CANCER BIOLOGY. II
Etiology and Therapy

Edited by

Cecilia M. Fenoglio, M.D.
Donald West King, M.D.

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College of Physicians and Surgeons of Columbia University
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3. Fenoglio CM, Goodman R, King DW (Eds): Developmental Genetics (1976)
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Contributors

Joseph H. Burchenal, M.D., Memorial Sloan-Kettering Cancer Center, New York, N.Y.

Judah Folkman, M.D., Department of Surgery, Harvard Medical School, Children's Hospital Medical Center, Boston, Mass.

Robert C. Gallo, M.D., Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Md.

Howard Green, M.D., Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass.

Kevin Lafferty, M.D., National University of Canberra, Canberra, Australia

Elliot Osserman, M.D., Department of Medicine, Institute for Cancer Research, College of Physicians and Surgeons of Columbia University, New York, N.Y.

Janet D. Rowley, M.D., Department of Medicine, University of Chicago, Chicago, Ill.

Richard T. Smith, M.D., Department of Pathology, University of Florida College of Medicine, Gainesville, Fla.

George J. Todaro, M.D., Viral Leukemia and Lymphoma Branch, National Cancer Institute, Bethesda, Md.

Peter K. Vogt, Ph.D., Department of Microbiology, University of Southern California, Los Angeles, Calif.

I. Bernard Weinstein, M.D., Institute for Cancer Research, College of Physicians and Surgeons of Columbia University, New York, N.Y.

Foreword

The Cancer Biology seminar held in Aspen July 20–25, 1975 was supported by the National Cancer Institute under Grant #5R13 CA 15961-02. It is clear that multiple etiologic factors are important in the development of cancer, including viral, immunologic, hormonal and environmental (physical and chemical) agents. Cancer is a complex disease with multifactorial ramifications, and its etiology cannot be a simple one.

A strong emphasis on environmental factors has been emphasized in this conference; the study of viruses has contributed greatly to molecular and cellular biology and may play an important role in the etiology of leukemia. The participants, both those in basic biology research and those in clinical practice, emphasize the necessity for close cooperation as they approach the problems of etiology, diagnosis and treatment.

Donald West King

CONTENTS

Introduction

The problems of etiology, diagnosis and therapy in cancer are enormous. Each field has its own specialized vocabulary, impeding communication between workers in different specialized areas. This monograph is the proceedings of an interdisciplinary seminar and as such may be of help to individuals holding single pieces of the enormous cancer puzzle.

Much of this seminar emphasizes external factors (viruses and carcinogens) which may play a role in the etiology of cancer. However, it is necessary to take a step away from the problem of neoplasia and define the growth controls which exist for non-neoplastic cells. Only then can we hope to determine if normal regulatory controls are present, absent or altered in neoplastic cell populations.

It is clear that external factors do have a regulatory function on the growth of the normal cell, and these factors affect various cells differently. (An example would be the lymphocyte mitogens which affect different classes of lymphocytes differently.) This differential response of cells to external stimuli would suggest that regulatory factors differ from one cell type to another; one would expect that disruption of these growth regulatory factors would result in a cancer of a single cell type.

These regulating systems are explored and defined by Howard Green, using the resting and growing state of the fibroblast as a model. He notes that as the cell shifts from the resting to growing state following mitogenic stimuli there is an increase in the protein synthetic machinery. This is reversible following withdrawal of the stimuli. The cellular regulation of the various components of the synthetic machinery differs, i.e., the regulation of the synthesis of mRNA and ribosomes differs. Replication of the genome also occurs following mitogenic stimuli; these features of cell growth are explored in the third volume of *Advances in Pathobiology.**

Clearly one group of external stimuli affecting cell growth is viruses. By applying some of the technics discussed in *Advances in Pathobiology 3, Developmental Genetics,* to the genetics of the RNA tumor viruses, fingerprints of mutant RNA, sequencing of RNA with localization of specific oligonucleotides of the genome and an analysis of DNA transcription may lead to advances in elucidating a possible viral etiology of cancer. The genome of these viruses can be studied using conditional and nonconditional viral mutants which represent deletions of the genetic material. This aspect

* A listing of the volumes in this series so far published is given on the verso of the title page of this book.

is discussed by Peter Vogt, who uses three functional classes of viral mutants. These mutants are defective in their ability to transform cells, to replicate in cells or to both transform and replicate in cells.

The RNA viruses are further explored in the discussions by Todaro and Gallo. Todaro points out that viral genetic material becomes incorporated into the host's own genetic machinery and then is transmitted with the host genes. These fragments of foreign genetic material (virogenes) which are normally repressed may become activated by intrinsic (genetic or hormonal) or extrinsic (chemical or viral) factors and lead to neoplastic growth.

Viral genes from one group of animals can lead to infective particles which can integrate into the DNA of other species and become part of the germ line, thus providing a mechanism by which stable interspecies transfer of genetic information can occur. The type C viruses are uniquely suited to this role, since they must incorporate into the host cellular DNA to replicate and they do not kill the cells they infect. The role of the type C RNA virus as a causative agent in leukemia is discussed by Gallo. He briefly surveys the problems encountered in trying to document proof that viruses are tumorogenic. He then details the replication and classification of these RNA viruses and concludes his discussion with the relationship of type C virus to human leukemia using a variety of experimental approaches.

A discussion of the viral etiology of cancer would be incomplete without a consideration of the DNA viruses. This subject is taken up at length in the following monograph, *Advances in Pathobiology 5.*

Another widely recognized group of external stimuli affecting cellular growth is a variety of chemical compounds. This aspect of etiology is presented in this monograph (also in no. 2 of the series) by Dr. Weinstein. In an historical survey he points out that in the 18th century, Sir Percival Potts demonstrated that coal tars were associated with human cancers and yet, in the 1970's, there are still 80,000 deaths a year from lung cancer—also related to coal tars. He surveys the current environmental situation, occupational and otherwise and points out that transplacental transport of certain compounds may affect the fetus. Following this background discussion he tackles the problem of how a chemical may serve as a carcinogen.

That viruses and chemicals do have effects on cells is well recognized, but undoubtedly these are only a fraction of a whole host of external factors which do. It is fascinating that something as simple as the geometric configuration of cells may also regulate their growth. This area is explored here by Folkman, who finds that growth in the two dimensional plane is virtually unlimited, whereas three dimensional growth is not. He relates this to a prime factor (vascularity) and ultimately to the cells' ability to obtain oxygen and nutrients and excrete wastes.

In considering etiologic agents of disease, it would be naive to discount genetic susceptibilities, but the relative importance of genetic versus environmental factors probably differs in each tumor. It is of interest that cells from tumors associated with specific etiologic agents have consistent chromosomal abnormalities (i.e., Burkitt's), whereas cells from tumors without specific etiologies have variable chromosomal alterations. These aspects of cancer biology are defined by Dr. Rowley.

Not only do external and genetic factors affect the mode of cellular growth but they may alter the cell membrane and produce characteristic antigenic changes (see *Advances in Pathobiology, no. 1*). These changes in cellular antigenicity provoke an immune response in the host, summarized here by Richard Smith, and, in an earlier volume, by John Marchalonis (*Advances in Pathobiology, no. 2*). In his overview, Dr. Smith deals with the nature of tumor antigens and the response they evoke in the host. These interactions are complex and involve an interaction between the tumor and the host defense systems. Different parts of this interaction may be duplicated in the laboratory but are less clear in the *in vivo* situation.

One of the initial problems in mounting an immunologic attack is the recognition of an intruder as a foreigner. As simple as one would think this would be, it is becoming evident that both a responding cell and a stimulating cell are required for such an identification to be made. The nature of the interaction and cooperation of these two types of cells (T lymphocytes and macrophages) are discussed by Dr. Lafferty.

In addition to the cellular effect on tumors, there is a host of soluble factors which may also have regulatory functions. Humoral antibody is certainly a major example of one of these (discussed by Dr. Smith), and lysozyme is now emerging as another important soluble mediator of cellular function. Dr. Osserman discusses the regulation of the effector function of macrophages by this material and compares it to the role of immunoglobulin on plasma cells.

By applying all that we know of etiologic factors and host responsiveness to tumors, we may hope in the future to better alter this response in a way which is beneficial to a given patient. However, preventive measures are not a reality for those already suffering from cancer. Fortunately for those people, significant advances have been made in cancer chemotherapy and these are summarized by Dr. Burchenal.

The continued close cooperation of cell biologists, virologists, endocrinologists, immunologists and clinicians will allow knowledge in one field to be available and applied in other fields. We may then be closer to the possibility of preventing and/or curing the disease most people fear.

The Editors

The Resting and Growing States of the Fibroblast

Howard Green, M.D.

What is greatly needed in the field of fundamental cancer research is some knowledge of how normal cells grow. Without it, the identification of causes of cancer (whether simple chemicals, viral genes or their products, or ionizing radiation) cannot be followed by any real progress with regard to their mechanism of action. This state of affairs has not prevented the development of successful therapy for some kinds of cancer, but it is difficult to imagine a general understanding of the disease and its therapy without detailed knowledge of the process of normal cell growth and its control.

The problem of cell growth can be conveniently divided into two parts. The first consists of the external regulating factors (mitogens or antimitogens) and their interaction with the target cells. In order that the growth of each differentiated cell type be regulated independently, these factors must be different for each cell type. Disruption of the basis for this part of the growth regulating mechanism should produce a cancer of only a single cell type. For example, specific chromosomal rearrangements such as those recently demonstrated in certain human leukemias lead to tumors of a single cell type. Presumably, the rearrangement affects a locus involved in the growth regulation of that cell type, but the same chromosomal rearrangement occurring in other cell types would produce no comparable disturbance.

A similar effect is probably responsible for the loss of neoplastic properties which occurs in the progeny of malignant teratoma cells. The process of differentiation evidently substitutes an intact regulating system characteristic of the differentiated cell type for the damaged one of the malignant stem cell. In this way, the process of differentiation leads to loss of neoplastic character.

The second part of the growth control system comes into operation after reception of the external growth controlling stimuli and prepares the cell for division or for the resting state. It seems likely that different cell types will

From the Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass.

have much the same mechanism for this part of the control system. In most cells, division follows automatically at an interval following replication of the genome, so that preparation for division is really preparation for DNA synthesis. Except for a small minority of cell types, once DNA synthesis has begun, the cell cannot reach a new resting state until it has completed DNA synthesis and divided.

In no case known does a mitogenic stimulus act by directly initiating DNA synthesis. The same is true of oncogenic viruses. A mitogenic stimulus acts in such a way that the target cell changes its biosynthetic pattern from that characteristic of the resting state to that characteristic of the growing state. DNA synthesis usually begins only after 11–24 hours of stimulation, depending on the cell type. In order to understand the mitogenic response, it is necessary to ask what are the changes that prepare the cell for DNA synthesis. For the fibroblast stimulated by serum, to pass from resting to growing state, these changes are summarized in Table 1. The three major components of the protein-synthesizing machinery increase in amount during the transition [8,19]. The amounts of ribosomes and tRNA increase proportionally, so that the ratio between the two is the same in resting as in growing cells. For 3T6 cells the ratio is about 25 molecules of tRNA per ribosome. Other mammalian cell lines may have a lower ratio (for V79 cells it is 18:1 [Mauck and Green, unpublished]), but for a line in which resting and growing states can be compared, the ratio is unchanged. This is quite different from the behavior of these two components in bacteria (Table 2). While bacteria regulate their ribosome content according to their growth rate, they do not regulate their tRNA content in this way; as a result, tRNA content is in excess at low growth rates [12]. It may be postulated that it is advantageous for the mammalian cell, which must be able to

TABLE 1. Increase in Components of the Protein-Synthesizing Machinery in Growing as Compared with Resting Cells

Component	Amount of Increase per Unit of DNA	Origin of Increase
ribosomes	1.6–2.5 fold	increased formation decreased destruction
tRNA	same as for ribosomes	increased formation decreased destruction
mRNA	greater than for ribosomes and tRNA (2.3–4.0 fold)	increased formation no decrease in destruction

TABLE 2. Number of Transfer RNA Molecules per Ribosome

(*a*) *In Salmonella growing at different rates* (*data of O. Maaloe and N. O. Kjeldgaard* [*12*])

Doubling time	*Transfer RNA molecules per ribosome*
25 min.	15.5
50 min.	41
100 min.	64
300 min.	159
(*b*) *In mammalian fibroblast line 3T6*	
resting	25
growing (Td = 15 hrs.)	25

maintain a resting state for long periods, to avoid the burden of an excess of tRNA.

Growing cells also contain more poly A(+) mRNA than resting cells [8]; as the difference is greater than for ribosomes, the ratio of mRNA to ribosomes in the growing state is higher by 50% or more. This change in ratio takes place quite early during the transition from resting to growing state, and is a convenient way of defining the boundary between the G_0 and G_1 states (Fig. 1).

The source of the increase in the various components is shown in Table 1. There is an increase in the rate of formation of all components during transition from resting to growing state. In addition, ribosomes and tRNA are more stable in growing than in resting cells (Table 3), so that conservation

TABLE 3. Lifetime of Cytoplasmic RNA of Resting 3T3 and 3T6 Cells

	Half-time	
	Resting	Growing
tRNA	36 hr	60 hr
rRNA 18S	70 hr	∞
28S	50 hr	∞
mRNA, poly(A)(+)	9 hr	9 hr

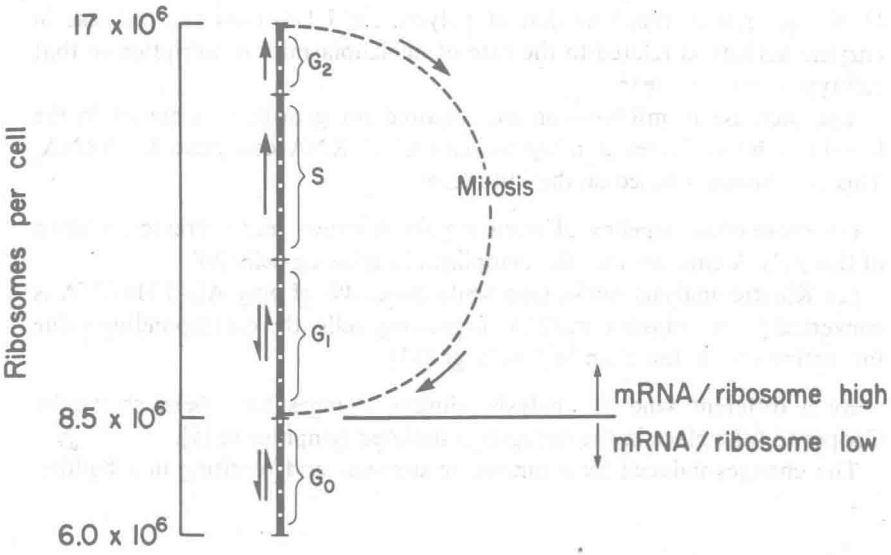

FIG. 1. The Division Cycle and the Resting State (G_0)

contributes to their accumulation in growing cells [1]. There is no difference between resting and growing cells in the stability of poly A(+) mRNA (1), so the increased content of growing cells must be due entirely to a greater rate of formation.

The origins of the increased rate of formation of the three components are summarized in Table 4. The rates of transcription of pre-rRNA and pre-tRNA increase very quickly when resting fibroblasts are stimulated to prepare for division [4,13,14]. On the other hand, the over-all rate of transcription of HnRNA, the precursor of cytoplasmic mRNA, does not increase during transition from resting to growing state [13]; this rate is fixed in relation to the amount of DNA. In other cell types the amount of RNA polymerase II has been found to be much less subject to change than the amount of polymerase I [16]. The mitogen-stimulated lymphocyte appears to be exceptional for it increases appreciably its level of polymerase

TABLE 4. Over-all Transcription Rates (per unit of DNA) of Major Classes of RNA during Transition of 3T6 Cells from Resting State to the Initiation of DNA Synthesis

preribosomal RNA	increased
pretransfer RNA	increased
HnRNA	not increased

II, though not as much as that of polymerase I [7]. How the increase in enzyme activity is related to the rate of nucleoplasmic transcription in that cell type is not yet clear.

The increase in mRNA content required for growth is achieved in the fibroblast by an increase in the amount of HnRNA converted to mRNA. This conclusion is based on the following:

(1) Pulse-chase labeling of nuclear poly A shows that a greater fraction of this poly A emerges into the cytoplasm in growing cells [9].

(2) Kinetic analysis shows that while about 4% of poly A(+) HnRNA is converted to cytoplasmic mRNA in growing cells, the corresponding value for resting cells is less than half as large [11].

By a different kind of analysis, similar changes have been shown by Cooper to take place in the mitogen-stimulated lymphocyte [5].

The changes induced by a mitogenic stimulus and resulting in a buildup

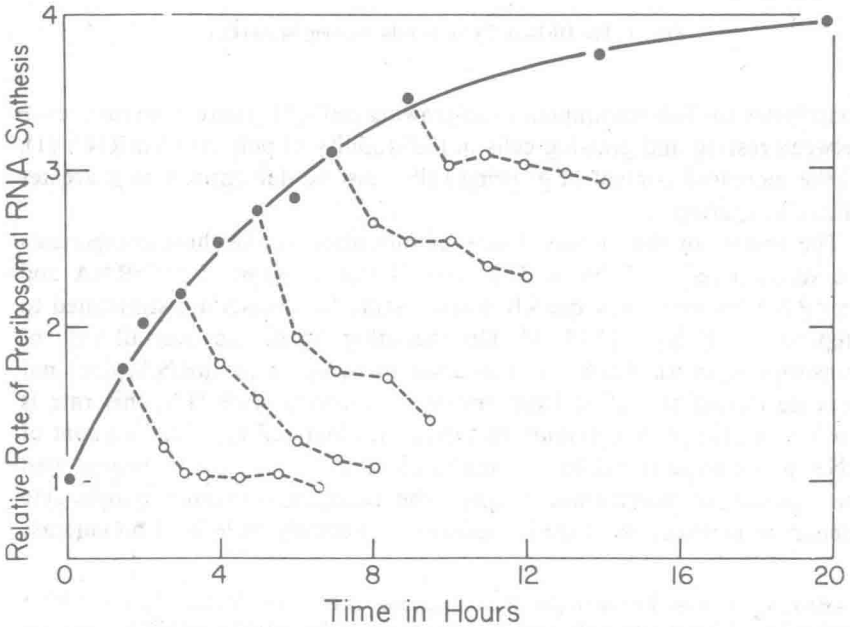

FIG. 2. Rate of Preribosomal RNA Synthesis after Withdrawal of Serum Stimulus. Solid line and solid circles show the increase in rate of synthesis after addition of serum-rich medium to resting 3T6 cultures at time zero. Open circles and hatched lines show the decline in rate when the serum-rich medium was removed and the original was replaced. Measurements of rate of incorporation of tritiated UTP were made after detergent treatment and in the presence of α amanitin at 0.25 μg/ml to suppress HnRNA synthesis (data of Mostafapour and Green [15]).

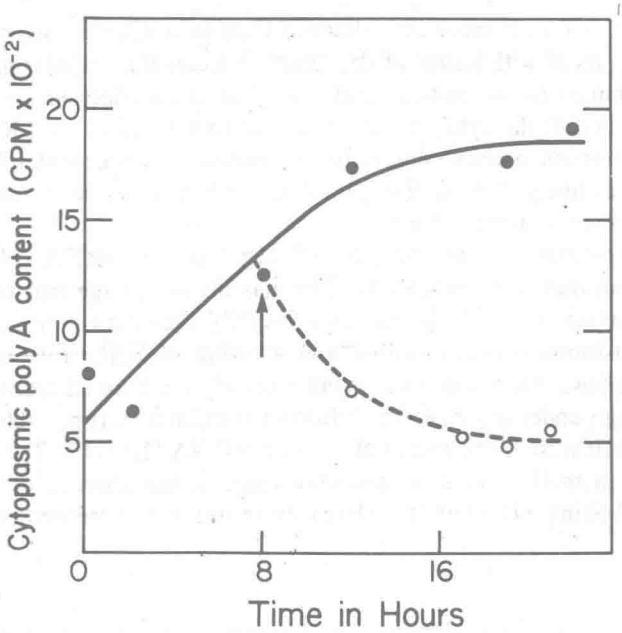

FIG. 3. Decline in Cytoplasmic Poly A Content after Withdrawal of Serum. Solid line and solid circles show rise in cytoplasmic poly A content after serum stimulation. Open circles and hatched line show decline in poly A content after withdrawal of the high serum medium (arrow). Measurements were made by hybridization of the poly A with tritiated poly U (data of Mostafapour and Green [15]).

of the protein-synthesizing machinery are reversed following withdrawal of the stimulus, but the rates of the reversal are different for the different components. Figure 2 shows the effect on pre-rRNA synthesis of withdrawing serum after varying periods of stimulation. In less than 30 minutes there was a definite drop in the synthetic rate. On the other hand, especially after long periods of serum stimulation, it required a long time for the rate to decline to the resting level. As in the case of the increase due to serum stimulation, a response begins quickly, but there is considerable inertia in the system and it may require many hours for the cell to reach a new equilibrium rate [15].

As would be expected from its behavior during shiftup following mitogenic stimulation, cytoplasmic poly A(+) mRNA content is adjusted downward more rapidly than ribosome content after withdrawal of the serum stimulus. Figure 3 shows the results of measurement of cytoplasmic poly A content (a good measure of poly A(+) mRNA content) following introduction and withdrawal of a serum stimulus. Within 8 hours after serum

stimulation, 3T6 cells more than doubled their cytoplasmic poly A content. Within 8 hours of withdrawal of the serum stimulus the cytoplasmic poly A content declined to the resting level. The rate of this decline can be compared with that of the cytoplasmic ribosomes (measured as total RNA (Fig. 4)). The return of mRNA content to the resting level is considerably faster [15]. This is likely due to the fact that mRNA turns over much more rapidly than ribosomes (Table 3).

These experiments show that the cell regulates its mRNA content differently from that of its ribosomes. This has the advantage that the rate of protein synthesis can be adjusted more rapidly than ribosome content. In simple transitions between resting and growing state the rate of protein synthesis follows mRNA content much more closely than ribosome content [8,15], though under more severe shiftdown conditions it is possible for cells to reduce their rate of utilization of existing mRNA [2,3,6,17,18].

Changes in mRNA content can take place in the absence of ribosome synthesis. Resting 3T6 cells stimulated by serum in the presence of fluoro-

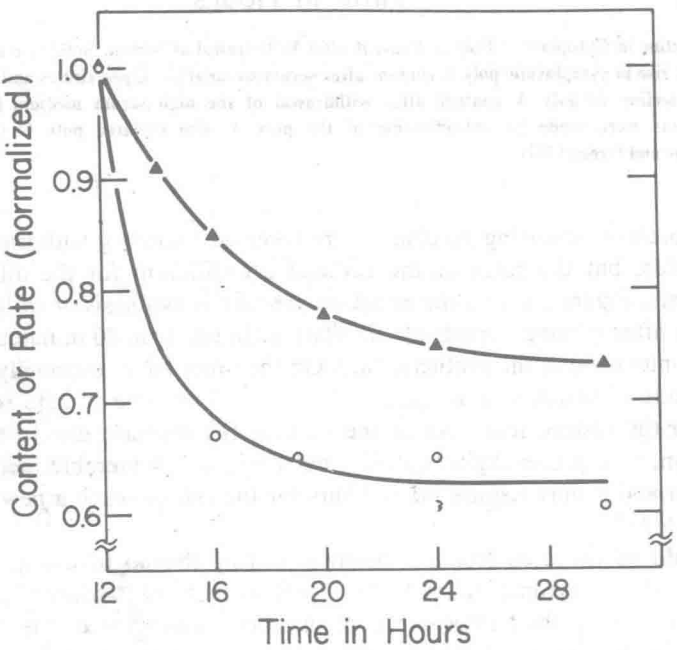

FIG. 4. Comparison of Declines of Ribosome and mRNA Content after Withdrawal of a Serum Stimulus. Circles, cytoplasmic poly A content; triangles, total cellular RNA (data of Mostafapour and Green [15]).

uridine are prevented by the drug from synthesizing ribosomes, but they nevertheless increase their cytoplasmic mRNA content [10].

Relations between the different parts of the program which prepares for growth can be studied by the use of mutants which cannot carry out a specific part of the program. For example, temperature-sensitive mutants in ribosomal RNA synthesis or aminoacylation of tRNA are known and could be employed for this purpose. Among the cell cycle mutants, those which result in failure of the cells to synthesize DNA are sometimes referred to as mutants for the initiation of DNA synthesis. This designation is likely to be incorrect in most cases. Any mutation which interferes with preparations for DNA synthesis will arrest the cell in the G_0 period, but from the complexity of these preparations it is obvious that most of these mutations will be unrelated to the DNA replication process. Study of such mutants may bring to light interesting aspects of the preparative program.

REFERENCES

1. Abelson HT, Johnson LF, Penman S, Green H: Cell 1: 161, 1974.
2. Baenziger NL, Jacobi CH, Tach RE: J Biol Chem 299: 3483, 1974.
3. Bandman E, Gurney T Jr: Exp Cell Res 90: 159, 1975.
4. Bombik BM, Baserga R: Proc Natl Acad Sci USA 71 2038, 1974.
5. Cooper HL: *In* Clarkson B and Baserga R (Eds): Control of Proliferation in Animal Cells, Cold Spring Harbor Conferences on Cell Proliferation, Vol. 1: 1974, p 769–783.
6. Engelhardt DL: J Cell Physiol 78: 333, 1971.
7. Jaehning JA, Stewart CC, Roeder RG: Cell 4: 51, 1975.
8. Johnson LF, Abelson HT, Green H, Penman S: Cell 1: 95, 1974.
9. Johnson LF, Williams JG, Abelson HT, Green H, and Penman S: Cell 4: 69, 1975.
10. Johnson LF, Penman S, Green H: J Cell Physiol (in press).
11. Levis RW, Johnson LF, Abelson HT, et al: (in preparation).
12. Maaløe O, Kjeldgaard NO: Control of Macromolecular Synthesis: A Study of DNA, RNA, and Protein Synthesis in Bacteria. New York, W. A. Benjamin, 1966.
13. Mauck JC, Green H: Proc Natl Acad Sci USA 70: 2819, 1973.
14. Mauck JC, Green H: Cell 3: 171, 1974.
15. Mostafapour M-K, Green H: J Cell Physiol (in press).
16. Roeder RG, Chou S, Jaehning JA, Schwartz LB, et al: *In* Markert CL (ed): Isozymes III, Developmental Biology. New York, Academic Press, 1975. p 27–44.
17. Rudland PS: Proc Natl Acad Sci USA 71: 750, 1974.
18. Soeiro R, Amos H: Science 154: 662, 1966.
19. Stanners CP, Becker H: J Cell Physiol 77: 31, 1971.