J. theor. Biol. (2001) **208,** 399–402
doi:10.1006/jtbi.2000.2228, available online at http://www.idealibrary.com on IDEAL®

Revisiting the Relationship of the Mammalian G1 Phase to Cell Differentiation

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(*Received on* 10 *October* 2000, *Accepted in revised form on* 28 *October* 2000)

It is widely held that mammalian cells make a decision in the G1 phase of the division cycle either to proceed through the cell cycle or to differentiate and cease growth and division (Hass, 1994; Herwig & Strauss, 1997; Loyer *et al*., 1996; Rifkind *et al*., 1996; Sherr, 1995; Wiman, 1993; Zavitz & Zipursky, 1997). This belief was originally derived from the general observation that differentiated cells are overwhelmingly (if not completely) cells with a G1-phase amount of DNA. Simply put, it is currently envisioned that cells make a decision in the G1 phase of the division cycle to differentiate. After differentiation the cells remain "in the G1 phase" and do not initiate DNA synthesis. Cells that are undergoing differentiation do not enter S, G2, or M phases. Cells that were in the S, G2, and M phases when the initiation signal was received pass through these phases, decide to differentiate in the G1 phase, and then do not initiate another S phase. In this way, the current model proposes that a population of differentiated cells is produced, with all of the cells having a G1-phase amount of DNA.

The proposed existence of a G1-phase decision point for differentiation has provided support for the general idea that there are important cell cycle controls residing in the G1 phase of the division cycle. The proposal of a restriction point, the entry of cells into G(0), and the general idea of G1-phase arrest under different starvation or inhibition regimens are implicitly supported by, and in turn support, the G1-phase differentiation model. The G1-phase differentiation decision is thus one more G1-phase control related to the general problem of cell-cycle control.

For a number of years I have been questioning the idea of G1-phase events (Cooper, 1979, 1982, 1987, 1988, 1997, 1998a, b, 1998c, 2000; Cooper *et al*., 1999). To summarize a number of these papers, they point out that the G1-phase control model has been primarily derived from experiments based on the assumption that arresting cells with a G1-phase amount of DNA is equivalent to demonstrating a specific G1-phase event. These papers note that the assumption of an equivalence of arrest with a G1-phase amount of DNA and arrest at a point in the G1 phase is not correct. Furthermore, these papers argue against the proposal that arresting cells with a G1-phase amount of DNA and releasing these cells to grow produces a synchronized culture in which G1-phase events can be studied (Cooper, 1998a). It has also been demonstrated that artifacts can be introduced by G1-phase arrest "synchronization" procedures (Cooper, 1998). Most recently, we have experimentally demonstrated that one of the key models of G1-phase-specific events, the phosphorylation of Rb protein in the G1 phase, is

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0022–5193/01/040399 + 04 $35.00/0

not a G1-phase event but is related to the general conditions of growth throughout the cell cycle (Cooper, 2000; Cooper *et al*., 1999).

I now turn to another foundation stone in the structure of G1-phase events, the idea that the differentiation decision is made in the G1 phase of the division cycle and is thus supportive of G1-phase events in general.

In contrast to the current G1-phase decision model, I propose that when a cell population receives a signal to differentiate, cells in all phases of the division cycle respond and begin the differentiation process. This differentiation process is *independent* of cell-cycle phase. Cells in all phases begin the differentiation process. The production of a differentiated cell, and thus the observation of cell differentiation, is relatively a long process; it usually takes many hours until the differentiated cell is observed. But the differentiation signal has two messages for the cell. One is for the cell to change into whatever differentiated cell is to be made, and the second message is cease S-phase initiation.

Let us examine the implications of this two-part differentiation result. It is proposed here that part of the differentiation signal is the signal to cease initiation of S phases. It should also be noted that inhibition of material that is made in all phases of the cell cycle, and not restricted to synthesis or expression within any particular phase, can lead to cessation of initiation of S phases. Thus, inhibition of initiation is not necessarily related to a G1-phase specific event. Cells in S or G2/M phases complete these phases and thus produce a population of cells with a G1-phase amount of DNA. The time for these cell-cycle processes to be completed is relatively short, being approximately 10–24 hr. These differentiation processes sometimes require a time of the order of 70–100 hr until cell differentiation can be observed. This temporal difference in cycle completion and differentiation implies that one would find differentiated cells all with a G1-phase amount of DNA. We now get a differentiated population with all cells having a G1-phase amount of DNA. But this population was derived with the original assumption that differentiation was initiated in all cells, and was independent of cell-cycle phase.

It therefore, appears as if only cells with a G1-phase amount of DNA differentiate, but an alternative explanation or conclusion is that the cell arrest with a G1-phase amount of DNA and differentiation are independent events.

In summary, the observation that differentiated cells all have a G1-phase amount of DNA could be accounted for by five processes: (i) cells in all phases of the cell cycle can initiate differentiation upon receiving a differentiation signal; (ii) cells take a relatively long time to exhibit differentiated characteristics, (iii) induction of differentiation leads to the cessation of S-phase initiation, (iv) cells in S and G2/M phases proceed through the cycle to division even though S-phase initiations are inhibited, and (v) the time for passage through S, G2, and M phases is significantly less than the time until cell differentiation can be observed or measured. The finding that differentiated cells all have a G1-phase amount of DNA does not prove the existence of a G1-phase decision at which cells decide whether to differentiate, because it is equally consistent with the hypothesis that cells begin to differentiate from any stage of the cell cycle.

I propose that there is no necessary relationship between the G1 phase and differentiation. I propose that differentiation can occur from all phases of the division cycle. The widely held belief of an association or relationship between the G1 phase and cell differentiation is merely a trivial result of the times required to complete each of these processes—a relatively short time for the cell-cycle-arrest process and a relatively long time for the differentiation process.

This proposal is testable. For example, one may ask "in a particular differentiation system, using very sensitive methods and flow cytometric cell sorting, can one observe differentiation markers appearing in cells in all phases of the division cycle?". This experimental test should be carried out shortly after the initiation of differentiation, so that cells in S and G2 will not have had a chance to divide. Any appearance of differentiation markers in the S- and G2/M-phase cells would thus indicate a differentiation decision without passage through the G1 phase. Whether

or not one can find a satisfactory experimental test of the suggestion made here, the essential point of this proposal should not be missed. It is to suggest that the primary observation that supports the belief that there is a G1-phase decision point for differentiation has an alternative explanation. The proposal made here also suggests that the burden of proof to contradict the ideas presented here should be placed on those who believe that there is a G1-phase decision point. This is because the explanation for the observation of G1-phase DNA in differentiated cells has not been considered in the genesis of the basic proposal of an association of G1 phase with differentiation. The classic explanation of the association of differentiation and cells with a G1-phase amount of DNA has an alternative explanation that has not been considered. Until the explanation presented here is eliminated, one must be cautious in associating differentiation with any particular phase of the cell cycle.

There are two ways to look at the proposal presented here. From the point of view of cell differentiation, we can see that the observation of differentiated cells all with a G1-phase amount of DNA does not rigorously prove that there is a G1 phase associated decision to differentiate. Differentiation could be independent of the division cycle.

From the point of view of the cell cycle, however, a more important message emerges. The G1-phase decision point for differentiation is a part of the larger view of the cell cycle that postulates important points in the G1 phase at which cells decide whether to proceed through the cell cycle or to take some other path. For example, the decision to enter quiescence or the G(0) phase has been proposed to take place in the G1 phase, at some point usually referred to as the restriction point (Zetterberg & Larsson, 1985). This general view of the G1 phase as having a number of important G1-phase specific functions has been reanalysed on the basis of new experimental results and reinterpretations of earlier experiments (Cooper, 1979, 1982, 1988, 1998a, b, c, 2000). The conclusion of this analysis presented here is that the experimental evidence supporting G1-phase events is not as strong as usually believed.

The critique presented here is thus part of a larger analysis of the G1 phase in which it has been concluded that there are no clearly demonstrable G1-phase specific events, decisions, restriction points, or processes (Cooper, 2000). Whatever occurs in the G1 phase occurs in all phases of the division cycle. With the removal of the "G1-phase differentiation decision point" from the canon of G1-phase specific events, another support of the G1-phase control model may be eliminated.

REFERENCES

COOPER, S. (1979). A unifying model for the G1 period in prokaryotes and eukaryotes. *Nature* **280,** 17–19.

COOPER, S. (1982). The continuum model: statistical implications. *J. theor. Biol.* **94,** 783–800.

COOPER, S. (1987). On G0 and cell cycle controls. *Bioessays* **7,** 220–223.

COOPER, S. (1988). The continuum model and c-myc synthesis during the division cycle. *J. theor. Biol.* **135,** 393–400.

COOPER, S. (1997). G1 and S phase gene expression cannot be analyzed in mammalian cells synchronized by inhibition. *Microb. Compar. Genom.* **2,** 269–273.

COOPER, S. (1998a). Mammalian cells are not synchronized in G1-phase by starvation or inhibition: considerations of the fundamental concept of G1-phase synchronization. *Cell Prolif.* **31,** 9–16.

COOPER, S. (1998b). On the interpretation of the shortening of the G1-phase by overexpression of cyclins in mammalian cells. *Exp. Cell Res.* **238,** 110–115.

COOPER, S. (1998c). On the proposal of a G0 phase and the restriction point. *FASEB J* **12,** 367–373.

COOPER, S. (2000). The continuum model and G1-control of the mammalian division cycle. *Prog. Cell Cycle Res.* **4,** 27–39.

COOPER, S., YU, C. & SHAYMAN, J. A. (1999). Phosphorylation–dephosphorylation of retinoblastoma protein is not necessary for passage through the mammalian division cycle. *IUBMB Life* **1,** 27–39.

HASS, R. (1994). Retrodifferentiation and cell death. *Crit. Rev. Oncol.* **5,** 359–371.

HERWIG, S. & STRAUSS, M. (1997). The retinoblastoma protein: a master regulator of cell cycle, differentiation and apoptosis. *Eur. J. Biochem.* **246,** 581–601.

LOYER, P., ILYIN, G., CARIOU, S., GLAISE, D., CORLU, A. & GUGUEN-GUILLOUZO, C. (1996). Progression through G1 and S phases of adult rat hepatocytes. *Prog. Cell Cycle Res.* **2,** 37–47.

RIFKIND, R. A., RICHON, V. M. & MARKS, P. A. (1996). Induced differentiation, the cell cycle, and the treatment of cancer. *Pharmacol. Ther.* **69,** 97–102.

SHERR, C. J. (1995). Mammalian G1 cyclins and cell cycle progression. *Proc. Assoc. Am. Physicians* **107,** 181–186.

WIMAN, K. G. (1993). The retinoblastoma gene: role in cell cycle control and cell differentiation. *Faseb J.* **7,** 841–845.

ZAVITZ, K. H. & ZIPURSKY, S. L. (1997). Controlling cell proliferation in differentiating tissues: genetic analysis of negative regulators of G1 → S-phase progression. *Curr. Opin. Cell Biol.* **9,** 773–781.

ZETTERBERG, A. & LARSSON, O. (1985). Kinetic analysis of regulatory events in G1 leading to proliferation or quiescence of Swiss 3T3 cells. *Proc. Natl Acad. Sci. U.S.A.* **82,** 5365–5369.