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MicroOpinion

Regulation of DNA synthesis in bacteria: analysis of the Bates/Kleckner licensing/initiation-mass model for cell cycle control

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Summary

Bates and Kleckner have recently proposed that bacterial cell division is a licensing agent for a subsequent initiation of DNA replication. They also propose that initiation mass for DNA replication is not constant. These two proposals do not take into account older data showing that initiation of DNA replication can occur prior to the division event. This critical analysis is derived from measurements of DNA replication during the division cycle in cells growing at different, and more rapid, growth rates. Furthermore, mutants impaired in division can initiate DNA synthesis. The data presented by Bates and Kleckner do not support the proposal that initiation mass is variable, and the proposed pattern of DNA replication during the division cycle of the K12 cells analysed is not consistent with prior data on the pattern of DNA replication during the division cycle.

The study of bacterial growth and division has a long and exciting history. Starting with the experiments of Schaechter, Maaløe and Kjeldgaard in 1958 (Schaechter *et al*., 1958), a large amount of data led to a description of the pattern of DNA replication in bacteria (Helmstetter, 1967; 1968; 1969; 1996; Cooper and Helmstetter, 1968; Donachie, 1968; Helmstetter and Cooper, 1968; Helmstetter and Pierucci, 1968; 1976; Helmstetter *et al*., 1968; Cooper, 1969; 1990; 1991; 1997a,b; 2004; Helmstetter and Leonard, 1987). These data led to the proposal that the mass of the cell at initiation is constant, and that the act of cell division does not control or regulate the subsequent initiation of DNA replication.

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Bates and Kleckner (Bates and Kleckner, 2005) presented experiments suggesting that initiation mass is not constant, and that initiation of DNA replication is dependent on a prior cell division. According to them (Bates and Kleckner, 2005), their study 'confirms and extends earlier findings showing that, in slowly growing cells, cell mass at the time of initiation and thus also the mass to origin ratio are far from constant and can vary over a range of at least 1.5-fold' and they go on to propose that the 'occurrence of cell division may *per se* license the chromosome(s) for the next round of replication initiation', stating that 'the exact timing of initiation after cell division would then be determined by other factors such as DnaA concentration' and that 'this model is simple and direct' and 'easily accommodates the fact that in fast-growing cells, which contain multiple origins, all of those origins fire synchronously'.

These two proposals will now be analysed with respect to previously published work.

Does cell division 'license' initiation?

Almost 40 years ago the pattern of DNA replication in bacteria was determined using the membrane-elution method (Helmstetter, 1967; 1969; Cooper and Helmstetter, 1968; Helmstetter *et al*., 1968; Cooper, 1991). Using prelabelling and backwards analysis it was shown that the time for DNA replication (the C period) and the time between replication termination and cell division (the D period) were relatively invariant.[1] Over a wide range of growth rates, with numerous measurements, it was

[1]Bates and Kleckner used the eukaryotic terminology – S, G1 and G2 – when referring to the bacterial division cycle. This departs from the widely used bacterial B, C, D terminology. There is no simple analogy between the two terminologies. In eukaryotic cells there are true 'gaps' before and after the S phase, but in bacteria, depending on the growth rate, these gaps in DNA synthesis disappear and DNA synthesis can be continuous during the division cycle. It is more clarifying to continue to use separate nomenclature, with G1, S and G2 reserved for eukaryotes and B, C and D reserved for prokaryotes (Helmstetter, 1996).

shown that *Escherichia coli* strain B/r has a C period of 40 min and a D period of 20 min. Many subsequent data support this model (Helmstetter, 1996). These results are inconsistent with the licensing proposal of Bates and Kleckner. Initiation of DNA replication can occur prior to the cell division that is proposed to license that initiation and therefore cell division cannot be the causal 'licensing agent' of initiation.

Consider cells growing with an 80 min interdivision time, with C and D periods of 40 and 20 min respectively. This means that the B period (the period between birth and initiation of the C period) is necessarily 20 min, as the sum of B + C + D must equal 80 min. Now consider these cells growing in a series of media enabling progressively more rapid growth and, thus, shorter interdivision times. As the interdivision times decrease, the B period shortens. Thus, cells growing with a 70 min interdivision time have a 10 min B period. At a 60 min interdivision time the B period disappears, as initiation of replication is coincident with division. As cells grow even faster, initiations that would normally occur following a cell division now occur *prior* to that cell division. The initiation of the C period moves to earlier times in the division cycle as growth rates increase (Cooper and Helmstetter, 1968; Helmstetter and Cooper, 1968). When the interdivision time is less than 30 min the initiation of DNA replication now moves prior to the previous cell division. Thus, cell division cannot control or license a subsequent initiation. Bates and Kleckner studied cells growing slowly at 30°C in three different media, with 90, 125 and 300 min interdivision times (minimal medium with glucose, succinate, or alanine as the carbon source) and thus did not study the rapid growth patterns that would have tested the licensing proposal.

Even more critical for the proposal that cell division licenses a subsequent initiation of DNA replication is the existence of mutants impaired in cell division that continue to synthesize DNA (Lutkenhaus *et al*., 1980; Robinson *et al*., 1984; Begg and Donachie, 1985; Donachie, 1993; 2001; Boyle *et al*., 1997; Begg *et al*., 1998; Wang *et al*., 1998; Addinall *et al*., 2005). A prediction of the Bates/Kleckner licensing model is that if cell division were not allowed to proceed, DNA synthesis would not continue. The existence of mutants impaired in division that do synthesize DNA is inconsistent with the Bates/Kleckner licensing model.

Do the data of Bates and Kleckner eliminate constant initiation mass?

The second Bates/Kleckner proposal regarding control of DNA replication is that initiation mass is not constant (Bates and Kleckner, 2005). Others have also suggested that the initiation mass is variable (Wold *et al*., 1994), but a reanalysis of the original data demonstrated that they are consistent with a constant initiation mass (Cooper, 1997a). The Bates/Kleckner proposal (Bates and Kleckner, 2005) of variable initiation mass is based on the size measurements of *E. coli* K-12 growing at three different growth rates. The cell size was determined by measuring cell lengths by microscopy. Length measurements do not necessarily provide an accurate measure of cell mass, particularly when considering cells growing in different media. Cell mass is a function of cell length *and* cell diameter, not length alone, and the cell diameter varies according to growth rate (Cooper, 1989; Cooper and Denny, 1997). Even if we consider the mass data based solely on cell length measurements as valid, it is of interest to look at the original Bates/Kleckner data in more detail (Table 1). Only three growth rates are presented, and thus it is difficult to have enough data to determine whether initiation mass is constant or variable. Objectively, it seems to me that the data are consistent with a constant mass at initiation, because two out of three measurements indicate a constant mass.

Table 1. Cell sizes at birth and initiation as a function of interdivision times (from Bates and Kleckner, 2005).

Doubling time (min)	Birth sizes	Size at initiation
90	1.0	1.0
125	1.4	1.5
300	1.1	1.2

It is of interest to consider the data that lead to the constant mass hypothesis. The story starts with the classical work of Schaechter, Maaløe and Kjeldgaard (Schaechter *et al*., 1958). In this article they presented the following results:

(i) They determined cell mass in a culture by measuring the optical density of a culture, but only after calibrating the optical density measurements using dry weight measurements of cells. They showed that their absorbance measurements gave an accurate and reliable measure of cell mass in their growth conditions and over a wide range of growth rates.

(ii) They showed that over a large number of growth rates, the cell size measurements fit a straight line when plotted on semi-logarithmic co-ordinates against the reciprocal of the interdivision time. The reciprocal of the interdivision time is the growth rate, i.e. cell size increased exponentially as growth rate increased.

(iii) The cell size and DNA content (a measure of the DNA pattern) were independent of temperature. Even though the absolute growth rate changed with temperature, the cell size was the same at different temperatures.

This substantial body of data by itself does not indicate constant mass at initiation; what is required is knowledge of the pattern of DNA synthesis during the division cycle. This was accomplished by the prelabelling, backwards, membrane-elution method. The pattern of DNA replication during the division cycle is based on the observation that, with numerous data points, the C period and the D period are constant over a wide range of growth rates. As already noted, these periods are approximately 40 and 20 min, respectively, in *E. coli* B/r.

The next step was the combining of the Schaechter, Maaløe, Kjeldgaard results (Schaechter *et al*., 1958) with the constant C/D DNA replication model. If one assumes that the initiation mass was constant (Donachie, 1968; Helmstetter *et al*., 1968), and that C and D were constant, one can predict the results of the Schaechter, Maaløe, Kjeldgaard experiment (Schaechter *et al*., 1958). A complete derivation of this idea was presented previously (Cooper, 1991) and will not be reiterated here. Conversely, the data of Schaechter, Maaløe and Kjeldgaard along with the DNA model indicate a constant initiation mass.

It is not an imperative or absolute biological requirement that the initiation mass be constant. One could imagine that mass is merely a surrogate measure for some other molecule that is present at a constant fraction of cell mass. One could further imagine that cells growing at different rates could have different observed initiation masses, even though the initiator molecule may be present at a constant amount at initiation. Thus, lowering the concentration of hypothetical 'initiator' at some growth rate would lead to an observed increase in the initiation mass.

On the DNA patterns observed by Bates and Kleckner

Aside from the theoretical/historical/scientific arguments made above, we can examine the basic methods and results obtained by Bates and Kleckner. First let us consider the DNA period measured by Bates and colleagues (Bates *et al*., 2005) and compare this to previous results. They write 'The calculated life spans of the DNA replication periods in the two rounds of replication analysed in Fig. 4 were 47 min and 49 min respectively. There is little published data available for cell cycle parameters in *E. coli* K-12 strains at 30°C. However, the values obtained here are qualitatively reasonable, as they are within 30% of earlier measurements obtained by flow cytometry analysis of cells of the same background growing at a comparable doubling time but at 37°C (64 min; Allman *et al*., 1991)'.

As already noted, cells growing at a higher temperature have a shorter interdivision time than cells growing at a lower temperature. One would therefore expect the cells growing with a 90 min interdivision time at 30°C to grow with a shorter interdivision time at elevated temperatures. *E. coli* B/r grows with a 45 min interdivision time at 37°C and, thus, one would expect the K12 strain to grow at this rate as well. As noted in the prior discussion of the experiments of Schaechter, Maaløe and Kjeldgaard, the pattern was unchanged by temperature (that is, all parameters changed the same proportion as temperatures were altered), and one would expect the C period at 37°C to be approximately 23.5–24.5 min. A perusal of the various C periods attributed to *E. coli* K12 strains calculated by Helmstetter (Helmstetter, 1996) gives values of 41, 39, 39, 44, 55, 37, 40, 53, 41, 43, 43, 52, 46, 48, 62, 64 and 77 min at 37°C. Thus one would must conclude that the cells used to get the 47–49 min C period (grown with proline as the carbon source; D. Bates, pers. comm.) actually have a C period that is extraordinarily short. One can also consider the ratio of C period to D period for other *E. coli* K12 strains. As calculated by Helmstetter (Helmstetter, 1996) the ratio of C to D varies from 1.0 to 2.4, with an average of 1.8. If one considers cells growing very slowly (300 min interdivision time) this ratio is 0.29. This value suggests that the measured C period may be too short.

Flow cytometry is used to determine the DNA content of asynchronous and synchronized cells. It is of interest to compare the results of Allman (Allman *et al*., 1991) with regard to the flow-cytometric patterns observed when one has a significant D period. When a strain of *E. coli* K12 was grown at 37°C with a doubling of 113 min (presumably with glycerol as the sole carbon source) the D period was determined to be 40 min. Flow cytometry revealed a distinct D period peak, as expected. Similar results were observed by Michelsen *et al*. (Michelsen *et al*., 2003). For cells with interdivision times of 90, 125 and 300, Bates and Kleckner observed D periods of approximately 30, 55 and 210 min (see Fig. 6 in Bates and Kleckner, 2005). The expected peak in DNA content around the D period was not seen in any asynchronous culture (Bates *et al*., 2005).

The relationship of bacterial cell cycle to the eukaryotic cell cycle

A subtle point about the model of Bates and Kleckner is its relationship to studies on eukaryotic cells. One of the currently dominant aspects of the control of the eukaryotic cell cycle is the 'licensing' of DNA replication, and that cell division starts a cascade of events leading eventually to the initiation of S phase and the ultimate cell division. I have repeatedly pointed out that eukaryotic cell division is not the start of a series of sequential events leading to initiation of DNA synthesis (Cooper, 1979; 1987; 1991; 1998; 2000; 2003; Cooper and Shayman, 2001). For this

reason it is important to know how the bacterial cell cycle is controlled, as these cells have served as a valuable model for eukaryotic cell cycle control.

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