Tissue culture — use or abuse?

T.M. Dexter reviews the techniques and resources currently available for tissue culture and discusses their future uses.

MOST mammalian cells can now be grown *in vitro* for periods ranging from several days to many years. The period of growth depends on whether the cells are normal or transformed, their stage of development (embryonic versus adult), the tissue, the cell lineage and the species. The increase in culture systems available (both cell lines and primary cultures) in the last twenty or thirty years and the relative ease with which cells can be cultured in quantities sufficient to enable biological, biochemical and molecular studies to be performed, means that more and more people are using tissue culture as a means to an end. A corollary of this is that more people are also abusing tissue culture, paying little or no attention to the physiological significance of the cells that they are growing or whether the cells are normal or not, in their search for the ever elusive "citation classic" on growth regulation, oncogenesis or gene expression!

Objectives

For cell biologists, the objectives of tissue culture are fairly simply defined. Growth, differentiation and development of normal cells are complex processes, requiring both non-specific and specific nutritional and regulatory molecules. One of the aims of tissue culture is to try and dissect these requirements, examining each and every variable in a controllable manner, free of systemic influences. This involves using "pure" cell populations, preferably clones, *in vitro*. Comparisons can then be made with cells undergoing aberrant developmental programmes such as during tumorigenesis.

A first step, of course, was to define the 'non-specific' requirements essential for the survival and growth of many different cell types, such as temperature, osmolarity and *p*H of the medium, the essential amino acids, vitamins, carbohydrates and trace elements. This work gave rise to the recipes now used for the synthetic growth media supplied by many companies. That the basic formulations of these media have remained unchanged for many years is a reflection of the pioneering efforts in this field by Eagle, Ham, Dulbecco, Fischer and numerous others. However, the successful growth of cells in these media invariably requires the presence of serum — a fickle cocktail of components.

Protein free media

Further elaboration of these synthetic media has led to the development of protein-free media which will support the growth of several different permanent cell lines[1]. While this work was of excellence in its approach to defining the nutritional requirements of cells, it was disappointing in that while transformed cells grew quite happily in such synthetic media for extended periods, non-transformed (normal) cells still generally required the presence of serum or serum components. (By transformed cells, I mean cells which were grown from normal tissue and became progressively adapted for growth in tissue culture, or cells which were taken from tumour tissue and showed an ability to grow *in vitro*. Invariably, such cells are aneuploid and have been selected for their ability to grow autonomously *in vitro* with minimal nutritional supplementation. Whether the cells are tumorigenic is another matter! By normal cells, I mean primary explants or continuously growing cells which maintain a diploid karyotype, a consistent phenotype and are non-tumorigenic. In only relatively few instances has it been determined if these cultured cells can function *in vivo* like the corresponding normal cells).

Following these efforts, extensive work has gone into defining the serum components essential for the maintenance of normal cells *in vitro*. These include corticosteroids, insulin, thyroid and pituitary-hormones, various growth factors, "spreading" factors and transferrin[2]. The mode of action of many of these supplements, and the nature of the cell responses mediated by them, are still largely unknown. Interestingly, however, the transferrin receptor has recently been implicated in cell proliferation[3] and transferrin has been found to be an essential component for the serum-free growth of many cell types, including haemopoietic progenitor cells[4]. From such humble beginnings is biology made.

Specific versus non-specific

Like the essential amino acids and trace-elements, I suspect that many of these other serum agents, such as transferrin, insulin and the steroid hormones, will be "non-specific" for growth. Instead they will have a tissue and lineage restriction only in terms of the quantities of the elements required. Rather more exciting are the current studies being undertaken with the so-called growth factors. These include nerve growth factor (NGF), epidermal and fibroblast growth factors (EGF and FGF), angiogenesis factor and various haemopoietic cell growth factors — many with a marked tissue or cell lineage restriction in their action. This is a topical and rapidly expanding field, too vast to be adequately touched on here, and the reader is referred to a good review on these factors[5].

The importance of these growth factors (many of them commercially available) is two fold. Many if not all normal cells demonstrate an absolute dependence on these growth factors for survival and proliferation. Commercially available sera may either not contain the growth factors, or contain them in too low a concentration to permit growth. Also, normal cells are usually grown in xenogeneic-sera; for example, human cells are grown in in fetal calf serum and murine cells in horse serum. Some of these growth factors, such as the haemopoietic cell growth factors are species specific in their activity. Thus, the inability to grow many normal cells may be due to a deficiency or an absence of the appropriate growth factor. Furthermore, the growth of 'transformed' cells in the absence of serum (and of these growth factors) may represent a fundamental process in the progression toward malignancy. Investigations are now underway to determine whether this independence arises as a result of the autonomous production of the essential growth factors by the tumour cells.

Thus, tissue culture is entering a new era of growth, although obviously many questions and problems remain. Also apparent is a realization of the limitations imposed by tissue culture, and of its prime requirement, namely that the culture cells must reflect their origin. □

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