Regulators of Cell Cycle Progression

One of the most exciting developments of the last decade has been the elucidation of the molecular mechanisms that control the progression of eukaryotic cells through the division cycle. Our current understanding of cell cycle regulation has emerged from a convergence of results obtained through experiments on organisms as diverse as yeasts, sea urchins, frogs, and mammals. These studies have revealed that the cell cycle of all eukaryotes is controlled by a conserved set of protein kinases, which are responsible for triggering the major cell cycle transitions.

MPF: A Dimer of Cdc2 and Cyclin

Three initially distinct experimental approaches contributed to identification of the key molecules responsible for cell cycle regulation. The first of these avenues of investigation originated with studies of frog oocytes (Figure 14.12). These oocytes are arrested in the G_2 phase of the cell cycle until hormonal stimulation triggers their entry into the M phase of meiosis (discussed later in this chapter). In 1971, two independent teams of researchers (Yoshio Masui and Clement Markert, as well as Dennis Smith and Robert Ecker) found that oocytes arrested in G_2 could be induced to enter M phase by microinjection of cytoplasm from oocytes that had been hormonally stimulated. It thus appeared that a cytoplasmic factor present in hormone-treated oocytes was sufficient to trigger the transition from G_2 to M in oocytes that had not been exposed to hormone. Because the entry of oocytes into meiosis is frequently referred to as oocyte maturation, this cytoplasmic factor was called **maturation promoting factor (MPF)**. Further studies showed, however, that the activity of MPF is not restricted to the entry of oocytes into meiosis. To the contrary, MPF is also present in somatic cells, where it induces entry into M phase of the mitotic cycle. Rather than being specific to oocytes, MPF thus appeared to act as a general regulator of the transition from G_2 to M.

The second approach to understanding cell cycle regulation was the genetic analysis of yeasts, pioneered by Lee Hartwell and his colleagues in the early 1970s. Studying the budding yeast *Saccharomyces cerevisiae*, these investigators identified temperature-sensitive mutants that were defective in cell cycle progression. The key characteristic of these mutants (called *cdc* for *c*ell *d*ivision *c*ycle mutants) was that they underwent growth arrest at specific points in the cell cycle. For example, a particularly important mutant designated *cdc28* caused the cell cycle to arrest at START, indicating that the Cdc28 protein is required for passage through this critical regulatory point in G_1 (Figure 14.13). A similar collection of cell cycle mutants was isolated in the fission yeast *Schizosaccharomyces pombe* by Paul Nurse and his collaborators. These mutants included *cdc2,* which arrests the *S. pombe* cell cycle both in G_1 and at the G_2 to M transition (the major regulatory point in fission yeast). Further studies showed that *S.. cerevisiae cdc28* and *S. pombe cdc2* are functionally homologous genes, which are required for passage through START as well as for entry into mitosis in both species of yeasts. To avoid confusion resulting from the difference in genetic nomenclature between *S.. cerevisiae* and *S. pombe*, the protein encoded by both genes will be called Cdc2 in this text. Further studies of *cdc2* yielded two important insights. First, molecular cloning and nucleotide sequencing revealed that *cdc2* encodes a protein kinase—the first indication of the prominent role of

protein phosphorylation in regulating the cell cycle. Second, a human gene related to *cdc2* was identified and shown to function in yeasts, providing a dramatic demonstration of the conserved activity of this cell cycle regulator.

The third line of investigation that eventually converged with the identification of MPF and yeast genetics emanated from studies of protein synthesis in early sea urchin embryos. Following fertilization, these embryos go through a series of rapid cell divisions. Intriguingly, studies with protein synthesis inhibitors had revealed that entry into M phase of these embryonic cell cycles requires new protein synthesis. In 1983, Tim Hunt and his colleagues identified two proteins that display a periodic pattern of accumulation and degradation in sea urchin and clam embryos. These proteins accumulate throughout interphase and are then rapidly degraded toward the end of each mitosis (Figure 14.14). Hunt called these proteins cyclins (the two proteins were designated cyclin A and cyclin B) and suggested that they might function to induce mitosis, with their periodic accumulation and destruction controlling entry and exit from M phase. Direct support for such a role of cyclins was provided in 1986, when Joan Ruderman and her colleagues showed that microinjection of cyclin A into frog oocytes is sufficient to trigger the G_2 to M transition.

These initially independent approaches converged dramatically in 1988, when MPF was purified from frog eggs in the laboratory of James Maller. Molecular characterization of MPF in several laboratories then showed that this conserved regulator of the cell cycle is composed of two key subunits: Cdc2 and cyclin B (Figure 14.15). Cyclin B is a regulatory subunit required for catalytic activity of the Cdc2 protein kinase, consistent with the notion that MPF activity is controlled by the periodic accumulation and degradation of cyclin B during cell cycle progression.

A variety of further studies have confirmed this role of cyclin B, as well as demonstrating the regulation of MPF by phosphorylation and dephosphorylation of Cdc2 (Figure 14.16). In mammalian cells, cyclin B synthesis begins in S phase. Cyclin B then accumulates and forms complexes with Cdc2 throughout S and G_2. As these complexes form, Cdc2 is phosphorylated at two critical regulatory positions. One of these phosphorylations occurs on threonine-161 and is required for Cdc2 kinase activity. The second is a phosphorylation of tyrosine-15, and of the adjacent threonine-14 in vertebrates. Phosphorylation of tyrosine-15, catalyzed by a protein kinase called Wee1, inhibits Cdc2 activity and leads to the accumulation of inactive Cdc2/cyclin B complexes throughout S and G_2. The transition from G_2 to M is then brought about by activation of the Cdc2/cyclin B complex as a result of dephosphorylation of threonine-14 and tyrosine-15 by a protein phosphatase called Cdc25.

Once activated, the Cdc2 protein kinase phosphorylates a variety of target proteins that initiate the events of M phase, which are discussed later in this chapter. In addition, Cdc2 activity triggers the degradation of cyclin B, which occurs as a result of ubiquitin-mediated proteolysis. This proteolytic destruction of cyclin B then inactivates Cdc2, leading the cell to exit mitosis, undergo cytokinesis, and return to interphase. TOP

Families of Cyclins and Cyclin-Dependent Kinases

The structure and function of MPF (Cdc2/cyclin B) provide not only a molecular basis for understanding entry and exit from M phase, but also the foundation for elucidating the regulation of other cell cycle transitions. The insights provided by characterization of the Cdc2/cyclin B complex have thus had a sweeping impact on understanding cell cycle regulation. In particular, further research has established that both Cdc2 and cyclin B are members of large families of related proteins, with different members of these families controlling progression through distinct phases of the cell cycle.

As discussed earlier, Cdc2 controls passage through START as well as entry into mitosis in yeasts. It does so, however, in association with distinct cyclins (Figure 14.17). In particular, the G_2 to M transition is driven by Cdc2 in association with the mitotic B-type cyclins (Clb1, Clb2, Clb3, and Clb4). Passage through START, however, is controlled by Cdc2 in association with a distinct class of cyclins called **G_1cyclins** or **Cln's**. Cdc2 then associates with different B-type cyclins (Clb5 and Clb6), which are required for progression through S phase. These associations of Cdc2 with distinct B-type and G_1 cyclins direct Cdc2 to phosphorylate different substrate proteins, as required for progression through specific phases of the cell cycle.

The cell cycles of higher eukaryotes are controlled not only by multiple cyclins, but also by multiple Cdc2-related protein kinases. These Cdc2-related kinases are known as **Cdk's** (for *c*yclin-*d*ependent *k*inases). As the original member of this family, Cdc2 is also known as Cdk1, with other currently identified family members being designated Cdk2 through Cdk8.

These multiple members of the Cdk family associate with specific cyclins to drive progression through the different stages of the cell cycle (see Figure 14.17). For example, progression from G_1 to S is regulated principally by Cdk2 and Cdk4 (and in some cells Cdk6) in association with cyclins D and E. Complexes of Cdk4 and Cdk6 with the D-type cyclins (cyclin D1, D2, and D3) play a critical role in progression through the restriction point in G_1. Cyclin E is expressed later in G_1, and Cdk2/cyclin E complexes are required for the G_1 to S transition and initiation of DNA synthesis. Complexes of Cdk2 with cyclin A function in the progression of cells through S phase. As already discussed, the transition from G_2 to M is then driven by complexes of Cdc2 with cyclin B.

The activity of Cdk's during cell cycle progression is regulated by at least four molecular mechanisms (Figure 14.18). As already discussed for Cdc2, the first level of regulation involves the association of Cdk's with their cyclin partners. Thus, the formation of specific Cdk/cyclin complexes is controlled by cyclin synthesis and degradation. Second, activation of Cdk/ cyclin complexes requires phosphorylation of a conserved Cdk threonine residue around position 160. This activating phosphorylation of the Cdk's is catalyzed by an enzyme called CAK (for *C*dk-*a*ctivating *k*inase), which may itself be composed of a Cdk (Cdk7) complexed with cyclin H. Complexes of Cdk7 and cyclin H are also associated with the transcription factor TFIIH, which is required for initiation of transcription by RNA polymerase II (see Chapter 5). It thus appears that this member of the Cdk family may participate in transcription as well as cell cycle regulation.

In contrast to the activating phosphorylation by CAK, the third mechanism of Cdk regulation involves inhibitory phosphorylation of tyrosine residues near the Cdk amino terminus, catalyzed by the Wee1 protein kinase. In particular, both Cdc2 and Cdk2 are inhibited by phosphorylation of tyrosine-15, and the adjacent threonine-14 in vertebrates. These Cdk's are then activated by dephosphorylation of these residues by members of the Cdc25 family of protein phosphatases.

In addition to regulation of the Cdk's by phosphorylation, their activities can also be controlled by the binding of inhibitory proteins (called Cdk inhibitors or **CKIs**) to Cdk/cyclin complexes. In mammalian cells, two families of Cdk inhibitors are responsible for regulating different Cdk/cyclin complexes (Table 14.1). Members of the Cip/Kip family regulate all stages of progression through G_1 and S phase by inhibiting complexes of Cdk2, 4, and 6 with cyclins A, D, and E. In contrast, members of the Ink4 family are specific for complexes of Cdk4 and 6 with cyclin D, so the Ink4 CKIs only regulate progression through the restriction point in G_1. In yeast, different CKIs similarly regulate distinct stages of cell cycle progression by inhibiting specific Cdk/cyclin complexes. Control of Cdk inhibitors thus provides an additional mechanism for regulating Cdk activity. The combined effects of these multiple modes of Cdk regulation are responsible for controlling cell cycle progression in response both to checkpoint controls and to the variety of extracellular stimuli that regulate cell proliferation.

Growth Factors and the D-Type Cyclins

As discussed earlier, the proliferation of animal cells is regulated largely by a variety of extracellular growth factors that control the progression of cells through the restriction point in late G_1. In the absence of growth factors, cells are unable to pass the restriction point and become quiescent, frequently entering the resting state known as G_0, from which they can reenter the cell cycle in response to growth factor stimulation. This control of cell cycle progression by extracellular growth factors implies that the intracellular signaling pathways stimulated downstream of growth factor receptors (discussed in the preceding chapter) ultimately act to regulate components of the cell cycle machinery.

One critical link between growth factor signaling and cell cycle progression is provided by the D-type cyclins (Figure 14.19). Cyclin D synthesis is induced in response to growth factor stimulation as a result of signaling through the Ras/Raf/ERK pathway, and the D-type cyclins continue to be synthesized as long as growth factors are present. However, the D-type cyclins are also rapidly degraded, so their intracellular concentrations rapidly fall if growth factors are removed. Thus, as long as growth factors are present through G_1, complexes of Cdk4, 6/cyclin D drive cells through the restriction point. On the other hand, if growth factors are removed prior to this key regulatory point in the cell cycle, the levels of cyclin D rapidly fall and cells are unable to progress through G_1 to S, instead becoming quiescent and entering G_0. The inducibility and rapid turnover of D-type cyclins thus integrates growth factor signaling with the cell cycle machinery, allowing the availability of extracellular growth factors to control the progression of cells through G_1.

Since cyclin D is a critical target of growth factor signaling, it might be expected that defects in cyclin D regulation could contribute to the loss of growth regulation

characteristic of cancer cells. Consistent with this expectation, many human cancers have been found to arise as a result of defects in cell cycle regulation, just as many others result from abnormalities in the intracellular signaling pathways activated by growth factor receptors (see Chapter 13). For example, mutations resulting in continual unregulated expression of cyclin D1 contribute to the development of a variety of human cancers, including lymphomas and breast cancers. Similarly, mutations that inactivate the Ink4 Cdk inhibitors (e.g., p16) that bind to Cdk4, 6/cyclin D complexes are commonly found in human cancer cells.

The connection between cyclin D, growth control, and cancer is further fortified by the fact that a key substrate protein of Cdk4, 6/cyclin D complexes is itself frequently mutated in a wide array of human tumors. This protein, designated Rb, was first identified as the product of a gene responsible for retinoblastoma, a rare inherited childhood eye tumor (see Chapter 15). Further studies then showed that mutations resulting in the absence of functional Rb protein are not restricted to retinoblastoma but also contribute to a variety of common human cancers. Rb is the prototype of a tumor suppressor gene—a gene whose inactivation leads to tumor development. Whereas oncogene proteins such as Ras (see Chapter 13) and cyclin D drive cell proliferation, the proteins encoded by tumor suppressor genes act as brakes that slow down cell cycle progression. Additional examples of cell cycle regulators encoded by tumor suppressor genes include the Ink4 Cdk inhibitors that bind Cdk4, 6/cyclin D complexes and the important growth regulator p53, which was discussed earlier in this chapter.

Further studies of Rb have revealed that it plays a key role in coupling the cell cycle machinery to the expression of genes required for cell cycle progression and DNA synthesis (Figure 14.20). The activity of Rb is regulated by changes in its phosphorylation as cells progress through the cycle. In particular, Rb becomes phosphorylated by Cdk4, 6/cyclin D complexes as cells pass through the restriction point in G_1. In its underphosphorylated form (present in G_0 or early G_1), Rb binds to members of the E2F family of transcription factors, which regulate expression of several genes involved in cell cycle progression, including the gene encoding cyclin E. E2F binds to its target sequences in either the presence or absence of Rb. However, Rb acts as a repressor, so the Rb/E2F complex suppresses transcription of E2F-regulated genes. Phosphorylation of Rb by Cdk4, 6/cyclin D complexes results in its dissociation from E2F, which then activates transcription of its target genes. Rb thus acts as a molecular switch that converts E2F from a repressor to an activator of genes required for cell cycle progression. The control of Rb via Cdk4, 6/cyclin D phosphorylation in turn couples this critical regulation of gene expression to the availability of growth factors in G_1. TOP

Inhibitors of Cell Cycle Progression

Cell proliferation is regulated not only by growth factors but also by a variety of signals that act to inhibit cell cycle progression. For example, agents that damage DNA result in cell cycle arrest, presumably to allow time for the cell to repair the damage. In addition, cell contacts and a variety of extracellular factors act to inhibit rather than stimulate proliferation of their target cells. The effects of such inhibitory signals are also mediated by regulators of the cell cycle machinery, frequently via the induction of Cdk inhibitors.

A good example of the action of Cdk inhibitors is provided by cell cycle arrest in response to DNA damage, which is mediated by the protein p53 (discussed earlier in this chapter). The p53 protein is a transcriptional regulator that functions, at least in part, to stimulate expression of the Cdk inhibitor p21 (Figure 14.21). The p21 protein inhibits several Cdk/cyclin complexes, and its induction by p53 appears to represent at least one mechanism of p53-dependent cell cycle arrest following DNA damage. In addition to inhibiting cell cycle progression via its interaction with Cdk's, p21 may directly inhibit DNA replication. In particular, p21 binds to proliferating cell nuclear antigen (PCNA), which, as discussed in Chapter 5, is a subunit of DNA polymerase δ. Thus, p21 may play a dual role in cell cycle arrest induced by DNA damage, not only blocking cell cycle progression by inhibiting Cdk's but also directly inhibiting DNA replication in S phase cells.

The best-characterized extracellular inhibitor of animal cell proliferation is TGF-β—a polypeptide factor that inhibits the proliferation of a variety of types of epithelial cells by arresting cell cycle progression in G_1. This action of TGF-β appears to be mediated by induction of the Cdk inhibitor p15, which binds to Cdk4, 6/cyclin D complexes. In the resulting absence of Cdk4 activity, Rb phosphorylation is blocked and the cell cycle is arrested in G_1.

A different molecular mechanism is used to control cell cycle progression through the G_2 checkpoint, which prevents entry into mitosis in the presence of unreplicated or damaged DNA (Figure 14.22). Arrest of the cell cycle at this checkpoint is mediated by a protein kinase called Chk1, which is activated in response to DNA damage or incomplete replication. Chk1 phosphorylates the protein phosphatase Cdc25, thereby preventing Cdc25 from dephosphorylating and activating Cdc2. In the absence of Cdc2 activation, progression to mitosis is blocked and the cell remains arrested in G_2. TOP