SPECIAL ARTICLE

The Continuum Model: An Experimental and Theoretical Challenge to the G1 Model of Cell Cycle Regulation

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The dominant and current view of cell cycle regulation is that the controls for cell proliferation reside primarily in the G1 phase of the division cycle. This current or classical model is deeply ingrained in cell biology, and has been described very well [1, 2]; therefore we will only summarize below the basic support of the G1 model. Over the past decade, however, an alternative proposal, the continuum model, has emerged that suggests that there are no G1-specific events or controls, and that all of the evidence for the importance of the G1 phase can be explained in terms of a continuous process that occurs in all phases of the division cycle [3–7]. These early reports addressed previous experimental results and explained them in terms of the continuum model. In a sense, therefore, these analyses were theoretical rather than experimental. The basic ideas of the continuum model are presented in Fig. 1.

Recently there has emerged a set of experiments that has been stimulated by the ideas of the continuum model, and these experiments support the basic ideas of the continuum model. This means that the continuum model has been a stimulus to experimental tests, and therefore has an experimental as well as a theoretical base. We will describe these experiments below, and explain how they support the continuum model.

ANALYSIS OF THE G1 MODEL OF CELL CYCLE REGULATION ACCORDING TO THE CONTINUUM MODEL

One may divide essentially all of the support for the G1 model of cell cycle regulation into a number of groups or categories of experiments. These are:

Variability of G1. The G1 phase of the cell cycle is the most variable. The longer the G1 phase, the longer was the interdivision time of cells. This suggested that the G1 phase regulates the rate of cell proliferation. The idea was that if cells passed through the G1 phase very slowly, they would be slow-growing cells.

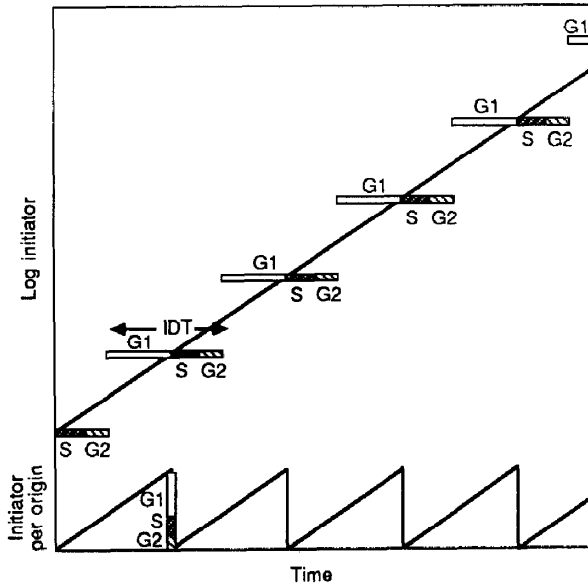[1] To whom reprint requests should be addressed.

Fig. 1. A schematic diagram of the continuum model. The lower sawtooth graph is the amount of initiator per origin, varying from 0.5 to 1.0; a value of 1.0 is the critical amount required for the initiation of DNA synthesis. At zero time the cells have just entered an S phase and the cells progress through S and G2. The amount of initiator per origin increases from 0.5 to 1.0 over one interdivision time (IDT). When the initiator per origin reaches 1.0 another S phase is initiated. Since there is a sudden doubling in the number of available origins of replication the amount of initiator per origin decreases immediately to 0.5. The concept of a doubling in "origins" is a formal concept, and in reality there are many origins of replication in the eukaryotic S phase. The G1 period is the time between the end of G2 and the start of S phase (here neglecting the short M period). If the rate of initiator synthesis decreased and S and G2 remained constant, then G1 would increase. If the rate of initiator synthesis increased, the G1 phase would decrease in length. As drawn in the upper graph, the synthesis of initiator is continuous. No synthesis of particular regulatory molecules occurs in any single phase of the division cycle.

G1 arrest. When cells are placed under nongrowth conditions, it is generally found that cells are arrested with a G1 content of DNA. This has been interpreted as indicating that there is some point or event in the G1 phase of the division cycle at which cells become arrested. The arrest point has sometimes been referred to as the "restriction point".

G1 events. When cells with a G1 amount of DNA are stimulated to resume growth, a number of different proteins appear to be made specifically within the G1 phase of the division cycle. This has been used to support the idea that there are G1-specific events.

G(0). The proposal of an out-of-cycle state, the G(0) phase, which a cell enters from the G1 phase of the division cycle, has supported the idea that the cell makes proliferative choices in the G1 phase of the division cycle. This is one of the G1-specific events that are believed to occur in the G1 phase of the division cycle. The importance of the G(0) phase is that it gives a general support to the notion that there exist other G1-specific events.
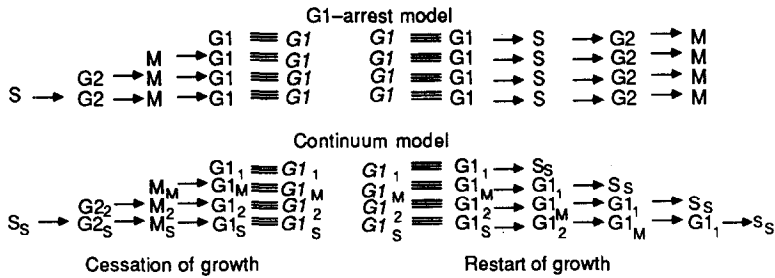
G1–arrest model

$$G1 \equiv G1 \qquad G1 \equiv G1 \rightarrow S \rightarrow G2 \rightarrow M$$
$$M \rightarrow G1 \equiv G1 \qquad G1 \equiv G1 \rightarrow S \rightarrow G2 \rightarrow M$$
$$G2 \rightarrow M \rightarrow G1 \equiv G1 \qquad G1 \equiv G1 \rightarrow S \rightarrow G2 \rightarrow M$$
$$S \rightarrow G2 \rightarrow M \rightarrow G1 \equiv G1 \qquad G1 \equiv G1 \rightarrow S \rightarrow G2 \rightarrow M$$

Continuum model

$$G1_1 \equiv G1_1 \qquad G1_1 \equiv G1_1 \rightarrow S_S$$
$$M_M \rightarrow G1_M \equiv G1_M \qquad G1_M \equiv G1_M \rightarrow G1_1 \rightarrow S_S$$
$$G2_2 \rightarrow M_2 \rightarrow G1_2 \equiv G1_2 \qquad G1_2 \equiv G1_2 \rightarrow G1_M \rightarrow G1_1 \rightarrow S_S$$
$$S_S \rightarrow G2_S \rightarrow M_S \rightarrow G1_S \equiv G1_S \qquad G1_S \equiv G1_S \rightarrow G1_2 \rightarrow G1_M \rightarrow G1_1 \rightarrow S_S$$

Cessation of growth   Restart of growth

Fig. 2. Comparison of the G1-arrest model of eukaryotic cell cycle regulation with the continuum model. In the G1-arrest model cells in all phases of the division cycle enter a resting phase with all cells having a G1 content of DNA. This resting phase, signified by the italicized G1, is entered from the G1 phase of the division cycle. When cell growth restarts, cells leave this special state, sometimes referred to as G0, and enter G1. The cells then proceed through the division cycle. In contrast, the continuum model proposes that there are two aspects that define the cycle phase of a cell. One is the DNA content of a cell (signified by the large letters), and the other is the amount of initiator per origin, indicated by the subscripts. When cells cease growing, the amount of initiator is held constant, and the cells pass through the phases of the cycle so all cells have a G1 amount of DNA. When these cells with a G1 amount of DNA are allowed to restart growth, the cells do not enter the division cycle all at the same time. Rather, they enter the cycle in the order that they entered the resting phase. There is no special unique state in which cells are resting. The cells have a G1 amount of DNA merely because cells in S, or G2, or M phase must complete these phases and produce two G1 DNA-containing cells even under deleterious conditions. There are no points in the G1 phase of the division cycle at which cells come to rest.

Transition probability. The variability of cell cycle interdivision times has been used to propose a model in which random transitions, generally associated with the G1 phase of the division cycle, are the regulatory agents for cell cycle proliferation. Although the transition probability model is quite different from the G1-event model, its proposal and wide acceptance as an alternative viewpoint have lent added weight to the idea that the important, cell cycle regulatory phase of the division cycle is the G1 phase.

The continuum model has reinterpreted these basic categories of support for a G1 model of the division cycle. We will describe this alternative explanation, and follow this with a description of the new experimental work that has been presented to support the continuum model.

Variability of G1. The continuum model proposes that the variable G1 is the result of a variation in the times between initiations of DNA synthesis. The G1 phase is what is left over when the time between initiations is greater than the time for S, G2, and M [3]. The continuum model proposes that the observed variability of G1 is a result of the variation in growth rate, rather than a cause of the variation in growth rate. This is illustrated in Fig. 1. If S and G2 are constant, as has generally been observed, then if the rate of initiator synthesis varies, large changes in the G1 phase of the division cycle are produced. If cells grow very rapidly, one can even eliminate the G1 phase of the division cycle [3]. This elimination of the G1 phase is difficult to explain with the G1-event model, but it falls naturally out of the continuum model.

G1 arrest. The continuum model proposes that cells are arrested with a G1

amount of DNA merely because the deleterious conditions that are used to produce nongrowing cells do not stop cells that are in the S, G2, or M phase of the division cycle from proceeding to the completion of DNA synthesis and the subsequent division [6]. The cells may have a G1 amount of DNA, but the continuum model proposes that they are not "in G1". This is illustrated in Fig. 2, where we see that the continuum model proposes that there is no special point or event in G1 at which cells are arrested when they are placed under conditions that do not support growth. The cells under a nongrowing condition have a G1 amount of DNA because cells, in general, do not stop in S, G2, or M phase when initiation of S phase is prevented. When growth is restarted, the cells are not synchronized, but enter the S phase in the same order as that in which they obtained a G1 DNA content during the inhibition of growth. The G1 model proposes that there is a unique state, within the G1 phase, at which cells come to rest. This is indicated by the italicized "G1" in Fig. 2.

G1 events. The events that are generally proposed to be G1 events have generally been found after stimulation of starved cells to resume growth. This perturbation leads to various changes in the metabolism of the cell that make various genes expressed at relatively high rates. Since the cells have a G1 amount of DNA, this is interpreted as support for the existence of G1 events. The continuum model suggests that these proposals are artifacts of the experimental approach [6, 7]. The continuum model suggests that the only reason that the cells have a G1 amount of DNA is that they are difficult to arrest in the S, G2, and M phases of the cycle. Thus, when cells are restarted, it only appears that the events that are observed are G1 events. According to the continuum model it is only a coincidence that cells with a G1 amount of DNA are the cells that are making a particular set of biochemical changes during the restarting of growth.

G(0). The continuum model proposes that the G(0) phase is an artifact of the prolonged starvation of cells under deleterious conditions [6, 7]. The variable conditions under which starved cells are found, and their variation in the resumption of normal growth, have been proposed to be an artifact of the starvation conditions and no indication that there is a G(0) state. The G(0) state is illustrated by the italicized "G1" in the G1 model in Fig. 2.

Transition probability. The continuum model can account for and explain all of the evidence for a transition probability regulating the cell cycle [5]. There is no need to invoke a random mechanism for the regulation of the cell cycle. The continuum model predictions fit all of the data better than the transition probability model [5], and in addition, explain the observed variability in terms of known biochemical events [5].

NEW EXPERIMENTAL SUPPORT FOR THE CONTINUUM MODEL

The continuum model proposes that the regulation of the rate of cell cycle progression, and the relative sizes of the various phases of the cell cycle, is due to the rate of synthesis of mass or some specific subset of mass (the hypothetical initiator) between the starts of S phases [3–7]. One of the predictions of the

continuum model is that the failure to synthesize the requisite material in the previous cycle will prolong the subsequent cell division. If this were found it would mean that at division the start of a new set of events, occurring in G1, was not the regulatory process for cell division. Rather, this finding would mean that there was a continuous process occurring during both the previous S and G2 phases and finishing in the subsequent G1 phase.

When rat 3Y1 fibroblasts were deprived of serum in S or G2 phase, the cells delayed entry into the S phase in the subsequent cycle [8, 9]. The delay also occurred when cells were prevented from adhering to the substrate [9] or were exposed to caffeine [10]. When the proliferating cells were deprived of serum [8] or exposed to caffeine [10] during G1 phase, they were inhibited from entering S phase. The cells arrested with a G1 amount of DNA at confluent cell density were also inhibited from entering S phase when incubated at a lower cell density under each of the deleterious conditions [8–10]. When cells expressing SV40 large T antigen proceeded through S and G2 phases in the absence of serum, subsequent entry into S phase was not markedly delayed after mitosis [11]. The proliferating cells expressing SV40 large T antigen, when deprived of serum in G1 phase, entered S phase normally [11]. The density-arrested cells entered S phase after reseeding at a lower cell density with serum-free medium containing EGF, and this effect was enhanced by addition of both insulin and transferrin [9]. The effects of the growth factors paralleled the growth-factor-mediated shortening of the delay in entry into S phase when the growth factors were added during the serum deprivation period in the previous cycle [9]. Recent analysis of the expression of c-*fos* and c-*myc* genes that were transiently and extensively induced in the cycling cells by change of culture condition from serum absence to serum presence, or after cycloheximide addition, or after serum exposure to density-arrested cells, has led to the proposal that the expression of these genes is continuously required, in low amounts, in all phases of the cell cycle to allow continued proliferation [12, 13]. These results have been interpreted as support for the continuous preparation for initiation of DNA synthesis occurring between the starts of S phase, and thus are completely consistent with, and supportive of, the continuum model.

Confirmation of these observations comes from time-lapse video analysis of cell cycle interdivision times. The initial finding that cell division in low serum proceeds normally for cells in the latter portion of the division cycle [14] was originally interpreted as support for the G(0) phase of the cell cycle. But analysis of the subsequent cycle revealed that the second mitosis, after a short treatment in low serum, is delayed [15]. This has been shown to be completely in accord with the predictions of the continuum model [16]. Recently the analysis of the cell cycle of an alga [17] has lent additional and strong support to the continuum model.

This brief analysis indicates that not only can the continuum model account for all of the past results supporting the proposal of G1-specific events, but the predictions of the continuum model have been tested and found to be supported by recent experimental results. We suggest that the continuum model is an
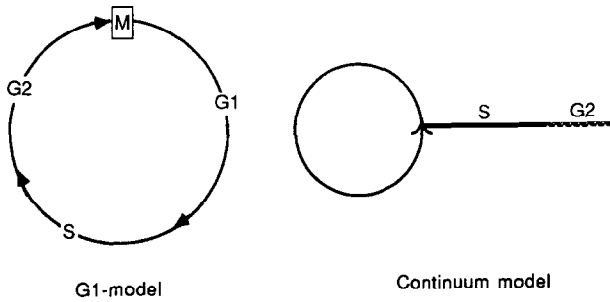
Fig. 3. The classical and continuum model icons for the eukaryotic division cycle. At the left is the circular representation of the division cycle. At the right is the icon for the continuum model. The main difference is that the continuum model proposes that termination of DNA replication and cell division do not affect or regulate the subsequent initiation of DNA replication. The classical icon implies that the end of each phase is the event that starts the next phase. This means that the end of M phase starts a series of G1 events that eventually leads to the initiation of DNA synthesis or S phase. In the continuum model cell division is the end of a process and the beginning of none.

important way to look at the division cycle, and that it allows a simplification of cell cycle analysis. We hope that more workers would take a look at the utility of the continuum model, and the various suggestions for its application [7].

The alternative ways of looking at the division cycle may be illustrated by comparing the classical icon of the division cycle (Fig. 3, left) with the icon for the continuum model (Fig. 3, right). The circular model of the division cycle implies that the end of each particular phase initiates a new phase of the division cycle. Thus, the end of the M phase starts a new G1 phase, and this G1 phase is a phase with particular properties that can be investigated. The continuum model, in contrast, sees the end of the S and G2 phases as the end of a process but this ending does not start any new processes. Cell division does not initiate any new series of events during the G1 phase that eventually lead to cell division. Preparations for division are continuous, as symbolized by the circle, and when S phase starts a cell division follows after a sequence of S, G2, and M phases. Neither termination of DNA synthesis nor the act of cell division regulates or alters the continuous preparations for the initiation of DNA synthesis that is occurring in all phases of the division cycle.

We hope that this presentation of the theoretical and experimental basis for the continuum model leads to a reinterpretation of both past and future experimental results in the light of this view of the division cycle. Although the great mass of papers heretofore have interpreted their results from the classical viewpoint, we feel that all of these results can be reinterpreted, reanalyzed, and shown to be consistent with the predictions of the continuum model.

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