Chapter 4

Sister Chromatid Differentiation and Exchange in Chinese Hamster Ovary Cells in Culture

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Introduction

This laboratory exercise is designed to (1) illustrate the semi-conservative mode of DNA duplication through the expression of sister chromatid differentiation (SCD), (2) illustrate the single-stranded nature (i.e., one DNA duplex per G_1 chromosome) of the macromolecular organization of eukaryotic chromosomes, (3) measure the mutagenic effects of chemical and/or physical agents through sister chromatid exchanges (SCEs), and (4) learn the basic cell culturing and cytogenetic techniques applied to chromosome studies.

Background

This exercise, in various forms, has been tested in our Mammalian Cell Culture course for a number of years. The prerequisite for this course is our sophomore-level Genetics; students in the latter class range from sophomores to seniors. It is not necessary for students to have completed a genetics course to succeed in this exercise as long as they have been exposed to the concepts of DNA duplication, mitotic cycle, and eukaryotic chromosome organization in other courses. Depending on facilities, knowledge of cell culturing, experience in laboratory work, and available time, this exercise can be adopted, in full or in part, in a variety of courses, including introductory biology, genetics, cell biology, molecular biology, and independent study.

Our students have been exposed to the basic principles and techniques of cell culturing before they do this exercise. However, this chapter includes some basic information on culturing Chinese hamster ovary (CHO) cells. More extensive information on animal cell culture can be found in Freshney (1987) and Jakohy and Paston (1979) and on Chinese hamster cell culturing systems in Gottesman (1985). Hsu (1972) is an excellent source of procedures for *in vitro* and *in vivo* mammalian chromosome preparations. Moreover, Hsu (1972) contains many original formulae for cytological reagents, fixatives, and stains for mammalian chromosome studies, especially for Chinese hamster ovary and bone marrow cells.

Adaptability

This exercise can be completed in a variety of options depending on cell culture facilities, technical know-how, and the time available. Therefore, the instructor can assess his/her own teaching environment and decide which option is best suited for that environment. These options are as follows:

Option 1: *The instructor prepares cell cultures and conducts the experiment. The students are provided with slides with cells to perform the FPG technique only (see Part 4 of Student Outline).*

Students can see the expected SCD and SCE results in one laboratory session. If this option is adopted, it is suggested that a 250-cm^2 flask be used for culturing cells, and that the shaking method of Terasima and Tolmach (1961) for collecting a high percentage of colchicine or colcemid arrested metaphase (C-metaphase) cells be used. The fixed cell suspension can then be stored in the dark at 4°C for a long time, provided the cell suspension is periodically washed in and replaced with fresh fixative. Only a very small drop of this cell suspension is needed for making a slide for the FPG technique. Thus the instructor does not have to start by culturing cells every semester.

This option can be done with or without the treatment of a mutagen. If a mutagen is included, at least two such large flasks should be set up. One flask serves as the control, and the other serves as the experimental flask in which cells are treated with a mutagen at a specific dosage. After students have processed the slides through the FPG steps, they can then collect data and analyze SCE results statistically.

Option 2: *The instructor prepares the initial cell culture flasks. The students, individually or as a group of three or four, do their own planning and carry out the experiment from Part 1 (step 2) through Part 4.*

This option will require the students/groups to follow their own schedules. Thus, it is an independent project with close supervision. They can research and study the mutagenic effects on SCE of a chemical or physical agent, in addition to learning the techniques this exercise demonstrates. The students will definitely learn more on how science works and how to manage the various logistic factors involved in a scientific experiment. On the other hand, the instructor should be prepared to answer a variety of interesting questions. To say the least, it is an excellent opportunity for the instructor to learn how to teach students to do science. However, it may also turn out to be either a rewarding or frustrating experience for both.

The students are expected to complete the experiment through Part 3 in 2 weeks. The entire class can then do the FPG steps together in one laboratory session. All together, this option will require at least 3 weeks to complete the experiment, exclusive of data analysis and writing the report.

Option 3: *The students will carry out the entire experiment from Part 1 through Part 4.*

The excitement of thawing an ampule of frozen cells and watching them grow into a healthy cell culture is an unforgettable experience for any undergraduate student. This option is highly recommended if cell culture facilities and technical know-how are available. However, the students should be given instructions and readings on the basic principles and techniques for managing mammalian cell cultures before they begin this exercise. Allow at least one more week for preparing cells in addition to the time required for Option 2.

Expendability and Resource Information

This laboratory exercise describes the techniques for studying SCD and SCE in cultured CHO cells. The techniques learned here can be applied to other plant and animal cell systems for *in vitro* and *in vivo* studies. The original FPG technique for studying SCE in CHO cells and human peripheral leucocytes in culture was reported by Korenberg and Freedlender (1974) and by Wolff and Perry (1974). Perry (1980) contributed a comprehensive summary of the work on SCE and included tables of positive tests for chemicals that increase the yield of SCEs in a variety of cell systems. Latt and Schreck (1980) provided a comprehensive review of the basic mechanisms of the formation of SCE, and it is a good reference for the various expressions of SCD and SCE after more than two rounds of BrdU substitutions. Goto et al. (1978) reported a detailed study of the factors

involved in differential Giemsa-staining of sister chromatids, and this is an excellent resource paper for understanding the many aspects of the FPG technique. It is recommended that this paper be read before attempts are made to improve SCD results, especially if modifications are made to the present protocol because of cell type changes or other local factors in the laboratory. These proposed changes can also be assigned to the students/groups as topics for independent projects.

SCE is a very useful system for mutagenicity testing in a wide variety of organisms. A wealth of information on the effects of various chemical, physical, and environmental agents on SCE can be found in the relevant scientific journals, annuals, and books listed in the Literature Cited. Recently, Loveday et al. (1990) reported the results of 46 chemicals tested for SCE in CHO cells in culture. If laboratory safety is a limiting factor, some of the early reports may serve as models for studying mutagenic effects on SCE in various cell systems in the teaching laboratory. These mutagens are relatively safely handled by undergraduates, and they are easy to apply. In addition, the cell systems are commonly available in the teaching laboratory. Hsu (1982) edited several comprehensive reviews of these cell systems for SCE studies by experts in the field, including the root-tips of *Vicia faba*, avian embryos, fishes, CHO cells, and human lymphocytes.

The following is a sample of original references: saccharine in CHO and human lymphocytes (Wolff and Rodin, 1978), caffeine in *in vivo* Chinese hamster bone marrow cells (Basler et al., 1979), formaldehyde in human lymphocytes (Kreiger and Garry, 1983; Popescu et al., 1979), caffeine and 8-ethoxycaffeine in *Vicia faba* (Kihlman, 1975), Neutral Red in the central mudminnow, *Umbra limi*, (Kligerman, 1979), 5-BrdU substitution in CHO cells (Morgan and Wolff, 1984), fluorescent light in human cells (Monticone and Schneider, 1979), Mitomycin-C in *in vivo* and *in vitro* systems (Kram et al., 1979; Moquet et al., 1987), UV light and its inhibition by caffeine (Kato, 1973), and the recent work employing the chick embryo system on the herbicide MPCA (Arias, 1992).

Materials

Cell Culturing and Cell Harvesting

CHO-K1 cell line (order one frozen ampule or cell culture flask From American Type Culture Collection)

Per group of four students, order or prepare the following (*Note:* all reagents and solutions for cell culturing must be made and kept sterile):

Ham's F-12 medium with glutamine, $NaHCO_3$, 15–20% fetal calf serum, penicillium-streptomycin (consult a cell culture catalog (e.g., Sigma) for specifications for your cell culturing environment) (100 ml)
1 N HCl solution (5 ml)
1 N NaOH solution (5 ml)
Hanks balanced salt solution (HBSS) without calcium and magnesium (200 ml)
KCl solution, 0.075 M (warm up before use) (100 ml)
BrdU solution, 10^{-3} M (dissolve from powder, freeze and store stock solution in higher concentration; dilute with HBSS before use) (5 ml)
Trypsin-EDTA solution (0.025%), 1X (store in freezer) (10 ml)
Colcemid working solution, 4 µg/ml in HBSS (5 ml)
Acetic-methanol fixative, 1:3 (25 ml glacier acetic acid to 75 ml absolute methanol prepared fresh and chilled before use) (100 ml)
Microscopic slides with frosted end (cleaned and stored in 70% histological-grade alcohol in the refrigerator, rinse in chilled distilled water before use) (1 box)

FPG Technique

Sorenson's buffer solution, pH 6.8 (prepared in bulk) (200 ml)
Hoechst 33258 working solution, 0.5 µg/ml (tubes must be wrapped in foil and refrigerated, store stock solution in higher concentration; order Hoechst 33258 from Eastman Kodak Co., Rochester, NY 14650) (3 ml)
4% Giemsa stain solution in Soreson's buffer at pH 6.8 (prepared fresh from Giemsa stock solution just before use in a coplin jar, discard used stain) (30 ml)

General Supplies and Equipment

Sterile pipets and associated pipettors, 1 ml, 5 ml, and 10 ml
Micropipettors, 10–100 µl size, and sterile tips
Aluminum foil, pre-cut to fit size of culture flask
Cell culture flasks, 25 cm^2, 75 cm^2, and 150 cm^2
Conical centrifuge tubes, 15 ml
Coplin jars with cover (at least 6 per group)
Rectangular staining dishes with cover (both the dish and cover can be used for photoreactivation and staining)
Slide tray with 20 slots (1 per group)
UV lamp, black-ray
Water bath
Incubators, regular or CO_2, depending on cell culturing system
Gas cylinder with 5% CO_2 to gas cell culture flask if regular incubator is used
Clinical centrifuge
Laminar-flow hood
Liquid nitrogen container
Lamp with a red safety bulb

Student Outline

Introduction

Sister chromatid exchange (SCE) is a symmetrical exchange at one point between sister chromatids that does not result in an alteration of overall chromosome morphology. The term SCE is generally applied to mitotic chromosomes, and its detection is dependent upon the presence of sister chromatid differentiation (SCD) of the colchicine or colcemid-arrested metaphase (C-metaphase) chromosomes in mitosis.

The current method for inducing SCD requires, first and foremost, successful substitution of the thymidine analogue 5-bromodeoxyuridine (BrdU) during the S-phase of mitosis for two consecutive mitotic divisions. After cell harvesting and slide making, cells are stained in the fluorescent dye, Hoechst 33258, to induce differential staining of sister chromatids which contain unequal numbers of DNA polynucleotide chains substituted with BrdU. However, at this point, SCD can only be detected by fluorescent microscopy, and the fluorochrome-stained slides can not be kept long without fading. An improved method for detecting SCD, the Fluorescence Plus Giemsa (FPG) technique, has been developed, which requires two additional steps. However, the slides are permanently stained, and they can be examined under a bright-field microscope commonly available

in the teaching laboratory. Figure 4.1 shows the BrdU method for detecting SCD and the expected FPG results.

Many modifications have been made in various laboratories since the original FPG technique was developed by Perry and Wolff (1974). We have modified the technique especially for teaching undergraduate students. The present protocol has been developed and tested for many years in the Mammalian Cell Culture Laboratory, Department of Biology, Valparaiso University.

The goals of this exercise are to (1) illustrate the semiconservative mode of DNA duplication through the expression of SCD, (2) illustrate the single-stranded nature (i.e., one DNA duplex per G_1 chromosome) of the macromolecular organization of eukaryotic chromosomes, (3) measure the mutagenic effects of a chemical or physical agent through SCE, and (4) learn the FPG technique.

The FPG Technique for Studying SCD and SCE

The following is a protocol for studying SCD and SCE in cultured CHO cells. It is divided into four parts. Please note that not all four parts will be done by all students individually. Please strictly follow the instructor's oral and/or written instructions. However, it is imperative that each student read and fully understand every step of this exercise whether or not he/she is assigned to do the work. Full participation in group discussions and in planning of experiments in every phase of this exercise is expected.

Directions: Prewarm all necessary reagents and culture media to room temperature in advance. Immediately after use, return and store all reagents and culture media to the refrigerator (4°C) or freezer (−10°C or lower) as instructed. Discard used reagents and culture media into the designated container only.

Part 1: Preparation of CHO Cells in Culture

1. Thaw an ampule of CHO cells and grow cells in a 25-cm^2 flask with 5 ml of the regular growth medium for CHO cells at 37°C until the cell culture has reached confluence (at which every cell is in contact with other cells in all directions).
2. Subculture (see Part 3, steps 2a to f) a confluent flask into a predetermined number of flasks with the regular growth medium.
   Note: Attention should be given to the initial cell number so that there is sufficient space for cells to complete two cell divisions. Under optimum conditions, the total cell cycle time of CHO cells in culture is about 12 hours. This means, in theory, one confluent flask will grow into two confluent flasks in 12 hours following a 1:2 split. However, it is expected that the novice will lose or damage some cells during the subculturing process. Therefore, the effect is to prolong cell cycle time, especially when cells are treated with chemical or handled roughly.
3. Examine cell culture flasks to see if cells have become attached to the growing surface at least 4 hours after subculturing, if the cell culture is to be used for experimentation on the same day. It is recommended that the newly subcultured flasks be left growing overnight and the experiment be started the next day.
   Note: In a healthy cell culture almost all cells should be attached and have become spindle-shaped. The color of the medium should have shifted to slightly more orange than orange-purple. These are signs of active cell growth and proliferation.

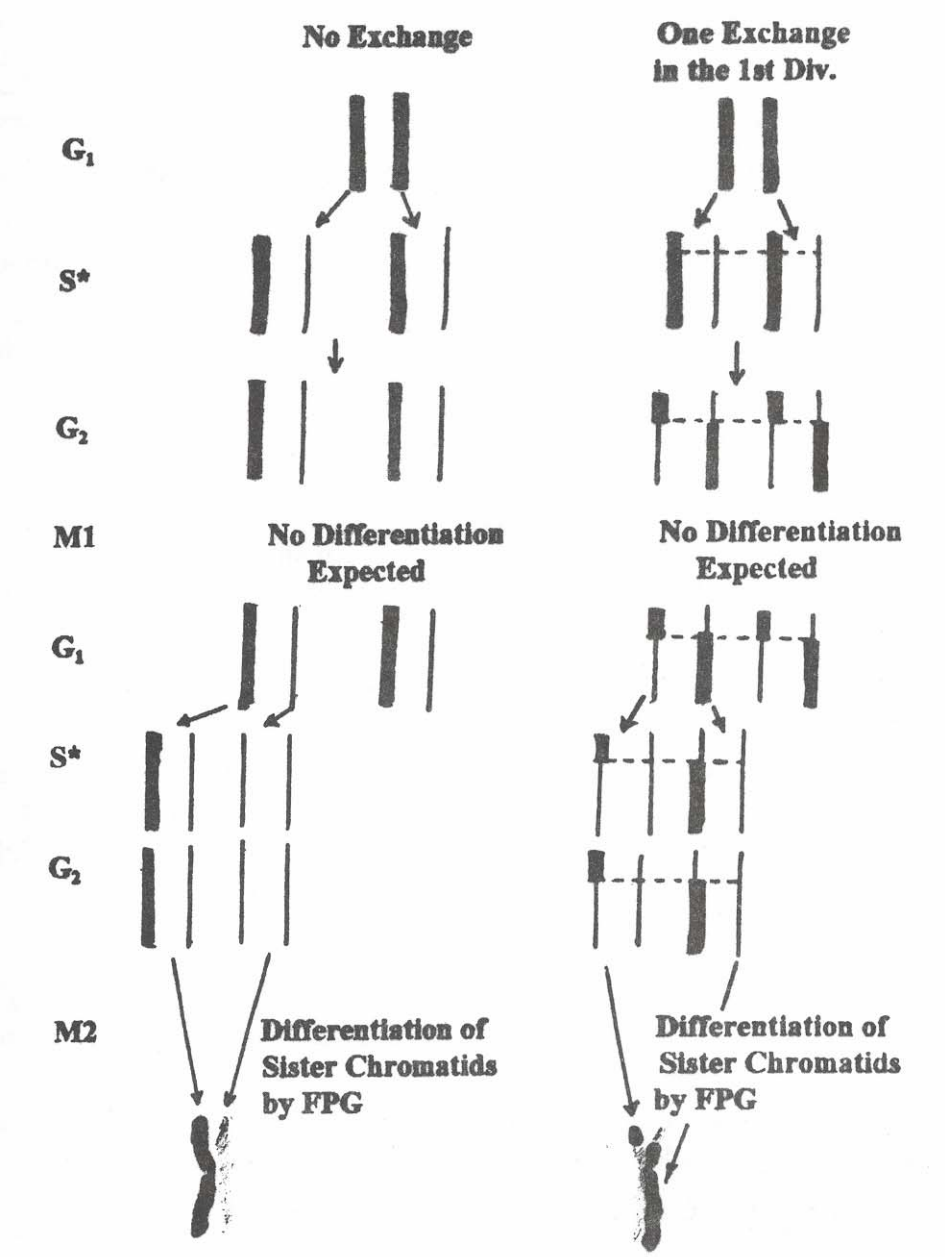

Figure 4.1. The BrdU method for detecting sister chromatid differentiation and exchange. Cells are cultured for two cycles in BrdU during DNA duplication (S*). Thick lines represent DNA polynucleotides with thymidine. Thin lines represent DNA polynucleotides substituted with BrdU. M1 (the first generation metaphase) has equal numbers of BrdU-substituted polynucleotides in the sister chromatids (unifilarly substituted). M2 (the second generation metaphase) has an unequal number of BrdU-substituted polynucleotides in the sister chromatids. One of the chromatids has two BrdU-substituted polynucleotides in the DNA duplex (bifilarly substituted). After the FPG treatment, the chromatid with the bifilarly substituted DNA duplex stains lighter than the other sister chromatid. These results are consistent with the semi-conservative mode of DNA duplication and with the single-stranded nature (one DNA duplex) of eukaryotic chromosomes. The diagram shows only one of the two expected M2 FPG chromosomes resulting from one G_1 chromosome in the second mitotic division.

Part 2: Treatment of BrdU and Mutagen

1. Decant old culture medium into the designated container inside the sterile tissue culture hood if the cell culture has been incubated overnight or longer. Replace it with fresh growth medium.
2. If planned, add the chosen mutagen to the desired final concentration per total volume of medium per flask.
   Note: All calculations should be done and verified by a fellow student well in advance.
3. If necessary, adjust the pH of culture medium by using sterile 1 N HCl or 1 N NaOH solution. Add drop by drop with a sterile 1 ml pipet until the color of the cell culture medium becomes orange-red (pH 7.2–7.4).
4. Wrap flasks with the pre-cut aluminum foil to prevent exposure of BrdU to light. Label the foil and the flask as well.
5. Preset the micropipettor or other suitable pipet to the mark directed by the instructor. Gather all necessary supplies and place them inside the hood. Turn off all lights. Turn on the red safety lamp placed inside the hood. Add BrdU from the 10^{-3} M working solution to reach the final concentration of 10 μM.
6. Gas each flask with 5% CO_2 for 20 seconds and quickly screw the cap tightly. Incubate culture flasks in the horizontal position.
   Note: Make sure the canted neck is pointed up and the flask is level so that the cell monolayer will be covered by the culture medium for the entire duration of the experiment. Sometimes it is difficult to tell if the flask is placed correctly on the shelf of the incubator if it has been wrapped with foil.

Part 3: Harvesting Cells and Making Slides

1. Twenty-eight hours after adding BrdU, add colcemid to each flask to reach the final concentration of 0.08 μg/ml of cell culture or as directed by the instructor. Incubate the flasks for an additional 1.5 hours to arrest cells in metaphase.
   Note: The amount is dependent on the concentration and freshness of the colcemid solution. If the working solution is 4 μg/ml, add 0.1 ml of this solution to 5 ml of cell culture.
2. At the end of the colcemid treatment, harvest cells and make slides.
   Note: Cell harvesting and slide making can be conducted outside the sterile hood, provided that none of the solutions used needs to be kept sterile. Unless otherwise instructed the HBSS and 1X trypsin solution should be kept sterile. The trypsin solution should be frozen after use. Make sure that the solutions are warmed or chilled in advance as directed.
   The following steps for cell harvesting and slide making are basic to all types of mammalian cell cultures for chromosome studies. *Note:* The amount added is based on a 25-cm^2 flask with 5 ml of medium.
   (a) Unwrap foil, decant old medium, and rinse the cell monolayer with Hanks balanced salt solution (HBSS) twice. The last HBSS must be completely removed from the cell culture flask. *Note:* The HBSS rinse is absolutely necessary for effective trypsinization, and it can not be skipped.
   (b) Add 2 ml of the 1X (0.025%) trypsin solution to cover the entire monolayer. Cap and remove the flask from the sterile hood and examine the monolayer on an inverted tissue culture microscope.
   (c) Observe carefully and wait until the trypsin has caused most of the cells to become rounded off, and then decant the trypsin solution quickly. Incubate the flask for 5 minutes.

(d) Add 3 ml of the cell culture medium with serum to stop the action of trypsin. Examine the flask and gently move it horizontally on the microscope stage to dislodge cells from the monolayer growing surface. Most, if not all, cells should be detached and floating in the medium if trypsinization is successful.

(e) After all or almost all cells have become dislodged, transfer the entire contents of the flask into a 15 ml conical centrifuge tube. Mark the tube at 5 ml if the original divisions are not clear. Centrifuge the tube at 800 rpm for 5 minutes.

(f) Carefully remove the supernatant with a pasteur pipet to about 0.1 ml above the cell button. Gently but thoroughly mix the cell button with the remaining solution. This can be achieved by blowing air through the pipet tip or tapping the tip of the centrifuge tube in the palm or on a soft pad.

(g) First, add the prewarmed 0.075 M KCl hypotonic solution drop by drop along the side of the tube to the 1 ml mark with constant mixing of all tubes involved. Then, quickly add to the 5 ml mark. Allow tubes to stand at room temperature for 12 minutes to complete the hypotonic treatment.

(h) At the end of the hypotonic treatment, centrifuge the tubes at 800 rpm for 5 minutes. Discard the supernatant, and mix the cell button with the remaining solution as described in the step f.

(i) Add the 1:3 acetic-methanol fixative, pre-chilled in the refrigerator, to each tube in the same manner as described in step g for multiple tubes. Allow the tubes to stand at room temperature for at least 15 minutes.
Note: This is the fixation treatment. Tubes with cells in the fixative can be stored in the refrigerator overnight or longer until the experiment is resumed. This step can also be planned as a break-point in the experimental schedule.

(j) At the end of fixation or storage after fixation, wash cells in the fixative twice as described in step f with no waiting time between. This means adding and removing the second and the third round of fixative in addition to the first round of fixative for the fixation treatment.

(k) Depending on the size of the cell button, add fixative drop by drop with constant mixing to the 0.2 ml (6 drops) mark. This is the *initial cell suspension* for making slides. *Note:* The cell suspension should look slightly cloudy but transparent.

(l) Make a test slide by delivering one drop of the initial cell suspension from a height of 8 to 12 inches above the slide. Dry the slide for 1 to 2 minutes on a slide warmer set at 55°C. Examine the slide, without staining, under a phase-contrast microscope, or under a bright-field microscope after staining in Giemsa. Cells should not appear crowded and overlapped.

(m) If necessary, dilute cell density by adding more fixative drop by drop, while looking at the color of the cell suspension, until the desired cell density is reached. If the cell density is too sparse, the initial cell suspension can be thickened by recentrifuging the tube, and the resultant cell button can then be mixed with less than 0.2 ml of the fixative. This is the *final cell suspension* from which multiple slides can be made.

(n) Transfer slides stored in 70% alcohol into clean, chilled deionized water. Use the final cell suspension to make multiple slides as described in step l. For best results, air dry slides in the dark overnight. For immediate use, dry slides on a slide warmer set at 55°C for 1–2 minutes. Store slides in the dark if the FPG treatment is not to be conducted immediately after drying to prevent interference of light with BrdU. *Note*: This can be used as another break-point if slides are air-dried in dark overnight or for several days.

Part 4: Preparing Slides by the FPG Technique

1. Stain the dried slides in the Hoechst 33258 fluorochrome stain, at 0.5 µg/ml in Sorenson's buffer at pH 6.8, for 15 minutes at room temperature.
2. Dip and rinse the stained slides several times in a 500- or 1000-ml beaker freshly filled with deionized water to thoroughly rinse off the fluorochrome stain from the surface of the slides. Place the slides at an angle inside a tray lined with paper towel to drain off the excess water.
3. It is not necessary to wait until the slides are completely dry to perform the following steps for *photoreactivation* by UV rays:
   (a) Put one slide in each slot, with the frosted end facing out, in a white slide tray with 20 slots. Flood the slides with 3 to 4 ml of Sorenson's buffer to cover the entire area of slide. Surface tension will help the buffer solution form a coating to prevent cells from drying.
   (b) Carefully put the slide tray on the slide warmer set at 55°C. Check the position of the tray to make sure the areas with cell drops are evenly exposed under the UV lamp.
   (c) Turn on the UV lamp over the slide warmer and expose the slides to the UV rays for 60 minutes. This is the duration for photoreactivation.
   (d) Turn off the UV lamp. Remove the slides from the tray, rinse the slides in fresh deionized water as in step 2 to rinse off the buffer solution and precipitates. For best results, air-dry slides before staining in Giemsa.
   (e) Stain slides in 4% Giemsa in Sorenson's buffer at pH 6.8 for 7 minutes. Dip the slides in fresh deionized water several times to rinse off the excess stain until the drip becomes clear.
   (f) For immediate use, dry slides for 1–2 minutes on the slide warmer set at 55°C or air-dry overnight.

   Note: The dried FPG slides can be studied without a coverslip under a bright-field microscope. However, care must be taken, in use or during storage, to prevent scratching of the surface of the slides. These slides can also be made permanent with a coverslip.

Notes for Instructor

Part 1: Preparation of CHO Cells in Culture

1. The original ampule from ATCC (American Type Culture Collection) comes with detailed information on the cell line and instructions for thawing and cell culturing. After thawing in the laboratory, cells may grow slowly at first, but they will eventually reach the confluent state. This first confluent flask can be subcultured into the desired number of flasks, depending on the plan for the exercise and the available facilities for cell culturing. After one or two subcultures, cells should have reached the log phase of growth. At this time a 1:4 split will grow to four confluent flasks in less then 30 hours. Cells can now be grown in mass, and subsequently frozen in liquid nitrogen in multiple ampules, each with 5×10^6 cells per ml of cells. These ampules can be issued to individual students or groups for laboratory work. In theory, cells can be stored in liquid nitrogen indefinitely. However, periodic checking and maintaining of cell lines is practiced in every cell culture laboratory. The advantage is that one does not have to order the cell line every semester if this is done.
2. Alternatively, individual students/groups can be issued a flask of cells at any state of growth. They are instructed to monitor cell growth and to prepare the experimental flasks according to their own schedules. They need to be reminded that serious planning should be done before they start any part of this exercise, and the cells will not wait for them. Therefore, the various break-points indicated in the protocol should be helpful to their planning.

3. A 1:5 split from a confluent flask is most desirable if the treatment of BrdU is done in the afternoon of the same day. One of the five flasks can be kept as the stock culture, if so desired. It is a good idea to require students to maintain their own stock culture during this exercise. A 1:8 split may be done in the evening, if cells are growing exponentially and the treatment of BrdU is to be performed the next day. Students need to be reminded that cells should never be overcrowded, which inhibits growth and proliferation. There must be ample space for cells to complete two rounds of DNA duplication. Otherwise, no SCD can be detected even if all the subsequent steps are performed without errors.
4. A healthy and vigorously growing cell culture is the prerequisite for success in this or any other experiment involving cell culture. There should be no compromise in this, and prudent advice to students is to start over with another healthy cell culture.

Part 2: Treatment of BrdU and Mutagen

1. Studies have shown that DNA substituted with BrdU undergoes photolysis when exposed to short-wavelength light and that light increases the yield of SCE. Therefore, every effort should be made to prevent the exposure of light to BrdU during the experiment and in storage.
2. The final concentration of BrdU given in the protocol is based on fresh stock solution. This concentration can be adjusted upward to insure that there are enough viable BrdU molecules for uptake during two rounds of DNA duplications, especially after the working BrdU solution has been frozen and thawed repeatedly. Although at high concentrations BrdU has been found toxic to cell cultures, it is safe to use in the range of 10 to 20 μM final concentration or 3 to 6 μg/ml of cell culture medium without noticeable effects in the teaching laboratory. The working solution is 10^{-3} M or 307 μg/ml. It is suggested that a 10^{-2} M stock solution be stored in the freezer and fresh working solution be made just before use.
3. Any mutagen should be handled carefully and in accordance with the latest federal and local regulations. Latex gloves and lab coats should be worn while doing lab work, and the experiment should be conducted in appropriate chemical or tissue culture hoods with adequate ventilation. However, it is not necessary to include a mutagen in this exercise if the primary purpose is to observe SCD and SCE only.

Part 3: Harvesting Cells and Making Slides

1. Timing is the most important factor here. For inexperienced students, 28 hours after adding BrdU is the minimum interval for their cell cultures to complete two cell cycles. More time is much better than too little. Therefore, the instructor may suggest to different students or groups to harvest cells at different times beyond 28 hours. This approach may work best for students who have different schedules, and it will not only insure that some students have the expected results at the end, but it will also provide additional opportunities to obtain third generation SCD and SCE results. These are the benefits of an open-ended experiment.
2. The concentration and duration of the colcemid treatment will affect the appearance of the C-metaphase chromosomes. The optimal combination of these two factors will give the C-metaphase chromosomes the best appearance for SCE and SCE studies, which means that the chromatids are not overly contracted and the exchanges are easily scored. Similarly, the duration of hypotonic treatment should not cause cells to burst. In short, there is no substitute

for conducting a pilot experiment in the local laboratory for testing the various factors involved in a long protocol like this one, in which every step affects the final outcome.

Part 4: The FPG Technique

1. The fluorochrome, Hoechst 33258, is used in microgram quantities. Depending on the freshness of the working solution, concentrations from 0.5 to 5 µg/ml, a ten-fold range, will not yield noticeably different results in the teaching laboratory, provided that the excess is properly rinsed from the slide, especially at higher concentrations. This is also consistent with the results reported in various research laboratories.
2. It is not necessary to use a slide tray to hold the fluorochrome-stained slides for photoreactivation as long as the buffer solution can be made stable on the slide for the entire period of photoreactivation. The cover of a standard histological staining dish works well for individual students to process two slides at a time. It is not necessary to use a slide grip to process slides through different solutions.
3. For best results, a black-ray UV lamp should be used for photoreactivation. An ordinary fluorescent light fixture with two 15 Watt BLB tubes will work well. It is best that the arm of the light fixture be adjustable so that the UV lamp can be easily placed just 1 to 2 inches above the slides. Remind the students that UV rays are harmful and mutagenic!

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