VARIATION IN S PHASE IN SYNCHRONOUS HUMAN CELL LINES

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SUMMARY

Growth parameters of diploid and trisomic human fibroblasts were determined. The rate of growth of both classes of cells was examined in asynchronous cultures, and diploid and trisomic cells had similar growth rates. Synchronous cultures were developed using simple mitotic selection. The patterns and length of the DNA synthetic period (S phase) were found to be altered in trisomy 21 cells when compared to diploid human or to heteroploid HeLa cells. Early S-phase synthesis was absent or reduced and the overall length of the S phase was extended. However, the trisomic cells have apparently normal rates of DNA chain elongation and normal replicon sizes.

Key words: trisomy 21; synchronous cells; cell cycle; S phase DNA synthesis.

INTRODUCTION

Most studies of the mammalian cell cycle have shown a single peak of incorporation of thymidine around the middle of the DNA synthesis (S) phase. More recently, however, studies with mitotically selected human diploid fibroblasts showed a relatively long S phase with three distinct peaks in the overall DNA synthetic pattern during S phase (1-3). In contrast to the pattern observed for human diploid fibroblasts, aneuploid HeLa cells have a single peak of thymidine incorporation during S phase. The HeLa cells also have a shorter S phase, longer G1 phase, and a similar generation time compared to diploid cells (1).

Since there is a clear difference in the cell cycles of HeLa and human diploid fibroblast cells, we attempted to examine the cell cycle of other types of human cells to determine if the cell cycle in aneuploid human cells would vary from the pattern shown by human diploid fibroblast cells. We examined the problem: "What does the cell cycle, and in particular, S-phase, look like in aneuploid human cells?" In the work reported here we used primary fibroblast cell strains developed from donors with Down's Syndrome (trisomy 21 syndrome) and compared their growth parameters and cell cycle to those of a cell strain derived from a diploid donor. We found that the pattern of thymidine incorporation during the S phase is altered in Down's syndrome cells.

MATERIALS AND METHODS

Cell culture. The human fibroblast cultures used in this study were from a normal diploid (E11), and from two different Down's Syndrome (trisomy 21) donors (M1 and P1). They were initiated at SUNY, Albany, and cultured in medium 199 (GIBCO) supplemented with 20% serum. A split ratio of 1:3, in the presence of trypsin, was used for all subcultivation. For growth studies, all cultures were early passage. Further details of culture conditions have been published previously (4).

Growth studies. Cells were trypsinized and replated in 25-cm² flasks at a density of 6.6 x 10⁸ cells per cm². At approximately 30, 48, 52 and 72 hr, cells were collected by trypsinization from three replicate flasks and counted electronically. Least squares regression analysis was used to calculate the slope of the growth curves, and the linearity of the log-normal slopes was evaluated.
by a BMD computer program for polynomial regression (5). Each curve in Fig. 1 represents the mean of three independent growth experiments, each one of which was done in triplicate. The variance estimates represent the range of nine points.

Synchrony. Synchronous populations were obtained by growing cells in 750-cm$^2$ roller bottles maintained at 0.2 rpm. To detach mitotic cells, the roller speed was increased to 200 rpm for 5 min, and then medium containing mitotic cells was collected from the roller bottles. Mitotic HeLa cells were collected once and the resulting mitotic population was plated into 20 dishes and pulse-labeled at hourly intervals after collection. Mitotic E-11, M-1 and P-1 cells were collected every hour, and each mitotic population was plated into a prescription bottle, so that after 20 hr there were 20 flasks, each containing a synchronous population ranging in age from 1 hr for the most recent selection to 20 hr for the earliest selection. This synchrony system has been used previously for the human diploid strain WI-38 (2) and for E-11 and another human diploid strain (1).

Thymidine incorporation. The cells were incubated for 1 hr with 2 μCi per ml $[^3]$H]thymidine (50 Ci per mmol), washed 3 times with cold SSC (0.15 M sodium chloride, 0.015 M sodium citrate), and trypsinized. One-half of the cells were removed and carrier DNA was added; this was sonicated and precipitated with 1.5 N HCl containing 6% pyrophosphate. The precipitate was filtered through Whatman GF/C filters which were washed with 4% perchloric acid, 70% ethanol, 95% ethanol and 100% ethanol and counted by liquid scintillation spectrometry. The other half of the cells were diluted in SSC and the cell number was determined in a Coulter Counter.

DNA replication rates and replicon sizes. DNA fork displacement rates were measured exactly as described by Povirk and Painter (6). Briefly, cells were pulse-labeled with $[^3]$H]thymidine (50 μCi per ml), BudR (10$^{-5}$ M) and FudR (10$^{-4}$ M) for 40 or 50 min and then subjected to various exposure doses of 313 nm light. Cells then were layered onto 0.5 ml of a lysis solution [0.02 M EDTA, 0.2 mM NaOH, 0.1% nonidet NP-40 (Shell Oil)] on top of a 5% to 20% alkaline sucrose gradient and centrifuged for 4 hr at 20,000 rpm in an SW27 Beckman rotor. Fractions were collected from the bottom of the gradients and the radioactivity in each fraction was measured. The length of the BudR-substituted DNA was calculated from the equation:

$$L = QM_b/b$$

where $QM_b$ is calculated from the distribution of radioactivity in the gradients and the exposure dose of 313 nm light to which the cells in the gradient had been exposed. $b/b_o$ is the extent to which thymidine was replaced by BudR. This method is described in detail by Kapp and Painter (7). The resulting DNA replication fork displacement rate was $L/$pulse label time).

Replicon sizes were estimated using the method of Kapp and Painter (7). Briefly, cells were exposed to 1000 rads of X-rays and then pulse-labeled for up to 50 min with $[^3]$H]thymidine, FudR and BudR. Cells were subjected to various exposure doses of 313 nm light, and the length of the labeled piece of DNA was measured as described above. Since X-rays, at this dose, inhibit initiation of replicons but have little effect on chain elongation, the length ($L$) increases normally with labeling time after exposure to X-rays. However, since new initiations do not occur, $L$ only grows until all replicons that were functioning before X-irradiation are completed. $L$ then remains constant. This plateau value for $L$ is a function of replicon size. It can be shown that replicon size = 1.4 × $L$, i.e. 1.4 times the size of the labeled DNA piece in Table 1B (7).

RESULTS AND DISCUSSION

Growth characteristics of human fibroblasts. Fibroblasts from two different donors with