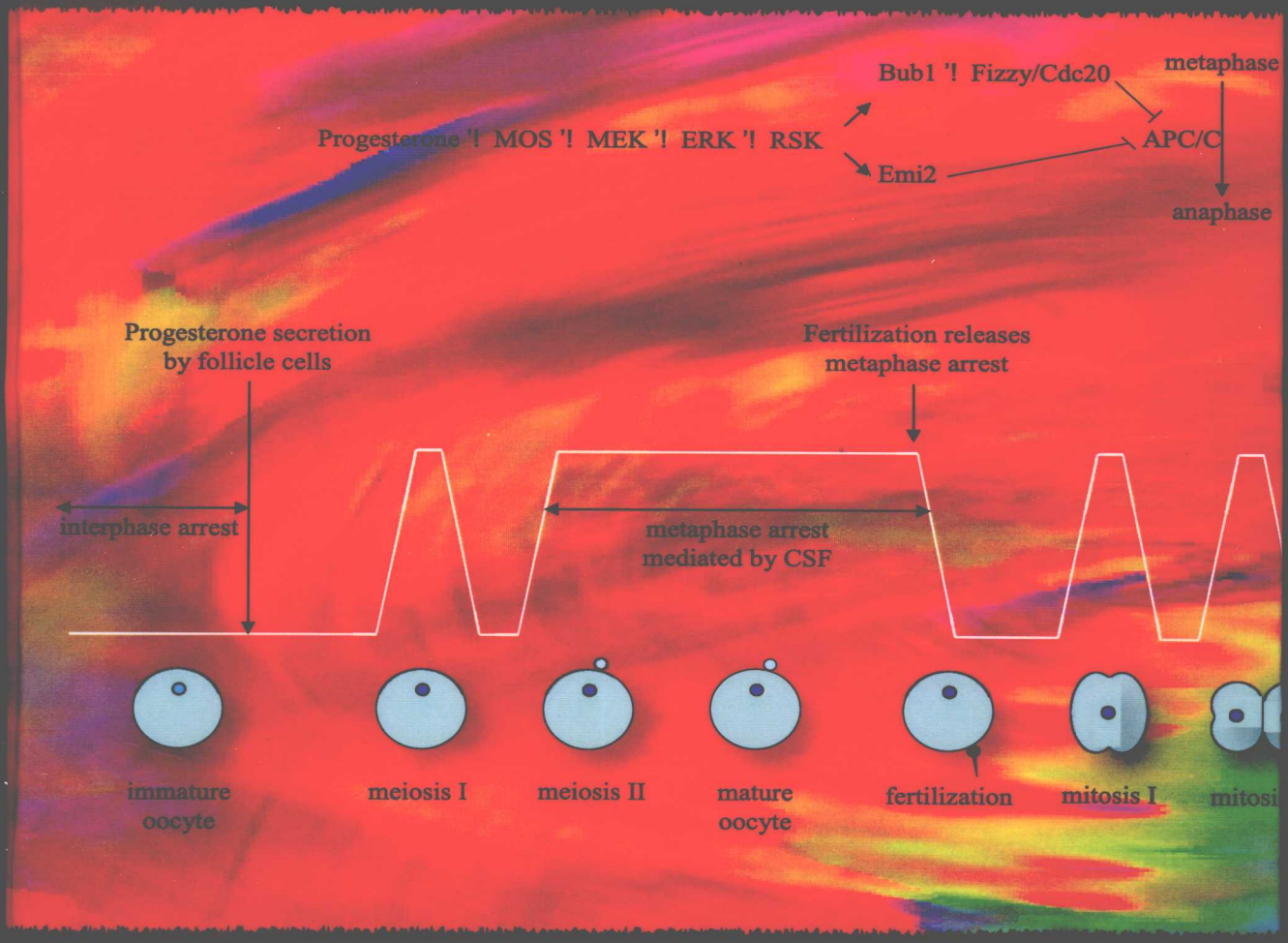
Progress in Cell Cycle Control Research



K. L. Chen
Editor

NOVA

PROGRESS IN CELL CYCLE CONTROL RESEARCH

K. L. CHEN
EDITOR

Nova Science Publishers, Inc.
New York

Library of Congress Cataloging-in-Publication Data

Progress in cell cycle control research / K.L. Chen (editor).
p. ; cm.
Includes bibliographical references and index.
ISBN 978-1-60456-797-7 (hardcover)
1. Cell cycle--Regulation. I. Chen, K. L.
[DNLM: 1. Cell Cycle. 2. Cell Cycle Proteins. QU 375 P9637 2008]
QH604.P76 2008
571.8'4--dc22 *2008023156*

Published by Nova Science Publishers, Inc. † New York

PROGRESS IN CELL CYCLE CONTROL RESEARCH

Preface

A cell cycle is an ordered and highly controlled set of events that leads to cell growth and proliferation. Cell cycle progression is driven by changes in the substrate specificity and subcellular localization of cyclin-dependent kinases (Cdks), which in turn are modulated by a collection of cyclins, Cdk-activating and Cdk-inhibiting kinases, and Cdk inhibitors (CDKIs). Regulation of the cell cycle is critical for the normal development of multicellular organisms and dysregulation of cell cycle could lead to cancer, a disease where normal cell growth and behavior are lost. Cell cycle regulation is tightly controlled by both synthesis and degradation of short-lived proteins, such as cyclins and CDKIs, and degradation of these proteins is mainly mediated by the ubiquitin-dependent proteasome pathway. This new book presents the latest research in the field from around the globe.

Chapter 1 - Cells of a given type tend to divide at a characteristic size. In most proliferating cells, it has been observed that cell growth and division are coordinated by linking the rate at which cells add mass to their division rate. The end result is a model that yields passive cell size control. However, to add an active mechanistic twist, experiments in a number of organisms have suggested that the proliferative potential of cells is closely linked to cell size. That is, cells become competent to divide only after achieving a minimum cell size. In these cases, it was found that cell growth was exponential and proportional to cell size. This creates somewhat of a circular paradox whereby cell size determines growth rate and growth rate determines cell size. Indeed, this is further complicated by the frequency with which the words "growth" and "proliferation" are used interchangeably. Comparison of oocytes to embryonic cells clearly illustrates the difference between growth and proliferation. Oocytes grow but don't divide. This generates cells that can be 10^5 times larger than somatic cells. In contrast, a fertilized ooccyte gives rise to embryonic cells that divide without growing yielding enormous numbers of cells in a relatively short period of time. Thus, a simple and direct connection between cell size, cell growth and cell cycle control remains to be discovered. Until recently, very little was known about the genetic pathways involved in cell size homeostasis or those that link cell growth to cell cycle control. However, the age of genomics and system-wide genetic screens have provided us with a fresh look at the long-standing riddle surrounding the relationship between cell size, cell growth, and cell cycle control. Herein, from a historical perspective, the paradox of cell size control is re-examined in the light of recent advances.

Chapter 2 - Cell cycle is dependent on correct cycling and activation of Cyclin Dependent Kinases (CDKs)/Cyclin pairs. Their protein levels and regulation mechanisms are altered in transformed cells and CDKs are considered attractive targets for cancer therapy. Data from knockout and siRNA experiments have shown a redundancy in CDKs functions. Recent clinical trials and preclinical studies have identified the possibility to use inhibitors specific for subclasses of cell-cycle CDKs (CDK1/2 or CDK4/6) as single agents for a few specific cancer types that show a more pronounced dependency on CDKs functions. Pan-CDK inhibitors are giving, instead, more promising results. They inhibit both cell-cycle CDKs and transcriptional CDKs by combining cytostatic and apoptotic effects in cancer cells. Apoptosis is induced through depletion of anti-apoptotic proteins, sensitive to inhibition of transcription because encoded by mRNAs with short half-lifes and rapid turnover. Recently, new crystal structures of transcriptional CDKs (CDK7 and CDK9) have provided molecular basis for targeting this subclass. Combinations of pan-CDK inhibitors with traditional chemotherapic agents have shown a synergistic cytotoxic effect in a variety of cancers and the restoration of susceptibility in cancers that developed resistance to traditional therapies. Flavopiridol, a pan-CDK inhibitor, is also being used in clinical trials in combination with Histone deacetylase (HDAC) inhibitors, which are also being developed as anti-neoplastic drugs. Evidence has been reported of cross-talk between CDKs and HDACs that indicates the existence of a complex cross-regulation mechanism. This cross-talk between CDKs and HDACs mediated by Retinoblastoma (pRb) and/or CDK inhibitors (CKI) is of particular pharmacological relevance. It engenders the expectation that the combined pharmacological inhibition of CDKs and HDACs might have a higher therapeutic profile than the single treatment.

Chapter 3 - In cancer cells, cell cycle regulatory genes are frequently altered by genetic or epigenetic mechanisms promoting cell proliferation. While genetic changes involve DNA mutation, deletion, and chromosomal translocation, epigenetic changes include DNA methylation and histone deacetylation. These changes often lead to overexpression of cyclins and suppression of cyclin-dependent kinases (CDKs).

Here, the authors focus on such aberrant changes in hematological malignancies.

For example, chromosomal translocation t(11;14), which causes overexpression of the cyclin D1 gene in human mantle cell lymphoma, is frequently accompanied by inactivation of p27KIP1. Epigenetic changes such as aberrant promoter methylation of the CDK inhibitors p15INK4b and p16INK4a has been shown to be associated with malignancies involving lymphoma and myelodysplastic syndrome (MDS).

In recent studies, many molecular targeted therapies have been developed that can restore the normal transcriptions of the key cell cycle control genes in hematological malignancies. For example, proteosome inhibitors and CDK inhibitors have been evaluated to suppress NF-kappaB and CDK in cancer cells and have been tested on malignancies involving multiple myeloma and mantle cell lymphoma. On the other hand, DNA hypomethylating agents and histone deacetylase inhibitors can restore the epigenetic alterations associated with chromatin structural change and have antiproliferative activity against cancers by activating tumor suppressor genes.

Drugs, such as bortezomib, 5-azatidine, 5-aza-2′-deoxycytidine, and vorinostat, have been approved by the US Food and Drug Administration (FDA) for the treatment of multiple myeloma, MDS, and cutaneous T-cell lymphoma, respectively.

In summary, genetic and epigenetic alterations of the cell cycle control genes play an important role in carcinogenesis, and their restoration by molecular targeted therapy offers a promising approach in the treatment of hematological malignancies.

Chapter 4 - The cell cycle is an ordered and highly controlled set of events that leads to cell growth and proliferation. Cell cycle progression is driven by changes in the substrate specificity and subcellular localization of cyclin-dependent kinases (Cdks), which in turn are modulated by a collection of cyclins, Cdk-activating and Cdk-inhibiting kinases, and Cdk inhibitors (CDKIs). Regulation of the cell cycle is critical for the normal development of multicellular organisms and dysregulation of cell cycle could lead to cancer, a disease where normal cell growth and behavior are lost. Cell cycle regulation is tightly controlled by both synthesis and degradation of short-lived proteins, such as cyclins and CDKIs, and degradation of these proteins is mainly mediated by the ubiquitin-dependent proteasome pathway. Indeed, the ubiquitin-proteasome degradation pathway plays an essential role in up-regulation of proliferation, down-regulation of cell death, and development of drug resistance in human cancer cells. Given that aberrant, mutated, and/or overexpressed versions of cell cycle regulatory proteins are also frequently involved in cancer, manipulation of the proteasome pathway that regulates the degradation of these proteins was expected to be exclusively damaging to the cancer cells. It has been found that human cancer cells are more sensitive to proteasome inhibition than normal cells. Both *in vitro* and *in vivo* experimental and clinical results have supported the potential use of proteasome inhibitors as novel anticancer drugs and chemosensitizers, although some side effects have been observed in clinical trials. Since these promising data made proteasome inhibitors highly attractive, it is expected that in the near future more specific, selective and potent proteasome inhibitors with reduced toxicity will be developed in laboratories and used in clinics.

Chapter 5 - Protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) are involved in the regulation of basic cell functions, such as proliferation, differentiation, and death. This review introduces the authors' preclinical studies of the roles of PKC and MAPK signaling in breast cancer cell-cycle regulation. PKC caused G_1 arrest in a breast cancer cell line via a mechanism involving a MAPK–c-jun NH_2-terminal kinase (JNK)–retinoblastoma (Rb) protein signaling pathway. Furthermore, the authors characterized a novel mechanism by which all-trans retinoic acid (ATRA), antineoplaston anticancer drugs, and catechin inhibited the growth of breast cancer cells via effects on intracellular pathways. ATRA decreased the expression of PKCα, reduced extracellular signal-regulated kinase (ERK) MAPK phosphorylation, and, consequently, caused G_1 arrest. Antineoplastons downregulated PKCα protein expression, resulting in the inhibition of ERK MAPK phosphorylation and Rb phosphorylation, leading to G_1 arrest. Catechin phosphorylated JNK and p38, which, in turn, inhibited the phosphorylation of cdc2, and regulated the expression of cyclin A, cyclin B1, and cdk proteins, thereby causing G_2 arrest. These results suggest that PKC and MAPK are essential for cell-cycle regulation in breast cancer cells. PKC and MAPK signaling thus represent promising targets for the development of novel therapeutic agents for breast cancer.

Chapter 6 - The fragile FHIT gene is among the very first targets of induced DNA damage. In early preneoplastic lesions, various insults such as oxidative stress induce DNA damage checkpoint. Loss of heterozygosity at the FRA3B/ Fhit common chromosome fragile region precedes or is coincident with activation of the checkpoint response in these early stages. Recent studies note a role of fragile gene Fhit in initiating cancer cells, which may open a new avenue to the treatment of early stages of cancer.

Chapter 7 - In metazoans, a complex, dynamically controlled, yet delicately balanced mechanism exists for the calibration of cell number and size in various organs. To a large degree, this homeostatic regulatory operation is cellularly determined by the integrity and functional competence of the cell cycle, whose orderly transition involves molecular scrutiny by conserved checkpoints, designated the G1/S and G2/M. Normally functioning cell cycle checkpoints prevent cells from inadvertently entering into a new cycle phase until the previous one has been successfully completed. In addition, the cell cycle checkpoints also provide cells with a highly responsive DNA damage sensing mechanism enabling the correction of genetic errors that might arise from insults imposed by exposure to external or internal hazards. Compared to normal cells, dysfunctional or total loss of cell cycle checkpoints leading to unrestricted proliferation is considered a major hallmark of tumorigenesis. Furthermore, cancer cells are usually less differentiated than the normal cells of the tissue where they arose, possibly reflecting cellular de-differentiation as another feature of malignant tumors.

We have been interested in developing anti-tumorigenic strategies that target the control of cell cycle beyond the conserved G1/S and G2/M checkpoints and which further incorporate the de-differentiation aspects of cancer cells. Herein, the authors report results of the authors' recent studies using the purine analog reversine as applied to the hormone refractory prostate cancer PC-3 cells. Reversine is a recently discovered novel purine with demonstrated ability to change differentiated muscle cells into multipotent progenitor cells, followed by their redirected differentiation into multiple phenotype depending on the composition of the culture media. Because of these attributes, the authors surmise that reversine might convert hormone refractory prostate cancer cells to the clinically manageable, hormone-responsive cells. Results of the authors' studies show that sub-μM reversine inhibited clonogenicity in cultured PC-3 cells, and that this suppressive effect was only partially reversible. Moreover, reversine-treated PC-3 cells also showed marked reduction in the expression of dual-specificity phosphatase cdc25c, accompanied by decline in plk1 (polo-like kinase 1) without a comparable change in the levels of plk3. Reversine treatment also altered the cellular location of cdc25c, restricting it largely to the extranuclear sites. Reversine treatment also induced cellular senescence in PC-3 cells. These results point to targets suitable for the development of anti-tumorigenic agents for malignant cells harboring flawed DNA damage sensing checkpoints.

Chapter 8 - Normal adult tissues are under homeostatic control, resulting a balance between cell proliferation and death. There are several factors involved in regulation of this balance, such as cytokines, hormonal receptor, transcription factors, oncogenes or suppressor tumoral genes. These factors exhort a negative regulation of cell cycle and assure dependence between two consecutively phases of cell cycle, keeping the DNA integrity along the replication and segregation processes.

The aim of present report was comparer the results obtained (by western blot, immunohistochemical, cellular culture or molecular analysis) with different cytokines (TNFα, IL-6, OSM and LIF) related with cell cycle progression with those obtained with several cycle regulators (p53, p21, Rb, bcl-2 family). The authors found an association between the expression of these proteins and increasing malignancy.

TNFα and IL-6 are two pro-inflammatory cytokine families, which have multiple biological properties and are involved in breast cancer development. IL-6 family comprises several members such IL-6, LIF or OSM, that are associated with cancer breast progression.

TNF-α seems to exert a key role in the promotion of many tumors as breast cancer. TNFRI is the major mediator of most TNF-α activities. These include apoptosis but also cell proliferation thought the NF-κB transcription factor activation. TNFRII has been described to mediate proliferation. Both TNF-α and IL-6 could also be related to different factors that favor tumor progression, such as estrogens synthesis or accumulation of mutated p53.

It has been reported that several cell cycle regulators as p53, p21 and Rb, as well as another proteins related with cell cycle control such bax and bcl-2 are affected in different tumors, altering the proliferation/apoptosis balance, and so producing an exceed of proliferation. In other hand, in breast tumor increases the expression of OSM, LIF, OSMRβ, LIFRβ, gp130, TNFα and TNF-RII. This increase may be associated with malignancy. IL-6 family exert their action through transducer receptor gp130, and gp130 expression increase with malignance, it might be a crucial point in the development of infiltrative adenocarcinoma. At the same time, IL-6 could act increasing bcl-2 expression, and thus altering the proliferation/apoptosis balance toward neoplastic cell proliferation. The increased bax immunoreaction observed only in infiltrating tumors and not so high as the increase in bcl-2 immunoreaction, might be interpreted as an attempt to hinder cell proliferation. Comparing the TNFα results with previous results for mp53, p21 and IL-6, the authors found an association between the expression of these four proteins and increasing malignancy.

Chapter 9 - Catalytic Therapy (CT) for treatment of malignancy is comprised of the combination of active agents, including a substrate molecule that is acted on by a catalyst. In the presence of oxygen, interaction of two drugs, the catalyst, this is most often an organic dye, with a substrate results in the generation of locally high concentrations of reactive intermediates. These reactive oxygen species subsequently act to deleteriously modify cancer cells. The components of this therapeutic regimen do not generate the high-energy intermediates until combined at the target site, rendering the treatment approach far less toxic. In current approaches to this therapy the effects are maximized through employing two agents that are preferentially taken up by tumor cells. Therefore treatment with low doses of the individual agents results in minimal injury to healthy cells, even those localized within the same environs.

Mechanistically the anti-tumor action of CT is similar to radiation and photodynamic (PDT) therapies in that a primary mechanism underlying the therapeutic effects is the degradation of critical tumor cell molecules by reactive oxidants. In CT a transition metal complex is employed as the catalyst and a second agent, often one that can support redox cycling, acts as the substrate. The selective accumulation of high concentrations of both of these substances within a tumor is a necessary prerequisite for the intense radical attack. This radical attack then rapidly induces the cell cycle disruption, apoptosis and tissue necrosis that

lead to tumor growth suppression. CT exhibits a unique advantage over light-based therapies such as PDT because exposure of the tumor cells to a light source is not required. This allows CT to be used for tumors inaccessible to fiber optic light sources, as well as making CT a practical alternative for diffuse and widely dispersed malignancies.

The first attempt at the development of CT for anti-cancer therapy was reported by Kimoto et.al. In these studies a killing effect on tumor cells coupled with increased life span of tumor bearing animals was achieved after treatment of murine tumors with a copper:glycylglycylhistidine catalytic complex using ascorbate as the CT substrate. Recently several promising new CT systems are been discovered. In one approach a combination of cobalt or iron phthalocyanine with sodium ascorbate has exhibited high levels of both in vitro and in vivo anti-tumor activity. In another approach using a combination of teraphthal (TP, cobalt (II) octa-4,5-carboxyphthalocyanine) and ascorbate, has progressed to Phase II clinical trials in Russia. This latter system has proven highly effective with a success rate similar to that of PDT, and its use is currently gaining popularity in Russian clinics. In fact a patent has been issued for enhancing the efficacy of CT through employing ultrasound. Recently the authors have demonstrated that significant cell cycle disruption and apoptosis is induced in cancer cells following CT treatment using porphyrins and extracts from medicinal herbs. Evolving from a handful of studies carried out in the 1980's and 90's, an array of novel substrate/catalyst couples are currently being explored for use in this rapidly expanding field. This review will address the methods and approaches, in vitro, in vivo and clinical findings and potential new applications for this new therapeutic approach.

Chapter 10 - With cancer cells being dedifferentiated rather than being proliferating as the major distinction from normal cells, tumors preserve its ability to redifferentiate. In areas endemic with bilharziasis, smoking and other urologic toxins, bladder carcinoma is the most prevalent cancer accounting for as many as 31% of all cancer cases. It is mostly of the squamous cell type, and arises in a background of the endemic schistosomiasis, preventable primarily by parasite elimination nationwide and/or by retinoids or cyclooxygenase 2 inhibitors. Intravesical chemotherapy and immunotherapy are in use for about 40 years, yet its exact role, the optimal dose and schedule of administration are still a matter of debate. After trans-urethral resection of the tumors (TURT, with or without intravesical therapy), the superficial bladder tumor recurrence rate is 30 - 70% within 12 months (Sabichi et al., 1998). Various chemotherapeutic combinations could not improve survival despite dose-escalation highlighting the importance of developing novel therapeutic approaches.

Chapter 11 - Here the authors review the complex molecular processes involved in skin development, growth and continual renewal, and how aberrations in cell cycle contribute to the development of skin cancers. Through a myriad of tightly orchestrated events involving developmental pathways and proteins including, Wingless (Wnt), Notch, Sonic hedgehog (Shh), bone morphogenetic proteins (BMP) and fibroblast growth factor (FGF), the pluripotent single-layer embryonic epidermis develops into committed epidermal cells, skin appendages and the neural system. Subsequent epidermal morphogenesis is characterized by asymmetric stem cell division, epidermal stratification, maturation into keratinized layers and cellular interaction with melanocytes, Langerhan's cells and Merkel cells. These sophisticated molecular and cellular processes are tightly regulated, the derangement of which can result in development of skin cancers. This paper focuses on major advances

achieved over the last two decades in our knowledge of deregulation of cell cycle control in the three commonest skin cancers, basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma (MM). In particular the authors review our current understanding of the role of ultraviolet irradiation (UVR), p53 and human papillomavirus (HPV) in the tumorigenesis of SCC and BCC, the Shh pathway in BCC, and abnormal cell cycle checkpoints in MM.

Chapter 12 - Tissue homeostasis requires a carefully orchestrated balance between proliferation, senescence and cellular death. Cellular proliferation is mediated by progression through the cell cycle with two major cell cycle checkpoints located at G1S and G2M. Retinoblastoma (Rb) protein family pRb/p105, p107 and pRb2/p130 are nucleoproteins essential in the suppression of G1S cell cycle progression. These proteins are involved in the negative control of the cell cycle, and their function is modulated primarily by posttranslational regulation of their phosphorylation status to binding multiple cellular proteins. In the G1 phase, hypophosphorylated pRb binds to transcriptional factors E2F protein family and suppresses its transcriptional activity. E2F protein family regulates the transcription of several genes whose products are required for either G1/S transition or DNA replication. Also, there are regulatory elements inhibitory to cell cycle progression including the INK4 and the CIP/KIP families of proteins that give rise to cell cycle arrest. Some of these prevent pRb phosphorylation, resulting in pRb activation and arrest of cell cycle progression. In contrast, various mechanisms exist to inactivate pRb function, resulting in unchecked cellular proliferation. Inactivation of pRb together with the tumor suppressor p53 has an established role in the implementation of cellular senescence and apoptosis. In addition, Rb protein family functions are essential for embryonic development and promote osteogenic, myogenic, adipogenic, thyroid, melanocytic and macrophage differentiation through interactions with tissue-specific transcription factors. Rb proteins, p107 and p130, may also have a role in cell cycle regulation and differentiation that, in part, overlaps the function of pRb. In this chapter the authors report state-of-the-art molecular findings of Rb protein family involved in cell cycle regulation. These findings, as well as others not yet elucidated, will allow us in the near future to clearly identify all cell cycle checkpoints, resulting in expanded knowledge of the molecular mechanism that can lead to tumorigenesis.

Chapter 13 - Signal transduction pathways often modulate cell proliferation by targeting the activity of cell cycle regulating proteins. One of the signaling pathways that can control the cell cycle is the mitogen-activated protein kinase (MAPK) signaling cascade. In mammalian cells, the classical MAPK pathway consists of sequential phosphorylation events leading to activation of a MAPK kinase kinase, a MAPK kinase, and a MAPK. MAPK in turn phosphorylates non-protein kinase substrates such as transcription factors, but it can also phosphorylate yet other protein kinases, referred to as MAPK-activating protein kinases (MAPKAPK). Eleven mammalian MAPKAPKs have been identified so far; six of them belong to the group of AGC protein kinases (RSK1, RSK2, RSK3, RSK4, MSK1, and MSK2), while the other five belong to the family of calmodulin-dependent kinases (MK2, MK3, MK5, MNK1, and MNK2). In this review the authors will discuss those MAPKAPKs that play a role cell-cycle regulation as well as the potential use of specific MAPKAPK inhibitors as therapy in conditions with abnormal cell cycle regulation.

Contents

In: Progress in Cell Cycle Control Research
Editor: K.L. Chen

ISBN 978-1-60456-797-7

Chapter 1

Sizing up Answers to a Long-Standing Riddle

Arkadi Manukyan, Huzefa Dungrawala, Noelle Zavala and Brandt L. Schneider[1]

Department of Cell Biology and Biochemistry,
Texas Tech University Health Science Center, USA

Abstract

Cells of a given type tend to divide at a characteristic size. In most proliferating cells, it has been observed that cell growth and division are coordinated by linking the rate at which cells add mass to their division rate. The end result is a model that yields passive cell size control. However, to add an active mechanistic twist, experiments in a number of organisms have suggested that the proliferative potential of cells is closely linked to cell size. That is, cells become competent to divide only after achieving a minimum cell size. In these cases, it was found that cell growth was exponential and proportional to cell size. This creates somewhat of a circular paradox whereby cell size determines growth rate and growth rate determines cell size. Indeed, this is further complicated by the frequency with which the words "growth" and "proliferation" are used interchangeably. Comparison of oocytes to embryonic cells clearly illustrates the difference between growth and proliferation. Oocytes grow but don't divide. This generates cells that can be 10^5 times larger than somatic cells. In contrast, a fertilized ooccyte gives rise to embryonic cells that divide without growing yielding enormous numbers of cells in a relatively short period of time. Thus, a simple and direct connection between cell size, cell growth and cell cycle control remains to be discovered. Until recently, very little was known about the genetic pathways involved in cell size homeostasis or those that link cell growth to cell cycle control. However, the age of

1 Corresponding author: Brandt L. Schneider, Department of Cell Biology and Biochemistry, Texas Tech University Health Science Center, Lubbock, TX 79430. Voice: (806) 743-2512, Fax: (806) 743-2990, email: brandt.schneider@ttuhsc.edu.

genomics and system-wide genetic screens have provided us with a fresh look at the long-standing riddle surrounding the relationship between cell size, cell growth, and cell cycle control. Herein, from a historical perspective, the paradox of cell size control is re-examined in the light of recent advances.

"That the role of size has been to some degree neglected in biology may lie in its simplicity."

John Tyler Bonner

Introduction

In biology, the size or the mass of organisms is probably the easiest recognized and most measured trait (discussed in (Bonner 2006; Haldane and Maynard Smith 1985; Hall et al., 2004)). Undoubtedly, part of the curiosity surrounding size arises from the incredible range found in nature. For instance, *Mycoplamsa*, with a size of 0.2-0.3 microns, are amongst the smallest known organisms (Morowitz and Tourtellotte 1962). In contrast, the largest giant sequoia is more than 80 meters tall with a mass of greater than 2000 tons (Bonner 2006). In relative terms, the sequoia is nearly 100 billion times larger than the *Mycoplasma*. This variability in size is nearly incomprehensible. Large size variations can also occur within a species. For example, the largest dog, an English mastiff, is nearly 300 times bigger than the smallest dog, a miniature Chihuahua. Interestingly, evidence suggests that differences in cell number accounts for the vast majority of size diversity. For instance, a 70 kg human weighs 2800 times that of 25 g mouse predominately because it has ~3000 times more cells (Baserga 1985; Conlon and Raff 1999). This suggests that in animals as diverged as man and mouse that the average cell size is remarkably similar. Indeed, this observation appears to hold true for most organisms (reviewed in (Conlon and Raff 1999)).

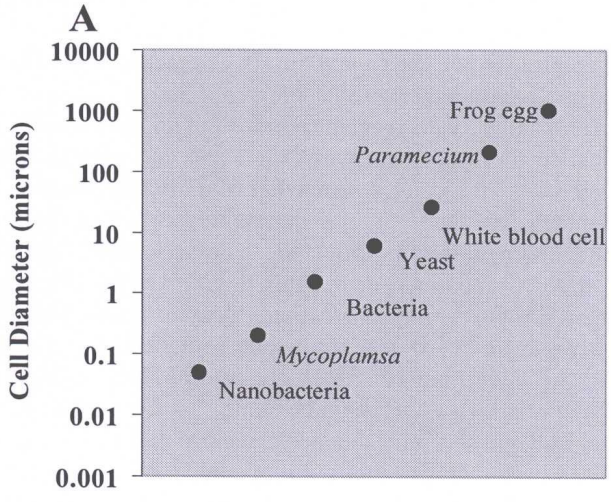


Figure 1. A. Relative size (microns in diameter) of cells found in nature.

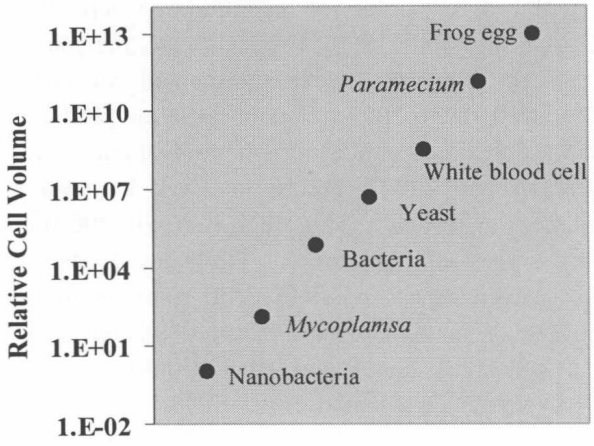


Figure 1. B. Relative volume (microns^3 =fL) of cells found in nature.

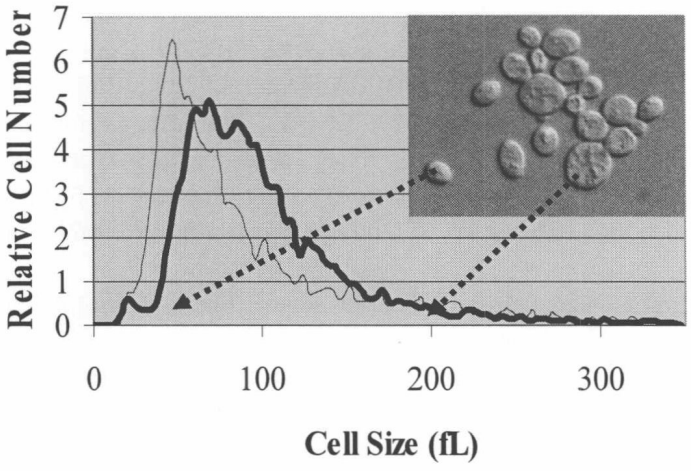


Figure 2. Coulter counter channelyzer plot illustrating the cell size distributions of wild type diploid yeast grown under optimal conditions (black line) or poor nutrient conditions (thin line). Inset is a picture of exponentially growing cells illustrating that while average cell size is uniform, small daughter and large mothers can be identified. Interestingly, the entire size distribution can be regenerated from the selection and propagation of either the smallest or largest cell.

Within a given lineage, the average size of cells remains remarkably similar and constant, yet cell size anomalies do exist (Alberts 1994; Altman and Katz 1961; Bonner 2006; Conlon and Raff 1999; Szarski 1976; Tessier 1939) (Figure 2). As Thomas Kuhn suggested, it is in the rigorous examination of anomalies where the most can be learned

(Kuhn 1962a; Kuhn 1962b). Indeed, analysis of the range of cell sizes found in nature yields amazement (Figure 1). Despite the fact that all organisms are composed of cells that are usually similar in size within a few orders of magnitude (Alberts 1994; Altman and Katz 1961; Bonner 2006; Conlon and Raff 1999; Tessier 1939), the range of possible sizes is astounding (Figure 1). Recently, a class of extremely small cells called Nanobacteria has been discovered (Urbano and Urbano 2007). Nanobacteria are predicted to be 20,000 times smaller in diameter and 8×10^{12} times smaller in volume than one of the largest known cells, a frog egg (Urbano and Urbano 2007) (Figure 1). Even between eukaryotes, cell size differences can be dramatic. For instance, a frog egg is nearly 200 times the size and 1.7×10^6 times the volume of a yeast cell (Figure 1). While these observations are useful for illustrating the range of physiological possible cells sizes, examination of the size of proliferating cells in culture or in vivo reveals that cell diameters can still vary 2-6 fold (Bauer and Thompson 2004; Clark and Ruehl 1919; Conlon et al., 2004; Cooper 2004; Cristofalo and Kritchevsky 1969; Dyachenko et al., 2006; Echave et al., 2007; Edgar and Nijhout 2004; Greenberg et al., 1977; Henrici 1928; Huntington and Winslow 1936; Jorgensen et al., 2002; Jorgensen et al., 2004a; Jorgensen and Tyers 2004; Mainland and Coady 1938; Mitchison 1971; Pendergrass et al., 1989; Prescott 1956a; Prescott 1976b; Rattan 1998; Zhang et al., 2002). Thus, while the majority of cells are quite similar in size, the largest and smallest cells can occupy a wide-size range (Figure 2). Importantly, this is the case for human, mouse, rat, fruit fly, protozoa, and bacterial cells and probably holds true for all cells (Bauer and Thompson 2004; Clark and Ruehl 1919; Conlon et al., 2004; Cooper 2004; Cristofalo and Kritchevsky 1969; Dyachenko et al., 2006; Echave et al., 2007; Edgar and Nijhout 2004; Greenberg et al., 1977; Henrici 1928; Huntington and Winslow 1936; Jorgensen et al., 2002; Jorgensen et al., 2004; Jorgensen and Tyers 2004; Mainland and Coady 1938; Mitchison 1971; Pendergrass et al., 1989; Prescott 1956; Prescott 1976; Rattan 1998; Zhang et al., 2005; Zhang et al., 2002; Anderson et al., 1969; John 1981; Prescott 1976b).

Examination of cell size distribution curves reveals that they are frequently skewed to the right (Figures 2-3). This means that even under steady-state conditions, cultures have abnormally large outliers. Remarkably, this pattern of cell size distributions is completely self-ordering. That is, isolated single cells that are either extremely small or very large will recapitulate the normal cell size distribution upon propagation (Figure 2). In addition, transferring cells from optimal to poor conditions shifts these curves to the left by generating smaller than normal cells (Figure 2). These observations illustrate the plasticity of cell size. How then is cell size modulated? Given the range of physiologically possible cell sizes, what determines the optimal cell size?

Coordination of growth with division will ensure cell size homeostasis. Remarkably, despite the implicit simplicity of this proposal, little is known about the molecular or biochemical pathways responsible for cell growth control. Nonetheless, the isolation of mutations that dramatically alter mean cell size clearly indicates that size is under genetic control (Figure 3). Moreover, while a number of recent genome-wide genetic screens have identified cell size control genes (discussed in a later section), the means whereby cell size homeostasis is established is still open for debate.