Programmed cell death results from a conserved cascade of events essential in the development and maintenance of tissue homeostasis. “Extrinsic” cell-death pathways initiate at the cell surface, leading to execution through substrate cleavage, and may involve mitochondrial amplification. Multiple “intrinsic” death pathways converge and require signaling through the mitochondria. Extrinsic cell death is integral to cell-mediated immunity and host immune surveillance/suppression of cancer. Caspase activation is highly regulated and defects at virtually all levels of death regulation are observed in cancer. This chapter focuses on the cell biology, biochemistry, and genetics of programmed cell death.

ORIGINS OF APOPTOSIS IN CAENORHABDITIS ELEGANS

Even after numerous reports in the early to mid-1900s of “programmed cell death” with characteristic morphological changes such as cell shrinkage and nuclear condensation and fragmentation, the importance of this process in normal cellular physiology went largely unexplored (1). However, with the description of the genetically controlled deletion of a subset of cells within the nematode C. elegans and the subsequent cloning of the genes responsible for this process, the field of programmed cell death or apoptosis gained popularity (2). The realization that apoptosis is an evolutionarily conserved, genetic event has sparked interest in understanding the regulation of the process in various model systems. Furthermore the deregulation of apoptosis in human disorders such as neurodegenerative disease and cancer has lead to the manipulation of these pathways in order to combat these diseases (3). It was, however, seminal work in C. elegans that laid the foundation for the central themes of apoptosis found throughout the animal kingdom.

While Richard Lockshin coined the term programmed cell death in 1964 (4), John Kerr is credited with early microscopic observations of cell death distinct from necrosis called “apoptosis” which he, Wyllie, and Currie perceived to be controlled by a series of con-
served events (1). However, Robert Horvitz was responsible for providing the first molecular clues of how apoptosis is controlled (5). The identification of *C. elegans* cell death abnormal or *ced* mutants with defective development (5,6) established three families of highly conserved proteins, which oversee apoptosis in most organisms (Fig. 1): caspases (*ced-3*), caspase-activating adaptor proteins (*ced-4*) and the bcl-2 family of proteins (*ced-9*). Caspases are the enzymes responsible for dismantling the cell and for eliciting the cellular phenotypes first described by Kerr, therefore their regulation is paramount when considering apoptotic pathways. In the worm, these three gene products act in a linear fashion to either suppress or activate *ced-3*, with *ced-9* acting as the only antiapoptotic protein in the pathway. *ced-9* inhibits *ced-4* function, which is required for *ced-3* caspase activation (7). Whereas the loss of *ced-3* or *ced-4* did not compromise the longevity of the organism, suppression of apoptosis by *ced-9* was crucial for its long-term survival (6). Subsequently, the lone BH3-only protein, egl-1, was placed genetically upstream of *ced-9* due to the ability of egl-1 to bind and negatively regulate *ced-9* (8). These four genes constitute the core apoptotic machinery in *C. elegans* required for the execution phase of cell death.

### INCREASED APOPTOTIC COMPLEXITY OF HIGHER EUKARYOTES

Cloning of the core apoptotic genes in *C. elegans* led to the discovery that higher eukaryotes adhered to this basic blueprint but had predictably evolved to include novel gene families to regulate further complexity (Fig. 1). Mammalian systems, being the most complex, contain 14 caspases (*ced-3*), 2 proapoptotic adaptor proteins (*ced-4*), at least 10 bcl-2 family proteins (*ced-9*), and a similar number of BH3-only proteins (egl-1) to date (9,10). BH3-only proteins antagonize the antiapoptotic members of the bcl-2 family in order to facilitate downstream adaptor-mediated caspase activation (11). However, unlike *C. elegans*, antiapoptotic bcl-2 proteins do not directly interact with adaptors but rather regulate adaptor assembly by influencing mitochondrial homeostasis (12). This pathway involving mitochondria and subsequent caspase activation is referred to as the intrinsic pathway and is the functional equivalent of the *C. elegans* cell death pathway (Fig. 1).

Following mitochondrial dysfunction, formation of a caspase activation complex known as the apoptosome initiates the death program. The apoptosome is comprised of the *ced-4* homolog, Apaf-1, along with procaspase-9, ATP, and cytochrome c, which has been extruded from the mitochondria. Activation of caspase-9 within the apoptosome in turn leads to the activation of caspase-3, the true mammalian *ced-3* homolog, committing the cell to death (13). The basic principle from *C. elegans* of bcl-2 mediated inhibition of adaptor-driven caspase activation is therefore represented at the mammalian level by the intrinsic pathway. In addition to the intrinsic pathway, however, mammals have evolved an alternative pathway—the extrinsic pathway—which is initiated at the cell surface by death receptor/death ligand interactions (14). Activation of this pathway also results in adaptor-driven caspase activation. The adaptor, FADD, and caspase-8 and -10, through a series of protein interactions with the death ligand-associated receptors, form a death-inducing signaling complex (DISC) which is sufficient for caspase activation (15). The last major difference between *C. elegans* and higher eukaryotes is the creation of another protein family, the inhibitor of apoptosis proteins, or IAPs (16). These proteins have evolved to bind to and negatively regulate caspases and will be discussed in more detail in a subsequent section (Fig. 1).