Review

Revisiting retinoblastoma protein phosphorylation during the mammalian cell cycle

S. Cooper* and J. A. Shayman**

* Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor (Michigan 48109-0620, USA), Fax +1 734 764 3562, e-mail: cooper@umich.edu
** Nephrology Division, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor (Michigan 48109-0676, USA), e-mail: jshayman@umich.edu

Received 16 October 2000; received after revision 13 November 2000; accepted 15 November 2000

Abstract. It is widely accepted that phosphorylation of the retinoblastoma (Rb) protein during the G1 phase of the mammalian division cycle is a major control element regulating passage of cells into S phase and through the division cycle. The experiments supporting G1-phase-specific Rb phosphorylation and the historical development of this idea are reviewed. By making a rigorous distinction between ‘growth cessation’ and the phenomena of ‘cell cycle exit’ or ‘G1-phase arrest’, the evidence for the G1-phase-specific phosphorylation of Rb protein is reinterpreted. We show that the evidence for G1-phase phosphorylation of Rb rests on few experiments and a chain of reasoning with some weak links. Evidence is reviewed that growth conditions regulate the phosphorylation of Rb. A growth-regulated control system that is independent of the cell cycle explains much of the evidence adduced to support cycle-specific phosphorylation of Rb. We propose that additional experimental evidence is needed to decide whether there is a G1-phase-specific phosphorylation of Rb protein.

Key words. G1 phase; cell cycle; G1-phase arrest; phosphorylation.

Why another review of retinoblastoma phosphorylation?

This is a review of the evidence related to the cell-cycle-specific phosphorylation of the Retinoblastoma Rb protein. As is well understood, reviews of scientific fields are usually summaries of the consensus view of a field. In general, a reviewer has an obligation to describe the main conclusions and key experiments in a field so that the contributions of the various workers to the current or consensus view are presented clearly and equitably. The essential activity of the normal review process is that it summarizes what the field believes, and not what one particular segment of the field believes.

* Corresponding author.

The evidence for control of cell growth and the cell cycle by G1-phase-specific phosphorylation of Rb protein has been presented in numerous reviews. It is not necessary to review and summarize the evidence that has been presented often and so ably by many others [1–4]. Therefore, this review has a somewhat different purpose – and this should be understood at the outset. We propose to reexamine the evidence that Rb protein is phosphorylated in a specific phase of the cell cycle. We do this in order to discern precisely what particular experimental evidence rigorously supports this widely held conclusion, and what evidence is merely consistent with this proposal. This reevaluation of the literature on the subject of cell-cycle-specific Rb phosphorylation reveals that the evidence for this phenomenon is not as strong as generally believed.
To anticipate our conclusion, we propose that essentially all of the data regarding G1-phase Rb phosphorylation is merely consistent with the notion or idea that there is a G1-phase phosphorylation event. We examine the chain of reasoning leading to the proposal of G1-phase phosphorylation and show that there is an alternative explanation for much of the data. Finally, we shall review the recent work on the in vitro sequence of phosphorylation steps and relate this work to the proposal that there is a G1-phase phosphorylation of Rb protein. As will be seen, it is possible to have sequential phosphorylation steps demonstrated in vitro without having these steps relate to the proposed in vivo phosphorylation of Rb protein during the mammalian cell cycle.

G1-phase phosphorylation of Rb protein is currently viewed as a paradigmatic example of G1-phase control of the mammalian division cycle. As much analysis of the cell cycle is based on the Rb model of cell-cycle-specific phosphorylation, it is important that the experimental basis for the Rb model be beyond reproach. Furthermore, as the Rb model is an example for future studies of control of the cell cycle in the G1 phase, it is important to consider what is required to rigorously conclude that there is a G1-phase event during the mammalian division cycle. Before presenting a detailed analysis of the Rb literature, we will first present some general ideas regarding the relationship of cell growth and the cell cycle. This analysis will serve as a foundation for discussing the evidence for cell-cycle specific phosphorylation of Rb protein.

The distinction between cell growth and the passage through the cell cycle

It has become popular to equate cessation of cell growth with the exit of the cell from the cell cycle. Resumption of cell growth has similarly been equated with reentering the cell cycle. There is an important distinction to be made between ‘cell growth’ and ‘passage through the cell cycle’. A similar distinction should also be made between cessation of growth and ‘exiting the cell cycle’.

In order to understand this distinction between cell growth and the cell cycle, consider a growing culture with the cells in the culture being at various points in the division cycle. Consider that the cells are growth arrested by some means so that the cells are now frozen at their particular points in the cell cycle. That is, a cell that was at the start of the cell cycle is now fixed with the characteristics of a cell at the start of the cycle. And a cell at the end of the cycle is now frozen as a cell near the end of the cell cycle. Upon releasing these cells for regrowth, we could agree, for this hypothetical and imaginary example, that resumption of growth was not a ‘reentering the cell cycle’. The cells were frozen at particular points in the cell cycle, and resumption of growth was merely the continuation of passage through the cell cycle from the point of arrest. In this example, the cells never left the cell cycle, and thus cannot be said to reenter the cell cycle upon resumption of cell growth.

A more important case is to have cells arrested at different points in the cell cycle, and yet have these cells alter some property of the cell so that the cells have an appearance of all being in the same part of the cell cycle. Specifically, arrest of cell growth will allow cells to accumulate with a particular amount of DNA similar to that in a particular phase of the cell cycle, for example the G1 phase. But as stated here, the cells can be arrested and fixed in particular phases of the cell cycle, with only a superficial appearance that the cells are arrested in the G1 phase of the cell cycle. To be explicit about the language of growth arrest, it is possible to have cells ‘arrested with a G1-phase amount of DNA’, and yet not be ‘arrested at a point in the G1 phase’. The implications of this apparently paradoxical distinction will become apparent in the discussion that follows.

Another distinction must be made explicit, and that is the distinction between growth of the culture which leads to an increase in cell numbers as well as total cell mass, and the growth of individual cells. Unless otherwise noted, the discussion here will always be related to individual cells increasing their cell mass during the division cycle.

With these distinctions in hand, we can look at the evidence for G1-phase-specific phosphorylation of Rb protein from a new perspective.

An industrial analogy to the cell cycle

Before presenting the cellular concepts, it is useful to present an industrial analogy to illuminate the distinction between the general process of cell growth and the specific subset of processes that comprise the cell cycle.

Consider an assembly line where workers assemble an automobile in a sequence of steps. When car production ceases due to a lack of demand, no new car assemblies are started, but cars on the assembly line are completed so no cars remain unfinished.

When the decision to cease production is given, all workers are immediately given layoff notices. However, the workers continue to work until there is no more work at their section of the assembly line. In practice, this means that workers at the start of the assembly line stop working first. Then workers in the middle of the assembly line stop working. Finally, workers at the end of the assembly line stop working. After the time required for complete car assembly, there is an accumulation of unemployed workers and an assembly line with no partly assembled cars.

Superficially, it now looks as if the idled employees in this factory are all in identical situations. One might even say that the workers are aligned at the same point in the work process and that when work resumes they will start...