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EUKARYOTIC CELL CULTURES

Basics and Applications

Edited by Ronald T. Acton
and J. Daniel Lynn

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PREFACE

The Second International Cell Culture Congress was structured as was the First Congress to bring together scientists from academia and industry to discuss the use of cell culture in support of bioscience. It was felt that a forum whereby state-of-the-art presentations were followed by informal workshops would provide opportunity for the greatest exchange of information. Within the atmosphere of the workshop, problems common to basic as well as applied research were discussed and directions for the future were brought to light. These proceedings reflect and epitomize those discussions.

Although it is difficult to cover all scientific disciplines utilizing cells in culture, we feel key areas were addressed at the Congress and are herein presented. Considerable emphasis has been given to the methods for establishing cells in culture and characterizing the cells once established as well as the improved technology for growing established cell lines. Examples of how recombinant DNA technology is being used to manipulate genes within mammalian cells, to clone mammalian genes and to insert them in prokaryotes has been included. Major emphasis has been given to the use of lymphocytes in culture for understanding immune responsiveness and the culturing of a variety of cell types as a means to understand disease states.

While not all encompassing, these presentations certainly reflect the ferment of activity that now exists within the scientific disciplines that utilize cells in culture. Moreover,

since all contributors had the opportunity to recently review their manuscripts, the contents of these proceedings are timely. The Congress and these proceedings could not have been possible without the cooperation of all participants as well as the organizing committee and staff of the Department of Microbiology. We are extremely grateful to all for working so diligently.

Ronald T. Acton, Ph.D.
J. Daniel Lynn, M.S.

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ESTABLISHING AND CHARACTERIZING CELLS IN CULTURE

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The human body is made up of approximately 10 trillion cells, each too small to be seen with the naked eye. Each cell contains enough DNA for one to three million genes, but probably not more than 50,000 different structural genes with the remainder being accounted for by redundancy and programming. In any specialized tissue such as bone, skin, thyroid, it is obvious that a limited number of genes are active and the majority are suppressed. Each tissue must perform its special functions and also be responsive to the whole body and to the demands of other tissues; for growth, response to disease, trauma, repair; to signals carried by blood, lymph and nerves. Coordination of all this is also programmed in the genes. A very complex system. With so many interactions going on simultaneously, it becomes very difficult to follow the minute details in a single cell by examining the whole animal.

Cell culture has provided an answer to this dilemma. By growing each cell type in culture, its physiology and pharmacology can be observed alone and in response to various added stimuli or other cells, their products, hormones, etc.

Use of cell cultures has spawned in the last thirty (30) years, an explosion of knowledge about life processes within the living cell and how they are controlled. The science of applied genetics has been called the most rapidly progressing area of human knowledge in the world today and a great deal of this progress can be attributed to the application of cell culture techniques.

Exploitation of these new techniques has only just begun. Human animal, plant and invertebrate cells are being manipulated in culture to study normal life processes, the changes that occur in disease, for early diagnosis, treatment and prevention of disease and the manipulation of genes for production of growth hormone and other products. The prospects for understanding and modifying the mechanisms of genetic control of aging and cancer were never better.

I will confine my remarks to some basic principles in establishing and characterizing animal cell cultures based on experience in operating the National Human Genetic Mutant Cell Repository and the Aging Cell Repository (1).

The great volume of cell culture research in the past has been carried out with cell types easiest to grow in vitro, i.e., fibroblasts, lymphoblasts and a few tumor cells.

Many investigators are now concentrating on cell culture of specialized epithelial cells which in general are more fastidious in their growth requirements and difficult to propagate in serial cultures. The majority of fatal cancers arise from epithelial cells. Others in this workshop will be describing new procedures and nutritional requirements for growth of new cell types.

My remarks will be confined to three common failures in cell culture laboratories which should be addressed no matter what cell type is being established in cell culture. They are: reproducibility of culture conditions, maintenance of sterility, and storage in liquid nitrogen.

Culture Conditions: When maintained in vitro, cells are very sensitive to changes in the culture conditions. Minor changes may cause them to gain or loose characteristics which they expressed when first established in cell culture. On the other hand, experiments using cell cultures take days, weeks or months to complete and have to be repeated several times for confirmation of results. This means that for any series of experiments, one must plan to stabilize all conditions including temperature of incubators, culture vessels and glassware preparation, pH of culture media, use of single lots of culture medium, trypsin growth supplements, and a single lot of fetal calf serum which has been pretested for toxicity, growth stimulation and sterility. Many cell culturists have observed that a lot of serum that supports excellent growth of one cell type will be only fair, poor, or toxic for another cell type. This is true to a greater or lesser extent for all the culture conditions mentioned above.

Sterility: Cell cultures provide excellent nutrition for molds, yeasts, bacteria, mycoplasma and viruses. Cell cultures must be opened to add fresh culture medium one, two or more times a week. This provides many opportunities for microbial contamination of cell cultures (2). Measures to prevent contamination are well established (3,4). They may be summarized as follows:

1. Pretest all culture media components for microbial contamination.

2. Transfer cell cultures in Hepa (high efficiency particulate air) filtered transfer hoods or rooms.
3. Use no antibiotics in cell culture media for maintaining stock cultures. Exceptions are made for first TC passage from potentially infected tissue biopsies and for short term biochemical experiments.
4. Treat every cell culture as if it were contaminated, i.e., employ measures to prevent the spread of the contaminant to other sterile cultures maintained in the laboratory. Two polices are paramount in achieving this goal:
   a. Transfer only one cell line at a time within the transfer enclosure, and:
   b. Disinfect the work surface, equipment and hands before introducing the next cell line.

If these measures are faithfully followed, cross contamination of cell lines will be eliminated.

Storage in Liquid Nitrogen: The third safeguard against uncontrolled change, contamination or loss of a cell culture is to store several ampules at an early passage in liquid nitrogen. The cells will remain unchanged for years and provide a standard seed stock for repetition or expansion of experiments by the original investigator or investigators in other laboratories (5).

Characterization of Cell Lines: Cells in culture tend to be fibroblast-like, epithelial-like or grow in suspended culture and it is not possible to make a precise identification upon inspection alone. New methods for identification of cells, cell components or products are published frequently. The traditional tests most used to identify cell cultures have been:

For Species of origin; the karyotype, (6) serology with species specific cytotoxic antibody, (7) and species specific fluorescent antibody (8).
For interspecies and more specific identity many assays are available: In Vitro lifespan (9),
Chromosome markers and polymorphisms (10, 11),
HLA pattern (12),
Isoenzyme pattern (13),
Organ specific products, e.g., secretion of collagen, albumen, insulin, and production of membrane antigens, are too numerous to list here
Clonal growth in soft agar (14), and
growth in nude mice (15) which is the most reliable indicator of malignant transformation.

I would like next to share some of the things we have learned in establishing and characterizing thousands of cell lines over the past 30 years. The mechanism of cross contamination of cell cultures was shown (4) to be via contamination of the environment during feeding and transfer of cell cultures. Prevention of cross contamination is accomplished by a combination of aseptic and antiseptic measures (4,16,17). We have confirmed the efficiency of these measures by re-examination of a group of 964 cell cultures expanded, characterized and stored in liquid nitrogen, before we knew how to detect non-cultivable strains of M. hyorhinis. By use of a specific immunofluorescence assay, 33 of the 964 cell cultures when retested were contaminated with M. hyorhinis. There was no evidence that the infection spread to other cell cultures in our laboratory. All 33 contaminated specimens were received from laboratories known to harbor M. hyorhinis (18).

During 9 years' operation of the Human Genetic Mutant Cell Repository and the Aging Cell Repository, we have processed and characterized over 5,000 biopsies or cell cultures and the 8th edition of the catalog contains 300 pages of listings and data (1). Included are fibroblasts and lymphoblasts from apparently normal individuals as well as from individuals with disorders of amino acid, carbohydrate, lipid, metal, nucleoside, or steroid metabolism and of connective tissue, muscle, bone, and unknown disorders. Included are many examples of twelve different categories of chromosome aberrations. Cell cultures with over 200 different translocations and inversions have been useful in gene mapping.

Submissions of new biopsies to the repositories and distributions to investigators are shown in Figure 1. Recipient use of cell cultures from the repositories are shown in Table 1.

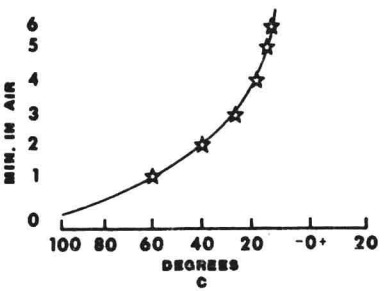

Fig. 1.