

Coupling the cell cycle to cell growth

A look at the parameters that regulate cell-cycle events

Erik Boye¹⁺ & Kurt Nordström²

¹Institute for Cancer Research, Montebello, Oslo, Norway, and ²Uppsala University Biomedical Center, Uppsala, Sweden

In order to multiply, both prokaryotic and eukaryotic cells go through a series of events that are collectively called the cell cycle. However, DNA replication, mitosis and cell division may also be viewed as having their own, in principle independent, cycles, which are tied together by mechanisms extrinsic to the cell cycle—the checkpoints—that maintain the order of events. We propose that our understanding of cell-cycle regulation is enhanced by viewing each event individually, as an independently regulated process. The nature of the parameters that regulate cell-cycle events is discussed and, in particular, we argue that cell mass is not such a parameter.

EMBO reports 4, 757–760 (2003)

doi:10.1038/sj.embor.embor895

Introduction

The classical way to study what has become known as the cell cycle is to observe, in a continuously growing culture of cells, the successive occurrence of chromosome replication, chromosome separation and cell division (Maaløe & Kjeldgaard, 1966). During exponential growth in a steady-state situation, each event is repeated at regular intervals, corresponding to the doubling-time of the culture.

In contrast to biochemical cycles such as the trichloroacetic acid (TCA) cycle, in which the product of one reaction is the substrate for the next, the cell cycle is composed of a series of biochemically unrelated reactions. In other words, the product of one round of the cell cycle (a newly divided cell) cannot be considered the substrate for the first event of the next (the initiation of DNA replication). Also, whereas a biochemical cycle is totally arrested if one of the reactions is inhibited, the obstruction of cell division does not preclude a new round of genome replication (see below), suggesting that the cell cycle is a composite of distinct and heterogeneous successive events. Finally, the 'substrate' and 'product' of most of these events are the same: one genome begets two genomes and one cell becomes two. Early investigators clearly made these distinctions

between the cell cycle and biochemical cycles (Hartwell & Weinert, 1989), but the former was originally termed a cycle (Howard & Pelc, 1951; Hartwell, 1978; Lee & Nurse, 1988) because, normally, DNA replication is followed by chromosome segregation and not by another round of DNA replication. Similarly, only under special circumstances does a cell divide twice without an intervening round of DNA replication.

Perturbations of the cell cycle

It is the exceptions to the norm, in other words the conditions under which the perturbation of cell growth fails to lead to the inhibition of later events, that give us insights into the differing natures of the cell cycle and biochemical cycles. For example, whereas the addition of penicillin to a culture of growing *Escherichia coli* cells inhibits cell division, the cells continue to grow and elongate into filaments, DNA replication continues, and the proper segregation of the daughter chromosomes is not affected (Fig. 1A). Similarly, *Schizosaccharomyces pombe* cells can go through mitosis without a preceding S phase, which means that they can divide without distributing a full genomic complement to each daughter cell (Fig. 1B). These observations suggest that the cell cycle is composed of at least two separate 'cycles': a DNA-replication cycle and a cell-division cycle for *E. coli*, and a DNA-replication cycle and a mitotic cycle for *S. pombe*. This conclusion is further supported by the following findings. There are conditional mutants in *Saccharomyces cerevisiae* that are unable to enter S phase but continue to initiate synchronous rounds of budding in the absence of DNA replication and mitosis (Hartwell, 1971). Moreover, in *S. pombe*, repeated rounds of uninterrupted DNA replication are observed on overproduction of the initiation protein Cdc18 (Nishitani & Nurse, 1995). Likewise, treatment of human cells with the protein kinase inhibitor staurosporine induces several consecutive rounds of chromosome replication (Stokke *et al.*, 1997). In meiosis, two consecutive reductive cell divisions, which entail carefully orchestrated chromosome pairing and segregation events, take place without intervening DNA replication, a process that is essential for the natural reproduction of almost all eukaryotic organisms, including humans.

On the basis of this evidence, we argue that the cell cycle should be treated as a series of distinct events (DNA replication, DNA segregation and cell division) that only seem to be parts of an obligatory cycle because of how they are tied together (Nordström *et al.*, 1991).

¹Department of Cell Biology, Institute for Cancer Research, Montebello, 0310 Oslo, Norway

²Department of Cell and Molecular Biology, Uppsala University Biomedical Center, Box 596, S-751 24 Uppsala, Sweden

*Corresponding author. Tel: +47 22934256; Fax: +47 22934580;

E-mail: eboye@labmed.uio.no

Submitted 25 February 2003; accepted 26 May 2003

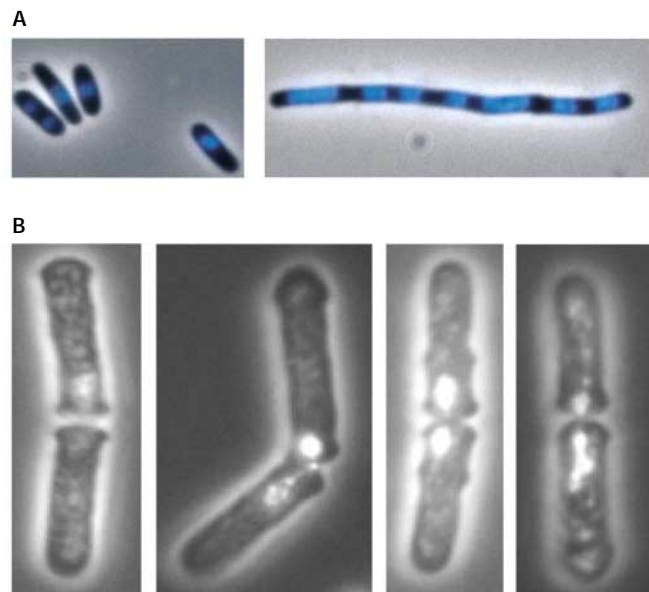


Fig. 1 | Examples of independence of cell-cycle events. (A) S phase can be independent of cytokinesis. *Escherichia coli* cells were grown exponentially in rich medium (left) or treated with ampicillin (right) for 90 min, fixed in ethanol and stained with DAPI (blue) for visualization of nucleoids. The images were obtained by phase-contrast and fluorescence microscopy. (B) Mitosis can occur independently of S phase. *Schizosaccharomyces pombe* *cdc10^{ts} rad3* mutant cells were incubated for 6 h at the non-restrictive temperature for *cdc10^{ts}*, a treatment that arrests the cells before S phase. Four different cells are shown. In the absence of the checkpoint protein Rad3 (the founding member of the ATR family of proteins), the cells proceed to mitosis and attempt to divide a single nucleus. The DNA is stained by DAPI (light foci located at the septum). Note the unequal amounts of DNA distributed to the two daughter cells. ATR, ataxia-tangiectasia mutated and Rad3-related; *cdc10^{ts}*, cell division cycle 10 temperature-sensitive mutant.

Moreover, these events run in parallel (Fig. 2A), possibly even being initiated at roughly the same time (Hartwell *et al.*, 1974; Murray & Hunt, 1993; Nordström & Dasgupta, 2001). For an illustration of this point, consider the use of the length of the G1 phase as a parameter by which to measure the effects of some treatment (mutation or treatment with chemicals) on the regulation of S-phase initiation. This implies that the treatment used does not affect the regulation of any cell-cycle event other than S-phase entry, particularly cell division, and this is usually difficult to verify. In such studies, S-phase entry should instead be compared to a general parameter, such as cell mass (which does not imply that mass is regulating the cell cycle (see below); mass is only a convenient general parameter for comparisons between cells in the same culture). Viewing cell-cycle events relative to a common parameter, rather than to each other, makes it possible to see connections and mechanisms of regulation more clearly. It is also important to realize that regulating a particular event of the cell cycle (in this case, DNA replication) does not automatically mean regulating the cell cycle as a whole. An interesting illustration of the independence of cell-cycle events can be derived from bacteria. At high growth rates, *E. coli* cell cycles are actually overlapping, so that the DNA replication of one cell cycle may occur before the cytokinesis event of the previous cycle (Cooper & Helmstetter, 1968).

Checkpoints

What makes the events of the cell cycle occur in a given order? Under constant growth conditions, the coupling between cell growth and individual cell-cycle events is sufficient to preserve their order. In most cases, the obstruction of one event leads to inhibition of a downstream event by activation of a checkpoint (Hartwell & Weinert, 1989). There is no need for checkpoints in a biochemical cycle, because a given reaction cannot start until the previous one, which provides the substrate, has finished.

Several cell-cycle checkpoints have been discovered. They include the inhibition of DNA replication when DNA is damaged, inhibition of mitosis when DNA has not been replicated or is damaged, and inhibition of cytokinesis when mitosis has not been properly executed (for a review, see Elledge, 1996). The first checkpoint to be discovered was the *E. coli* SOS response, which involves the inhibition of cell division in the presence of DNA damage (Defais *et al.*, 1971). The checkpoints are the active gatekeepers of the cell cycle and they preserve the order of events even in the face of perturbations of any of the processes involved. We argue that a linear representation of the different events that take place during growth, with checkpoints serving as cross-checks between them (Fig. 2B), is a true reflection of the cell cycle, and is more appropriate than the common circular representation.

Regulation

In a culture growing at steady state, the key events in each cell occur at a defined pace or frequency that is specified by the generation time (Maaløe & Kjeldgaard, 1966). However, this is simply a consequence of steady-state growth and does not yield any information about the mechanisms of regulation. The system is regulated by the conditions under which the cells are grown; that is, the growth medium determines the growth rate of a culture, which in turn dictates the frequency of all cell-cycle-related events (Jensen & Pedersen, 1990; Marr, 1991). This is trivial; to grow with a doubling time of x minutes, the cells must perform each required step once every x minutes.

It is not straightforward to identify the parameters regulating cell-cycle events. The finding that a change in one parameter also changes the cell cycle is not sufficient to prove that this parameter normally regulates the cell cycle. Frequently, cell mass is identified as such a regulator (for example, see Rupes, 2002), but we argue that this is based on a logical flaw. Let us take a closer look at the steady-state growth of a culture. All cell constituents double in amount from mitosis to mitosis, from one initiation of DNA replication to the next, or from cytokinesis to cytokinesis. Fig. 3 is a schematic, two-parametric representation of cells in such a culture. The ordinate shows DNA content per cell and the abscissa could be any general growth parameter, such as cell mass, cell size, number of ribosomes, cell surface area, number of messenger RNA molecules for a specific gene, and so on. Parameter values for cells in a given steady-state culture will always fall within the red area. Newborn cells appear at the bottom left and continue to increase in terms of this parameter throughout G1 phase. At a certain point, DNA replication (S phase) commences, and the DNA content is doubled. Thereafter, the parameter increases in G2 phase until the cells divide. Therefore, cells at a given point in the cell cycle have compositions identical to those of cells at the same point in a previous passage. The culture is not at steady state unless this criterion is fulfilled. It follows that in any given culture, the cells contain the same amount of any constituent that one might care to measure

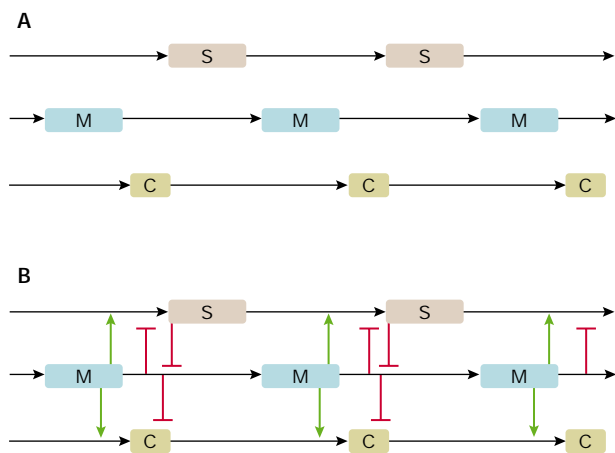


Fig. 2 | Representation of the cell cycle as being composed of three linear and repetitive series of S phase (S), mitosis (M) and cytokinesis (C). (A) In this simplified model, the three series of events would be regulated individually and coupled to cell growth in an unknown manner. (B) In reality, they are coupled to one another more directly through checkpoint mechanisms. Cues are given to inhibit another process (blocked arrows) or allow it to continue (arrows).

(the abscissa in Fig. 3) when they start DNA replication, and it is not surprising that all the cells initiate DNA replication or divide at a fixed mass, whether they are bacterial, yeast or mammalian cells. Although this has been observed repeatedly, this constancy is a tautology, and the interpretation of such observations to mean that cell mass has a regulatory function is misguided.

Indeed, it is important to discriminate between the correct observation that mass is constant during a given event of the cell cycle and the incorrect conclusion that mass is regulating this event. A correlation is distinct from a causative relationship. We argue that the numerous publications showing a constancy of mass at a certain point in the cell cycle under a given set of conditions do not provide evidence for mass as a regulatory parameter. Only when comparing individual cultures grown under different conditions can we critically test whether a parameter is constant at a certain point in the cell cycle, and such experiments have revealed that cell mass can be excluded as a regulator of S-phase entry: when the growth conditions of bacterial, yeast and mammalian cells are varied to give different average cell sizes, cell mass at S-phase entry is indeed variable (Churchward *et al.*, 1981; Rønning *et al.*, 1981; Wold *et al.*, 1994; Carlson *et al.*, 1999). In addition, crucial experiments have identified factors that promote the cell-cycle progression of *Drosophila* wing-tissue cells and rat cells *in vitro* without promoting cell growth (Neufeld *et al.*, 1998; Conlon *et al.*, 2001). Not only is it counterintuitive that a cell uses a crude and chemically ill-defined parameter such as cell mass to regulate its progress through the cell cycle, but it seems likely that a central molecular parameter—or set of parameters—that reflects the growth state of the cell is used for such vital and flexible regulatory processes. Candidates for such regulators include cell-cycle proteins themselves and low-molecular-weight signalling molecules (see below). Intuitively, it is likely that the onset of the key cell-cycle events is governed by the availability of many such components (Bernander, 1994; Tyson *et al.*, 1995).

Primary and secondary regulators of the cell cycle

Genetic approaches have identified a large number of genes in different systems whose protein products are involved in the execution of the key cell-cycle processes. These key processes may be inhibited by checkpoint mechanisms, which mainly function in the case of perturbations. However, no proteins have been proven to be the initial, or primary, regulators of cell-cycle events under normal conditions. For example, in *S. pombe* cells, overproduction of the DNA replication initiation protein Cdc18 promotes several reinitiations in rapid succession (Nishitani & Nurse, 1995), and the initiator protein DnaA does the same in *E. coli* (Løbner-Olesen *et al.*, 1989). Nonetheless, it is not obvious that the two proteins are rate-limiting for S-phase entry *in vivo*; they may simply be mediators of the regulatory signal. Thus, it is important to discriminate between the primary regulators (the parameters that normally regulate cell-cycle progression) and the secondary regulators (parameters that either can be made to regulate or that are actually involved in executing cell-cycle progression).

Another potential regulator is the Cdc2 protein of *S. pombe*, a cyclin-dependent kinase (CDK) that is involved in different protein phosphorylation events that are required to trigger both DNA replication and mitosis (Stern & Nurse, 1996). The kinase activity of Cdc2 is regulated by its phosphorylation, by the availability of its cyclin partners and by proteins that inhibit its activity, and there is good evidence that mitosis is initiated when the kinase activity reaches a certain level. Indeed, CDK activity has been referred to as the ‘cell-cycle engine’ and it is now firmly established that members of the CDK family choreograph cell-cycle changes in eukaryotes (for example, see Nigg, 1995). However, as in the case of Cdc18, it is not clear whether, under steady-state growth conditions, the Cdc2 protein has a regulatory function or is simply a transmitter of an upstream regulatory signal. In other words, there is as yet no direct evidence that Cdc2 activity couples cell-cycle events to cell growth.

In *E. coli*, factors that are thought to regulate DNA replication, chromosome partitioning or cell division are also directly involved in the execution of these processes. An effect caused by modulating the activity of such a factor may be due either to changes in a regulatory function or to interference with the process itself. In such cases, it is particularly difficult to distinguish between secondary and primary regulators. Other candidates for regulators of initiation of DNA

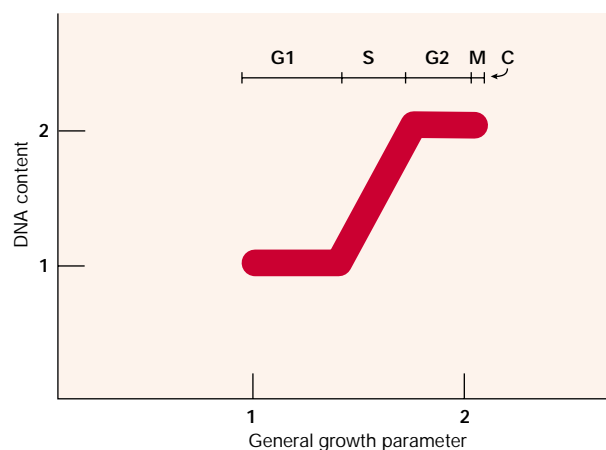


Fig. 3 | Two-parametric histogram of a cell culture growing under steady-state conditions. The cell cycle is composed of a G1 phase (G1), S phase (S), G2 phase (G2), mitosis (M) and cytokinesis (C).

replication in *E. coli* are the small signalling molecules guanosine tetraphosphate (ppGpp) and cyclic AMP, which provide links between the cell cycle and the general nutritional status of the cells (for a review, see Vinella & D'Ari, 1995). In another bacterium, *Caulobacter crescentus*, the CtrA protein has functions that may classify it as a regulator. CtrA controls several events, including the initiation of DNA replication, DNA methylation, cell division and flagellar biogenesis. In addition, it is a member of the response-regulator family of two-component signal-transduction systems and is activated by phosphorylation and inactivated by proteolysis (Domian *et al.*, 1997). This complex regulation of CtrA at the levels of synthesis, phosphorylation and degradation has parallels to the regulation of Cdc2 kinase/cyclin activity in eukaryotes, a form of regulation that may supply the system with the periodic oscillation believed to be necessary for the onset of the key cell-cycle events. However, even this does not reveal a clear link to cell growth. Interestingly, a free-running oscillator that drives G1 events independently of cyclin activity has been described (Haase & Reed, 1999), supporting the idea of parallel cell-cycle processes with their own, separate controls.

In summary, although several proteins have the ability to regulate the cell cycle under certain conditions, none of them have been shown to have a crucial role in regulating the cell cycle in a normal, unperturbed situation. Therefore, most of these proteins represent downstream effector molecules, leaving the initiators of cell-cycle regulation to be identified.

Conclusions

The cell cycle is a complex, multi-faceted process, consisting of a number of biochemically independent events that run in parallel. Checkpoints ensure that the order of events is preserved. Regulatory mechanisms acting positively or negatively on individual cell-cycle events have been identified, but it is important to discriminate between primary and secondary regulators. In particular, the parameter of cell mass can be excluded as an active regulator of the cell cycle. In fact, the nature of the connection between the cell-cycle events and cell growth has not been identified in any organism, and it is unlikely to be any simple parameter of general cell growth.

ACKNOWLEDGEMENTS

We thank O. Nielsen, K. Skarstad and T. Stokke for stimulating discussions. This work was supported by the Norwegian Cancer Society, the Norwegian Research Council, the Swedish Cancer Society and the Swedish Natural Science Research Council.

REFERENCES

Bernander, R. (1994) Universal cell cycle regulation? *Trends Cell Biol.*, **4**, 76–79.

Carlson, C.R., Grallert, B., Stokke, T. & Boye, E. (1999) Regulation of the start of DNA replication in *Schizosaccharomyces pombe*. *J. Cell Sci.*, **112**, 939–946.

Churchward, G., Estiva, E. & Bremer, H. (1981) Growth rate-dependent control of chromosome replication initiation in *Escherichia coli*. *J. Bacteriol.*, **145**, 1232–1238.

Conlon, I.J., Dunn, G.A., Mudge, A.W. & Raff, M.C. (2001) Extracellular control of cell size. *Nature Cell Biol.*, **3**, 918–921.

Cooper, S. & Helmstetter, C.E. (1968) Chromosome replication and the division cycle of *Escherichia coli* B/r. *J. Mol. Biol.*, **31**, 519–540.

Defais, M., Fauquet, P., Radman, M. & Errera, M. (1971) Ultraviolet reactivation and ultraviolet mutagenesis of lambda in different genetic systems. *Virology*, **43**, 495–503.

Domian, I.J., Quon, K.C. & Shapiro, L. (1997) Cell type-specific phosphorylation and proteolysis of a transcriptional regulator controls the G1-to-S transition in a bacterial cell cycle. *Cell*, **90**, 415–424.

Elledge, S.J. (1996) Cell cycle checkpoints: preventing an identity crisis. *Science*, **274**, 1664–1672.

Haase, S.B. & Reed, S.I. (1999) Evidence that a free-running oscillator drives G1 events in the budding yeast cell cycle. *Nature*, **401**, 394–397.

Hartwell, L.H. (1971) Genetic control of the cell division cycle in yeast. II. Genes controlling DNA replication and its initiation. *J. Mol. Biol.*, **59**, 183–194.

Hartwell, L.H. (1978) Cell division from a genetic perspective. *J. Cell Biol.*, **77**, 627–637.

Hartwell, L.H. & Weinert, T.A. (1989) Checkpoints: controls that ensure the order of cell cycle events. *Science*, **246**, 629–634.

Hartwell, L.H., Culotti, J., Pringle, J.R. & Reid, B.J. (1974) Genetic control of the cell division cycle in yeast. *Science*, **183**, 46–51.

Howard, A. & Pelc, S.R. (1951) Nuclear incorporation of ³²P as demonstrated by autoradiographs. *Exp. Cell Res.*, **2**, 178–187.

Jensen, K.F. & Pedersen, S. (1990) Metabolic growth rate control in *Escherichia coli* may be a consequence of subsaturation of the macromolecular biosynthetic apparatus with substrates and catalytic components. *Microbiol. Rev.*, **54**, 89–100.

Lee, M. & Nurse, P. (1988) Cell cycle control genes in fission yeast and mammalian cells. *Trends Genet.*, **4**, 287–290.

Løbner-Olesen, A., Skarstad, K., Hansen, F.G., von Meyenburg, K. & Boye, E. (1989) The DnaA protein determines the initiation mass of *Escherichia coli* K-12. *Cell*, **57**, 881–889.

Maaløe, O. & Kjeldgaard, N.O. (1966) *Control of Macromolecule Synthesis*. W.A. Benjamin, New York, USA.

Marr, A.G. (1991) Growth rate of *Escherichia coli*. *Microbiol. Rev.*, **55**, 316–333.

Murray, A.W. & Hunt, T. (1993) *The Cell Cycle*. Oxford Univ. Press, Oxford, UK.

Neufeld, T.P., de la Cruz, A.F., Johnston, L.A. & Edgar, B.A. (1998) Coordination of growth and cell division in the *Drosophila* wing. *Cell*, **93**, 1183–1193.

Nigg, E.A. (1995) Cyclin-dependent protein kinases: key regulators of the eukaryotic cell cycle. *Bioessays*, **17**, 471–480.

Nishitani, H. & Nurse, P. (1995) p65^{cdc28} plays a major role controlling the initiation of DNA replication in fission yeast. *Cell*, **83**, 397–405.

Nordström, K. & Dasgupta, S. (2001) Partitioning of the *Escherichia coli* chromosome: superhelicity and condensation. *Biochimie*, **83**, 41–48.

Nordström, K., Bernander, R. & Dasgupta, S. (1991) The *Escherichia coli* cell cycle: one cycle or multiple independent processes that are co-ordinated? *Mol. Microbiol.*, **5**, 769–774.

Rønning, O.W., Lindmo, T., Pettersen, E.O. & Seglen, P.O. (1981) The role of protein accumulation in the cell cycle control of human NHK 3025 cells. *J. Cell Physiol.*, **109**, 411–418.

Rupes, I. (2002) Checking cell size in yeast. *Trends Genet.*, **18**, 479–485.

Stern, B. & Nurse, P. (1996) A quantitative model for the cdc2 control of S phase and mitosis in fission yeast. *Trends Genet.*, **12**, 345–350.

Stokke, T., Smedhammer, L., Jonassen, T.S., Blomhoff, H.K., Skarstad, K. & Steen, H.B. (1997) Uncoupling of the order of the S and M phases: effects of staurosporine on human cell cycle kinases. *Cell Prolif.*, **30**, 197–218.

Tyson, J.J., Novak, B., Chen, K. & Val, J. (1995) Checkpoints in the cell cycle from a modeler's perspective. *Prog. Cell Cycle Res.*, **1**, 1–8.

Vinella, D. & D'Ari, R. (1995) Overview of controls in the *Escherichia coli* cell cycle. *Bioessays*, **17**, 527–536.

Wold, S., Skarstad, K., Steen, H.B., Stokke, T. & Boye, E. (1994) The initiation mass for DNA replication in *Escherichia coli* K-12 is dependent on growth rate. *EMBO J.*, **13**, 2097–2102.



Erik Boye



Kurt Nordström