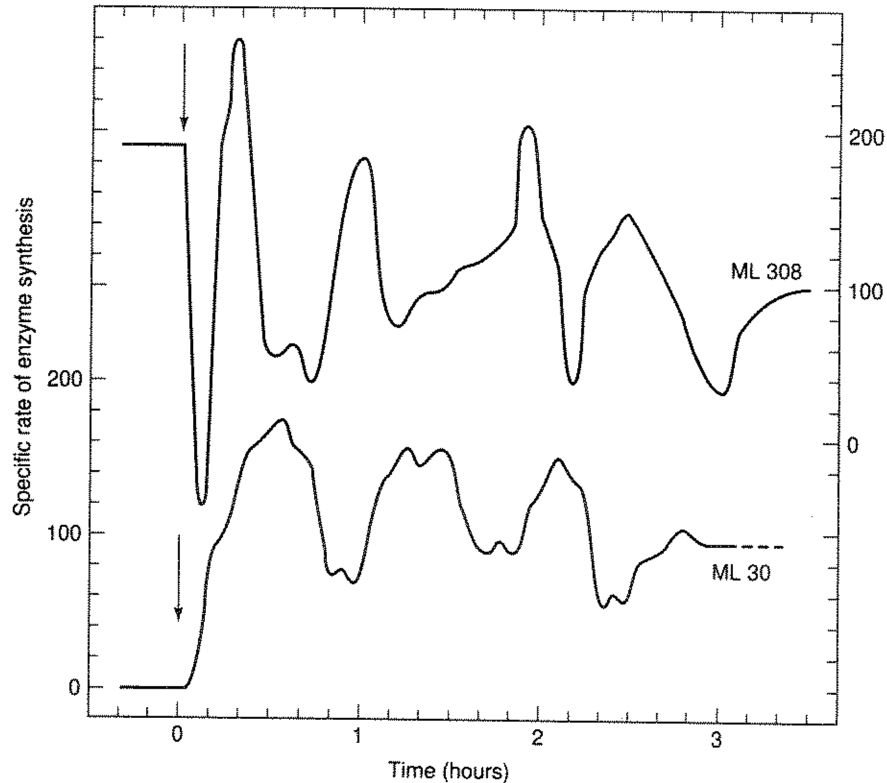

Figure 13.3 Oscillations of the specific rate of β-galactosidase synthesis in *E. coli* strains ML30 and ML 308. (Knorre, 1968).

13.2 Cell Cycle Control

The *cell-division cycle* is that process by which a cell duplicates its contents and then divides in two. The adult human must manufacture many millions of new cells each second simply to maintain the status quo, and if all cell division is halted, the individual will die within a few days. On the other hand, abnormally rapid cell proliferation, i.e., *cancer*, can also be fatal, as rapidly proliferating cells interfere with the function of "normal" cells and organs. Control of the cell cycle involves, at a minimum, control of cell growth and replication of nuclear DNA in such a way that the size of the individual cells remains, on average, constant.

The cell cycle is traditionally divided into several distinct phases (shown schematically in Fig. 13.4), the most dramatic of which is mitosis or *M phase*. Mitosis is characterized by separation of previously duplicated nuclear material, nuclear division, and finally the actual cell division, called *cytokinesis*. In most cells the whole of *M* phase takes only about an hour, a small fraction of the total cycle time. The much longer period of time between one *M* phase and the next is called *interphase*. The por-

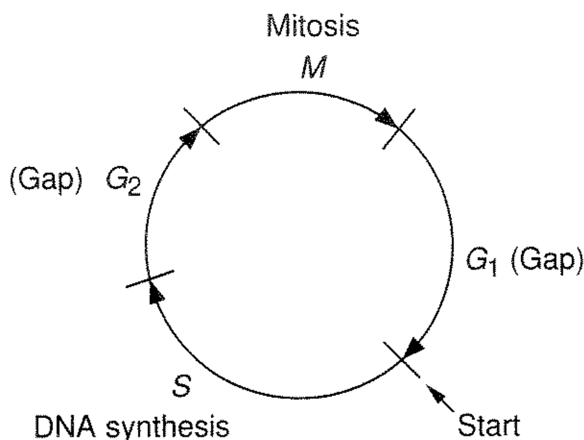

Figure 13.4 Schematic diagram of the cell cycle.

tion of interphase following cytokinesis is called G_1 *phase* (G = gap), during which cell growth occurs. When the cell is sufficiently large, DNA replication in the nucleus is initiated and continues during S *phase* (S = synthesis). Following S phase is G_2 *phase*, providing a safety gap during which the cell is presumably preparing for M phase, to ensure that DNA replication is complete before the cell plunges into mitosis.

There are actually two controlled growth processes. There is the chromosonal cycle, in which the genetic material is exactly duplicated and two nuclei are formed from one for every "turn" of the cycle. Accuracy is essential to this process, since each daughter nucleus must receive an exact replica of each chromosome. A less tightly controlled process, the cytoplasmic cycle, duplicates the cytoplasmic material, including all of the structures (mitochondria, organelles, sarcoplasmic reticulum, etc.). This growth is continuous during the G_1, S, and G_2 phases, pausing briefly only during mitosis.

In mature organisms these two processes operate in coordinated fashion, so that the ratio of cell mass to nuclear mass remains essentially constant. However, it is possible for these two to be uncoupled. For example, during *oogenesis*, a single cell (an *ovum*) grows in size without division. After fertilization, during *embryogenesis*, the egg undergoes twelve rapid synchronous mitotic divisions to form a ball consisting of 4096 cells, called the *blastula*.

There is strong evidence that these early embryonic divisions are controlled by a cytoplasmic biochemical limit cycle oscillator. For example, if fertilized (*Xenopus*) frog eggs are enucleated, they continue to exhibit periodic "twitches" or contractions, as if the cytoplasm continued to generate a signal in the absence of a nucleus. Enucleated sea urchin eggs go a step further by actually dividing a number of times before they notice that they contain no genetic material and consequently die.

The cell cycle has been studied most extensively for frogs and yeast. Frog eggs are useful because they are large and easily manipulated. Yeast cells are too small for these kinds of studies, but are suitable for cloning and identification of the involved genes and gene products. The budding yeast *Saccharomyces cerevisiae*, used by brewers and

bakers, divides by first forming a bud that is initiated and grows steadily during S and G_2 phases, and finally separates from its mother after mitosis.

13.2.1 The G_1 Checkpoint

The autonomous cell cycle oscillations seen in early embryos are unusual. Most cells proceed through the division cycle in fits and starts, pausing at "checkpoints" to ensure that all is ready for the next phase of the cycle. There are checkpoints at the end of the G_1, G_2, and M phases of the cell cycle, although not all cells use all of these checkpoints. During early embryogenesis, however, the checkpoints are inoperable, and cells divide as rapidly as possible, driven by the underlying limit cycle oscillation.

The G_1 checkpoint is often called *Start*, because here the cell determines whether all systems are ready for S phase and the duplication of DNA. Before Start, newly born cells are able to leave the mitotic cycle and differentiate (into nondividing cells with specialized function). However, after Start, they have passed the point of no return and are committed to another round of DNA synthesis and division.

As with all cellular processes, the cell cycle is regulated by genes and the proteins that they encode. There are two classes of proteins that form the basis of the cell-cycle control system. The first is the family of *cyclin-dependent protein kinases* (Cdk), which induce a variety of downstream events by phosphorylating selected proteins. The second family are the *cyclins*, so named because the first members to be identified are cyclically synthesized and degraded in each division cycle of the cell. Cyclins bind to Cdk molecules and control their ability to phosphorylate target proteins, but without cyclin, Cdk is inactive. In budding yeast cells there is only one major Cdk and nine cyclins, leading to a possibility of nine active cyclin–Cdk complexes. In mammals, the story is substantially more complicated, as there are (at last count) six Cdks and more than a dozen cyclins.

The critical chemicals for getting through the G_1 and G_2 checkpoints are known as *S-phase promoting factor* (SPF) and *M-phase promoting factor* (MPF) respectively. These are *heterodimers* because they consist of two essential subunits, a Cdk and a cyclin. A schematic diagram of the cell cycle is shown in Fig. 13.5.

The molecular events that constitute Start are most thoroughly understood for budding yeast. The major events triggered by Start are DNA synthesis and bud emergence. DNA synthesis appears to be triggered by a Cdk called Cdc28 (Cdc for cell-division-cycle; remember that in yeast there is only one major Cdk) in association with either cyclin Clb5 or cyclin Clb6. Bud emergence seems to depend on Cdk association with cyclin Cln1 or Cln2. These four cyclins are subject to rapid degradation, so their levels in the cell are controlled by their rates of transcription. Their transcription, in turn, is controlled by two transcription factors, SBF and MBF. As the cyclins accumulate and associate with Cdc28, they activate their own transcription factors, so their rate of accumulation increases. These positive feedback loops lead to autocatalytic production of the cyclins, ensuring that the cells pass Start decisively and irreversibly by an explosive activation of Start kinase. In addition, there is another activator, a complex of Cdc28

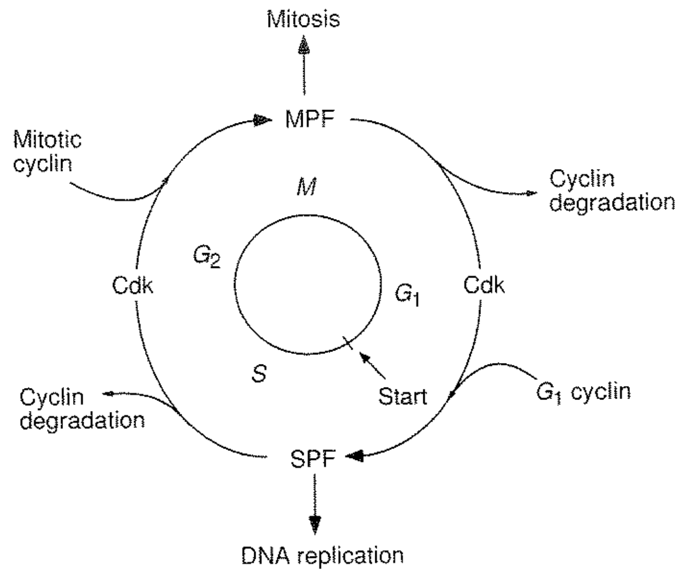

Figure 13.5 Schematic diagram of the primary chemical reactions of the cell cycle.

with cyclin Cln3, which is thought to activate SBF and MBF in a cell-size-dependent fashion, although the mechanism of this size dependence is not known.

A simple mathematical model of this process (following Tyson et al., 1995) can reveal how the G_1 checkpoint works. We assume that there is one transcription factor, SBF, denoted by S_a when active, and by S_i when inactive. The transcription factor is rendered active by a Cln cyclin (the only cyclin in the model), denoted by N, and by a starter kinase (Cdc28-Cln3), denoted by A. The transcription factor is rendered inactive by another chemical, a phosphatase, denoted by E. The cyclin is produced via activation of SBF and degrades naturally according to

$$\xrightarrow{S_a} \mathrm{N} \xrightarrow{k_2}, \tag{13.16}$$

so that

$$\frac{d[\mathrm{N}]}{dt} = \frac{k_1[\mathrm{S}_a]}{k_s + [\mathrm{S}_a]} - k_2[\mathrm{N}]. \tag{13.17}$$

The SBF is activated by both Cln and the starter kinase and is deactivated by the phosphatase via

$$\mathrm{S}_i \underset{E}{\overset{\mathrm{N+A}}{\rightleftarrows}} \mathrm{S}_a, \tag{13.18}$$

leading to the equation

$$\frac{d[\mathrm{S}_a]}{dt} = k_3([\mathrm{A}] + [\mathrm{N}])\frac{[\mathrm{S}_i]}{k_i + [\mathrm{S}_i]} - k_4[\mathrm{E}]\frac{[\mathrm{S}_a]}{k_a + [\mathrm{S}_a]}. \tag{13.19}$$

We assume that $[S_a] + [S_i] = C$, a constant. Then, in terms of the nondimensional variables $[S_a] = Cs, [N] = k_1/k_2 n, t = \tau/k_2$, we have

$$\frac{dn}{d\tau} = \frac{s}{\kappa_s + s} - n, \tag{13.20}$$

$$\frac{ds}{d\tau} = (\alpha + \lambda n)\frac{1-s}{\kappa_i + 1 - s} - \mu\frac{s}{\kappa_a + s}, \tag{13.21}$$

where $\alpha = \frac{k_3[A]}{k_2 C}, \lambda = \frac{k_3 k_1}{k_2^2 C}, \mu = \frac{k_4[E]}{k_2 C}, \kappa_s = k_s/C, \kappa_i = k_i/C, \kappa_a = k_a/C$.

The behavior of this system is readily exposed by its phase portrait. The nullclines are the curves $\frac{dn}{d\tau} = 0$ and $\frac{ds}{d\tau} = 0$. It is apparent that for the $\frac{ds}{d\tau} = 0$ nullcline, n is decreasing as a function of the control parameter α, for fixed s. In Fig. 13.6 are shown examples of the nullclines, $\frac{dn}{d\tau} = 0$ a dashed curve, and $\frac{ds}{d\tau} = 0$ as solid curves, for two different values of $\alpha = 2.5$ and 3.8. Other parameter values are $\kappa_s = 1.0, \kappa_i = 0.1, \kappa_a = 0.001, \lambda = 37.0, \mu = 4.0$.

The behavior of this model for the G_1 checkpoint is now easily described. There are either one or three steady solutions. The steady solution with s saturated (near 1) is a stable steady state that always exists, and corresponds to high levels of cyclin and activation of Start. For large values of the control parameter α, corresponding to large cell size, this is the only steady solution. However, for small cell size, and hence, small values of the control parameter α, there are three steady states, the smallest and largest being stable and the intermediate being an unstable saddle point.

We suppose that during G_1 phase with α small the system sits at the small steady-state solution. However, as the cell grows, the value of α increases (by an unknown mechanism), reaching a critical value at which the small steady-state solution disappears (through a saddle-node bifurcation), and the system quickly switches to the large steady state, at which production of cyclin is high, enabling the rapid production of S-phase promoting factor.

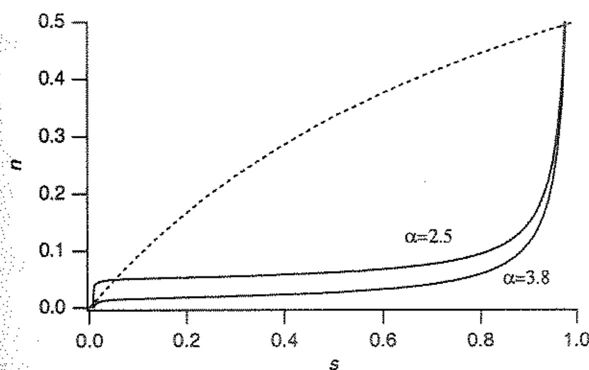

Figure 13.6 Nullclines for the system of equations (13.20), (13.21), with the nullcline $\frac{dn}{dt} = 0$ for (13.20) shown as a dashed curve and nullclines $\frac{ds}{dt} = 0$ for (13.21) shown as solid curves, shown for $\alpha = 2.5$ and 3.8.