Invertebrate Tissue Culture

RESEARCH APPLICATIONS

Edited by

Karl Maramorosch

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Preface

In the past decade there has been a tremendous surge in the applications of invertebrate tissue culture to various aspects of biomedical as well as agricultural research. The sudden growth of interest in the use of insect and snail cells coupled with the progress in techniques and new areas of investigation motivated a United States–Japan seminar in Tokyo, December 9–13, 1974, on the current status of invertebrate tissue culture in research. The seminar proved helpful in stimulating further research in several fields as well as in promoting the wider use of invertebrate tissue culture. The Tissue Culture Association (United States) enthusiastically endorsed the seminar, and financial support was obtained from the National Science Foundation, the Japan Society for the Promotion of Science, U.S. Department of Agriculture, and WRARI (Walter Reed).

The conference concerned itself with an inquiry into diverse areas of basic science linked by a common tool: the *in vitro* culturing of tissues and cells of invertebrate animals. The program covered areas of particular importance to genetics, embryology, endocrinology, parasitology, virology, plant pathology, entomology, and neurophysiology. The United States–Japan Cooperative Science Program should be congratulated for effectively serving a very useful purpose: promoting cooperation between outstanding scientists of the two countries. A small multidisciplinary group of researchers in such diverse fields as biology, medicine, biochemistry, agriculture, botany, entomology, genetics, insect pathology, plant pathology, and zoology could hardly have been assembled by any other organization. The goals of the seminar, to stimulate interdisciplinary research and to promote international cooperation, have been achieved to the satisfaction of the participants.

After the seminar, several participants from the United States and Japan were invited to contribute extended chapters, comprising their own as well as work done by others, to a book that would cover the

broad field and current status of basic research in invertebrate tissue culture. The invited authors were requested to combine authoritative reviews of research areas with a discussion of the latest developments in each field. Many of the authors pioneered the development of their areas of expertise. The contributors were requested to discuss in depth such topics as cell growth and differentiation, cloning of established cell lines, the breakthrough in molluscan tissue culture and the establishment of the first snail line, invertebrate endocrinology, ecdysone biosynthesis *in vitro*, the identification of distinct juvenile hormones from corpora allata and the production of peptide neurohormones by cultured insect brains, the use of *Drosophila* discs *in vitro* to study gene activity sites, and the applications of insect tissue culture to the study of intracellular parasites, symbionts, and arboviruses. The topics were chosen because of their particular importance in biomedical fields and because of their rapid current development. Insect pathogenic viruses in insect cell lines, extranous contaminants in invertebrate cell cultures, the uses of invertebrate cells in plant pathology, and a description of invertebrate cell lines complete this volume.

The text is quite detailed. It is aimed at an audience with at least an introductory knowledge of tissue culture. The presentation of the most recent results of original research, interpretations, and original conclusions makes this book a unique body of information and brings into sharp focus current findings and new dimensions of invertebrate tissue culture. An attempt was made to have all chapters well documented by tables, photographs, and up-to-date and complete bibliographies. By combining diverse areas currently under investigation, this book should be of interest to microbiologists, parasitologists, virologists, entomologists, geneticists, and medical researchers working in the field and to graduate students in related fields of biomedical research.

I am deeply indebted to the contributors for the effort and care with which they have prepared their chapters and to the National Science Foundation and the Japan Society for the Promotion of Science for supporting the seminar that brought together several authors of this book. Finally I would like to mention the important roles played by the staff of Academic Press in their cooperation and assistance throughout the planning and completion of this volume. It is my hope that this book will contribute both to basic scientific and medical problems and will benefit all who are interested in invertebrate tissue culture.

KARL MARAMOROSCH

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PART A

Cell Growth and Differentiation

1

Agar Suspension Culture for the Cloning of Invertebrate Cells

ARTHUR H. McINTOSH

I. Introduction

Established insect cell lines, like their vertebrate counterparts, usually consist of a heterogeneous population of cell types resulting from selective influences of the particular cell culture system. As more invertebrate cell lines become established and available to investigators, the need for the successful cloning of such cells becomes essential. The main advantage of working with cloned lines is that the variability due to the presence of diverse cell types would be avoided, although it is recognized that wide variations in clonal populations themselves do occur. It also should be emphasized that cloning should be done as early as possible, even before the cultures become established cell lines. If performed later it is possible that only a few cell types might remain.

The many techniques employed in the cloning of vertebrate cells, such as the capillary method (Sanford *et al.*, 1948), the microdrop technique (Lwoff *et al.*, 1955), the feeder layer method (Puck and Marcus, 1955), and the dilution method described by Paul (1961), could be applied to invertebrate cell lines. However, despite these available techniques there have been relatively few reports regarding the successful cloning of insect

cells. In this regard, Suitor *et al.* (1966) and Grace (1968) reported the successful cloning of *Aedes aegypti* and *Antheraea eucalypti* cell lines utilizing the capillary and dilution methods, respectively. The *A. aegypti* cloned line has been shown to be a moth line (Greene *et al.*, 1972). More recently McIntosh and Rechtoris (1974) have cloned the insect-established *Trichoplusia ni* cell line (TN-368) of Hink (1970) by an agar suspension culture technique. Nakajima and Miyake (1974) have reported the succesful cloning of several *Drosophila* cell lines using spent medium.

This chapter will be concerned with the agar suspension culture technique as a means of isolating clones from several established insect cell lines. In addition some of the criteria used in assessing transformation of vertebrate cells will be discussed with respect to invertebrate cell cultures.

II. Materials and Methods

1. *Cell Cultures and Media*

A description of the insect cell lines and media employed in this study is presented in Table I. The cultivation of the lepidopteran and homop-

TABLE I
Insect-Established Cell Lines

Cell line	Medium	Source
Trichoplusia ni (cabbage looper)	TC199-MK[a]	Hink (1970)
Carpocapsa pomonella 169 (codling moth)	TC199-MK	Hink and Ellis (1971)
Spodoptera frugiperda (fall army worm)	TC199-MK	Goodwin *et al.* (1970)
Agallia constricta (leafhopper)	TC199-MK	Chiu and Black (1967)
Drosophila melanogaster Line 2 (fruit fly)	TC199-MK	Schneider (1972)
Aedes albopictus (ATCC) (mosquito)	M and M[b]	Singh (1967)
Aedes albopictus (Webb)	M and M	Singh (1967)
Aedes aegypti	M and M	Peleg (1968)

[a] McIntosh *et al.* (1973).
[b] Mitsuhashi and Maramorosch (1964).

teran lines have been previously reported (McIntosh *et al.*, 1973; McIntosh and Rechtoris, 1974). The *Aedes albopictus* cell lines were obtained from Dr. Sonja Buckley, Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, Connecticut; *A. aegypti* from Dr. Victor Stollar, Rutgers Medical School, New Brunswick, New Jersey; and *Drosophila melanogaster* from D. B. Skowronski, State University of New York, Purchase, New York. The *D. melanogaster* line has been adpated to grow in TC199-MK and is now in its tenth passage in this medium.

2. Virus

Chikungunya virus was obtained from Dr. Sonja Buckley and was used to challenge *A. albopictus* (ATCC). Approximately 2×10^6 cells in a Falcon T-flask (25 cm^2) were inoculated with 10^4 PFU/ml. Cells were subcultured every 3–4 days by making a 1:5 split.

3. Agar Suspension Culture Technique

This technique has been described in a previous report (McIntosh and Rechtoris, 1974). Briefly the procedure consists of preparing base layers comprised of heart infusion agar and TC199-MK to which is added the cell suspension in 0.33% Special Agar-Noble as overlay. Plates are incubated at 28°–30°C for 10–14 days and colonies counted.

4. Agglutination with Concanavalin A

Seventy-two hour cultures of the insect cell lines were employed in these studies. The medium was removed from the flask and the cell surface washed twice with Hanks' balanced salt solution (HBSS). Cells were collected in 3–4 ml of HBSS by pipetting back and forth and counted in a hemacytometer (McIntosh *et al.*, 1973). The cell density was adjusted to 1×10^6 cells/ml with HBSS and 0.5 ml cells mixed with 0.5 ml of concanavalin A (Con A) (200 μg/ml) in a petri plate (60×15 mm). For controls, 0.5 ml of HBSS was substituted for Con A. The reaction was observed with an inverted microscope at final magnifications of $\times 60$ and $\times 100$.

5. Mycoplasma

All cultures were tested for the presence of mycoplasma by a previously described method (McIntosh and Rechtoris, 1974) with the exception