Journal of Cellular Biochemistry 54:405–414 (1994)

WI-38 Cell Long-Term Quiescence Model System: A Valuable Tool to Study Molecular Events That Regulate Growth

Kenneth J. Soprano

Department of Microbiology and Immunology and the Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

Abstract A number of cell culture model systems have been used to study the regulation of cell cycle progression at the molecular level. In this paper we describe the WI-38 cell long-term quiescence model system. By modulating the length of time that WI-38 cells are density arrested, it is possible to proportionately alter the length of the prereplicative or G-1 phase which the cell traverses after growth factor stimulation in preparation for entry into DNA synthesis. Through studies aimed at understanding the cause and molecular nature of the prolongation of the prereplicative phase, we have determined that gene expression plays an important role in establishing growth factor "competence" and that once the cell becomes "competent" there is a defined order to the molecular events that follow during the remainder of G-1. More specifically, we have determined that the prolongation represents a delay in the ability of long term quiescent cells to become fully "competent" to respond to growth factors which regulate progression through G-1 into S. This prolongation appears to occur as a result of changes during long term quiescence in the ability of immediate early G-1 specific genes (such as c-myc) to activate the expression of early G-1 specific genes (such as ornithine decarboxylase). While ODC is the first and thus far only growth associated gene identified as a target of c-myc (and the Myc/Max protein complex), it is likely that further studies in this model system will reveal other early G-1 growth regulatory genes. We anticipate that future follow-up studies in this model system will provide additional valuable information about the function of growth-regulatory genes in controlling growth factor responsiveness and cell cycle progression.

Key words: competence, progression, G-1, c-Myc, ODC, cell cycle, quiescence, WI-38 cell

The ultimate goal of any research that focuses on understanding the regulation of cell growth is the identification of *bona fide growth regulatory genes*. Since a considerable amount of evidence suggests that events which occur during the prereplicative or G-1 stage regulate mammalian cell growth [for review, see Baserga, 1985; Soprano and Cosenza, 1992], numerous laboratories including our own have set out to identify and analyze the patterns of induction of a wide variety of proto-oncogenes and growth associated genes during the prereplicative stage (G-1) [for review, see Denhardt et al., 1987]. The most common model system employed for such studies has been the NIH 3T3 cell, made quiescent by either serum deprivation or growth to high cell density followed by stimulation with serum or purified growth factors. While it is true that a considerable amount of information has been obtained about the patterns of expression of many growth associated genes, this model system does not permit one to easily distinguish between growth-associated genes that are *growth regulatory* and those that are merely *growth regulated*. For example, a number of these genes (e.g., c-fos and c-myc) can in fact be induced at stages of the cell cycle other than G-1 merely by treating the cells with fresh serum. Also, such inductions have been shown to occur even in response to treatments that are not mitogenic and, in some cases, actually inhibit cell proliferation. This suggests that the induction of at least some growth associated genes may be merely a direct consequence of the interaction of a growth factor with its receptor and may not play an important role in *regulating* growth.

Received August 16, 1993; accepted August 23, 1993.

Address reprint requests to Dr. Kenneth J. Soprano, Department of Microbiology and Immunology, Temple University School of Medicine, 3400 North Broad Street, Philadelphia, PA 19140.

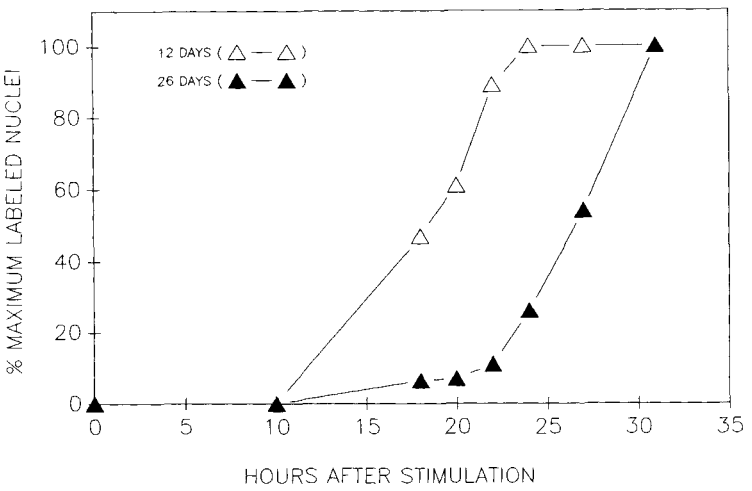

Fig. 1. Kinetics of entry into DNA synthesis following prolonged quiescence. WI-38 cells growing on glass coverslips were stimulated on either the 12th day after plating (△) or the 26th day after plating (▲) by replacement of the culture medium with complete DMEM supplemented with 10% fetal calf serum and ^{3}H-thymidine. At various times after stimulation, coverslips were harvested, fixed and processed for autoradiography as described by Owen et al. [1987, 1989a, 1990]. The number of labeled nuclei from at least 1,000 cells was determined per time point. The data show that WI-38 cells maintained in quiescence for an additional 14 days exhibit a 10-h prolongation of the prereplicative phase upon stimulation.

Some time ago, we hypothesized that it might be possible to distinguish molecular events that are *growth regulatory* from those that are merely *growth regulated,* if one could clearly uncouple initial events which occur merely as a result of the interaction of a ligand and its membrane bound receptor from events critical for G-1 progression, entry into DNA synthesis and subsequently mitosis. One way in which this can be accomplished is to examine growth-associated gene expression in a model system in which the length of the prereplicative (G-1) period after serum stimulation prior to initiation of DNA synthesis can be physiologically modulated. In this way the induction of a growth associated gene can be classified into one of two groups: (1) as a response to the stimulatory process (if the gene is induced at the same time regardless of when the cells enter S), or (2) as a response to the progression of cells out of G-0, through G-1 into S (if the gene is induced at different times that are dependent upon when the cells enter S). The WI-38 cell long-term quiescence model system constitutes just such a system.

WI-38 CELL LONG-TERM QUIESCENCE MODEL SYSTEM

This model was first reported by Augenlicht and Baserga in 1974. In the original description of this model system, Augenlicht and Baserga [1974] compared the lengths of the prereplicative periods of cells stimulated after remaining in a state of density arrest for various prolonged periods of time. They found that the time it took for the cells to reenter S phase (the prereplicative period) increased in proportion to the length of time in quiescence. For example, cells stimulated 8–12 days after plating required 10–14 h to enter DNA synthesis, while cells stimulated 18–25 days after plating entered S phase 20–30 h after stimulation. They demonstrated that the prolongation of the prereplicative period was not simply due to the depletion of the medium of nutrients such as amino acids or vitamins since the cells could be refed biweekly throughout the time in quiescence with fresh medium supplemented with 0.3% serum without altering their kinetics of entry into S phase. We and others have repeated these experiments with WI-38 cells of different passage and with other strains of normal human diploid fibroblasts as well as concanavalin A-stimulated lymphocytes, mouse 10T1/2 cells, Swiss 3T3 cells, and monkey CV-1 cells [Gunther et al., 1974; Miska and Bosman, 1980; O'Farrell and Yanez, 1989; Owen et al., 1987, 1989a, 1990]. Figure 1 illustrates the differences in the kinetics of entry into S of WI-38 cells which have remained in density-dependent growth arrest for extended periods of time. The only differences observed between our more recent studies and those of Augenlicht and Baserga is that while all cells exhibit a quiescence-dependent prolongation, there is, as one might

expect, variation in the precise time of entry into S, depending on the age of the cells.

It should be noted that the lengthening of the prereplicative stage with time in growth arrest exhibited by WI-38 cells has several in vivo counterparts [for review, see Baserga, 1985]. For example, after partial hepatectomy, hepatocytes from older rats take longer to enter S than do hepatocytes from younger rats. The same results have been reported after mitogenic stimulation of salivary glands by isopreterenol treatment of rats of various ages. Thus, the WI-38 cell long-term quiescence model system would appear to offer an in vitro tissue culture system that very closely approximates the response in vivo of cells and tissues which exist for extended periods of time in a nonproliferating state and are then induced to proliferate by injury, exposure to carcinogens, tumor viruses, etc.

EVENTS THAT OCCUR DURING PROLONGED QUIESCENCE

One of the most obvious criticisms of a model system which requires cells to remain for an extended period of time in a growth arrested state is that during this time the cells gradually deteriorate and therefore do not resemble a physiologically functioning and meaningful biological entity. However, this is not the case. Biochemical and molecular studies have shown that the quiescence is not a state of overall gradual deterioration, but rather a highly viable, metabolically active state. Our laboratory previously examined a number of biochemical and molecular parameters in WI-38 cells that were density arrested for various lengths of time [Ferrari et al., 1988; Owen et al., 1990]. A summary of our results is diagramed in Figure 2. We found that at least in WI-38 cells, density-dependent growth arrest occurs as a two stage process. Cells density arrested for 7–10 days (~ 12 days after plating) entered a state referred to as early G-0. In early G-0, the cells stopped bulk DNA synthesis as determined by percentage of cells labeled with ^{3}H-thymidine. However, these cells had not yet undergone any dramatic biochemical changes with respect to RNA or protein content compared with their actively proliferating counterparts. In contrast, as the cells remain in a state of density arrest for an additional 7–10 days (20 days after plating), they leave early G-0 and enter into late G-0. Late G-0 cells exhibited a decrease in total cell protein and RNA. However, the most interesting finding was that while levels of proteins and gene products commonly associated with G-1 progression decreased during late G-0 (e.g., c-myc, p53, ODC), the levels of other proteins such as fibronectin, collagen, and insulin receptor actually increased. Clearly, long-term quiescent cells are still metabolically active and exhibit both decreases and increases in a number of specific biochemical and molecular processes. Moreover, when a quiescence-associated change in a biochemical process does occur, it happens in a two-step fashion.

GROWTH FACTOR REQUIREMENTS: COMPETENCE/PROGRESSION

As a first step in using this model system to identify and understand growth regulatory events, we set out to determine the qualitative and quantitative growth factor requirements necessary to stimulate G-1 progression and entry into S in long-term quiescent WI-38 cells [Owen et al., 1989a]. We found that stimulation of long- and short-term density-arrested WI-38 cells does not involve different individual growth factors or different concentrations of the same growth factors. The same defined medium supplemented with EGF, IGF-1, and dexamethasone at the same concentrations stimulated DNA synthesis with an efficiency and kinetics similar to that elicited by FCS in both short- and long-term quiescent WI-38 cells. Moreover, the use of different growth factors or higher concentrations of individual growth factors did not alter the time required by long-term cells to enter S after stimulation. *However, the time during the prereplicative period for which these growth factors are needed is different.* As illustrated diagramatically in Figure 3, we found that with extended time in density-dependent growth arrest, WI-38 cells lose the ability to respond immediately to one or more growth factors contained in serum (e.g., EGF and IGF-1) [Owen et al., 1989a,b]. Consequently, entry into DNA synthesis is delayed by the length of time required for the cell to become responsive. Two groups of experiments lead us to this conclusion: those in which serum was withdrawn at various times after stimulation and those in which individual growth factors were withheld for various lengths of time at the time of stimulation. Short-term quiescent WI-38 cells can, do and in fact must respond either immediately or very early during the G-0/G-1 transition to both EGF and IGF-1, since a delay in the addition of either growth

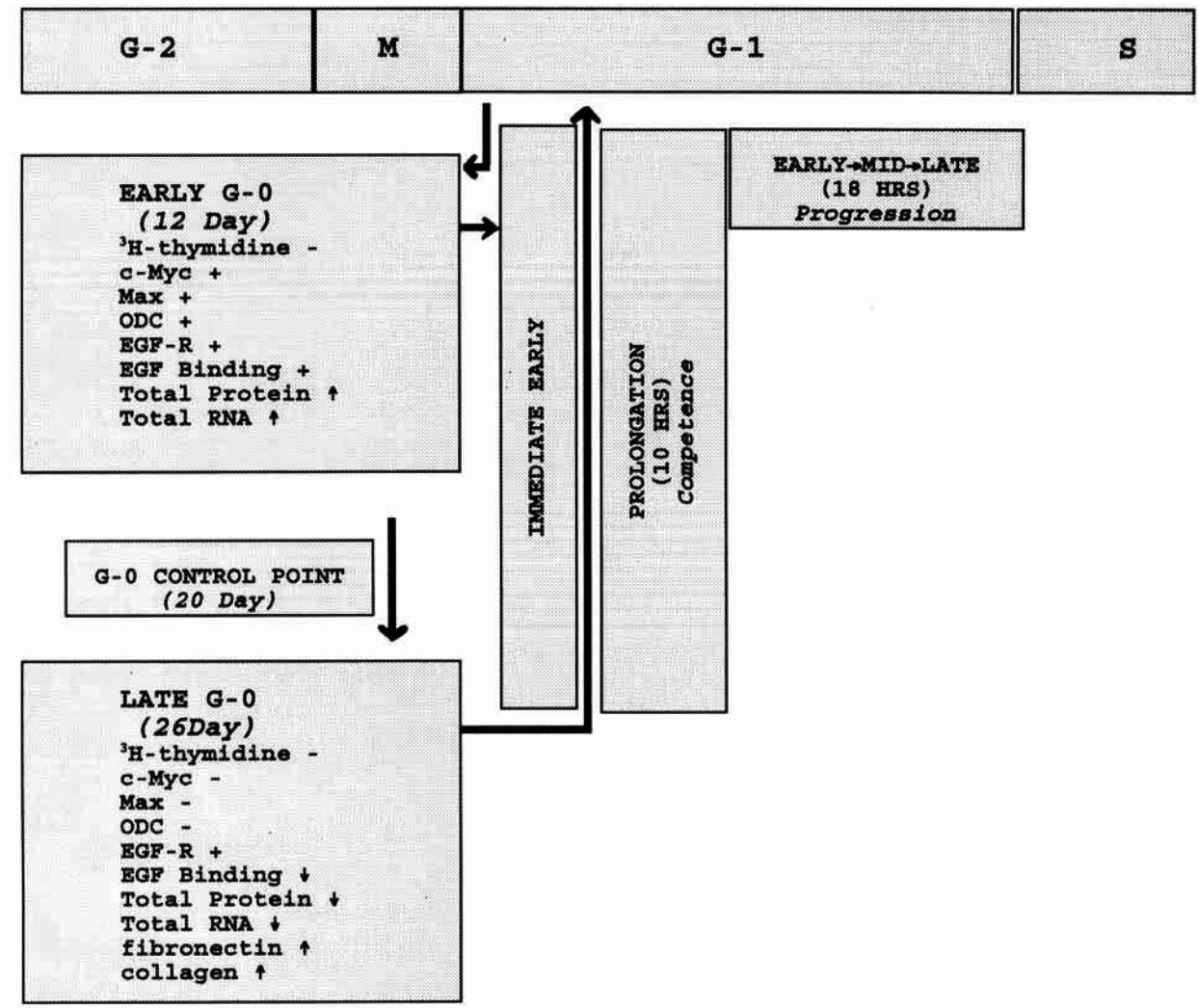

Fig. 2. Diagramatic representation of the properties of WI-38 cells during early (short-term or 12 day) and late (long-term or 26-day) quiescence.

factor results in a delay in the initiation of DNA synthesis (i.e., a prolongation of the prereplicative phase). Likewise, our finding that no additional prolongation occurred in long-term quiescent WI-38 cells when addition of IGF-1 was delayed up to 10 h or when addition of EGF was delayed up to 3 h suggested that long-term quiescent WI-38 cells were unable to respond to EGF during the first 3 h after stimulation and could not respond to IGF-1 during the first 10 h after stimulation (the length of the prolongation of the prereplicative phase).

It is very tempting to compare these results to the more extensively characterized mouse 3T3 competence-progression model system [Pledger et al., 1977, 1978]. This model proposes that growth factors contained in the platelet portion of serum (i.e., PDGF) induce biochemical and molecular changes that permit density-dependent growth arrested 3T3 cells to become "competent" to respond to additional growth signals contained in the plasma portion of serum (PPP, somatomedin C, or IGF-1). These growth factors permit the "progression" of competent cells through G-1 to S. In a situation somewhat analogous to that exhibited by WI-38 cells, the prereplicative phase can be prolonged by delaying the time at which the cells are exposed to each of the individual growth factors. The failure of long-term quiescent WI-38 cells to respond immediately to one or more of the growth factors contained in fetal calf serum is consistent with the possibility that EGF is a competence growth factor for WI-38 cells and that long-term quiescent cells are deficient in their ability to respond immediately to EGF and

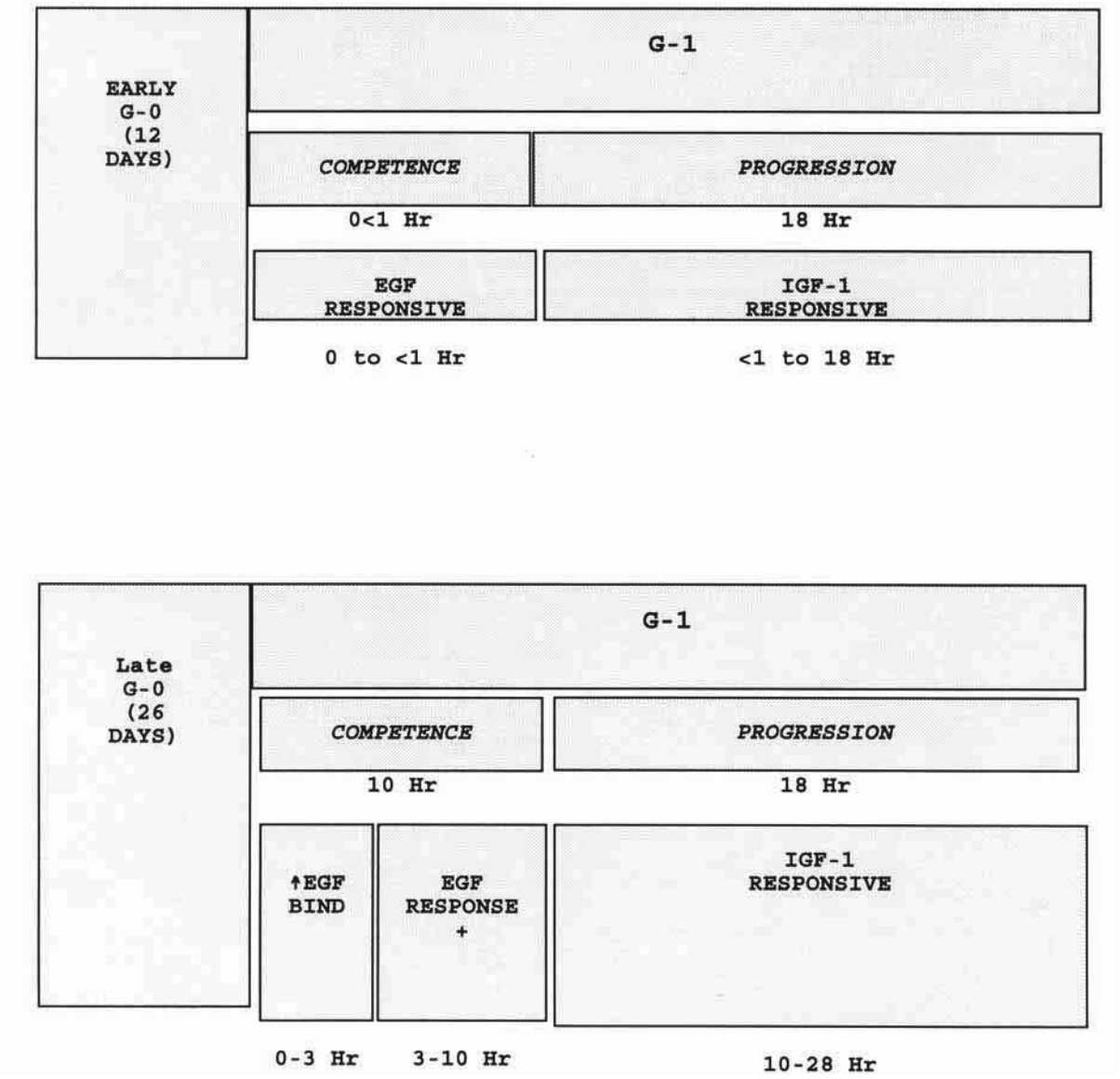

Fig. 3. Diagramatic representation of the growth factor responsiveness of early (short-term or 12-day) and late (long-term or 26-day) G-0 WI-38 cells.

become fully "competent" to respond to progression growth factors (i.e., IGF-1).

Most recently, we have completed studies which show that short-term quiescent WI-38 cells bind substantially higher amounts of EGF than long-term growth arrested WI-38 cells [Donigan et al., 1993]. When short-term growth arrested cells are treated with EGF, ligand binds maximally to the cell surface receptor within 2 min of cellular contact and the EGF-receptor complex internalizes rapidly thereafter. Long-term growth-arrested cells, however, although also binding ligand maximally within 2 min, do so at much lower levels and internalize EGF-receptor complexes more slowly. We also determined that the amount of EGF receptor remains constant during long term quiesence. Therefore, it would appear that the reduction in EGF responsiveness that occurs during long-term growth arrest results from a change in the nature and/or location of the EGF receptor such that they bind substantially less ligand and internalize what little they bind at a slower rate. Interestingly, we have found that long-term quiescent WI-38 cells require approximately 3 h to achieve internalized levels of EGF comparable to those measured within 2 min of treatment of short-term quiescent WI-38 cells. This is consis-

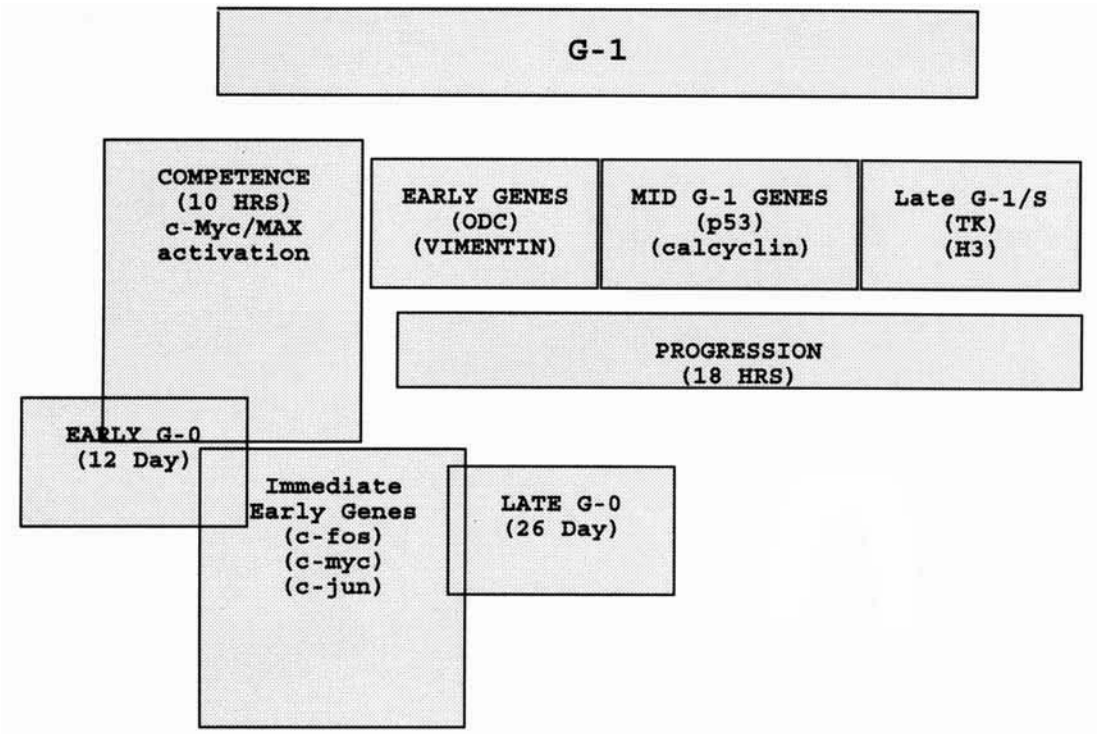

Fig. 4. Diagramatic representation of the temporal patterns of growth-associated gene expression following growth factor stimulation of early (short-term or 12-day) and late (long-term or 26-day) G-0 WI-38 cells.

tent with our previous finding that long-term quiescent WI-38 cells are unable to respond to EGF during the first 3 h after stimulation. It is tempting to speculate that a critical level of EGF must be bound and internalized in order to initiate "competence," the signal transduction pathway which induces the molecular events which subsequently allow the cell to respond to "progression" growth factors such as IGF-1 (see Fig. 3). It is logical to hypothesize that the combination of events involved in competence and those involved in progression *regulate* the prereplicative phase.

MOLECULAR EVIDENCE FOR A DEFINED ORDER OF EVENTS THAT OCCUR AS A CELL PROGRESSES OUT OF QUIESCENCE THROUGH G-1 INTO S

Identifying and understanding the nature of the unique molecular events that must occur in order for long-term quiescent WI-38 cells to re-enter the cell cycle should provide valuable information about the corresponding *growth regulatory* events that must occur in order for tumor formation to occur in vivo. Our laboratory therefore set out to map, at the molecular level, the location of the prolongation of the prereplicative phase exhibited by WI-38 cells stimulated after long-term growth arrest. We analyzed the changes in the patterns of expression of a number of representative immediate early G-1 (c-fos, c-jun, c-myc), early G-1 (ornithine decarboxylase, vimentin, c-ras), mid G-1 (p53, calyclin) and late G-1/S (thymidine kinase and histone H3) growth-associated genes [Owen et al., 1987; Cosenza et al., 1988; Carter et al., 1991]. As shown in Figure 4, we found that the time of induction and/or maximal accumulation of all the transcripts analyzed except the immediate early genes (c-fos, c-jun, c-myc) and c-Ha-ras was delayed by a period of time nearly equal to the length of the prolongation of the prereplicative phase. Moreover, once early G-1 growth associated gene expression occurred (ODC and vimentin), the pattern of expression and the time required to progress through G-1 and enter S was the same in all cell groups regardless of how long they were growth arrested. We concluded that the prolongation occurs at a point closely following the induction of the immediate early genes upon stimulation and suggested that certain molecular events must occur in cells stimulated after prolonged periods before early G-1 genes become expressed. However, once

these events occur, there do not appear to be any additional delay points along the path to S. It is clear from these data that, at least in this model system, *growth-associated gene expression is temporally and coordinately regulated with respect to time of entry into DNA synthesis.*

If one compares these molecular data with the previously described kinetic data following growth factor stimulation, it is possible to imagine that "competence" at the molecular level represents the induction by competence growth factors such as EGF of both (1) the immediate early G-1 genes (many of which represent transcription factors), and (2) the genes which the immediate early genes regulate (some of which may be involved in mediating membrane associated and signal transduction responses to progression growth factors such as IGF-1). Once critical levels of these gene products are achieved, the cell is molecularly "competent" to respond to the "progression" growth factors. This transition between competence and progression is identified at the molecular level by the induction of the early G-1 genes such as vimentin and ODC. Once these genes are expressed, the time required to complete G-1 and enter S, just like the time between treatment of the competent cell with the progression growth factor and the time of entry into S is constant.

Thus, by analysis of the quiescence dependent changes in kinetics of entry into S following growth factor treatment and the differences in time of growth associated gene expression as a function of length of the prolongation of the prereplicative phase, we have been able to provide the most definitive molecular evidence to support a hypothesis first proposed by Hartwell and supported at least on a theoretical basis, by many others [see Baserga, 1985; Pardee, 1987; Prescott, 1987; and Hartwell and Weinert, 1989, for reviews] that there is a defined order to the molecular events that occur as a cell progresses out of quiescence through G-1 into S.

CONTROL POINT WITHIN G-0

Thus far, we have looked at the regulation of cell cycle progression from the point of view of the events which must occur upon growth factor stimulation (i.e., competence and progression). In fact, competence and progression have traditionally always been discussed in terms of the stimulated cell. However, our data in the WI-38 cell model system suggest that it is also necessary to discuss competence and progression in terms of the quiescent cell. The fact that long-term quiescent WI-38 cells, unlike short term quiescent WI-38 cells and 3T3 cells, cannot become immediately competent suggests that the status of the cell during quiescence determines the nature of subsequent molecular events which must occur in order for the stimulated cells to become competent and subsequently to progress through G-1, synthesize DNA and divide. In other words, the long-term quiescence WI-38 cell model shows that not all quiescent cells are equal and therefore, the events that constitute "competence" are also not always the same, depending on how long the cell has been growth arrested. This suggested that a new, previously unidentified control point exists in long-term quiescent WI-38 cells. This point must be traversed before this defined order of G-1 specific molecular events can be initiated (and before G-1 progression can occur). Cells growth arrested for short periods (early G-0 cells) already have traversed this point at the time of stimulation. However, in cells growth arrested for extended periods (late G-0 cells), the cells are blocked at this control point and cannot pass through it until certain events occur, which are necessary for the cells to be able to fully respond to competence growth factors such as EGF. Although at this time we do not know the identity or nature of all the events that mediate passage through this G-0 control point, one strong possibility involves the release and/or activation of EGF receptors present in long term quiescent cells but presumably sequestered in a location which prevents ligand binding. Additional logical possibilities would include the various substrates and kinases which constitute the EGF signal transduction pathway.

MOLECULAR NATURE OF COMPETENCE: A ROLE FOR THE c-Myc/Max PROTEIN COMPLEX

Once the long-term quiescent WI-38 cell has become capable of responding to the competence growth factor, what events occur to make the cell "competent?" As discussed above, we had previously localized the prolongation of the prereplicative phase to a point early in G-1, after induction of immediate early response genes such as c-fos, c-jun, and c-myc, but before maximal expression of early response genes such as ornithine decarboxylase (ODC). We had also shown that once the stimulated long term cells pass through this prolongation or block, they

progress through G-1 into S, with the same kinetics as short term quiescent WI-38 cells (Cosenza et al., 1988). We therefore reasoned that induction of early response genes such as ODC could be considered as a molecular marker for passage through the prolongation or "competence." In addition, because ODC is the first and apparently the rate-limiting enzyme in the biosynthesis of polyamines, molecules which appear to be indispensable for mammalian cell growth, ODC is a logical representative gene for a study such as this [for review, see Pegg, 1988; Tabor and Tabor, 1984]. We compared changes in the interaction of nuclear proteins with the human ODC promoter which occur during prolonged quiescence as well as following stimulation of short and long term quiescent WI-38 cells. Our results show that the c-Myc/Max protein complex can regulate human ODC expression and suggest that the activity of this protein complex is altered during prolonged quiescence. More specifically, we showed both in vivo and in vitro, that ODC expression could be regulated by the interaction of c-Myc and Max with a CACGTG element between −491 and −474 of the human ODC 5′ regulatory region. To our knowledge, ODC is the first growth associated gene shown to be regulated by the c-Myc–Max complex. Moreover, the fact that the ODC activity is known to be essential for mammalian cell proliferation [for review, see Pegg, 1988; Tabor and Tabor, 1984] makes this finding even more significant since it demonstrates a role for this complex in regulating cell growth.

Evidence that the c-Myc/Max protein complex plays a role in regulating the expression of the human ODC gene during quiescence was provided by several different approaches. The first indication of this came from the presence of a CACGTG element within the portion of the human ODC promoter which consistently displayed a specific protein–DNA interaction with short term quiescent nuclear extracts but not with extracts prepared from long term quiescent cells. This was confirmed by our finding that pretreatment of the short term quiescent cell extracts with antibodies to either c-Myc or Max reduced the amount of complex formed.

We also performed studies using antisense oligodeoxynucleotides specific to c-Myc to test directly, in vivo, whether ODC could be a c-Myc/Max regulated gene. We were able to dramatically reduce (by ~80%) c-Myc mRNA and protein levels by antisense treatment. This reduction in c-Myc protein was reflected in a reduction in the amount of complex capable of binding to the −491 to −474 CACGTG element of the human ODC promoter and consequently, in the steady-state level of ODC mRNA.

If reduction in c-Myc levels causes a corresponding reduction in ODC expression presumably via reduced human ODC promoter activity, and if ODC is a c-Myc-Max regulated gene, it follows that an increase in c-Myc levels should lead to an increase in ODC promoter activity. Transcriptional analysis involving the use of the CAT reporter gene linked to the ODC promoter showed this to be the case.

Finally, additional evidence that c-Myc and Max can interact with this element in the human ODC promoter was provided by studies with purified recombinant proteins. We showed that purified NH_2 truncated c-Myc and full length Max proteins could interact with each other and the −491 to −474 region of the human ODC promoter. Interestingly, the type of interaction observed was dependent on the relative concentration of each protein. With constant amounts of Max and increased amounts of c-Myc, more c-Myc:Max heterodimer and less Max:Max homodimer formed and bound to the probe. This provides a clue about what might occur during quiescence. Previous studies from our laboratory have already shown that basal levels of c-Myc mRNA are maintained in short-term quiescent WI-38 cells but are reduced to below levels of detection (by RNAse protection) in long-term quiescent cells [Owen et al., 1987, 1989b; Ferrari et al., 1988]. In contrast, Max mRNA and protein levels have been shown to be extremely stable in a number of model systems [Blackwood et al., 1992]. We also have found no change in Max mRNA levels during prolonged quiescence or following stimulation. Although we have not measured c-Myc or Max protein levels in our model, it is tempting to speculate that as c-Myc levels become reduced during long term quiescence, the amount of Myc:Max heterodimers decrease and the amount of Max:Max homodimers increase. A number of reports suggest that Max:Max homodimers are transcriptionally inactive since Max lacks a transcriptional activation domain [Blackwood et al., 1992; Kato et al., 1992]. Thus it is possible that as the amount of these homodimers increase during long-term quiescence (when c-Myc levels are low), c-Myc/Max-regulated growth-associated gene expression (including ODC expression) in

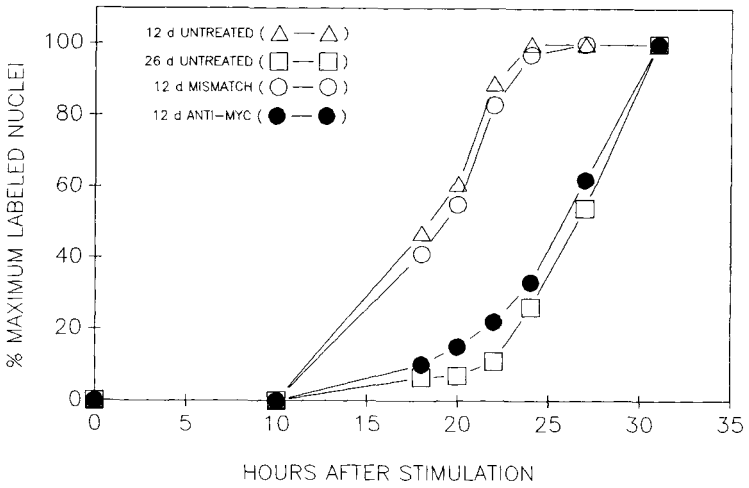

Fig. 5. Kinetics of entry into DNA synthesis following treatment of early (short-term or 12-day) G-0 WI-38 cells with antisense oligodeoxynucleotides specific to c-myc. WI-38 cells growing on glass coverslips were treated on day 11 after plating with 10 μM of either an 18 base oligodeoxynucleotide specific to amino acids 2–7 of human c-myc or a mismatched 18-base oligodeoxynucleotide in which the c-myc sequence was changed at three positions. The cells were stimulated on the 12th day after plating by replacement of the culture medium with complete DMEM supplemented with 10% fetal calf serum and ^{3}H-thymidine. At various times after stimulation, coverslips were harvested, fixed and processed for autoradiography as described by Owen et al. [1987, 1989a, 1990]. The number of labeled nuclei from at least 1,000 cells was determined per time point. The data show that early (short-term) G-0 cells in which c-myc levels have been reduced exhibit a prolongation of the prereplicative phase comparable to that exhibited by late (long-term) G-0 WI-38 cells.

long-term quiescent cells decreases. Conversely, one would predict that upon stimulation of long term quiescent WI-38 cells, c-myc levels increase, c-Myc:Max heterodimer level increases, and as a consequence, "competence genes" (i.e., genes necessary for the initiation of G-1 progression, including ODC) become expressed.

Highly provocative in vivo kinetic evidence for this model was provided by studies which showed that upon reduction of c-Myc levels in short-term quiescent WI-38 cells by c-Myc antisense oligodeoxynucleotide treatment, a prolongation of the prereplicative phase occurred upon serum stimulation comparable to that observed in long term quiescent WI-38 cells. Representative kinetic data from this experiment is shown in Figure 5.

A similar model has already been proposed by Kato et al. [1992] and Berberich and Cole [1992] based on indirect evidence from in vitro studies assaying the binding of recombinant proteins to an oligomer containing an optimal binding site for c-Myc and Max and a variety of co-transfection studies using c-Myc and Max expression plasmids and a number of chimeric reporter plasmids. Our data provide the first direct evidence for this model, showing both regulation of G-1 progression and transcriptional regulation of an important growth regulated gene by the c-Myc/Max complex.

CONCLUSION

We have described a unique model system to study regulation of mammalian cell growth. By an analysis of events that occur in this model system at the kinetic, biochemical and molecular level, we have been able to show that regulation of gene expression is intimately involved in the process by which a cell becomes competent to respond to growth factors and progress through the cell cycle to synthesize DNA and divide. While many of our observations in this model system are similar if not identical to those made in other model systems, the WI-38 long-term quiescence model has provided one extremely important piece of information about growth regulation that could not be obtained from any of the other models. Because of the prolonged period of time that long-term quiescent WI-38 cells require to become competent, we were able to identify a target for the c-myc gene. Although it has been known for a long time that c-myc plays an important growth-regulatory role, its precise function and target(s) has remained unknown. We now know, through our studies with the WI-38 model system, that c-myc is responsible, at least in part, for the transcriptional activation of the ODC gene, a gene essential for cell growth. Obviously, c-myc has more than one target and ODC is

regulated by more than one transcription factor. However, the value of the WI-38 cell long term quiescence model system as a tool for finding these additional growth regulatory factors has clearly been established. We anticipate that future follow-up studies in this model system will provide additional valuable information about the function of growth-regulatory genes in controlling cell cycle progression.

ACKNOWLEDGMENTS

This work was supported by grants from the W.W. Smith Charitable Trust (C9104) and the National Institutes of Health Public Health Service (CA 56309). The author also acknowledges the invaluable assistance of five former graduate students: Drs. Thomas Owen, Stephen Cosenza, Ruth Carter, Anne Donigan, and Angel Peña. The progress we have made in developing and understanding the WI-38 cell long term quiescent model system is directly the result of their hard work, effort and stimulating discussion over the past 7 years.

REFERENCES

Augenlicht LH, Baserga R (1974): Changes in the G-0 state of WI-38 fibroblasts at different times after confluence. Exp Cell Res 89:255–262.

Baserga R (1985): "The Biology of Cell Reproduction." Cambridge: Harvard University Press.

Berberich SJ, Cole MD (1992): Casein kinase II inhibits the DNA-binding activity of Max homodimers but not Myc/Max heterodimers. Genes Dev 6:166–176.

Blackwood EM, Luscher B, Eisenman RN (1992): Myc and Max associate in vivo. Genes Dev 6:71–80.

Carter R, Cosenza SC, Peña A, Lipson K, Soprano DR, Soprano KJ (1991): A potential role for c-jun in cell cycle progression through late G-1 and S. Oncogene 6:229–235.

Cosenza SC, Owen TA, Soprano DR, Soprano KJ (1988): Evidence that the time of entry into S is determined by events occurring in early G-1. J Biol Chem 263:12751–12758.

Denhardt DT, Edwards DR, Parfett CL (1987): Gene expression during the mammalian cell cycle. Biochim Biophys Acta 865:83–125.

Donigan AM, Cavalli RC, Peña A, Savage CR, Soprano DR, Soprano KJ (1993): Epidermal growth factor receptors lose ligand binding ability as WI-38 cells progress from short-term to long-term quiescence. J Cell Physiol 155: 164–170.

Ferrari S, Calabretta B, Battini R, Cosenza SC, Owen TA, Soprano KJ, Baserga R (1988): Expression of c-myc and induction of DNA synthesis by pletelet-poor plasma in human diploid fibroblasts. Exp Cell Res 174:25–33.

Gunther GR, Wang JL, Edelman GM (1974): The kinetics of cellular commitment during stimulation of lymphocytes by lectins. J Cell Biol 62:366–377.

Hartwell LH, Weinert TA (1989): Checkpoints: Controls that ensure the order of cell cycle events. Science 246:629–634.

Miska D, Bosmann HB (1980): Existence of an upper limit to elongation of the prereplicative period in confluent cultures of C3H10T1/2 cells. Biochem Biophys Res Commun 93:1140–1145.

Kato GJ, Lee WM, Chen L, Dang CV (1992): Max: Functional domains and interaction with c-myc. Genes Dev 6:81–92.

O'Farrell M, Yanez S (1989): Variation in the length of the lag phase following serum stimulation of mouse 3T3 cells. Cell Biol Int Rep 13:453–462.

Owen TA, Cosenza SC, Soprano DR, Soprano KJ (1989b): Evidence that stimulation of growth following long term density arrest of WI-38 cells proceeds via a pathway independent of protein kinase C and of cAMP dependent protein kinase. Oncogene Res 4:137–147.

Owen TA, Cosenza SC, Soprano DR, Soprano KJ (1987): Time of c-fos and c-myc expression in human diploid fibroblasts stimulated to proliferate after prolonged quiescence. J Biol Chem 262:15111–15117.

Owen TA, Soprano DR, Soprano KJ (1989a): Analysis of the growth factor requirements of WI-38 cells after extended periods of density-dependent growth arrest. J Cell Physiol 139:424–431.

Owen TA, Carter R, Whitman MW, Soprano DR, Soprano KJ (1990): Evidence that density-dependent growth arrest is a two-stage process. J Cell Physiol 142:137–148.

Pardee AB (1987): The yang and yin of cell proliferation: An overview. J Cell Physiol (Suppl) 5:107–110.

Pegg A (1988): Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. Cancer Res 48:759–774.

Peña A, Reddy CD, Wu S, Hickok N, Reddy EP, Yumet G, Soprano DR, Soprano KJ (in press): Regulation of human ornithine decarboxylase expression by the c-myc/max protein complex. J Biol Chem.

Pledger WJ, Stiles CD, Antoniades HN, Scher CD (1978): An ordered sequence of events is required before BALB/c 3T3 cells become committed to DNA synthesis. Proc Natl Acad Sci USA 75:2839–2843.

Pledger WJ, Stiles CD, Antoniades HN, Scher CD (1977): Induction of DNA synthesis in BALB/c 3T3 cells by serum components: Reevaluation of the commitment process. Proc Natl Acad Sci USA 74:4481–4485.

Prescott DM (1987): Cell reproduction. Int Rev Cytol 100:93–128.

Soprano KJ, Cosenza SC (1992): Cell cycle and cell growth control. In Stein G, Stein J, Lian J (eds): "Molecular and Cellular Approaches to the Control of Proliferation and Differentiation." Orlando, Florida: Academic Press, pp 73–114.

Tabor CW, Tabor H (1984): Polyamines. Annu Rev Biochem 53:747–790.