Regulation of G1 Cell Cycle Progression: Distinguishing the Restriction Point from a Nutrient-Sensing Cell Growth Checkpoint(s)

David A. Foster, Paige Yellen, Limei Xu, and Mahesh Saqcena

Abstract

Most genetic changes that promote tumorigenesis involve dysregulation of G1 cell cycle progression. A key regulatory site in G1 is a growth factor–dependent restriction point (R) where cells commit to mitosis. In addition to the growth factor–dependent “R,” which maps to a site about 3.5 hours after mitosis, there is another checkpoint later in G1 that is dependent on nutritional sufficiency that has also been referred to as R. However, this second site in late G1 can be distinguished both temporally and genetically from R. We are proposing that the late G1 regulatory site be more appropriately referred to as a “cell growth” checkpoint to distinguish it from R. This checkpoint, which likely has an evolutionary relationship to the yeast cell cycle checkpoint START, is regulated by signals governed by mTOR, the mammalian target of rapamycin. This review summarizes evidence that distinguishes R from the proposed cell growth checkpoint. Since both checkpoints are dysregulated in most, if not all, human cancers, distinguishing between these 2 distinct G1 regulatory checkpoints has significance for rational therapeutic strategies targeting oncogenic signals.

Keywords: cell cycle, restriction point, cyclins, Rb, mTOR, TGF- β , START, cancer, quiescence

Introduction

While there are many genetic alterations that contribute to human cancers, a majority of these mutations are in genes that encode proteins that regulate progression through the G1 phase of the cell cycle.^{1–3} Although much is known about the control of G1 cell cycle progression, there remains notable confusion with regard to what is commonly referred to as the restriction point (R). There are 2 distinct sites in G1 that have been called R: one relatively early in G1,^{4,5} and another at a site later in G1 closer to the beginning of S phase.^{2,3,6} Significantly, there are distinguishable genetic alterations in cancer cells that facilitate passage through both of these “R” sites. The 2 sites referred to as R can be clearly distinguished by examining complementing genetic alterations that lead to cell transformation. A model is presented here where the first R represents the sensing of appropriate growth factor signals that suppress exit from the cell cycle into quiescence as originally described by Pardee.⁷ It is proposed that the second R site in late G1 is a nutrient-sensing “cell growth” checkpoint that

may be evolutionally related to a G1 checkpoint in yeast known as START.⁸ Importantly, there is an apparent need to dysregulate both R and the cell growth checkpoint in virtually all human cancers.

Cooperating Genetic Changes in Cancer Cells

Most of the genetic changes observed in cancer cells involve genes that control G1 cell cycle progression.³ It was first reported by Weinberg *et al.* in 1999 that the tumorigenic conversion of normal human epithelial and fibroblast cells could be achieved with a combination of genes that expressed telomerase, activated Ras, and SV40 early region genes encoding both large and small T antigens.^{9,10} While the telomerase requirement indicated a need to acquire immortality and avoid cell senescence, all of the other genes have been implicated in progression through G1 (Table 1). Ras is activated in response to a wide variety of growth factors, and therefore, activating Ras mutations confer a constitutive growth factor signal in the absence of growth factors. SV40 large T

antigen binds and inactivates the p53 and Rb tumor suppressor gene products, both of which suppress passage through G1 checkpoints. p53 monitors genomic integrity and suppresses passage from G1 into S if the genome is damaged.¹¹ Rb is involved throughout much of G1 and is a target of G1 cyclin-dependent kinases (CDKs) that promote G1 cell cycle progression.^{2,12} SV40 small t antigen inhibits protein phosphatase 2A (PP2A), which has also been implicated as a tumor suppressor.¹³ PP2A is a Ser/Thr phosphatase that regulates many aspects of cell physiology including G1 cell cycle progression.¹⁴ More recently, Hahn *et al.* demonstrated that human cells immortalized with telomerase could be transformed without viral genes using a combination of activated Ras, suppressed expression of p53 and Rb, and, to substitute for the loss of

Department of Biological Sciences, Hunter College of The City University of New York, New York, NY, USA

Corresponding Author:

David A. Foster, Department of Biological Sciences, Hunter College of the City University of New York, 695 Park Avenue, New York, NY 10065
 Email: foster@genectr.hunter.cuny.edu

Table 1. Genetic Requirements for the Transformation of Human Cells

Genetic Requirements for the Transformation of Human Cells with Viral Genes		
Gene	Molecular Target	Cell Cycle Target
Ras	Growth factor signals	Restriction point
SV40 large T	p53	G1/S checkpoint(s)
	Rb	All G1 checkpoints
SV40 small t	PP2A	Cell growth checkpoint
Genetic Requirements for the Transformation of Human Cells without Viral Genes		
Gene	Molecular Target	Cell Cycle Target
Ras	Growth factor signals	Restriction point
P53 null	p53	G1/S checkpoint(s)
Rb null	Rb	All G1 checkpoints
Myc	Gene expression	Cell growth checkpoint
PTEN loss	Akt/mTOR	Cell growth checkpoint

Note: The genetic requirements for transforming human cells with (upper section) and without viral genes (lower section) are presented along with the molecular target of the genetic alteration and where in the cell cycle the genetic alteration impacts. Telomerase expression, which is required in both cases, is not included because it does not impact directly on G1 cell cycle progression. These data are mostly from the work of Hahn and Weinberg.^{9,10,15}

PP2A, elevated the expression of Myc and suppressed PTEN (phosphatase and tensin homolog deleted on chromosome 10) expression.¹⁵ Circumventing the loss of PP2A provided further insight into the requirements for transforming human cells because the many and complex roles of PP2A make its impact on tumorigenesis difficult to evaluate. In contrast, the roles of Myc and PTEN on G1 cell cycle progression are much better understood.

The implication from the above studies is that increased Myc and suppressed PTEN complement an activated Ras gene to transform cells. While there are several downstream targets of Ras,¹⁶ activation of the Raf protein kinase cascade leads to elevated expression of cyclin D¹⁷ (Fig. 1A). PTEN and Myc are both part of a highly complex signaling network that involves the protein kinase mTOR, the mammalian target of rapamycin. Ultimately, signals that elevate mTOR kinase activity result in the elevated expression of Myc and the activation of cyclin E (Fig. 1B). PTEN is a phosphatase that removes a phosphate from phosphatidylinositol (PI)-3,4,5-tris-phosphate (PIP3) to generate PI-4,5-bis-phosphate (PIP2). The reverse reaction, catalyzed by PI-3-kinase

(PI3K), has been widely implicated in cell cycle progression and survival.¹⁸ Thus, the loss of PTEN, which is commonly observed in many human cancers,¹⁸ leads to elevated levels of PIP3. PIP3 serves to recruit proteins with plextrin homology (PH) domains, most notably phosphoinositide-dependent kinase 1 (PDK1). PDK1 phosphorylates Akt, another PH domain kinase that is a key regulator of G1 cell cycle progression.¹⁹ Akt suppresses the tuberous sclerosis complex (TSC1/2), which is a GTPase-activating protein that suppresses the GTPase Rheb.²⁰ Rheb contributes to the activation of the mTOR complex 1 (mTORC1).²¹ Rheb also activates phospholipase D1 (PLD1),²² which generates the phosphatidic acid necessary for the assembly of mTORC1.^{23,24} Rheb has also been reported to stimulate the dissociation of the inhibitory FKBP38 from mTORC1.²⁵ mTOR has been widely implicated in cancer cell survival and proliferation.²⁶ Thus, the finding that transformation of human cells results in the activation of mTOR suggests that the activation of mTOR is able to cooperate with Ras signaling in the transformation of human cells.

There is a large body of evidence revealing the complementary genetic

alterations needed for cell transformation and tumorigenesis.^{27,28} These studies have revealed that there are specific non-redundant signals controlling G1 cell cycle progression that need to be dysregulated during tumorigenesis. Much of the current literature on G1 cell cycle progression is not entirely consistent with the evidence obtained from studies on cell transformation and tumorigenesis. The remainder of the review will focus on reconciling the control of G1 cell cycle progression with the genetic evidence for dysregulation of G1 cell cycle progression in cancer cells.

The Restriction Point (R)

If cells are deprived of growth factors prior to R, they exit the cell cycle into a state of quiescence known as G0. One of the rationales for G0 (exiting the cell cycle exit, rather than arrest) is that it takes more time to transition from G0 to S phase than it does to transition from the end of mitosis (start of G1) to S phase.^{29,30} Zetterberg *et al.* have carefully mapped the time for postmitotic cells to reach R to about 3 to 4 hours, a time course that was remarkably constant for virtually all cell lines examined.^{4,31,32} Therefore, it was suggested that G1 be divided into 2 portions: G1-pm for G1-postmitotic, and G1-ps for G1-pre-S⁴ (Fig. 2). Whereas G1-pm is relatively constant, G1-ps is variable, and it is this variability in the duration of G1-ps that contributes to much of the confusion. Pardee's early studies used Swiss 3T3 cells, which have a relatively short G1 of about 5.5 hours.^{33,34} Consistent with Zetterberg's work, R was approximately 3.5 hours after mitosis^{33,34} and, therefore, approximately 2 hours prior to S phase. And 2 hours prior to S is where most texts place R; that is, R is positioned relative to S phase entry rather than relative to the end of mitosis.^{2,6} However, for most cell lines, G1 is significantly longer than the 5.5 hours observed for Swiss 3T3 cells. Therefore, since R is consistently 3.5 hours after mitosis,⁴ the time from R until S phase is much longer than the 2 hours.

Figure 1. Complementary signaling pathways activated in human cancer cells. Two signaling pathways are shown that are commonly activated in cancer cells. It is proposed that activation of these 2 signaling pathways promotes progression through different regulatory points in G1 of the cell cycle. **(A)** The Ras pathway involves a kinase cascade whereby activated Ras proteins recruit and activate Raf, which then phosphorylates and activates MEK, which then phosphorylates and activates MAP kinase (MAPK). This leads to increased expression of cyclin D and passage through R. This pathway is ordinarily activated by growth factors that prevent G1 cell cycle exit to quiescence. **(B)** The mTOR pathway is complicated and has many inputs. The PI3K input involves the generation of PIP3 from PIP2, which recruits and activates PDK1, which then phosphorylates Akt at Thr308. Akt can then phosphorylate and suppress the GAP activity of TSC1/2. Suppression of TSC1/2 results in elevated activation of the GTPase Rheb, which then leads to a complex activation of mTORC1 via the activation of PLD1 and suppression of FKBP38 whereby elevated PLD activity generates the phosphatidic acid necessary for the formation of mTORC1 complex and FKBP38 dissociates from mTORC1. mTOR stimulates ribosomal subunit S6 kinase (S6K), which stimulates the translation of many transcripts including those for Myc.⁶³ In addition, mTORC1 suppresses TGF- β signals in a manner that is poorly understood. Suppression of TGF- β signals leads to elevated cyclin E-CDK2 activity and subsequently higher levels of cyclin E. This pathway is also impacted by the AMPK, which, in combination with the tumor suppressor LKB1, activates TSC1/2 and suppresses mTOR under conditions where ATP levels are low and AMP levels are high. AMPK was also shown to phosphorylate Raptor, a protein associated with mTORC1, leading to the inactivation of mTORC1.⁶⁷ Akt is also phosphorylated by mTORC2 at Ser473 in response to insulin and IGF1 in a PLD-dependent manner. Phosphorylation at this site has been correlated with altered substrate specificity and kinase activity for Akt. A common theme in this complex signaling network is that it is highly sensitive to the presence of nutrients needed for cell growth.

Also contributing to the confusion are studies on the yeast cell cycle. Hartwell *et al.* described a site in late G1 where cells arrest in the absence of sufficient nutrients for the cell to double its mass.⁸ This site was called START and

has been referred to as the equivalent of the mammalian R.⁶ However, START is not sensitive to growth factors; the yeast cells are responding to nutrient availability. This significant difference may provide an important lead for resolving

the confusion as to what R actually represents and where R is located in G1. Of significance, passing START is dependent on TOR,³⁵ which has widely been implicated in sensing nutritional needs in both yeast and mammalian cells.^{36,37} Blenis' group, as well as others, demonstrated that rapamycin treatment in mammalian cells leads to cell cycle arrest in G1^{38,39} at a site that is consistent with START. The studies described by Blenis' group indicate that suppressing mTOR results in arrest late in G1. Importantly, cells arrested with rapamycin were smaller than the untreated cells,^{38,39} consistent with a role for mTOR as a nutritional sensor that restricts cell growth in the absence of sufficient nutrients. Thus, the site in G1 where rapamycin arrests cells prior to cell growth may be more equivalent to the yeast START, which is dependent on TOR and nutrient availability.

Existing data are consistent with R and START being distinguishable from each other in that R is a growth factor-sensitive site where cells receive instructions to avoid cell cycle exit into quiescence, and START senses whether there is sufficient nutrition for a cell to double in size prior to committing to replicate the genome and divide. The ability of rapamycin, which inhibits the nutrient sensing mTOR, to induce arrest in late G1 indicates that START may be conserved in mammalian cells, but not as R. Instead, START may be conserved in mammalian cells as a distinct checkpoint that senses nutritional sufficiency. This mTOR-dependent checkpoint could more appropriately be referred to as a "cell growth" checkpoint. Importantly, signals that regulate mTOR and cell growth apparently need to be dysregulated in tumorigenesis.

Regulation of G1 Progression

The proposal of a cell growth checkpoint warrants a brief overview of what is known about the control of G1 cell cycle progression. There are several in-depth reviews on this subject^{1,3,12,40,41}; therefore, the discussion will be restricted

Figure 2. Mammalian cell cycle. The mammalian cell cycle originally described as consisting of 2 parts: mitosis and interphase. During interphase, the genome is duplicated and was called S phase for synthesis. G1 and G2 were proposed as the gaps between mitosis and S phase and between S phase and mitosis, respectively. G1 is usually where critical decisions are made as to whether to enter a resting quiescent stage known as G0 or to continue cycling and commit to replicating the genome and mitosis. The point in G1 where this growth factor-dependent decision is made is known as the restriction point (R). G1 has been described as consisting of 2 parts on either side of R, where the first part of G1 is known as G1-pm for postmitotic, and the second part is known as G1-ps for pre-S.⁴

Figure 3. Rb and G1 cell cycle progression. Rb exists in different states of phosphorylation: unphosphorylated, hypophosphorylated, and hyperphosphorylated. The hypophosphorylated state occurs after phosphorylation by cyclin D-CDK4/6, and this leaves Rb associated with E2F family transcription factors such that E2F is unable to activate transcription. Rb becomes hyperphosphorylated after the activation of cyclin E-CDK2. At this point, E2F dissociates from E2F and can initiate the transcription of genes required for progression into S phase. One of the genes activated by E2F is cyclin E generating a positive feedback loop to aid in the progression through G1-ps.

only to controlling progression through R and the proposed cell growth checkpoint. Key regulators of cell cycle progression are the cyclins, proteins that interact with and activate specific CDKs. There are 2 major classes of G1 cyclins: cyclin D and cyclin E. Cyclin D interacts with either CDK4 or CDK6, and cyclin E partners with CDK2. Cyclin D, partnered with either CDK4 or CDK6, phosphorylates the retinoblastoma tumor suppressor Rb to generate what has been

called hypophosphorylated Rb.³ Hypophosphorylated Rb exists in a complex with E2F family transcription factors that are critical for progression into S phase. The association of E2F with Rb is dependent on the hypophosphorylation provided by cyclin D-CDK4/6,⁴² and therefore, the suppression of E2F by Rb is dependent on cyclin D-CDK4/6. However, phosphorylation of Rb by cyclin D-CDK4/6 also leads to the dissociation of histone deacetylases from

Rb leading to the derepression of cyclin E gene expression.⁴³ Cyclin E-CDK2 is suppressed by the CDK inhibitor p27^{Kip1},^{12,44} but p27^{Kip1} also interacts with cyclin D-CDK4/6 and may be required for its activity.¹² As cyclin D levels increase, more p27^{Kip1} is sequestered, and thus, ultimately, cyclin E-CDK2 loses its inhibitory p27^{Kip1} to the more abundant cyclin D-CDK4/6. It has been proposed that the sequestration of p27^{Kip1} by cyclin D-CDK4/6 contributes to the activation of cyclin E-CDK2.¹² Activated cyclin E-CDK2 phosphorylates Rb to generate a hyperphosphorylated Rb, which liberates E2F to initiate the transcription of genes needed for progression into S phase, including cyclin E. Since the phosphorylation of Rb by cyclin D-CDK4/6 is required prior to the phosphorylation of Rb by cyclin E-CDK2,⁴⁵ it has been proposed that Rb serves as a cell cycle clock that controls progression through critical regulatory points in G1 mediated by its sequential phosphorylation,² which is shown schematically in Figure 3.

The location of R according to both Zetterberg and Pardee is about 3.5 hours after mitosis,^{4,34} a point in G1 where cyclin D levels increase. Growth factors that facilitate passage through R stimulate increases in cyclin D levels.^{46,47} Activated Ras, which mimics growth factor signals, also stimulates an increase in cyclin D levels.⁴⁸ Importantly, the transformation of cells by Ras is dependent on cyclin D.⁴⁹ Thus, there is a clear correlation between cyclin D levels and passage through R. Whether the hypophosphorylation of Rb by cyclin D-CDK4/6 has any role in passing R is not clear in that it has been reported that hypophosphorylation of Rb occurs after R passage.³² However, in the absence of Rb, cells apparently do not exit the cell cycle when deprived of growth factors,⁵⁰ indicating that inactivating Rb may impact on the ability to enter quiescence.

After progression through the cyclin D-dependent portion of the cell cycle, cyclin E becomes activated, and Rb becomes hyperphosphorylated by cyclin

E-CDK2. At this point, Rb no longer suppresses E2F, and E2F can then stimulate the expression of many genes needed for progression into S phase. Another substrate of cyclin E-CDK2 is its inhibitor, p27^{Kip1}. Phosphorylation of p27^{Kip1} targets it for ubiquitination and degradation by the proteasome. At this point, cyclin D is no longer required for sequestering p27^{Kip1} as a means of activating cyclin E-CDK2. This creates a positive feedback loop whereby cyclin E-CDK2 suppresses Rb and liberates E2F, which then increases the level of cyclin E such that cyclin E-CDK2 can continue to suppress Rb and keep p27^{Kip1} targeted for degradation. The cells can now progress through the last part of G1 into S phase. This regulatory point of the cell cycle has been referred to as R in several reviews and texts.^{2,3,6} However, this site is also where START of the yeast cell cycle is located.

As indicated above, START is a site in the yeast cell cycle that senses nutrition⁸ and is dependent on TOR.³⁵ Moreover, passage through the growth factor-dependent R occurs long before the increase in cyclin E levels and increased activity of CDK2.³¹ Thus, there is a clear distinction as to what R represents and where R exists in G1. Of interest is the regulation of cell cycle progression by transforming growth factor- β (TGF- β), which suppresses cell cycle progression late in G1 and also increases levels of cyclin E-CDK2 inhibitor p27^{Kip1}.^{5,51} Therefore, TGF- β can suppress cell cycle progression at the cyclin E-dependent point late in G1 that is equivalent to START. Defects in p27^{Kip1} expression are also common in human cancer.⁵² A critical aspect of TGF- β -induced cell cycle arrest is the suppression of Myc expression,⁵³ which is consistent with Myc being a downstream target of mTOR and PLD signals, both of which suppress TGF- β signaling⁵⁴⁻⁵⁶ (Fig. 1). Transformation studies with phorbol esters also implicate a late G1 checkpoint mediated by TGF- β . Tumor-promoting phorbol esters cooperate with activated Ras, but not elevated

Myc expression, to transform primary rodent fibroblasts.⁵⁷ This would implicate the tumor promoters as progression factors for passage through G1-ps and the putative cell growth checkpoint. The tumor-promoting effects of phorbol esters are the result of suppressing protein kinase C δ (PKC δ) expression,^{58,59} and significantly, PKC δ is required for the TGF- β signals that induce cell cycle arrest.⁵⁵ Thus, the effect of tumor-promoting phorbol esters can be linked to their ability to facilitate passage through the G1-ps checkpoint mediated by TGF- β . In summary, genetic studies that reveal a requirement for elevated mTOR signaling in cell transformation, coupled with the ability of mTOR to suppress TGF- β signals, strongly indicate that this late G1 checkpoint is distinguishable from the site regulated by cyclin D, which is elevated in response to growth factor signals and activating mutations to Ras.

A Mammalian Cell Growth Checkpoint

START was originally described as a commitment point late in G1 where nutritional sufficiency and cell size were established prior to committing to replicate the genome and cytokinesis.^{8,60} Cyclin E was discovered as being able to complement G1 cyclin mutants in *S. cerevisiae*, thereby facilitating passage through START.^{61,62} The connection between cyclin E and mTOR via TGF- β signaling⁵⁴⁻⁵⁶ links cyclin E to nutritional sensing in that mTOR is activated by amino acids and is suppressed by low ATP levels.^{36,63} The lack of essential amino acids, similar to rapamycin treatment, results in G1 cell cycle arrest.^{7,34} Importantly, re-entry of Swiss 3T3 cells into the cell cycle upon restoring amino acids is significantly faster (2 hours) than when cells have entered quiescence upon being deprived of growth factors (12 hours).³⁴ Exiting quiescence and entering S phase generally takes much longer than G1, which is part of the rationale for postulating G0.^{29,30} The

difference between recovery from amino acid starvation versus serum deprivation also clearly distinguishes R from the mTOR-dependent checkpoint.

Signals activated by insulin and insulin-like growth factor-1 (IGF1) also provide a response to nutritional sufficiency.⁶⁴ Stiles *et al.*⁶⁵ provided a model for commitment and progression of cells from G0 into S phase whereby transient exposure to platelet-derived growth factor (PDGF) was sufficient to get cells to "commit" to cell cycle entry, but "progression" through the rest of G1 required continuous treatment with IGF1. This early study is consistent with a model where PDGF is needed for entrance into the cell cycle and IGF1 required for passage through G1-ps. PDGF stimulates increased expression of cyclin D,⁴⁶ while IGF1 activates PI3K and both mTORC1 and mTORC2.⁶⁶ Thus, the commitment progression model for G1 cell cycle progression is consistent with a need to pass through both a growth factor cyclin D-dependent R and an mTOR and cyclin E-dependent cell growth checkpoint.

Shaw *et al.* have recently reported that the AMP-dependent kinase (AMPK) phosphorylates Raptor directly and is required for suppression of mTORC1.⁶⁷ As AMPK has been widely implicated in sensing metabolic capability in the cell, a "metabolic checkpoint" mediated by AMPK and mTORC1 has been proposed.⁶⁸ A checkpoint mediated by AMPK and mTORC1 would be considered part of the cell growth checkpoint (Fig. 1). It is also worth noting that PI3K signaling, which contributes to mTOR activation, has also been linked to the regulation of growth and metabolism.⁶⁹ Thus, a significant body of evidence is emerging that implicates mTOR as a critical mediator of cell cycle progression through a late G1 checkpoint that can be distinguished both functionally and temporally from R. A model for control of G1 cell cycle progression is shown in Figure 4. This model for G1 cell cycle progression is consistent with the complement of genetic changes that occur in cancer cells and with the genetic

Figure 4. G1 cell cycle checkpoints. Progression through G1 of the cell cycle is shown as having 3 major obstacles for which specific genetic alterations need to occur during tumorigenesis. After completing mitosis, the first obstacle is passing R where cells avoid quiescence. Passage of this site is dependent on growth factor instructions or, in the case of a cancer, a mutation to Ras or another component of the Ras pathway that leads to cyclin D expression. After passing R, the next obstacle involves the activation of signals that indicate sufficient nutrients for the cell to double in mass. We refer to this site as the “cell growth” checkpoint. A focal point of this checkpoint is mTOR, which suppresses TGF- β signaling, which stimulates the expression of p27^{Kip1}, which suppresses cyclin E-CDK2.

requirements for the transformation of human cells (Table 1).

Remaining Issues

While the model provided in Figure 4 is consistent with the genetic changes that occur in cancer, there are still unresolved issues. Foremost among these are the overlapping actions of signals that facilitate passage through R and the cell growth checkpoint. Ras activates multiple downstream targets in addition to the Raf/MEK/MAP kinase pathway.¹⁶ And significantly, Ras can activate PI3K,⁷⁰ which feeds into the mTOR pathway. Ras also activates Ral-GDS,⁷¹ which leads to elevated PLD activity, and similarly feeds into the mTOR pathway. The activation of RalA has been reported to be important for the transformation of human cells by activated Ras.⁷² Human cancer cells with activating mutations to Ras were dependent on RalA and PLD

activity for survival,^{73,74} indicating that RalA and PLD are important Ras targets for maintaining cell transformation. These studies reveal that in addition to activating Raf/MEK/MAP kinase signals and elevating cyclin D expression, Ras is also capable of activating signals that target mTOR. Ras can also stimulate Myc expression,⁷⁵ which is also a target of mTOR signals. Thus, logically, Ras should be able to dysregulate both R and the cell growth checkpoint. However, many studies have found that introduction of both Ras and Myc genes is required for the transformation of either mouse or human cells.^{9,10,15,76} Therefore, while Ras is able to induce Myc expression under some circumstances, Ras could not transform primary cells without Myc. In addition, cyclin D-null cells were resistant to transformation by Neu and Ras but not to transformation by Myc.⁴⁹ Therefore, although Ras can

activate multiple signaling pathways in different cell contexts, it is likely that the most critical target of Ras is the Raf/MAP kinase pathway to stimulate cyclin D and passage through the growth factor-dependent R.

Another troublesome issue is the surprising finding that cyclin E knockout mice develop somewhat normally.^{77,78} However, while the cyclin E-deficient cells are apparently capable of dividing during development, cells from the cyclin E-null mice are resistant to transformation,⁷⁷ supporting the premise that cyclin E is a critical target of signals activated during tumorigenesis. In an excellent review,⁴⁴ Hwang and Clurman have addressed the inconvenient truths raised by the cyclin E knockout mice. It is possible that cyclin E represents a mechanism for promoting cell cycle progression not utilized during development but is able to drive G1 cell cycle progression under oncogenic stimulation.

We have proposed that the cell growth checkpoint in G1-ps is related to the yeast cell cycle checkpoint known as START. However, passing START in the budding yeast cell cycle may involve multiple checkpoints prior to progression to S phase.⁷⁹ In addition to checking for nutritional abundance, cell size is also evaluated. START also represents the point in the cell cycle where pheromone sensitivity is lost in that pheromone-induced cell cycle arrest occurs before START, but not after.⁷⁹ Thus, the nutrient sensing mediated by mTOR in mammalian cells may reflect a subset of checkpoints involved in START. It is also possible that the cell growth checkpoint proposed here may represent more than one checkpoint and involves multiple inputs informing the cell that it is competent to replicate its genome.

Summary and Conclusions

Genetic evidence from cancer and cell transformation studies reveals a need to dysregulate distinct sites in G1 that are regulated sequentially by cyclin D and then cyclin E. The site in G1, where

cyclin D levels increase, is very close to the site in G1, where cells avoid quiescence, and is dependent on growth factor instructions. In contrast, the site in G1 where cyclin E-CDK2 becomes active occurs after growth factor independence has been achieved³¹ and is regulated by mTOR and TGF- β signals. The mTOR dependence indicates that this regulatory site in late G1 is where nutrient and energy status is evaluated prior to committing to cell growth and cytokinesis. While both of these sites have been referred to as R in the literature and in texts, these 2 sites can be distinguished both temporally and genetically.

It is clear from the genetics of cancer that much of what is dysregulated in human cancer involves defects in the control of G1 cell cycle progression. A key defense against inappropriate progression through G1 into S phase is apoptosis or cell senescence. Therefore, suppression of oncogenic signals, in principle, can resurrect these defenses and result in apoptosis or senescence.⁸⁰ While many genetic alterations have been discovered in human cancers and cancer cell lines⁸¹ and a major effort to identify still more genetic alterations in cancer cells is ongoing,⁸² these mutations may funnel through a relatively small number of regulatory points in G1 of the cell cycle. Understanding these key regulatory sites may allow for rational targeting of relatively few signals that override the key regulatory points in G1 that apparently are necessary in most, if not all, cancers.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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