FACTORS TO CONSIDER IN PERFORMING SURVIVAL STUDIES WITH INSECT CELLS

THOMAS M. KOVAL

George Washington University School of Medicine and Health Sciences, Washington, D.C. 20037, and National Council on Radiation Protection and Measurements, 7910 Woodmont Avenue, Suite 1016, Bethesda, Maryland 20814

(Received 31 October 1986; accepted 4 March 1987)

SUMMARY

Insect cell lines are not well-suited to colony formation in liquid medium following low-density cell plating. The present studies demonstrate that the time of addition of fetal bovine serum to the culture medium and the number of $\gamma$-irradiated feeder cells added to each plate are important factors in developing a useful colony formation assay. TN-368 lepidopteran and WR69-DM-1 dipteran cell lines were used for these experiments. Both cell types display increased plating efficiencies if serum is added to the medium one or more days prior to plating as compared to adding serum immediately before plating. Growth curves obtained by seeding cells at higher densities also indicate that cell growth is slightly better if serum is added one or more days before seeding. These findings are especially important for survival and toxicity studies because the results demonstrate that even seemingly minor factors involved in cell survival assays may benefit treated cells to a greater degree than untreated control cells, thus providing an erroneous assessment of cell survival.

Key words: insect cell culture; cell survival; colony formation; serum; feeder cells; toxicity studies.

INTRODUCTION

It is beneficial to optimize culture conditions when performing survival studies with in vitro cell systems. For example, investigators working with cultured mammalian cells most often make their culture medium without serum and then add the serum to small portions of the medium lot immediately before use (3). This is done to obtain maximum growth promoting ability from the serum since it is believed by many that this growth promoting ability of serum-supplemented medium is diminished even upon short term storage at 4°C (3). This is even more applicable in cell survival and mutagenesis studies when working with very low cell densities because cell requirements at these densities are much more fastidious.

The description of the use of x-irradiated feeder cells by Puck and Marcus (11) and Fisher and Puck (1) over 30 years ago provided a quantitative means of studying the effect of potentially toxic agents on cell survival with cell cultures having very low colony-plating efficiencies. It was later demonstrated that 254 nm UV light-irradiated cells could also serve as a feeder layer, but not cells that had been fixed in ethanol for 10 min, air-dried for 30 min, or exposed to three cycles of freezing and thawing (12). In addition, cells x-irradiated with doses sufficient to completely suppress DNA synthesis were less potent as a feeder layer than cells x-irradiated with much lower (although still sufficient to prevent cell multiplication) doses (12). These studies concluded that metabolically active cells were required to provide feeder activity. Although these and other early studies delineate conditions necessary for obtaining functional feeder layers, optimal feeder effect is generally determined empirically by individual investigators based on their laboratory resources, specific cell system, and research needs.

Examination of the survival of cultured insect cells after treatment with a variety of physical and chemical agents is an important assay in this laboratory (6-8). The endpoint measured is colony formation in liquid medium. The focus of these studies is the determination of mechanisms of cell damage and repair. The shape of survival curves generated from colony formation experiments and the surviving cell fraction at a given dose are important factors in speculating on and testing for potential molecular repair mechanisms (8,9). While performing such experiments, it was noted that ostensibly small variations in the time of addition of serum to the plating medium could result in significant deviations in the colony-forming ability of the cells. The use of feeder cells can also have a significant impact on the colony-forming ability of cells. This study therefore addresses the timing of addition of serum to otherwise complete medium at both clonal and subculture seeding densities, and the use of x-irradiated feeder cells to enhance the colony-forming abilities of both untreated and UV light-irradiated insect cells. The colony forma-
tion technique provides a powerful quantitative tool for assessing the toxicity of various agents in cultured insect cells.

MATERIALS AND METHODS

Cell lines. The TN-368 cells are a continuous line of fibroblastlike cells derived from minced adult ovaries of the cabbage looper, _Trichoplusia ni_ (2). They have been in culture for more than 15 y. The cells grow in a loosely attached monolayer and generally require only moderate agitation to detach them from the bottom surface of polystyrene or glass flasks. Cultures are maintained in polystyrene tissue culture flasks (Corning) and kept in a humidified incubator at 28° C. The line has a doubling time of approximately 19 h and is normally maintained by subculturing three times per week at a 1:10 ratio in TNM-FH medium (KC Biological, Lenexa, KS). Further details concerning the TN-368 cells have been described elsewhere (5).

The WR69-DM-1 cells (Schneider’s line 1) are a continuous line of _Drosophila melanogaster_ cells derived by enzymatic dissociation of Oregon-R embryos on the verge of hatching (13). At the time of this study, the cells had been subcultured more than 600 times at a split ratio of at least 1:10. The line is composed of mostly epithelial cells, but a significant number of fibroblastlike cells are also apparent. The cells grow in a relatively loosely attached monolayer and are detached from the bottom surface of culture vessels by a few flushings with medium. Cultures are maintained in polystyrene tissue culture flasks (Corning) and kept in a humidified incubator at 28° C. The line is normally maintained by subculturing three times per week at a 1:10 ratio in Schneider’s medium (KC Biological) supplemented with 15% fetal bovine serum (Dutchland, Denver, PA) and without antibiotics.

Serum addition to medium. Serum was stored at −80° C and thawed, one 500-ml bottle at a time, as needed. Once thawed, unused serum was kept refrigerated. Such refrigerated serum was generally used within 1 to 2 wk during these experiments. Both TNM-FH and Schneider’s media were prepared as usual, except that serum was omitted. Serum used to supplement Schneider’s medium was heat inactivated at 56° C for 30 min. For a given experiment, serum was removed from the refrigerator and the appropriate amount added to a given volume of medium. This medium containing serum, as well as the stock bottles of serum and medium, were kept at room temperature, approximately 24° C, until serum was added to medium at the various times indicated. Once the final batch of complete medium was prepared (immediately before initiating the experiment), cells were prepared and seeded and all the samples were incubated at 28° C. Therefore, temperature should not be a factor and the only difference between the several batches of complete medium was the time at which serum was added.

Growth curves. Exponentially growing cells were suspended in the medium in which they had been growing, counted, and seeded into 25-cm² flasks containing the same volume of medium to which serum had been added either 24 h or immediately before seeding. Flasks were incubated at 28° C. A Coulter counter was used to perform cell counts on two replicate flasks per medium (serum added 24 h or immediately before seeding) at each sampling. These flasks were then discarded.

γ-Irradiations. A 2.1 kCi ¹³⁷Cs source was used for all γ-irradiations. Feeder cells were irradiated at a dose rate of approximately 4.8 Gy/min. Dosimetry was performed using a 0.11-cc air ionization chamber (P.T.W. Co.) having an accuracy within 0.05% and LiF thermoluminescent dosimeters (Harshaw) having an accuracy within 3 to 5%. TN-368 cell feeder layers were irradiated to a final dose of approximately 1600 Gy and WR69-DM-1 with approximately 340 Gy.

Ultraviolet irradiations. Four 15-W germicidal lamps were used to perform 254-nm irradiations. Cells were irradiated at a fluence rate of 1.3 to 1.4 J/m²/s, as measured by an IL500 Research Radiometer (International Light, Inc.) and a J-225 short wave UV intensity meter (UV Products,