review article

A unifying model for the G1 period in prokaryotes and eukaryotes

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A model to explain the cell division cycle in both prokaryotes and eukaryotes is presented. No specific 'G1 functions' take place during the G1 period, which is merely part of a larger period for the preparation of DNA synthesis which began at the previous initiation of DNA synthesis. A G1 period exists merely because the doubling time of the cells is greater than the sum of the S and G2 periods.

THERE was a time, a few years ago, when the division cycles of prokaryotes and eukaryotes could be clearly distinguished. The division cycle of eukaryotes consisted of three periods—G1, S and G2—whereas prokaryotes had only C and D periods (analogous to the S and G2 periods, respectively) but did not clearly exhibit a G1 period. The S and C periods are analogous when defined as the time of DNA synthesis, and the G2 and D periods are analogous when defined as the time between the termination of DNA synthesis and mitosis or cell division. The analogy between the S and G2 periods and the C and D periods was strengthened by the observations that the lengths of time for these periods were quite invariant with large changes in the growth rate of the various cells[1–10]. More recently, however, it has been shown that prokaryotes can have a G1 period[11] and that there are eukaryotic cells which appear to be devoid of a G1 period[10,12–15]. Is there then any common, unified basis for understanding eukaryotic and prokaryotic division cycles?

There have been two different ways of analysing the division cycle of cells, one based on the classic results with eukaryotes and one based on the results obtained with prokaryotes. I believe that this schism is unnecessary and that there is a simple and encompassing view which allows both types of division cycle to be discussed in the same terms. My main premise is that there is no physical reality to the G1 period which requires that it have any particular function; instead the G1 period is merely part of a larger period involved in the preparation for DNA synthesis. This leads to a common, unified model for the cellular division cycle.

Bacterial division cycle

The bacterial division cycle is illustrated schematically in Fig. 1. The C and D periods, analogous to the S and G2 periods, are 40 and 20 min, respectively. That is, it takes 40 min to replicate the DNA of the cell, and there is a period of 20 min between termination of replication and cell division. Figure 1 illustrates the relative rates of DNA synthesis in the cells (the rate being proportional to the number of DNA growing points). Note that for cells growing with a doubling time greater than 60 min there is a G1 period as well as the S and G2 periods analogous to those in the eukaryotic division cycle. Bacterial cells, however, can grow at rates faster than 60 min per doubling. In these cases Fig. 1 illustrates that there is no G1 period. Instead DNA synthesis occurs during cell division, having started in the previous division cycle. Where the doubling time is less than 40 min DNA synthesis will commence before the completion of previously started rounds of DNA replication. These division cycle patterns are not exhibited by eukaryotes.

The particular pattern of DNA synthesis in the division cycle of a prokaryote therefore is determined solely by the frequency with which DNA replication is initiated. Once initiated, the 'clock' regulating the constant C and D periods determines the subsequent cell division. It is currently assumed that the preparations for DNA synthesis are occurring in the time gap between successive initiations of DNA synthesis. But the preparations for DNA synthesis are occurring continuously and not just in the period designated G1, which will appear when the time for the preparation of initiation of DNA synthesis (equivalent to the doubling time of the cells) is greater than the sum of the C(S) and D(G2) periods.

The nature of the factor(s) responsible for the initiation of DNA synthesis in bacterial cells is not known. Although it is quite reasonable to discuss the phenomenon in terms of a 'hypothetical initiator', circumstantial evidence points to the initiator being synthesised in the same manner as, or in parallel with, the synthesis of cell mass. Therefore, either the cell initiates DNA synthesis when a predetermined cell mass is reached or when some specific initiator, which is a constant fraction of cell mass, is present in the cell. In formal terms, DNA synthesis is initiated when the cell contains a given amount of initiator per origin of DNA to be initiated[16].

Eukaryotic division cycle

In contrast to the bacterial division cycle, the eukaryotic cycle is generally seen to fall into three divisions, a G1 period before DNA synthesis, the S phase defined as the period of DNA synthesis, and the G2 period, defined as the period between DNA synthesis and mitosis plus cell division. (In the following

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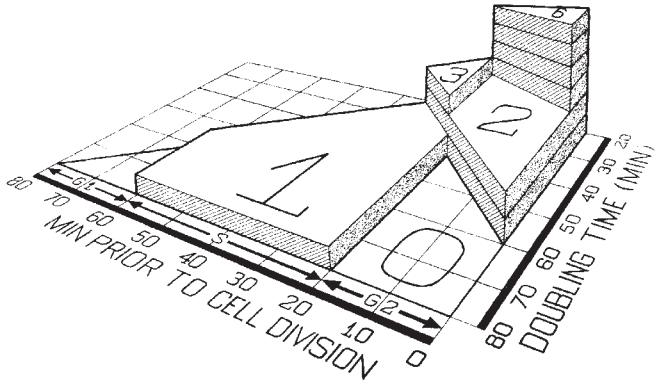

Fig. 1 Theoretical pattern of DNA synthesis during the division cycle of bacteria growing at different growth rates. The vertical axis is the rate of DNA synthesis during the division cycle. Zero DNA synthesis indicates that there is a 'gap' in DNA synthesis and this can be divided into the G1 and G2 periods as indicated. G1 and G2 periods are found when the doubling time is greater than the sum of the S and G2 periods, in this case with doubling time greater than 60 min as the S and G2 periods in *Escherichia coli* B/r are 40 and 20 min, respectively[1]. As the graph extends forward to larger and larger doubling times the G2 and S periods remain constant, and the G1 period grows. This portion of the figure is compatible with both prokaryotic and eukaryotic observations. Bacteria can grow with doubling times less than 60 min, however, and the pattern of DNA synthesis during the cycle is altered so that there is no G1 period and the late gap is shortened. The synthesis of DNA is necessarily initiated before cell division, but the time between the termination of a round of DNA replication is unchanged and remains at 20 min. Thus, between 60 and 40 min doubling times the initiation of DNA synthesis occurs during the period between termination of DNA synthesis and cell division (that is, in the bacterial G2(D) period). At doubling times of less than 40 min DNA synthesis is continuous with no 'gaps' in synthesis, and multiply-forked DNA molecules appear. Eukaryotic cells usually have doubling times which exhibit a G1 and a G2 (as in the cultures with doubling times greater than 60 min in this figure).

discussion it is assumed that there is a very small and negligible mitosis and cell division period, so the cycle is divided into only three parts.) To summarise a large amount of data, it seems that the S and G2 periods are fairly constant time periods and therefore are excellent analogues of the C and D periods of bacteria[10].

A generally current view of eukaryotic cells is that they, in contrast to prokaryotic cells, have a defined G1 period in which occur events preparatory for DNA synthesis[14,17,18]. This view is difficult to reconcile with reports that there are eukaryotic cells which are devoid of a G1 period ("G1-less cells")[14,15]. How can one have cells which have the G1 events but which do not have G1? I will answer this by presenting a simple description of the division cycle, of both prokaryotic and eukaryotic cells, which suggests that the eukaryotic G1 period has no functional significance and can disappear if the cell grows so fast that the S and G2 periods occupy the entire division cycle. In this model, the 'G1 period' is merely part of a larger period of preparation for the initiation of DNA synthesis.

Description of the general model

Consider three reactions which are observed to occur at various times in the G1 period of cells growing at such a growth rate that they have a substantial G1 period. In Fig. 2 they are indicated as a, b and c. The current (eukaryotic) assumption, that G1 is a period of preparation for S, is illustrated in Fig. 2*A*. If these cells are now grown at a rate where G1 grows appreciably smaller, S and G2 being relatively constant, then the rate of sequential appearance of the various G1 functions will increase rapidly as indicated in Fig. 2*B*. In the limiting case where G1 disappears, this model produces a paradox in that there is no G1 period during which the G1 function can take place.

The alternative model, proposed here, is illustrated in Fig. 2*C*. Here the functions a, b and c occur at precisely the same time as in Fig. 2*A* with regard to the observed G1 period but these functions are occurring as part of a longer preparation period which stretches from the initiation of S to the next initiation of S. There is no experimental or operational distinction between Fig. 2*A* and 2*C*. However, if the cells now grow at a faster rate so that G1 is appreciably shortened, then as indicated in Fig. 2*D*, the three functions a, b and c can occur earlier than G1—even occurring in S or G2 as shown in Fig. 2*D*. Where the doubling time is so short that there is no G1, the 'G1 functions' would all take place in either the S or G2 periods.

Illustrative applications of the model

Prescott[10] has summarised the large amount of experimental data on the timing of the G1, S and G2 periods in eukaryotic cells. He has even noted that "These studies lead to the conclusion that the initiation of DNA replication is tied to the attainment by a cell of a crucial mass". He has also noted how this is similar to the bacterial model briefly described above (Fig.

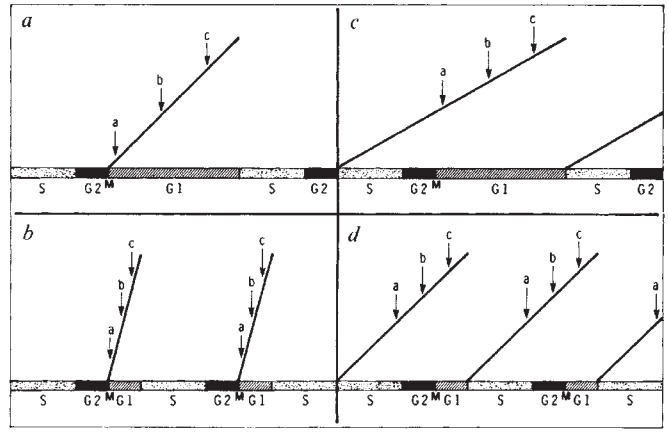

Fig. 2 Comparison of the eukaryotic view of the preparation for DNA synthesis (*A*, *B*) and the prokaryotic view of the preparations for DNA synthesis (*C*, *D*). *A* and *C* are two different interpretations of the observed preparations (a, b, c) for DNA synthesis as experimentally measured during G1. In *A* they are all part of a period of preparation for S which takes place in G1. In *C* they are part of a larger period of preparation for S which takes place from one initiation to the next, but with a, b and c only fortuitously occurring during the G1 period. *B* and *D* show how each of the models visualises the preparatory events when the rate of growth increases and the G1 period decreases in size. Although this figure is drawn as though there are different events occurring during the period of preparation for DNA synthesis, note that there is no firm and compelling evidence that the initiation of DNA synthesis occurs after a sequence of discrete reactions has occurred. The alternative view, that initiation occurs when some unitary factor has accumulated to some crucial level, is also compatible with this figure with the events a, b and c indicating the achievement of a particular level of the 'hypothetical initiator substance'.

1). I do not wish to repeat his analysis, and rather than review the mass of data on the nature, timing and events occurring in the G1 period, I will show with a few examples how the bacterial model serves to rationalise and explain the data on the nature of the G1 period better than the current eukaryotic model.

For example, Tobey *et al.*[19] have described a specific f1 histone phosphorylation event as occurring in G1 and therefore as a G1-specific event. When cells are growing more rapidly, it would be predicted (although it has not been specifically predicted or stated before, presumably because the possibility has not been studied) that the G1 events would merely occur more

rapidly leading to shorter G1 periods as seen in Fig. 2*B*. In contrast, the bacterial view presumes that the G1 events occurring in G1 are found in G1 only because G1 exists as an observable phenomenon when the doubling time of the cells is greater than the sum of the S and G2 periods. At a faster growth rate the events which may be observed previously to occur in the G1 period may now occur in the G2 or even the S period. This is clearly illustrated in Fig. 2*D*. The existence of a G1 event does not support or refute the view proposed here, but study of this phosphorylation reaction in cultures growing at different growth rates might lead to an experimental test of the unified model.

A clearer example is the analysis of eukaryotic cells which do not have a G1 period. Liskay and Prescott[15] have studied such a cell, and have obtained variants from the G1-less cell which contain a G1. They observed that the S and G2 periods did not change and that the appearance of a G1 was entirely accounted for by the increase in the doubling time of the variant cells. I suggest that this is an experimental verification of the idea that the G1 period is produced when the doubling time of a culture is greater than the sum of the S and G2 periods. When Liskay and Prescott selected for cells which had a G1 period, they were actually selecting cells which grew slower and which had less frequent initiations of DNA synthesis. The appearance of the G1 period was not due to any change in the genetic control of the G1 period itself, but merely due to the selection of mutants which had a slower growth rate without any concomitant change in the rate of DNA synthesis (S) or the rate of preparation for cell division following DNA synthesis (G2). Fusion experiments between the slow growing variant and the parental G1-less cell revealed that the variant was a "deficient condition' which is just what one would expect to produce a slow growing cell. Work by Liskay[14] had shown that the G1-less cells, when fused to temperature-sensitive mutants which had defects in their 'G1 functions', were able to complement the mutants. Therefore, the 'G1-less' cells still exhibited the G1 functions. This would normally seem paradoxical unless one subscribes to the model exhibited in Fig. 2*C* and *D* which shows that there is no special G1 period and no special G1 function. Assuming that there are functions which occur sequentially in preparation for DNA synthesis, the model proposed in Fig. 2*C* and *D* explains this apparent paradox since it is merely an accident of the cellular growth rate whether the function appears in the G1 period or not. In contrast to the model proposed here, Liskay and Prescott[15] have interpreted the G1-less cells as having 'full expression' (constitutive expression?) of the G1 functions. This is an *ad hoc* explanation which says that G1 functions can be expressed at other times. But it retains the notion of G1 functions.

Implications of the model

The model proposed here presents a unified view of the prokaryotic and eukaryotic division cycles. The main thesis is that the G1 period is merely an artificial construct of the observation, in slow growing prokaryotes and in most eukaryotic cells, of a period of cell growth and synthesis before the S period. The model described above proposes that it is more profitable to look at G1 as part of a larger period, from one initiation of DNA synthesis to the next initiation of DNA synthesis, during which the preparations for DNA synthesis are taking place.

How do eukaryotes and prokaryotes differ?—With regard to the division cycle only, the only difference between prokaryotes and eukaryotes seems to be the apparent inability of eukaryotes to synthesise DNA, that is, have an S period, at the time of cell division. This is possible for prokaryotes because the two nucleoids of the bacterial cell are self-contained and can function while the cell divides. I suggest that this is not the case for eukaryotes, where the completion of the S period produces a single nucleus with twice the diploid DNA content and which separates into two nuclei at mitosis and cell division. It is very likely that mitosis, and the associated condensation of DNA into chromosomes, is not compatible with continued DNA synthesis, and for this reason there is never any DNA synthesis during cell division in eukaryotes. If eukaryotic cells could produce two 'interphase' nuclei following the S period (for example, if the nuclei underwent an immediate mitosis without cell division), then it would be possible for DNA synthesis to occur in these nuclei while the cells were undergoing cell division. This would be analogous to bacterial cells growing with a doubling time of less than the sum of the C and D periods (60 min in Fig. 1).

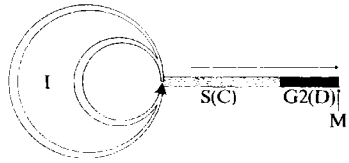

Fig. 3 A schematic representation of the cellular division sequence indicating the period for preparation of initiation, I, which is followed by the S(C) and G2(D) periods finally resulting in M (mitosis). The frequency of initiation is determined by the rate at which preparations for initiation occur, and this is indicated by the two different-sized circles, each representative of a different rate of growth. Note that the preparations for initiation occur continuously and do not start at M.

The cell division sequence—A corollary of my hypothesis is that there is no such thing as the division cycle. The word 'cycle' implies that at cell division something is initiated, the cell cycle. Actually, nothing starts at cell division but it is merely the end of a sequence of events which start with the accumulation of some initiation potential, and which was followed in succession by the initiation of DNA synthesis, the preparations for cell division following termination of DNA synthesis, and the final cell division (Fig. 3). The final cell division is the end of the process and the beginning of nothing. These ideas were expressed in relation to the bacterial cell division a decade ago[20].

The ideas described above were developed about a decade ago in discussions with many colleagues. One who deserves special mention is C. E. Helmstetter of Roswell Park Memorial Institute. At that time it was expected that the analysis of eukaryotic cells would eventually follow the logic of the bacterial analysis and therefore an explicit description of a unified model was unnecessary. This has not been the case, hence this brief article. This work was supported by grant DCM 77-14883 from the NSF.

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