Articles

G_1 Events and Regulation of Cell Proliferation

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Cells prepare for S phase during the G_1 phase of the cell cycle. Cell biological methods have provided knowledge of cycle kinetics and of substages of G_1 that are determined by extracellular signals. Through the use of biochemical and molecular biological techniques to study effects of growth factors, oncogenes, and inhibitors, intracellular events during G_1 that lead to DNA synthesis are rapidly being discovered. Many cells in vivo are in a quiescent state (G_0), with unduplicated DNA. Cells can be activated to reenter the cycle during G_1. Similarly, cells in culture can be shifted between G_0 and G_1. These switches in and out of G_1 are the main determinants of post-embryonic cell proliferation rate and are defectively controlled in cancer cells.

THE G_1 PHASE OF THE CELL CYCLE IS A FUNCTIONAL PERIOD during which cells prepare for S phase, which is marked by beginning of DNA, histone, and some enzyme syntheses. Cell biological and biochemical studies carried out during the past quarter century reveal that most post-embryonic cells require many hours to transit a series of G_1 subphases, starting either from a G_0 nonproliferating quiescent state or from the previous cell cycle. Metabolic inhibitors, mutants, and the powerful techniques of molecular biology have provided many insights into the numerous biochemical reactions that are required during this long interval.

Control of post-embryonic cell proliferation occurs before S phase. [See the article in this issue by O'Farrell *et al.* regarding developmental controls (*1*).] Proliferation is defined as the increase in cell number resulting from completion of the cell cycle, as contrasted to growth, which is the increase in cell mass. Extracellular factors determine whether a quiescent cell will begin to proliferate and also whether a normal proliferating cell in G_1 will continue to cycle or will revert to quiescence. Cell-cycle events become largely independent of extracellular factors after cells enter into S phase, where they will go on to divide and produce two daughter cells. These later processes, such as mitosis, depend on intracellularly triggered controls [see Hartwell and Weinert (*2*) and Murray and Kirschner (*3*) in this issue].

In cancer, the control of proliferation is deranged. Studies comparing cancer and normal cells have helped to dissect and identify significant regulatory events. In addition to proliferation control, cell differentiation is also initiated in G_1. There is often an inverse relation between differentiation and proliferation. Deranged differentiation is a hallmark of cancer; regulation of differentiation is discussed in the article by O'Farrell *et al.* (*1*).

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This article is an overview of current ideas, illustrated with a few results, some of which are controversial. Papers published before 1985 are mainly referenced through reviews (*4*), and some published up to 1988 are referenced by brief summary articles (*5*). Only a few of the multitude of primary articles can be cited owing to space limitations.

This review is limited to fibroblastic cells in culture, which are responsive to proliferation stimulation. Most of these studies on G_1 have been with 3T3 mouse cells. Work that parallels and extends the results with fibroblasts has also been done with hematopoietic cells (*6*). Furthermore, the methods of genetics have revealed new information regarding G_1 events in yeast (*5, 7*), and these results can readily be extended to mammalian cells. Epithelial cells have been more difficult to culture; much has been discovered about their growth modifications by steroids and retinoids acting on intracellular receptors (*8*).

G_1, Cell Cycle, and G_0

G_1 was originally defined as a time interval, a gap between the readily observed events of mitosis and DNA synthesis. It is an interval requiring many hours in most cells, during which cells grow and inhibitors and mutations are effective in blocking proliferation. This kinetic definition is not useful for understanding mechanisms and has been the basis for some controversy because G_1 has a negligible duration in early embryos and in a few cultured cells (*9*). It has even been suggested that G_1 does not exist in that it is not important. An idea that reconciles the short G_1 times with essential biochemical events is that functional phases of the cycle overlap (*4, 10*). The G_1 events necessary for eventual onset of S phase can begin during the previous cycle, at the same time and in the same cell as other events such as DNA synthesis or preparation for mitosis (Fig. 1). Thus the observed kinetic G_1 interval between M and S phase depends on how much progress was made during the previous cycle. Cells with no apparent G_1 phase can accomplish most of these events before mitosis, but their G_1 interval appears if by inhibiting protein synthesis they are allowed to complete fewer processes. Preparation for mitosis may also begin in S and continue during the kinetically defined G_2 period. Two cycles have been proposed to be linked, one consisting of G_1-S, and the other of G_2-M (*4*). Their connections can be disrupted experimentally, thereby separating G_1-S from G_2-M (*11*), as discussed by Hartwell and Weinert in this issue (*2*).

Cells in vivo, for example hepatocytes and neurons, can remain healthy for very long periods in the nonproliferating or quiescent state often called G_0. Cells in culture can also be in a G_0 state. These G_0 arrested cells have an unduplicated DNA content, as do cycling cells in G_1. But G_0 and G_1 cells differ in a great many other properties (*4*). G_0 cells decrease in size because their protein and RNA molecules are degraded and are not rapidly resynthesized; macromolecular syntheses are about one-third as rapid in G_0 as in

proliferating cells. Enzymes and transmembrane transport activities are low in G_0 cells, and ribosomes are monosomal rather than polysomal (*4*). Some new RNAs and proteins appear after mammalian and yeast cells become quiescent (*12*). The distinction between G_0 and G_1 is central for cell proliferation, because the rate of increase of a population of cells is primarily dependent on the fraction of cells that are in cycle as contrasted to those in G_0.

Fibroblasts in culture move out of cycle and into G_0 within 1 hour after being placed in suboptimal conditions such as in medium with little serum (*13*). After G_0 cells are stimulated with additional serum to reenter the cycle during G_1, they require extra metabolism and therefore more time to reach S than do cells that have just finished mitosis. For example, 3T3 cells require at least 12 hours to pass from G_0 to S, whereas M to S in cycling cells requires about 6 hours.

Subphases of G_1

G_1 events require many hours and they appear to occur sequentially in a cause-effect relation. But very few biochemical "landmarks" have been identified that permit positions in G_1 to be fixed. Temperature-sensitive mutant cells are arrested at different positions in G_1. These mutations specifically block progression through G_1 and thereby provide a means to sequence metabolic events (*4*). Thus G_1 has been divided by several investigators into subphases (*14*), depending on the effects of limiting growth factors, nutrients, or inhibitors, as measured by time to reach S phase after the block is removed. These subphases are placed in sequence and are referred to here as competence, entry, progression, and assembly. Starting from G_0 these are separated by C, V, and R points, ending at S (Fig. 1). The roles assigned to the growth factors in this overview apply only to fibroblasts.

Competence. Untransformed BALB/c 3T3 mouse fibroblasts do not progress to S from G_0 if they are provided with either platelet-derived growth factor (PDGF) or plasma (which lacks PDGF). If they are first given PDGF briefly and subsequently provided with plasma, they progress to S phase, but not vice versa. Thus, these PDGF-treated cells carry out some initial processes called competence (*4, 14*). Competent cells require as much time to reach S as do G_0 cells (minimum of 12 hours for 3T3 cells). Other competence factors such as bombesin are active for other cells (*15*), but not all cells show a competence response. Very early events include changes in chromatin structure, increased transport of nutrients through the membrane, and production of novel mRNAs (*4, 5*).

Entry (G_{1a}). Competent cells progress to S phase after they are given plasma, which provides factors, such as epidermal growth factor (EGF) and insulin (*4, 16*). Competent cells incubated with plasma in a medium lacking essential amino acids move toward S up to a point named V. After the amino acids are provided, the cells at the V point require only 6 hours to reach S phase (as compared to 12 hours from competence). This length of time is very similar to the duration of G_1 (M to S) for the cycling cells, about 6 hours. Little is known about biochemistry during the G_0 to V period. It may be a time of recovery from G_0. Turnover of proteins and nucleic acids permits some synthesis of new macromolecules; polysomes and glycolytic enzymes increase.

Progression (G_{1b}). The only growth factor required (by 3T3 cells) to progress to S after the V point is insulin-like growth factor–1 (IGF-1) (*16*). This further progression requires net protein synthesis, as contrasted to "entry." This is shown by the requirement for amino acids, and also by an extreme sensitivity to inhibitors of protein synthesis; inhibition by only 50 to 70% causes exponentially growing cells, which originally were in all parts of the cycle, to continue to cycle until they stop with an unduplicated DNA content. Thus there is a specific requirement for rapid protein synthesis during this middle part of G_1; enzymes required for DNA synthesis are made during progression (*4*), and an important regulatory protein must also be made (*17*).

Assembly (G_{1c}). Serum and rapid protein synthesis are found not to be required during the final 2-hour transit to S phase, a surprisingly long interval (*13*), nor for the remainder of the cycle. Little is known about the intracellular events at the end of G_1. Movement of enzymes into the nucleus and their organization into a complex that catalyzes DNA synthesis might require considerable time (*18*).

Signaling Pathways from the Cell Membrane to the Nucleus

A series of signals to initiate DNA replication are transmitted from outside the cell to the nucleus (*4, 15, 19*). A generalized transduction sequence is given in Fig. 2, to serve as a frame of reference. Steps are numbered sequentially in time; they extend over the dozen hours required to reach S phase.

Step 1. Growth factors are the most frequently investigated stimulators of cell proliferation (*4*). They are small proteins; EGF is a single peptide chain of 53 amino acids. Other factors inhibit mammalian cells' growth; they include transforming growth factor–β (TGF-β) (*20*), interferons (*21*), and tumor necrosis factor (*22*).

Step 2. Growth factors combine with their specific receptors, which are proteins that extend across the plasma membrane. For example, the EGF receptor is a single peptide chain of 1186 amino acids. About half of these lie outside the membrane, 23 traverse the membrane, and the rest are in the cytoplasm. These receptor molecules dimerize when EGF binds to their extracellular sites. Their intracellular domains are thereby brought together, and their proximity activates autophosphorylation by the receptor's intracellular tyrosine kinase (*23*). The PDGF receptors are composed of the three combinations of two different subunits (*24*).

Step 3. Subsequent phosphorylation of tyrosines, serines, and threonines on other proteins may further transmit the signal (*19*). Other kinases are activated by less direct mechanisms. Some growth factors activate phospholipase C, which hydrolyzes inositol phospholipids to produce diacylglycerol and inositol phosphates (*15, 24*). Diacylglycerol activates protein kinase C. Phorbol ester also activates protein kinase C and thereby stimulates gene transcription and cell proliferation. Calcium ion is also required; it is released from intracellular stores by inositol trisphosphate, and EGF activates Ca^{2+} uptake into cells (*15, 25*). Other kinases are involved; cyclic adenosine monophosphate (cAMP)–dependent protein kinases are important for cell proliferation in yeast, and possibly mammalian cells. Calmodulin is a Ca^{2+}-binding protein that regulates other protein kinases (*26*). Protein phosphorylation is important in signal transmission, but most of the phosphorylated products and their functions are not well defined; as an example, an 80-kD protein is specifically phosphorylated by protein kinase C (*27*). Initial phosphorylations are often on tyrosine residues, but later phosphorylations are on serines and threonines. Thus, a cascade of kinases is activated that can react with these different amino acid residues (*19, 28*). One of these kinases is the product of the *raf* oncogene, which codes for a serine-threonine kinase (*29*). The kinases are balanced by phosphatases; these enzymes are also activated by growth factors (*30*). For example, the quantity of a phosphoprotein is increased when protein kinase C is stimulated by phorbol ester, but also when protein phosphatase activity is inhibited by okadaic acid (*31*). The enormous number of studies of roles of protein phosphorylation in cell signaling have been brought together recently (*19*).

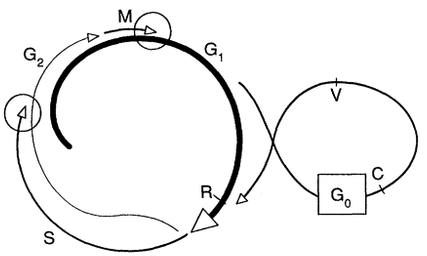

Fig. 1. A functional cell cycle. The conventional cell cycle is shown on the left. It is modified to indicate that G_1 activities, those preparatory for S phase, may begin during the previous cycle, concurrently with G_2 and mitotic events. Similarly, early preparation for mitosis (G_2) may overlap with S. Processes linking completion of M to continuation of G_1 and completion of S to continuation of G_2 are indicated by circles. On the right is indicated exit from G_1 into the G_0 quiescent state and reentry into the cycle. The critical points C, V, and R are also marked.

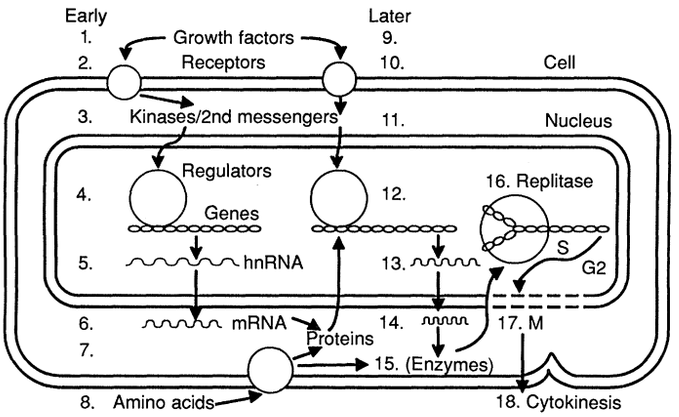

Fig. 2. Events in cell proliferation. This figure is a brief generalized depiction of sequential events that extend over the many hours from growth factor stimulation to S, numbered in the order of their occurrence.

Growth factors can activate different sequences in different cells and can cause different end effects (*15, 19*). Thus, TGF-β inhibits growth of some cells and stimulates growth of others (*17*). Thrombin activates Chinese hamster embryo fibroblast (CHEF) cells through a protein kinase C sequence; alternatively, EGF acts by a Ca^{2+}-calmodulin mechanism. Both factors activate the Na^+/H^+ antiport (*32*). Such results make generalizations difficult at present. Second messengers are a heterogeneous group of molecules that include phosphoproteins, Na^+, Ca^{2+} and H^+ ions, diacylglycerol, inositol phosphates, cyclic nucleotides, prostaglandins, and polyamines whose concentrations change after activation by growth factors (*15, 26*).

Step 4. What happens next to activate gene expression is under very active investigation. A model for gene induction was derived long ago for bacterial β-galactosidase; reversible binding of a repressor protein to a specific regulatory DNA region is changed by a galactoside ligand, and this activates the gene (*19*). Similarly, kinases activated by second messengers may modify binding of preexisting transacting "regulator" proteins of eukaryotic cells to specific promoter DNA sequences, thereby activating (or inactivating) transcription of adjacent genes (*5, 33*). Such specific DNA protein interactions are determined by retardation by proteins during electrophoresis of deoxynucleotide sequences, and also by ability of proteins to protect these sequences from a degradative enzyme. One example is the interaction of Fos and Jun proteins, which bind to one another. Jun but not Fos binds weakly to a DNA sequence in the upstream promoter regions of many genes; the combined proteins bind much more tightly. Protein kinase C phosphorylates Jun protein, and this activates *jun* gene transcription, thus creating autoregulated production of the Jun protein (*34*).

Step 5. Activation of genes is determined as appearance of new mRNA molecules. Heterogeneous nuclear RNAs (hnRNA) are produced initially; these are processed to shorter mRNAs and exported to the cytoplasm. Competent cells produce immediate-early mRNAs including *fos*, which appears in a few minutes, and *myc*, which appears several hours later. Some of these mRNAs turn over rapidly; their amounts peak for only a short time. They appear even when protein synthesis is inhibited, showing that their transcriptions do not require production of new proteins (*4, 5*). That some of them are necessary for progression is demonstrated by countereffects of their antisense oligodeoxynucleotides or of antibody to their proteins (*5, 35*).

Step 6. Many immediate-early mRNAs, including ones generated by PDGF, have been cloned by subtractive or differential hybridization (*4, 5*). These mRNAs are only a small fraction, about 1%, of the 10,000 mRNAs in a growing cell (*36*). Also only about 1% of the 1000 major proteins that can be identified on two-dimensional gels by labeling with [^{35}S]methionine are produced differentially in growing versus G_0 cells (*5, 37*). Phosphorylation of a few proteins also changes (*5, 19*), and, of these few, only a fraction may be primary modifiers of proliferation. Most molecules are made continuously for maintenance or "housekeeping" functions (*4*). The increases of some mRNAs and proteins are evident after cells are started from G_0, because their initial quantities may be much lower than in cycling cells owing to their degradation in G_0. For example, the mRNA and activity of thymidine kinase (TK) are low in G_0 and are increased by a factor of 40 in S phase (*38*). All molecules in a cell must double in each cycle, to produce a new cell, but these small increases between M and S may not be readily observed, particularly if the molecule under study is not degraded at the end of the previous cycle.

Step 7. The functions of most immediate-early proteins are not known. At least some of them, such as Fos, Jun, Myc, and Krox-20 (*39*), may be transactivating factors that bind to DNA sequences to activate genes later (*5*).

Step 8. Gene transcriptions that are initiated later in G_1 depend on protein synthesis and require that essential amino acids be brought into the cell by specific transport systems (*4*). Other genes are activated later, in at least two stages over a dozen hours, by second (EGF) and third (IGF-1) growth factors (*14, 15*), which again stimulate second messengers (*4*). These may interact with and activate the transactivating gene regulators that were formed earlier. For simplicity, only one of these sequences (in steps 9 to 15) is shown in Fig. 2.

Step 14. Few studies have identified the mRNAs and proteins that appear in mid- or late G_1. The *ras* gene can be activated in mid-G_1; its role there is indicated because antibody to Ras protein blocks progression if it is microinjected in the first but not the latter half of G_1. Ras is a guanosine triphosphate (GTP)–binding G protein whose GTPase activity is controlled by an accessory protein; its involvement in G_1 not clear (*19*).

Step 15. Several enzyme activities increase in mid-G_1, including transin (which is a protease), ornithine decarboxylase (which catalyzes polyamine synthesis), hydroxymethylglutaryl coenzyme A reductase for isoprenoid synthesis, and a 68-kD nuclear protein that is an RNA helicase; the p53 and p68 proteins also increase (*40*). Other proteins appear late in G_1, including enzymes involved in DNA synthesis: ribonucleotide reductase, dihydrofolate reductase, thymidylate synthase, thymidine kinase, DNA polymerase (*4*), and cyclin [also named proliferating cell nuclear antigen (PCNA)] (*4, 9, 39*). The mRNAs for a few of these enzymes also increased.

Step 16. Enzymes are produced on ribosomes in the cytoplasm; they must move to the nucleus to catalyze DNA synthesis. Enzyme migration is observed at the end of G_1. Within the nucleus, enzymes do not function in isolation but coalesce into a multienzyme

complex, whose molecular weight may be larger than that of a ribosome. Such a "replitase" complex has been purified tenfold and includes enzymes required for DNA replication such as DNA polymerase (*41*), enzymes that catalyze precursor syntheses, such as ribonucleotide reductase and dihydrofolate reductase, and TK, which is an enzyme for salvage of thymidine (*18*). These processes of migration and organization might occupy some of the time during the assembly period at the end of G_1.

A final event is required to start DNA synthesis even after the enzymes are assembled and when an ample supply of precursors is provided, as for instance to permeablized cells into which they can enter freely. Progress has been made recently with cell-free systems capable of initiating DNA synthesis (*42*). Initiation requires an enzyme that changes the conformation of DNA at the replication origin. These cell-free systems will allow the identification of the components required to initiate the final DNA biosynthetic reaction. This reaction, together with histone synthesis (*4, 5*), culminates the many hour–long chain of G_1 events. The cycle is completed by mitosis (step 17) and cytokinesis (step 18), which are discussed in McIntosh and Koonce (*43*) in this issue.

Control of Cell Proliferation

Cell proliferation is a tightly controlled process in higher organisms. The great majority of cells are quiescent in an adult vertebrate organism, and their DNA is unduplicated, as is the case for G_0 cells in culture. Cells can remain quiescent for long times or can increase rapidly in number, for example, during embryogenesis and in wound healing. A cell population in vivo proliferates at a rate dependent on the fraction of its cells that are in cycle rather than on the cycling time, which has a constant duration independent of external conditions (*4*). The cycle duration of cells in culture can be changed by various treatments (*4*). Physiological control of growth initiation is external; it is created by other cells as required in a multicellular organism. External controls switch the intracellular machinery between quiescence and G_1. Extracellular factors affect single-cell organisms in a different way, but control is exerted before DNA synthesis. Bacterial growth rates depend on the nutrient supply, such as the carbon source. Bacteria evolved to grow as rapidly as possible, and they cycle continuously under conditions of adequate nutrition. Conditions of extreme inadequacy or toxicity cause some bacteria to differentiate into spores. The growth of yeast cells is similar to that of bacteria, in that it is stopped by inadequate nutrition and is little affected by other cells. However, yeast cell growth is arrested by mating factors, which stop them before they duplicate their DNA (*5*). The circadian control of a dinoflagellate's proliferation also is executed before S phase (*44*). Only a few controlling events are known that critically determine proliferation, as contrasted to the myriad of housekeeping processes. The latter have minimal effects on proliferation when their activities are increased. Controls are exerted on the housekeeping processes to maintain a balance of cell metabolism. If one of these reactions is drastically inhibited, with drugs or by mutational blocks, cell proliferation will of course be stopped; but these are not physiological mechanisms of control.

How cell proliferation is regulated may be considered by asking how an all-or-none, off-or-on response is generated biochemically from the continuously graded stimulus that can be provided by growth factors. Which processes in the chain of signaling events throw the switch? One mechanism for obtaining a stepwise response to an extracellular signal depends on requiring a controlling molecule to reach a critical amount or concentration for activation of the next step in the signaling chain. This critical quantity might be required to titrate a constant amount of an inhibitory factor or to saturate a binding site such as a genetic promoter sequence. Concentration dependence is abrupt if several regulatory molecules must combine for activity. Multiple subunit bindings such as the Fos-Jun interaction provide an example (*34*).

Few critical regulatory processes have been identified. One is at the restriction point, R (*17*). Kinetic experiments suggest that a protein must accumulate to a critical amount in order for a cell to pass the R point. G_1 transit is highly sensitive to overall inhibition of protein synthesis, suggesting that this protein's rapid turnover requires rapid protein synthesis for its net accumulation. Furthermore, kinetics suggest that this protein is more stabilized in related, tumorigenic cells. A protein with a molecular mass of 68 kD was identified on two-dimensional gels as having the several required properties: (i) it is synthesized in G_1; (ii) it is unstable, with a half-life of a few hours; and (iii) it is stabilized and overproduced in tumor cells. This 68-kD protein is proposed to be a key regulatory element for proliferation (*37*).

Diverse mechanisms control times of appearance and amounts of gene products. Amounts of molecules depend on their degradation rates and not only on syntheses (*45*). Many molecules are rapidly degraded; ornithine decarboxylase and p53 proteins have half-lives of a half-hour or less. Thymidine kinase and topoisomerase II are particularly unstable at the end of the cell cycle; TK is degraded while still on the ribosome (*46*). The mRNAs for Fos and Myc similarly have short half-lives. Evidence is accumulating that TK production is regulated differently in various types of cells.

The enzymatic activity of TK and its mRNA are very low in G_0 cells; both rise dramatically near the onset of S phase. They are higher in cycling cells after division, and their relative increases are thus much smaller. These activities and initiation of DNA synthesis are controlled similarly in G_1. Thus TK provides alternative markers for the onset of S phase (*38*). A great advantage is that one can study control of production of this single gene's mRNA with molecular techniques, in contrast to the difficulties of investigating mechanisms that turn on the complex S phase events.

The transcription of TK is controlled by promoter sequences at the 5′ end of the gene; negatively regulating sequences have also been identified at greater distances. Regulatory proteins that bind to these sequences can retard their electrophoretic movement in gels. There is little evidence yet that binding of any regulatory protein has a role in progression through G_1. A DNA sequence upstream from the TK gene has been reported to be differently retarded by nuclear proteins obtained from cells that are commencing S phase as compared to the proteins from cells in G_1 (*38*). This "reverse approach," working backward from a very late G_1 event to the molecules that modulate to it, revealed an unexpected level on which control is exerted. A relatively small 3- to 5-fold increase in transcription was determined by run-on measurements, as compared to total TK mRNA, which rises 30-fold (*38*). Considerable regulation must therefore be post-transcriptional. Processing of TK hnRNA to its final product appears suddenly in the nucleus at the onset of S phase; TK mRNA also then appears in the nucleus and cytoplasm. Other examples of controlled hnRNA processing suggest that this is a general process (*47*).

Cancer Cells and Regulation of Proliferation

Regulation of cell proliferation is defective in cancer cells, which replicate in vivo at incorrect times and incorrect locations in the body. Cancerous cells appear spontaneously and can be induced with carcinogens or viruses that carry transforming genes (viral oncogenes). Cancer arises in a multistep process; cells progress to

stages of greater malignancy, characterized by more rapid growth, immortalization, invasiveness, metastasis, and angiogenesis. The *ras* oncogene has been implicated in this progression (*48*). Their genetic changes are mainly chromosomal rearrangements and deletions, although point mutations are also recognized as in *ras* (*49*).

Many aberrations in G_1 processes are observed in cancer cells. The switching mechanism between quiescence and proliferation is defective; cancer cells continue to cycle under conditions insufficient for normal cell proliferation (*4, 17*). Control is not totally eliminated, since many cultured cancer cells can be forced into quiescence if external conditions are made more extreme. In tumors in vivo, many cells are arrested with an unduplicated DNA content; they cycle only occasionally, which partly accounts for the relatively slow growth of many tumors (*4*).

The escape of cancer cells from extracellular growth control is shown by their ability to grow in culture in low concentrations of serum and in the absence of growth factors such as EGF. They also can grow in suspension, without requiring attachment on extracellular matrix (*50*). Their growth is arrested at higher cell density than that of normal cells (*4*). But they do not restart growth when more serum is added, as is the case for normal cells; rather they require nutrients that have been exhausted from the medium (*51*). Is quiescence of a tumor cell actually the same as the G_0 state of a normal cell, or might cancer cells stop at the V point owing to a suboptimal nutrient supply? In support of this possibility, some macromolecules are present in arrested tumor cells that are absent in normal cells in G_0. These may include a nucleolar antigen, the M1 subunit of ribonucleotide reductase, protein p68, an antigen named Ki, and *myc* mRNA (*52*).

Cancer cells provide valuable tests of the physiological significance of proposed growth-regulating processes. Alteration of a process in a cancer cell suggests that it is important in proliferative control. As examples, cells from cancers often have a greater number of EGF receptors than their normal counterparts, suggesting that EGF stimulation can limit normal cell growth in vivo. Many but not all tumors overexpress or alter cellular proto-oncogenes relative to normal cells (*53*). Retroviral oncogenes can alter functions at many sites along the signaling pathway (Fig. 2) (*5, 54*). Some transformed cells produce TGF-α, which replaces EGF as a growth factor. The v-*sis* gene codes for the B chain of PDGF, thereby eliminating the requirement for a competence factor. Overexpression of c-*myc* also eliminates the PDGF requirement (*55*). At the next level, the v-*erb* B gene codes for a truncated EGF receptor, which diminishes the EGF requirement. The v-*src* gene codes for a kinase that phosphorylates many other proteins, some of which may function as second messengers. The v-*ras* gene codes for a 21-kD protein that is involved in mid-G_1 in a yet unclear way. Other oncogenes such as *fos*, *jun*, and *myc* code for proteins that move to the cell nucleus and most likely activate proliferation-related genes. Retroviral transformed cells overproduce and stabilize the p68 protein whose production has been proposed to cause 3T3 cells to pass R (*37*). Thus, various steps in G_1 that are normally initiated by growth factors can be activated or short-circuited by viral oncogenes; thereby growth is less restricted. This loss of control does not permit the conclusion that the altered step limits proliferation of normal cells.

The oncogene products of DNA viruses, such as large T antigen of SV40 and the E1A protein of adenoviruses, appear to cancel the proliferation controls in G_1 (*5*). They recently have been shown to complex with the protein encoded by the normal cell's retinoblastoma gene protein, thereby removing this block in the proliferative pathway. The transforming genes of other DNA virus, including human papilloma virus, probably act in a similar way (*51*). A homolog of large T antigen has been identified in normal human cells. It is a 68-kD protein with RNA-dependent adenosine triphosphatase activity and proposed helicase activity (*56*).

One expects much to be learned about balanced control of proliferation from oncogenes and tumor-suppressor genes. The importance of genes that negatively regulate G_1 events and cell proliferation has quite recently come to the forefront (*57, 58*). For many years, fused normal and tumorigenic cells were known to form hybrids that have the normal phenotype. Thus, normal cells express a tumor-suppressing activity.

A similar conclusion is reached from studies of the gene involved in retinoblastoma. The protein coded for by the normal retinoblastoma gene appears to be a critical regulatory molecule. Mutations and deletions of this gene are found in retinoblastomas and in other tumors, suggesting that it is a suppressing anti-oncogene. Its transduction into tumor cells is reported to suppress tumorigenicity. This protein is underphosphorylated during most of G_1 and becomes further phosphorylated in late G_1 or early S phase. This additional phosphorylation may release its suppressing action on control genes so as to permit progression through G_1 (*59*).

The p53 protein is of unusual interest. It was originally identified by being immunologically coprecipitated with T antigen from SV40-transformed cells. The p53 gene was originally classified as an oncogene because it transformed NIH 3T3 cells. But this originally investigated gene may have been mutated; mutated p53 has been found in many cancers (*60*). The p53 gene of normal cells may actually be a tumor suppressor that has lost a suppressing role in its mutated form. The latter can dimerize with the normal p53 protein to produce a nonsuppressing complex (*61*).

Conclusions

Our understanding of processes required to initiate DNA replication and to control normal and defective proliferation has been greatly increased owing to techniques of molecular biology. We expect numerous exciting discoveries, based on these methods. Detailed biochemical studies will be required to substantiate many of the processes that have been proposed from the experiments summarized here, such as the roles of various oncogenes and the p68, p53, and retinoblastoma proteins.

A fundamental general concept that should prove fruitful is that regulatory constituents are in a dynamic steady state. The rapid, large changes in internal molecules effected by modest alterations of external factors, as contrasted to sluggish responses of stable molecules, show that the steady-state mechanism is particularly suitable for control (*17*). Production of a regulatory factor ideally should be highly responsive to extracellular stimuli. A fine-tuned response can be obtained if the molecule is dynamically turning over, as are many substances both large and small. Balance of synthesis and degradation then determines a steady-state level that is rapidly changed if either rate is altered. Examples of dynamic regulation occur at many levels; quantities of RNAs and proteins are determined by their rates of both synthesis and degradation (*45*), and amounts of growth factor receptors are determined by their rates of production and downregulation.

Several methods are available to determine whether proposed elements affect proliferation. Introduction into cells of genes, mRNAs, proteins, antisense messages, or antibodies can be used to examine modifications of cell growth (*5, 35*). Recent results with yeast cell-cycle mutants and transfer of genes between yeast and mammalian cells demonstrate that studies of yeast will aid in understanding the mammalian cell cycle as discussed in this issue by Murray and Kirschner (*3*). These studies already show that genes controlling G_2 of yeasts also affect G_1, and phosphorylations are cell

cycle–dependent (*5, 62*). Genetic exchanges between yeast and mammalian cells are valuable because their cycles are quite similar and because mammalian cell mutants have not yet provided as much information as has been hoped; they are difficult to produce (owing to diploidy) and complex to study. Mammalian genes that suppress temperature-sensitive cell-cycle mutations in yeast and mammalian cells (*63*) are being isolated. Finally, these recent fundamental discoveries about growth regulation should provide both theoretical and practical approaches to cancer treatment.

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64. We thank S. Cravo Rains for excellent secretarial assistance. This work was supported by PHS grant 24571.