Control Mechanisms in Animal Cells

Specific Growth Factors

Editors

Luis Jimenez de Asua, M.D., Ph.D.
Robert Shields, D.Phil.
Rita Levi-Montalcini, M.D.
Stefano Iacobelli, M.D.

Control Mechanisms in Animal Cells

Specific Growth Factors

Editors

Luis Jimenez de Asua, M.D., Ph.D.
Group Leader
Regulatory Events of the Cell Cycle Unit
Cell Biology Group
Friedrich Miescher-Institute
Basel, Switzerland

Rita Levi-Montalcini, M.D.
Professor and Director
Laboratory of Cellular Biology
CNR
Rome, Italy

Robert Shields, D.Phil.
Colworth Laboratory
Unilever Research
Sharnbrook, Bedford, England

Stefano Iacobelli, M.D.
Chief
Laboratory of Molecular Endocrinology
Catholic University
Rome, Italy

Raven Press ■ New York

Raven Press, 1140 Avenue of the Americas, New York, New York 10036

Made in the United States of America

Library of Congress Cataloging in Publication Data

Main entry under title:

Control mechanisms in animal cells.

"Based on papers delivered at the Round Table on the Regulation of the Initiation of DNA Synthesis as well as the EMBO Workshop on Specific Growth Factors held in Rome in October 1979."

Includes bibliographical references and index.

1. Cells-Growth-Regulation-Congresses. 2. Deoxyribonucleic acid synthesis-Congresses. 3. Cellular control mechanisms-Congresses. I. Jimenez de Asua, Luis.

QH604.C65 591.87'61 79-5293

ISBN 0-89004-509-7

Preface

This volume contains chapters based on papers delivered at the Round Table on the Regulation of the Initiation of DNA Synthesis as well as the EMBO Workshop on Specific Growth Factors held in Rome in October 1979.

The term "growth factor" is used to describe a number of extremely active molecules that have cellular receptors of high affinity and act mitogenically. As such, the term embraces conventional hormones as well as other molecules that are not usually thought of as hormones. However, the mechanisms of action of the growth factors appear to be similar, so that results obtained with one factor may be relevant to the study of another.

The purpose of this volume is to highlight certain areas of active research rather than to present a comprehensive overview of the field. Although it is still not possible to give a review as to how growth factors work, some features are becoming clear. The introduction to this volume is an attempt to draw some of these common threads together and to point out areas of knowledge that are firmly established and areas in which future research is needed.

Another purpose of this book is to show that seemingly disparate fields (such as the possible action of growth factors inside the cell and the action of SV40 antigen) may have features in common.

The material in this volume should therefore act as a catalyst for scientists to examine work going on in fields other than their own. For example, endocrinology and tumour biology may have a common ground because many tumours produce growth factors ectopically. This secretion of growth factors by tumours may lead to the discovery of new molecules that play important roles in normal growth and development.

This volume should provide a valuable source of material for molecular endocrinologists, developmental biologists, and those interested in oncogenic transformation.

Luis Jimenez de Asua
Rita Levi-Montalcini
Robert Shields
Stefano Iacobelli

Contributors

K. Adachi
Department of Biochemistry
Kanazawa Medical University
Kahoku-gun, Ishikawa 920-02, Japan

S. C. Aitken
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

F. S. Ambesi-Impiombato
CNR Endocrinology and Experimental Oncology Center
Institute of General Pathology
Faculty of Medicine
University of Naples
Naples, Italy

S. Barlati
CNR Laboratory of Genetic Biochemistry and Evolution
University of Pavia
27100 Pavia, Italy

D. C. Bennett
The Salk Institute
San Diego, California 92138

S. Biocca
CNR Laboratory of Cell Biology
00196 Rome, Italy

J. Brachet
Department of Molecular Biology
University of Brussels
Brussels, Belgium

R. R. Bürk
Friedrich Miescher-Institute
CH-4002 Basel, Switzerland

P. Calissano
CNR Laboratory of Cell Biology
00196 Rome, Italy

D. Cirillo
Department of Human Anatomy
University of Turin School of Medicine
10126 Turin, Italy

J. Conscience
Friedrich Miescher-Institute
CH-4002 Basel, Switzerland

H. G. Coon
Laboratory of Cell Biology
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

J. E. De Larco
Laboratory of Viral Carcinogenesis
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

T. Ebendal
Department of Zoology
Uppsala University
S-751 22 Uppsala, Sweden

J. E. Errick
Department of Molecular, Cellular, and Developmental Biology
University of Colorado
Boulder, Colorado 80309

M. Gabbay
Weizmann Institute of Science
Rehovot, Israel

D. Gospodarowicz
Cancer Research Institute
University of California Medical Center
San Francisco, California 94143

A. Graessmann
Institute for Molecular Biology and Biochemistry
Free University of Berlin
1 Berlin 33, West Germany

M. Graessmann
Institute for Molecular Biology and Biochemistry
Free University of Berlin
1 Berlin 33, West Germany

F. Grummt
Max-Planck-Institute for Biochemistry
D-8033 Munich, West Germany

R. T. Hamilton
Cell Biology Laboratory
The Salk Institute
San Diego, California 92138

K. O. Hedlund
Department of Zoology
Uppsala University
S-751 22 Uppsala, Sweden

R. Hinnen
Friedrich Miescher-Institute
CH-4002 Basel, Switzerland

R. W. Holley
Molecular Biology Laboratory
The Salk Institute
San Diego, California 92138

S. Iacobelli
Laboratory of Molecular Endocrinology
Catholic University
00168 Rome, Italy

C. O. Jacobson
Department of Zoology
Uppsala University
S-751 22 Uppsala, Sweden

P. James
Specialized Center for Thrombosis Research
Temple University School of Medicine
Philadelphia, Pennsylvania 19140

L. Jimenez de Asua
Friedrich Miescher-Institute
CH-4002 Basel, Switzerland

L. K. Johnson
Cancer Research Institute
University of California Medical Center
San Francisco, California 94143

T. Kano-Sueoka
Department of Molecular, Cellular, and Developmental Biology
University of Colorado
Boulder, Colorado 80309

W. R. Kidwell
Laboratory of Pathophysiology
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

P. Leuthard
Friedrich Miescher-Institute
CH-4002 Basel, Switzerland

A. Levi
Weizmann Institute of Science
Rehovot, Israel

A. Levi
CNR Laboratory of Cell Biology
00196 Rome, Italy

L. A. Liotta
Laboratory of Pathophysiology
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

M. E. Lippman
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

P. Longo
Laboratory of Molecular Endocrinology
Catholic University
00168 Rome, Italy

P. C. Marchisio
Department of Human Anatomy
University of Turin School of Medicine
10126 Turin, Italy

B. R. McAuslan
CSIRO Molecular and Cellular Biology Unit
North Ryde, N.S.W. 2113, Australia

W. Meier
Friedrich Miescher-Institute
CH-4002 Basel, Switzerland

P. Mignatti
Institute of Genetics
University of Pavia
27100 Pavia, Italy

D. Monard
Friedrich Miescher-Institute
CH-4002 Basel, Switzerland

C. Mueller
Institute for Molecular Biology and Biochemistry
Free University of Berlin
1 Berlin 33, West Germany

L. Naldini
Department of Human Anatomy
University of Turin School of Medicine
10126 Turin, Italy

C. Natoli
Laboratory of Molecular Endocrinology
Catholic University
00168 Rome, Italy

R. A. Newman
Imperial Cancer Research Fund
London WC2A 3PX, England

S. Niewiarowski
Specialized Center for Thrombosis Research
Temple University School of Medicine
Philadelphia, Pennsylvania 19140

M. Nilsen-Hamilton
Cell Biology Laboratory
The Salk Institute
San Diego, California 92138

K. Nishikawa
Department of Biochemistry
Kanazawa Medical University
Kahoku-gun, Ishikawa 920 02, Japan

G. Norrgren
Department of Zoology
Uppsala University
S-751 22 Uppsala, Sweden

C. Okitsu
Department of Biochemistry
Kanazawa Medical University
Kahoku-gun, Ishikawa 920 02, Japan

A. M. Otto
Friedrich Miescher-Institute
CH-4002 Basel, Switzerland

D. Paul
Department of Pharmacology and Toxicology
Hamburg University Medical School
Hamburg 20, West Germany

W. J. Pledger
Department of Pharmacology
University of North Carolina School of Medicine
Chapel Hill, North Carolina 27514

F. O. Ranelletti
Laboratory of Molecular Endocrinology
Catholic University
00168 Rome, Italy

K. M. V. Richmond
Friedrich Miescher-Institute
CH-4002 Basel, Switzerland

M. A. Ritter
Imperial Cancer Research Fund
London WC2A 3PX, England

B. Rucinski
Specialized Center for Thrombosis Research
Temple University School of Medicine
Philadelphia, Pennsylvania 19140

S. Rucker
Fels Research Institute
Temple University School of Medicine
Philadelphia, Pennsylvania 19140

P. S. Rudland
Ludwig Institute for Cancer Research
Haddow Laboratories
Sutton, Surrey SM2 5BX, England

D. Salomon
Laboratory of Pathophysiology
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

G. H. Sato
Department of Biology
University of California, San Diego
La Jolla, California 92093

N. Savion
Cancer Research Institute
University of California Medical Center
San Francisco, California 94143

J. Schlessinger
Weizmann Institute of Science
Rehovot, Israel

Y. Shechter
Weizmann Institute of Science
Rehovot, Israel

R. Shields
Unilever Research
Bedfordshire MK44 1LQ, England

D. A. Sirbasku
Department of Biochemistry and Molecular Biology
University of Texas Medical School at Houston
Houston, Texas 77030

G. Steck
Friedrich Miescher-Institute
CH-4002 Basel, Switzerland

G. J. Todaro
Laboratory of Viral Carcinogenesis
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

D. Tramontano
CNR Endocrinology and Experimental Oncology Center
Institute of General Pathology
Faculty of Medicine
University of Naples
Naples, Italy

M. Ulrich
Friedrich Miescher-Institute
CH-4002 Basel, Switzerland

K. G. Varma
Fels Research Institute
Temple University School of Medicine
Philadelphia, Pennsylvania 19140

I. Vlodavsky
Cancer Research Institute
University of California Medical Center
San Francisco, California 94143

M. J. Warburton
Ludwig Institute for Cancer Research
Haddow Laboratories
Sutton, Surrey SM2 5PX, England

W. Wharton
Department of Pharmacology
University of North Carolina School of Medicine
Chapel Hill, North Carolina 27514

M. S. Wicha
Laboratory of Pathophysiology
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

Y. Yoshitake
Department of Biochemistry
Kanazawa Medical University
Kahoku-gun, Ishikawa 920 02, Japan

I. Yuli
Weizmann Institute of Science
Rehovot, Israel

Introduction

Robert Shields

What are growth factors? A facetious answer is that they are molecules that make cells grow and divide, but this definition covers many nutrients as well as substances considered to be true growth factors. Probably the best answer comes from Gordon Sato: Growth factors are hormones; they share with hormones their high specific activities, which are due in turn to their high-affinity receptors in the cell.

Where do growth factors come from? Conventional hormones are synthesized by glands and carried by the blood to their target cells, which may be far removed from their site of origin. Very few growth factors have been shown to have a unique site of synthesis. While the insulin-like growth factor IGF (formerly known as NSILA) is probably made in the liver (not generally regarded as a gland), NGF appears to be made systemically. Many other growth factors appear in certain organs in high concentrations (FGF in the pituitary and EGF in the submaxillary gland), but it is unclear where they are synthesized (they too may be systemic). And what about the platelet-derived growth factors discussed by Pledger and Wharton and Paul et al.? Where are they made?

The absence of any well-defined locale for their synthesis means that the classic technique of endocrinology—removal of the gland—is impossible. Their function must be studied in tissue culture. This is well illustrated in Sato's chapter. He shows that growth factors are interesting not merely because they help cells grow and sometimes differentiate, but also in a practical sense. By their judicious use it may be possible to culture any differentiated tissue in a functional state. This is illustrated by many examples in Sato's chapter and also in that of Ambesi-Impiombato et al., who show how to grow normal functioning thyroid cells *in vitro*.

While most of the growth factors discovered to date make cells grow, inhibitory growth factors do exist. Probably the best known negative factor is interferon, and the chapter by Holley describes another molecule that may turn out to be a chalone. Also, it should be remembered that growth factors have many actions besides promoting (or inhibiting) growth. Some (NGF is a good example) promote differentiation. Neither do growth factors work in isolation. Many act in conjunction with other hormones, and the target cell shape and the proximity of other cells also plays a role. This having been said, what is known about how growth factors work?

There seems to be general agreement that the initial interaction of peptide

growth factors with their target cells occurs at specific cell surface receptors which are evenly distributed over the cell surface. After interaction with the hormone, the hormone–receptor complexes form small aggregates which then coalesce into larger "patches" on the cell surface and are subsequently internalized. The disappearance of the receptor from the surface means the cell becomes temporarily refractile to further stimulus by the same hormone (down regulation). Despite intensive research in a number of laboratories, it is still not known whether peptide hormones act entirely at the cell surface or whether some of their effects require internalization. What is known is that the mere possession of receptors and the internalization of the hormone–receptor complex is not sufficient for the cell to be responsive (Gospodarowicz et al.).

At least three possible mechanisms (not necessarily exclusive) for peptide hormone action can be entertained: (a) The hormone acts at the cell surface to produce its effects either directly (e.g., by interacting with membrane transport sites) or indirectly via second messengers that act within the cell. (b) The hormone is internalized and acts inside the cell to produce its effects (either directly or via one or several subsidiary messenger molecules). According to this model the receptor is merely a vehicle for transporting the hormone. (c) The receptor itself (or fragments or derivatives of the receptor) are the active species. The function of the hormone is then to internalize the receptor.

Several chapters in this volume discuss these models. That by Schlessinger et al. shows that the rapid effects of EGF and insulin on membrane transport do not involve large scale patching or internalization of their receptors. Indeed, many of the so-called "early events" following mitogenic stimulation occur too rapidly for hormone internalization to be involved. Whether all the effects of growth factors may be explained by their action at the cell surface is unclear. While there is fairly convincing evidence that the protease thrombin does not need to be internalized to be mitogenic, the enzyme appears to act by cleaving its own receptor, and these fragments could act inside the cell (1). Recent studies using inhibitors of receptor internalization have shown that the mitogenic effects of EGF are undiminished if internalization is prevented (3). Against this, it should be pointed out that the degree of EGF receptor internalization and the mitogenic effects of the peptide are closely paralleled (2). So while it is clear that at least some of the effects of protein hormones are a result of cell surface events, internalization of the hormone may be required for others.

Several cellular events are known to be initiated after the binding of macromolecules to their receptors and their internalization. Among these are the control of cholesterol transport following internalization of low-density lipoproteins and their receptors and the toxic effects of ricin and cholera toxin. Against this background the idea that some of the actions of peptide hormones requires their internalization seems quite reasonable. The internalization and intracellular distribution of NGF and EGF are discussed in chapters in this volume (Biocca et al., Marchisio et al., and Gospodarowicz et al.). It is shown

that the internalization of NGF and EGF leads to down regulation and the internalized hormones become localized in and around the cell nucleus, where they could possibly exert some of their effects. This nuclear localization of growth factors may explain reports that it is possible to extract growth factors from the nuclei of tumour cells (Nishikawa et al.).

There is some precedent for the action of peptide growth factors in the cell nucleus. The T antigen of SV40, when microinjected, can act as a growth factor for quiescent cells. It too has a largely nuclear location (see Graessman et al., *this volume*), although it must be admitted that it is unknown whether the molecules detected by immunofluorescence are the same ones that are acting mitogenically. The direct microinjection technique used in these studies will no doubt prove to be very valuable in the study of growth factor–cell interactions. It has already proved possible to demonstrate that EGF microinjected directly into cells is nonmitogenic (A. Graessman, *personal communication*), which shows that either EGF works at the cell surface or that it must be suitably modified or bound to its receptors before it will work in the cell. So while it is clear that while some macromolecules bind to receptors, are internalized, and then act inside the cell, it is not yet clear whether this applies for growth factors. It may even be the case that the mitogenic signal is delivered from the cell surface, but changes such as differentiation (which require altered gene expression) require factor internalization.

One possibility that has been rather overlooked is that the presence of the growth factor inside the cell may not be as important as the presence of its receptor. The function of peptide growth factors may be to promote internalization of the receptor which acts within the cell. Steroid hormones seem to work in this way; most of the responses to steroids depend on the translocation of the receptor to the nucleus (see Iacobelli et al. and Aitken and Lippman, *this volume*). Could it be that the steroid receptor acts on the nucleus in the same way as the T antigen of SV40? Once growth factor receptors have been purified it may be possible to answer this question.

Many different mitogens act on a variety of cell types to produce a rather similar range of metabolic responses (dubbed "pleiotypic" by Gordon Tomkins). These events include rapid changes in nutrient and ion transport, changes in protein synthesis and degradation, and ultimately cell division itself. A central question raised by Robert Holley is which of the metabolic events induced by growth factors are necessary for cell division to occur. Do peptide growth factors act on the cell membrane to produce a single pleiotypic mediator which orchestrates intracellular events? Do the changes in membrane transport allow in nutrients rate limiting for DNA synthesis? Are there several "second messengers" that interact on some central cellular process to form an initiator of DNA synthesis? Or do growth factors merely "jazz up" the cell machinery which makes DNA synthesis more probable?

While the idea of a single pleiotypic mediator is attractive, no candidate for such a molecule has yet stood the test of time; however, Grummt provides a new

possible mediator. A fruitful way to search for such a substance is to examine an intracellular event which occurs rapidly, before the growth factor is internalized. The study of the phosphorylation of the ribosomal protein S6, which correlates well with the growth factor-induced stimulation of protein synthesis, may provide such a system (Nilsen-Hamilton and Hamilton). Even if no single mediator is discovered, at least such experiments may reveal part of the mechanism of the increase in protein synthesis, which is one of the very few cellular events that appears to be uniquely correlated with DNA synthesis.

An alternative approach to the problem of how mitogens work is to focus on the kinetics of cell division. The cell cycle seems to be adequately described as being controlled by two random events separated by a lag (Shields). Viewed in this way the control of cell division reduces to how growth factors control the probability of undergoing these transitions and what the events between these transitions are and what influence growth factors have on them. These problems are discussed in a number of chapters in this volume. What appears clear is that these two transitions are differentially affected by different growth factors, some influencing the first transition (which makes the cell competent), some affecting commitment of competent cells, and some affecting both transitions and events between (Pledger et al., Jimenez de Asua, Richmond, and Otto et al.). If it is accepted that growth factors influence the *probability* of a cell's dividing, then a cell may be regarded as similar to a car. If conditions are suboptimal (the oil thick, the spark plugs dirty, the battery old, and the brakes seized), then the probability that the car will proceed will be low (but finite). The improvement of any one of these parameters will increase the probability of motion. Viewed in this way there is no unique path leading to cell division and growth factors may be additive, synergistic, or inhibitory. The function of growth factors may be to "jazz up" many features of cell metabolism that interact to make the probability of division high; the search for a unique pathway to growth may therefore prove fruitless. This may be the reason why attempts to uniquely correlate events at the cell membrane with cell division have been unsuccessful.

Although the normal sites of synthesis of many growth factors are unknown it has become clear that a number of tumour cell lines produce growth factors in culture. Why is this? An attractive explanation is that cells that produce their own growth factors have an advantage over other cells. The production of growth factors may then be one of the events that leads to the successful establishment of a tumour (Todaro and De Larco and Bürk). It seems unlikely that tumour cells are producing entirely novel growth factors, but rather that these factors are another example of ectopic hormone production by tumours. A study of the proteins released from cultured tumour cells may reveal many as yet undiscovered factors that are normal developmental and growth hormones. These ectopically produced growth factors may be responsible for many of the characteristics of transformed cells, such as their morphology and their ability to grow in agar (Todaro and De Larco and Barlati and Mignatti). If these factors act on the cells that produce them and are internalized and transported to the

perinuclear area (like other growth factors), it may be possible to extract growth factors from the nuclei of tumour cells (Nishikawa et al.).

Tumour cells might also gain a selective advantage not by manufacturing their own growth factors but by increasing the supply of growth factors by promoting tumour vascularization. This may be done by secreting a tumour angiogenesis factor (TAF) which is a growth factor for endothelial cells. Alternatively, tumours could release a chemotactic agent that encourages migration of endothelial cells, with division occurring subsequent to migration. There is no reason to suppose that such a factor need be a protein; an ion could be active (McAuslan).

Many of the growth factors discussed in this volume have been discovered by their actions on cells cultured *in vitro*. While it is quite clear that a number of tumours are hormone dependent *in vivo*, hormone dependency cannot always be demonstrated *in vitro*. This could be due to shortcomings of the *in vitro* technique, or, more interestingly, the hormone *in vivo* may elicit the production of a second substance that acts as the ultimate growth factor. Two contributions in this book give examples of such a situation (Sirbasku and Kano-Sueoka and Errick): In one case a high-molecular weight factor (estromedin) mediates the effects of estrogens; in another case (MGF) is shown to be phosphoethanolamine. It should be remembered that the idea of secondary factors mediating the effects of hormones is not new. For instance, glucocorticoids mediate some of the physiological effects of ACTH, and growth hormone (an *in vivo* growth factor par excellence) exerts many of its trophic effects via somatomedin.

The term "growth factor" implies that such substances are concerned only with promoting cell division. This is an understatement. Many of these factors can cause or modify differentiation in suitable target tissues. It might be thought that differentiation was too complicated to study in pure cell culture, as cell–cell interaction and cell factor interaction are often involved. However, a number of chapters in this volume lead me to suspect that the study of differentiation *in vitro* might not be as intractable as had first been thought. The approach (which proved so successful with the mitogenic action of growth factors) has been to produce clonal lines of cells which differentiate in pure culture. Initially, only tumour cells could be cloned. This had the doubtful advantage that their differentiation potential was limited and the advantage that one part of differentiation (e.g., neurite extension in neuroblastomas or globin induction in Friend cells) could be studied in isolation. The disadvantage was that it is unclear whether these events had any relevance to differentiation of non-tumourous tissue *in vivo*. Conscience and Meier show that it is possible to "map" the state of differentiation of Friend cells so that one can be reasonably sure that the induction of globin synthesis seen in these cells represents events occurring during the normal development of erythroid cells. Having a clonal cell line that differentiates *in vitro* does not mean that the problem of what causes differentiation will soon be solved. Hinnen and Monard describe attempts to find out the glial factor (GF) acting on a single cell type (neuroblastoma) elicits

a simple response (neurite extension). The possibility that NGF causes membrane depolarization is interesting, as it immediately suggests how growth factors can elicit pleiotropic responses through changes in ion transport and intracellular pH. NGF does not appear to be the only factor that causes neurite growth; the situation *in vivo* may be rather more complicated (Jacobson et al.).

Cell–cell interactions have long been claimed to be involved in differentiation. Kidwell et al. show that the function of one cell in this putative interaction may be to produce a suitable support on which differentiation of the other cell type occurs. Thus it may be possible to study the differentiation of a single cell type if it is grown on the correct substrate. Rudland et al. describe just such a system where a single stem cell type produces both myoepithelial and alveolar cells. This promising system may provide the basic tool to study the action of growth factors on both the growth and development of the mammary gland. Finally, anyone who believes that a suitable *in vitro* system is the limiting factor in understanding differentiation should read the chapter by Brachet. Although much of the phenomenology of oögenesis and the events following fertilization in amphibia is well documented, after many years of study we still have little understanding of the mechanisms involved.

REFERENCES

1. Cunningham, D. D., Carney, D. H., and Glenn, K. C. (1979): In: *Cold Spring Harbor Conference on Cell Proliferation, Vol. 6,* pp. 199–215. Cold Spring Harbor Laboratories, Cold Spring Harbor, New York.
2. Fox, C. F., Vale, R., Peterson, S. W., and Das, M. (1979): in *Cold Spring Harbor Conference on Cell Proliferation, Vol. 6,* pp. 143–157. Cold Spring Harbor Laboratories, Cold Spring Harbor, New York.
3. Maxfield, F. R., Davies, P. J. A., Klempner, L., Willingham, M. C., and Pastan, I. (1979): *Proc. Natl. Acad. Sci., USA*, 76:5731–5735.

Contents