining confluence in lesser duration yielding more mitotic cells in less duration. These cells were shown to be in culture for more that ten months with no diminishing growth-rate, cellular changes or colonial morphological properties. Also, the efficient reaction to trypsinization makes easier the subsequent passages and maintenance of the culture cell line.

The interphase partner HPBL is the most commonly used sample for postnatal chromosome investigations due to its ease of handling and collection. The resting peripheral blood lymphocytes are the most ideal for mutagenic studies and they can be induced into premature chromosome condensation very easily. For studies such as cell-cycle kinetics where cells blocked at specific check points of the cell cycle are used,, PCC performed on “pure lymphocyte cultures” have a distinct advantage over the conventional “whole blood cultures”. This considerably reduces the processing of cultures such as lysis of erythrocytes, etc prior to fusion. A simple harvest of the synchronized cells and suitable washing is sufficient to obtain the interphase lymphocytes being utilized for the studies.

Polyethylene Glycol (PEG) is widely used as

a fusogen for cell hybridizations. As PEG is highly toxic to the cells due to its mode of action, a 50% concentration is preferred. The RPMI medium is found to support growth of hybrid cells and is commonly used for fusion protocols. The medium in the presence of the fusogen is found to play an important role in the induction of fusion. The RPMI 1640 is found to be better for fusion when compared to DMEM and for post-fusion processing. In general, the incidence of premature chromosome condensation reaches a maximum within 1 hour after the fusion with polyethylene glycol (Johnson and Rao 1970). Prolonging the duration of incubation of the fused cells for more than 1 hour does not increase the efficiency of PCC.

Since the hybrids are sensitive and fragile, it is important that the duration of hypotonic treatment is reduced from conventional cytogenetic protocol of twenty minutes to five minutes. Solid 3% Giemsa staining results in the differential staining of mitotic and the interphase cells. Thus the mitotic partner is stained darker when compared to the interphase partner.

It has been observed that gross genotoxicity can be detected and quantified by the extent of fragmentation of the condensed DNA as obtained by PCC (Waldren et al. 1974). However, the G1 stage PCC provides us with an ideal tool by which unaltered expression of genetic damage can be analyzed with maximum accuracy. The G2 stage PCC results in the condensed DNA morphology to study specific and finer damages such as chromatid breaks and gaps.

REFERENCES

Charles A, Waldren, Robert TJ 1974. Analysis of interphase chromosome damage by means of premature chromosome condensation after X- and Ultraviolet- irradiation. *Proc Nat Acad Sc*i, **71:** 1137-1141.

Durante M, Furusawa Y, Gotoh E 1998. A simple method for simultaneous interphase- metaphase chromosome analysis in biodosimetry. *Int J Radiat Biol,* **74:** 457-462.

Garcia CL, Carloni M, Pena NPDL, Fonti E, Palitti F 2001. Detection of DNA primary damage by premature chromosome condensation in human peripheral blood lymphocytes treated with methyl methane sulphonate. *Mutagenesis,* **16:** 121-125.

Hittleman WN, Rao PN 1974. Premature chromosome condensation: I. Visualization of X-ray induced chromosome damage in interphase cells. *Mutat. Res*, **23:** 251-258.

Johnson RT, Rao PN 1970. Mammalian Cell Fusion: Induction of Premature Chromosome Condensation in Interphase Nuclei. *Nature*, **226:** 717-722.

Pontecorvo G 1975. Production of mammalian Somatic cell hybrids by means of polyethylene glycol treatment. *Somatic Cell Genetics***, 1:** 397-400.

Schmiesing JA, Gregson HC, Zhou S, Yokomori K 2000. A Human Condensin Complex Containing hCAP-C–hCAP-E and CNAP1, a Homolog of *Xenopus* XCAP-D2, Colocalizes with Phosphorylated Histone H3 during the Early Stage of Mitotic Chromosome Condensation. *Molecular and Cellular Biology,* **20:** 6996–7006.

Solomon FDP, Venkatachalam P, Jeevanram RK 1996. *IGC Report, IGC-173*: *1-52*

Sunkara PS, Wright DA, Rao PN 1979. *Mitotic Factors From Mammalian Cells Induce Germinal Vesicle Breakdown and Chromosome Condensation in Amphibian Oocytes*. Departments of Developmental Therapeutics and Biology, The University of Texas, *Annual Report* 2001-2002.

Waldren CA, Johnson RT 1974. Analysis of interphase Chromosome Damage by Means of Premature Chromosome Condensation after X- and Ultraviolet-Irradiation. *Proc Nat Acad Sci,* **71:** 1137-1141.

Yoshitaka O, Lee SC, Herbert W, Avery AS 1974. Prophasing of interphase nuclei and induction of nuclear envelops around metaphase chromosomes in HeLa and Chinese Hamster Homo- and Heterokaryons. *T J of Cell Bio,* **62:** 104-113