The Continuum Model of the Eukaryotic Cell Cycle: Application to G1-phase control, Rb phosphorylation, Microarray Analysis of Gene Expression, and Cell Synchronization

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ABSTRACT

The Continuum Model proposes that control of the cell cycle is based on a continuous, phase-independent, accumulation process. Accumulation occurs between the initiations of S phases. Thus the G1 phase is merely a period during which an accumulation process begun in the previous S and G2 phases are completed. The origins of the Continuum Model are reviewed, and experimental data supporting the continuum model are described. Recent support of the continuum model comes from analyses of (i) time-lapse studies of interdivision times of presumed synchronized cells, (ii) studies on Rb phosphorylation during the cell cycle, (iii) microarray measurements of gene expression during the cell cycle, and (iv) the effects of short- and long-term starvation and growth arrest on cell-cycle progression. The Continuum Model is a better explanatory exemplar for understanding the regulation of the cell cycle than the current G1-phase dominant model where phenomena such as G0, restriction points, and G1-phase specific events are proposed to be controlling elements of the cell cycle.

Key words: G1 phase, cell cycle, synchronization, restriction point, continuum model, retinoblastoma, lovastatin, G0.

The current view of control of the eukaryotic cell cycle is dominated by the presence of G1-phase controls and G1-phase phenomena. The standard or consensus model of cell-cycle control proposes that elements specific to the G1-phase are important in regulating the interdivision time and determining whether a cell will grow and divide, or enter some resting/differentiation state. For example, the decision to enter a non-growing "G0" phase is believed to be decided during the G1-phase. Further, numerous biochemical changes are proposed to occur specifically and uniquely in the G1 phase.

The Continuum Model of the cell cycle, in contrast, proposes that G1-phase is devoid of unique control elements. The Continuum Model proposes that the G1-phase is not the repository of any specific controls regulating the eukaryotic cell cycle. The Continuum Model is briefly reviewed below, primarily to bring the literature on this alternative description of cell-cycle regulation to the students of, and researchers on, the eukaryotic cell cycle.

The Continuum Model had its genesis over 35 years ago in a discussion of the implications of the finding that the S and G2 phases of bacteria (usually termed the C and D periods) are invariant as growth rate varies [22,27]. Since a large number of previous studies had revealed a similar relationship for eukaryotic cells—a relatively invariant S and G2 phases and a more variable G1 phase [26]—it was suggested that the bacterial control model, which necessarily did not include any G1-phase control elements, may also apply to eukaryotic cells [26].

A decade later this conjecture was used to present a unified control model for both eukaryotic and prokaryotic cells when it was shown, in separate experimental systems,

that eukaryotic cells may grow without a G1-phase and prokaryotic cells may have, when grown slowly enough, a G1-phase (termed, in bacteria, the B phase) [2].

The term “Continuum Model”, describing this unified view of cell-cycle control is derived from two different concepts. One was the fundamental idea that the control of the cell cycle was based on a *continuous* and unitary accumulation phenomenon, rather than a sequence of different processes. The current view of the cell cycle is a sequential event model where cells have to perform separate discrete events in a particular order in order to complete a cell cycle. The second origin of the term “Continuum Model” is rooted in the idea that the patterns of DNA replication observed during varied bacterial cell cycles and eukaryotic cell cycles form a *continuum* of patterns [2,4].

Shortly after the unified Continuum Model was proposed, these ideas were applied to a critique of the postulated G0 phase [4]. If growing cells are placed in a situation where they grow extremely slowly, and the S and G2 phases are relatively invariant in time, then the fraction of cells with a G1-phase amount of DNA will inevitably increase. It will thus appear that cells are “arrested” in G1 phase. Experimentally this is indicated by a large percentage of cells having a G1-phase amount of DNA when cells are subjected to FACS analysis [19]. But of course this is only an appearance of “G1 arrest”. The cells may actually be progressing continuously through the cell cycle and thus the cells are not necessarily arrested at any particular point in the cell cycle [19].

One of the key events leading to the current G1-dominated view of the eukaryotic cell cycle was the postulation of a unique “restriction point” in the G1 phase [30]. The

restriction point has become one of the foundational ideas of the G1-phase control model. The restriction point is proposed to be a point in the G1-phase where cells that are placed in non-growth conditions come to rest. A common point was found after growth arrest by different conditions; this was termed the restriction point.

A more detailed analysis of the restriction point has revealed many problems with the proposal. For example, even in the original work the cells released from growth arrest were not synchronized as would be expected if cells were arrested at a particular point within the cell cycle [20]. But despite these flaws in the original proposal that cells are arrested at a particular point in the cell cycle—or in a special out-of-cycle phase termed the "G0 phase"—the restriction point idea lives on. There are literally hundreds if not thousands of papers that propose to "synchronize" cells by growth arrest using serum starvation or related whole-culture methods. An analysis of whole-culture synchronization methods indicates that these approaches cannot synchronize cells [21]

The problematic origins of the current G1-phase model of cell cycle regulation have not deterred many researchers and reviewers from promoting the importance of G1-phase controls. Because these are so well known, these ideas will not be reviewed here. Rather, the recent developed supporting evidence for the Continuum Model will be reviewed in order to bring to the field a series of neglected observations that deserve consideration.

Time-lapse analysis of synchronization methods. A positive examination of the effect of proposed whole-culture synchronization methods has been performed using time-lapse analysis of lovastatin treated cells [18]. Lovastatin has been proposed to be a

general synchronizing agent. Not only was it found that cells treated with lovastatin were not synchronized, but a detailed examination of prior work proposing either synchronization or arrest “in the G1-phase” [29,31,32] are actually inconsistent with these interpretations [18].

Rb phosphorylation and the cell cycle. One of the archetypal G1-phase events is the proposed G1-phase specific phosphorylation of the retinoblastoma protein [23,25]. The literature on this topic is enormous, and suffice it to say that the current view of G1-phase dependent Rb phosphorylation is indicated by research papers, numerous review articles, and even pictures in commercial catalogues. However, a restudy of the Rb system has revealed that if one grows cells without overcrowding then Rb phosphorylation/dephosphorylation during the cell cycle is not a necessary requirement for correct passage through the cell cycle. More to the point, experiments on NIH3T3 cells have presented an explanation of why so many believe in the G1-phase Rb phosphorylation phenomenon [23,25]. Simply put, if cells are even slightly overgrown, one has a mixed population of cells, some growing some not growing. The cells with a G1-phase amount of DNA from these two populations gives both phosphorylated and dephosphorylated Rb while the growing cells (with S and G2 phase DNA contents) have only phosphorylated Rb. The readers should read both papers on Rb phosphorylation [23,25] in order to appreciate the arguments presented.

Microarray analysis of gene expression during the cell cycle. Microarray studies of gene expression during the cell cycle have proposed that numerous genes are expressed in a cell-cycle-specific manner. In disparate systems such as primary fibroblasts, HeLa cells, yeast, arabidopsis, NIH3T3 cells, and the prokaryotic

Caulobacter, hundreds of genes have been reported to be expressed in a cell-cycle-specific manner. A detailed reanalysis of the raw data [1] on gene expression in primary fibroblasts has revealed that not only can random noise explain the data but the data are not reproducible by a number of criteria and the internal evidence indicates that the cells were not synchronized [24,33]. In the case of yeast cells [35] the data is not accountable at this time by random noise, but comparisons of whole-culture synchrony methods (critiqued above) with selective methods indicate that the data are far from reproducible [24,34]. A complete review of the data from numerous systems reveal that the microarray data is not supportive of numerous genes being expressed in a cell-cycle specific manner [24].

Whole culture synchronization. It is arguably correct to say that almost all of the experiments on the eukaryotic cell cycle are based on whole-culture synchronization methodologies. That these methods do not work has been shown by a rigorous theoretical analysis, as well as analyses of published experimental data [6,9,10,12,17,20,21,24]

Selective synchronization by the eukaryotic "baby machine". The continuum model predicts that selective synchronization can lead to well-defined synchronized cells. This prediction has been born out with the development of the "baby machine" for eukaryotic cells [17,28,36].

The Zetterberg-Larsson Experiments. Time-lapse analyses by Zetterberg and Larsson have led to the proposal that the G1-phase can be divided into two phases. One phase, the post-mitotic (pm) phase, occurs for approximately 3.5 hours following division. The remainder of the G1-phase is the pre-synthetic (ps) phase. The point

demarcating the pm and ps phases was identified, in these experiments, by observing that a short (1 hr) period of cell starvation led to a long-delay in division (8 hr) in the pm cells, but no division delay in the ps cells. As it turned out, as predicted by the Continuum Model, division of the ps cells were delayed in the subsequent division, after one un-delayed division. A complete analysis of the Zetterberg-Larsson experiments indicates that these results are predicted by the Continuum Model, and in fact support the basic tenet of the continuum model, that an accumulation process continues from one cell cycle into the next cell cycle [6,12]

Differentiation and the G1 phase. The observation that the cells in the differentiated tissue of multicellular organisms are predominantly cells with a G1-phase amount of DNA has been cited as support that cells decide to differentiation when they are in the G1-phase of the cell cycle. Thus, cells move through the cell cycle, and when a differentiation signal is received the cells that are in G1 phase proceed to differentiate and do not initiate S phase. This leads to differentiated cells with a G1-phase amount of DNA. In contrast, the Continuum Model explains the observed G1-phase DNA contents of differentiated cells without postulating any phase-specific decision process [15]. Cells in all phases of the cell cycle can proceed to differentiation. But associated with differentiation is a signal to inhibit initiation of future S phases. Since differentiation takes such a long time relative to the time for S and G2 phases, the S and G2 phases are completed before differentiation is observed. Thus, without a G1-phase specific decision point, differentiated cells are found with a G1-phase amount of DNA according to the Continuum Model.

Detailed reviews of the continuum model. A more detailed exposition of the Continuum Model is not presented here because of space limitations, but further information and references can be found in relevant publications.[2-16,18,20,21,23-25,34]

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Key papers on this subject may be read at www.umich.edu/~cooper

References

1. R.J. Cho, M. Huang, M.J. Campbell, H. Dong, L. Steinmetz, L. Sapinoso, G. Hampton, S.J. Elledge, R.W. Davis and D.J. Lockhart, Transcriptional regulation and function during the human cell cycle, Nat Genet 27 (2001), 48-54.
2. S. Cooper, A unifying model for the G1 period in prokaryotes and eukaryotes, Nature 280 (1979), 17-19.
3. S. Cooper, The central dogma of cell biology, Cell Biol Int Rep 5 (1981), 539-549.
4. S. Cooper, The continuum model: application to G1-arrest and G(O). in Cell Growth. Edited by Nicolini C: Plenum Press; 1981:315-336.
5. S. Cooper, The continuum model: statistical implications, J Theor Biol 94 (1982), 783-800.
6. S. Cooper, On G0 and cell cycle controls, Bioessays 7 (1987), 220-223.
7. S. Cooper, The continuum model and c-myc synthesis during the division cycle, J Theor Biol 135 (1988), 393-400.
8. S. Cooper, Bacterial Growth and Division. San Diego: Academic Press; 1991.
9. S. Cooper, G1 and S phase gene expression cannot be analyzed in mammalian cells synchronized by inhibition, Microb Comp Genomics 2 (1997), 269-273.
10. S. Cooper, Mammalian cells are not synchronized in G1-phase by starvation or inhibition: considerations of the fundamental concept of G1-phase synchronization, Cell Prolif 31 (1998), 9-16.
11. S. Cooper, On the interpretation of the shortening of the G1-phase by overexpression of cyclins in mammalian cells, Exp Cell Res 238 (1998), 110-115.
12. S. Cooper, On the proposal of a G0 phase and the restriction point, FASEB J 12 (1998), 367-373.
13. S. Cooper, The continuum model and G1-control of the mammalian cell cycle, Prog Cell Cycle Res 4 (2000), 27-39.
14. S. Cooper, Toward a standard system for the mammalian cell cycle., ASM News 66 (2000), 71-75.
15. S. Cooper, Revisiting the relationship of the mammalian G1 phase to cell differentiation, J Theor Biol 208 (2001), 399-402.
16. S. Cooper, Cell cycle analysis and microarrays, Trends in Genetics 18 (2002), 289-290.
17. S. Cooper, Minimally disturbed, multi-cycle, and reproducible synchrony using a eukaryotic "baby machine" Bioessays 24 (2002), 499-501.
18. S. Cooper, Reappraisal of G1-phase arrest and synchronization by lovastatin, Cell Biol Int 26 (2002), 715-727.
19. S. Cooper, How the change from FLM to FACS affected our understanding of the G1 phase of the cell cycle, Cell Cycle 2 (2003), 157-159.
20. S. Cooper, Reappraisal of serum starvation, the restriction point, G0, and G1-phase arrest points, FASEB J 17 (2003), 333-340.
21. S. Cooper, Rethinking synchronization of mammalian cells for cell-cycle analysis, Cell Mol Life Sci 6 (2003), 1099-1106.
22. S. Cooper and C.E. Helmstetter, Chromosome replication and the division cycle of Escherichia coli B/r, J Mol Biol 31 (1968), 519-540.

23. S. Cooper and J.A. Shayman, Revisiting retinoblastoma protein phosphorylation during the mammalian cell cycle, Cell Mol Life Sci 58 (2001), 580-595.
24. S. Cooper and K. Shedden, Microarray Analysis of Gene Expression during the Cell Cycle, Cell & Chromosome 2 (2003), 1-12.
25. S. Cooper, C. Yu and J.A. Shayman, Phosphorylation-dephosphorylation of retinoblastoma protein not necessary for passage through the mammalian cell division cycle, IUBMB Life 48 (1999), 225-230.
26. C. Helmstetter, S. Cooper, O. Pierucci and E. Revelas, On the bacterial life sequence, Cold Spring Harb Symp Quant Biol 33 (1968), 809-822.
27. C.E. Helmstetter and S. Cooper, DNA synthesis during the division cycle of rapidly growing Escherichia coli B/r, J Mol Biol 31 (1968), 507-518.
28. C.E. Helmstetter, M. Thornton, A. Romero and K.L. Eward, Synchrony in human, mouse, and bacterial cell cultures, Cell Cycle 2 (2003), 42-45.
29. K. Keyomarsi, L. Sandoval, V. Band and A.B. Pardee, Synchronization of tumor and normal cells from G1 to multiple cell cycles by lovastatin, Cancer Res 51 (1991), 3602-3609.
30. A.B. Pardee, A restriction point for control of normal animal cell proliferation, Proc Natl Acad Sci U S A 71 (1974), 1286-1290.
31. S. Rao, M. Lowe, T.W. Herliczek and K. Keyomarsi, Lovastatin mediated G1 arrest in normal and tumor breast cells is through inhibition of CDK2 activity and redistribution of p21 and p27, independent of p53, Oncogene 17 (1998), 2393-2402.
32. S. Rao, D.C. Porter, X. Chen, T. Herliczek, M. Lowe and K. Keyomarsi, Lovastatin-mediated G1 arrest is through inhibition of the proteasome, independent of hydroxymethyl glutaryl-CoA reductase, Proc Natl Acad Sci U S A 96 (1999), 7797-7802.
33. K. Shedden and S. Cooper, Analysis of cell-cycle-specific gene expression in human cells as determined by microarrays and double-thymidine block synchronization, Proc Natl Acad Sci USA 99 (2002), 4379-4384.
34. K. Shedden and S. Cooper, Analysis of cell-cycle-specific gene expression in *Saccharomyces cerevisiae* as determined by Microarrays and Multiple synchronization methods., Nuc Acids Res 30 (2002), 2920-2929.
35. P.T. Spellman, G. Sherlock, M.Q. Zhang, V.R. Iyer, K. Anders, M.B. Eisen, P.O. Brown, D. Botstein and B. Futcher, Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization, Mol Biol Cell 9 (1998), 3273-3297.
36. M. Thornton, K.L. Eward and C.E. Helmstetter, Production of minimally disturbed synchronous cultures of hematopoietic cells, Biotechniques 32 (2002), 1098-1105.