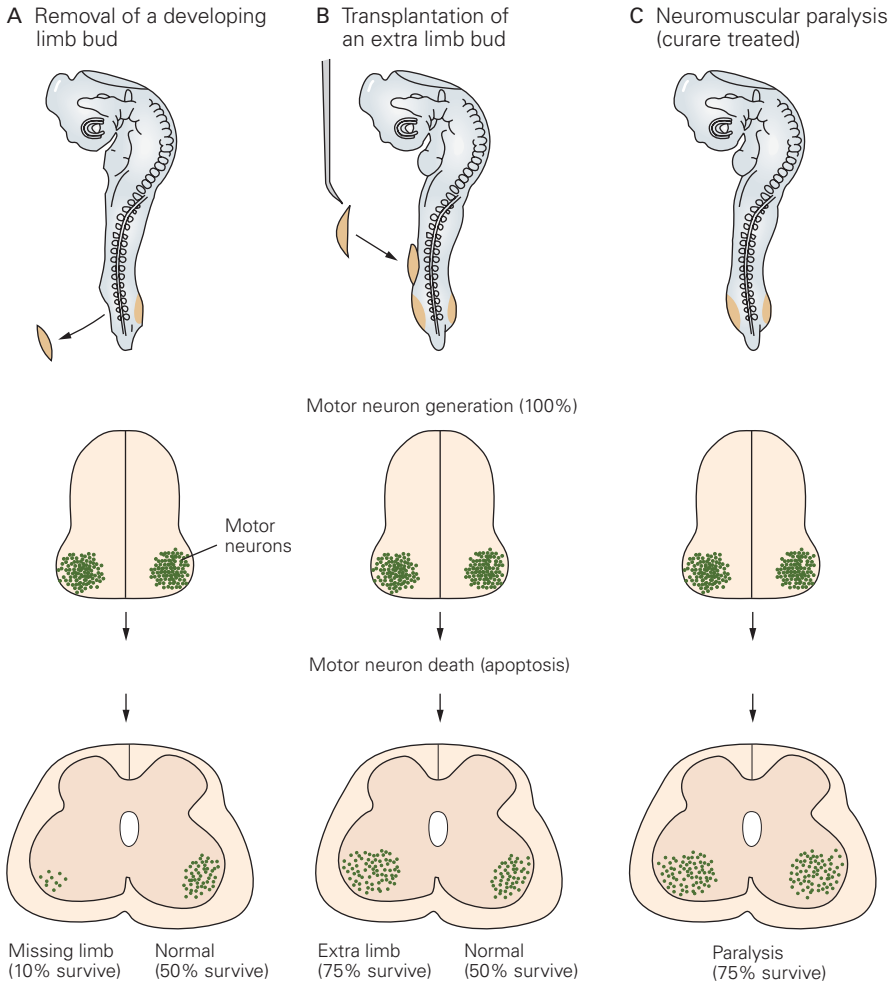

Figure 46–14 **The survival of motor neurons depends on signals provided by their muscle targets.** The role of the muscle target in motor neuron survival was demonstrated by Viktor Hamburger in a classic series of experiments performed on the chick embryo. (Adapted from Purves and Lichtman 1985.)

A. A limb bud was removed from a 2.5-day-old chick embryo soon after the arrival of motor nerves. A section of the lumbar spinal cord 1 week later reveals few surviving motor neurons on the deprived side of the spinal cord. The number of motor neurons on the contralateral side with an intact limb is normal.

B. An extra limb bud was grafted adjacent to a host limb prior to the normal period of motor neuron death. A section of the lumbar spinal cord 2 weeks later shows an increased number of limb motor neurons on the side with the extra limb.

C. Blockade of nerve-muscle activity with the toxin curare, which blocks acetylcholine receptors, rescues many motor neurons that would otherwise die. Curare may act by enhancing the release of trophic factors from inactive muscle.

dozen secreted factors that promote neuronal survival. The best-studied are related to NGF and are called the neurotrophin family.

There are four main neurotrophins: NGF itself, brain-derived neurotrophic factor (BDNF), and neurotrophins-3 and -4 (NT-3 and NT-4). Other classes of proteins that promote neuronal survival include members of the transforming growth factor β family, the interleukin-6–related cytokines, fibroblast growth factors, and even certain inductive signals we encountered earlier (BMPs and hedgehogs). Other neurotrophic factors, notably members of the glial cell line–derived neurotrophic factor (GDNF) family, are responsible for the survival of different types of sensory and sympathetic neurons (Figure 46–16).

Neurotrophins interact with two major classes of receptors, the Trk receptors and p75. Neurotrophins promote cell survival through activation of Trk receptors. The Trk family comprises three membrane-spanning tyrosine kinases named TrkA, TrkB, and TrkC, each of which exists as a dimer (Figure 46–17).

Much is now known about the intracellular signaling pathways activated by binding of neurotrophins to Trks. As with other tyrosine kinase receptors, the binding of neurotrophins to Trk receptors leads to dimerization of the Trk proteins. Dimerization results in phosphorylation of specific tyrosine residues in the activation loop of the kinase domain. This phosphorylation leads to a conformational change in the receptor and to phosphorylation of tyrosine residues that serve as docking sites for adaptor proteins. The adaptors then trigger production of second messengers that both promote the survival of neurons and trigger their maturation. These divergent biological responses involve different intracellular signaling pathways: neuronal differentiation largely via the mitogen-activated protein kinase (MAPK) enzymatic pathways and survival largely via the phosphatidylinositol-3 kinase pathway (Figure 46–18).

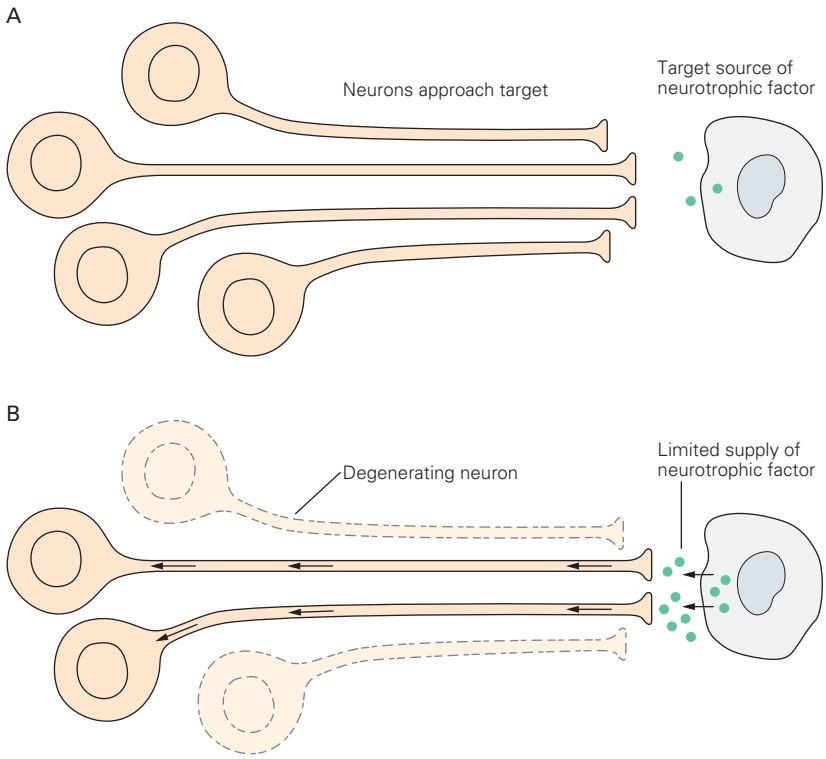

Figure 46–15 The neurotrophic factor hypothesis.

A. Neurons extend their axons to target cells, which secrete low levels of neurotrophic factors. (For simplicity, only one target cell is shown.) The neurotrophic factor binds to specific receptors and is internalized and transported to the cell body, where it promotes neuronal survival.

B. Neurons that fail to receive adequate amounts of neurotrophic factor die through a program of cell death termed apoptosis.

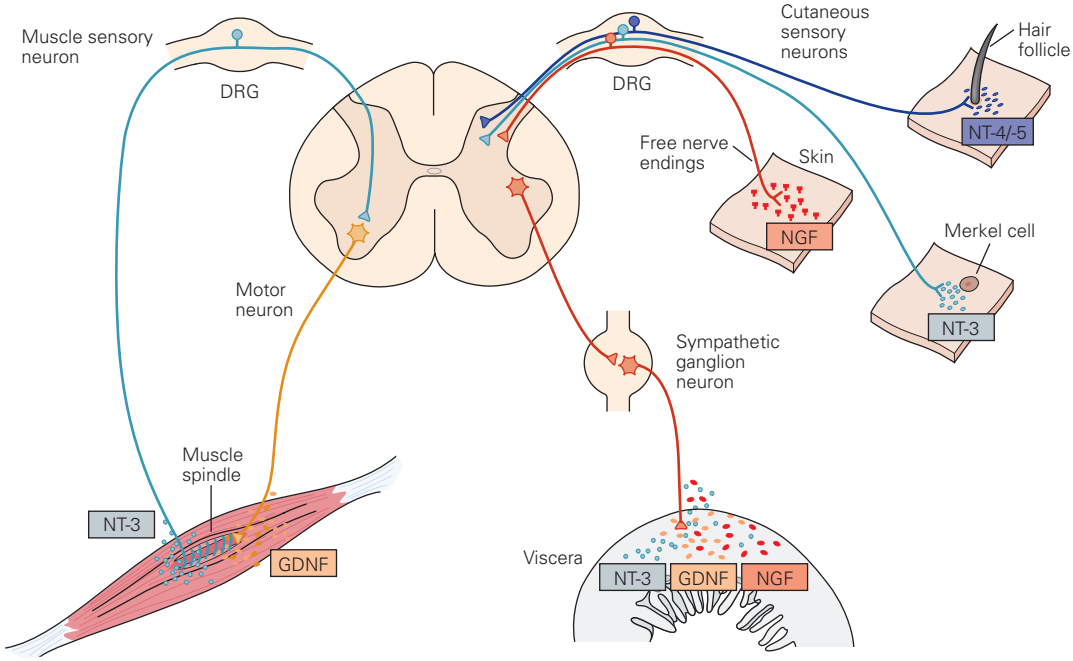

Figure 46–16 Particular neurotrophic factors promote the survival of distinct populations of dorsal root ganglion neurons. Proprioceptive sensory neurons that innervate muscle spindles depend on neurotrophin-3 (**NT-3**); nociceptive neurons that innervate skin depend on nerve growth factor (**NGF**) and neurturin; mechanoreceptive neurons that innervate Merkel cells depend on NT-3; and those that innervate hair follicles depend on neurotrophin-4 and -5 (**NT-4/-5**) and brain-derived neurotrophic factor. Motor neurons depend on glial cell line–derived neurotrophic factor (**GDNF**) and other factors. Sympathetic neurons depend on NGF, NT-3, and GDNF. (Adapted from Reichardt and Fariñas 1997.)

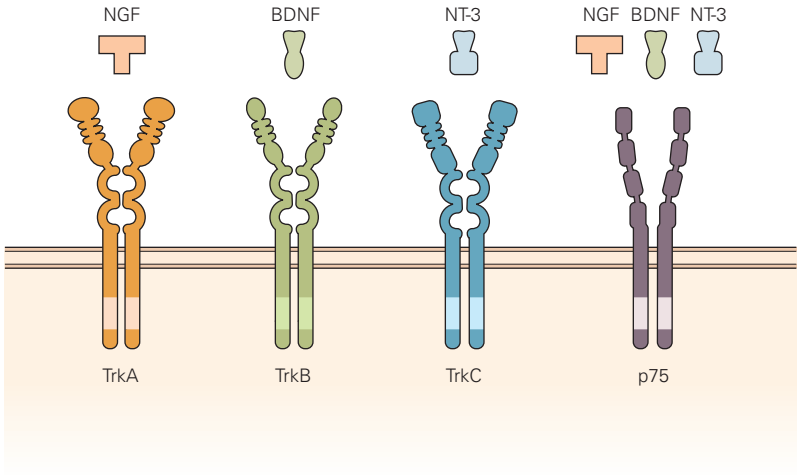

Figure 46–17 **Neurotrophins and their receptors.** Each of the three main neurotrophins interacts with a different transmembrane tyrosine kinase receptor (**Trk**). In addition, all three neurotrophins can bind to the low-affinity neurotrophin receptor p75. (Abbreviations: **BDNF**, brain-derived neurotrophic factor; **NGF**, nerve growth factor; **NT-3**, neurotrophin-3.) A fourth neurotrophin, NT-4, is not shown. (Adapted from Reichardt and Fariñas 1997.)

In contrast to the specificity of Trk receptor interactions, all neurotrophins bind the receptor p75 (Figure 46–17). In some cases, p75 works along with Trk receptors, tuning the affinity and specificity of Trks for their neurotrophin ligands and thereby contributing to neuronal survival. However, p75 leads a double life. It can also bind unprocessed precursors of neurotrophins, called proneurotrophins, and it can associate with other membrane receptors called sortilins. Binding of proneurotrophins to the p75/sortilin complex promotes neuronal death. Receptor p75 is a member of the tumor necrosis factor (TNF) receptor family and promotes cell death by activating proteases of the caspase family, which we discuss below.

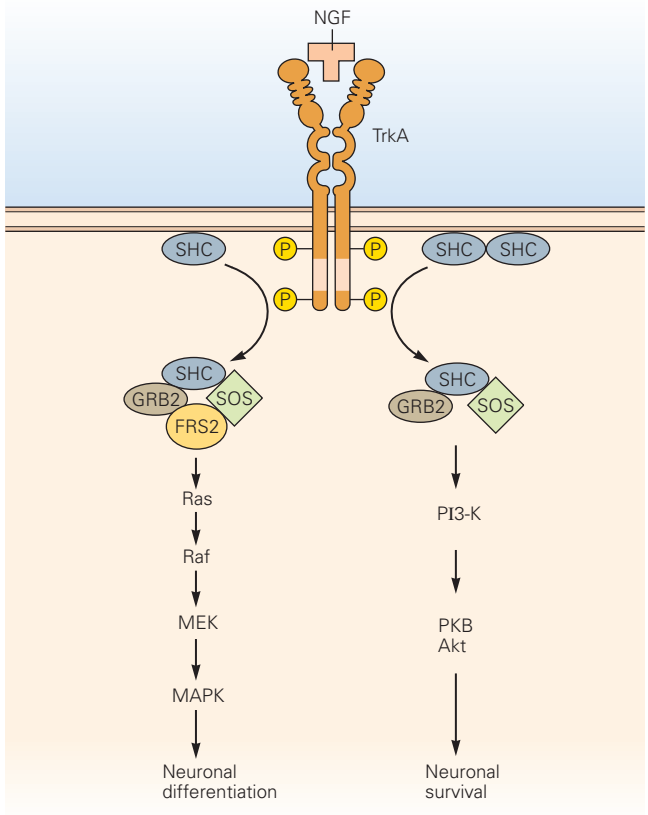

Figure 46–18 **Binding of nerve growth factor to the TrkA receptor activates alternative intracellular signaling pathways.** The binding of nerve growth factor (**NGF**) induces dimerization of the TrkA receptor, which triggers its phosphorylation at many different residues. Phosphorylation of TrkA results in the recruitment of the adaptor proteins SHC, GRB2, and SOS. The additional recruitment of FRS2 to this complex (*left*) activates a Ras kinase signaling pathway that promotes neuronal differentiation. In the absence of FRS2 (*right*), the complex activates a phosphatidylinositol-3 kinase (**PI3-K**) pathway that promotes neuronal survival. (Abbreviations: **Akt/PKB**, protein kinase B; **MAPK**, mitogen-activated protein kinase; **MEK**, mitogen-activated/ERK kinase; **P**, phosphate.)

Neurotrophin signaling is relayed from the axon terminal to the cell body of the neuron through a process that involves internalization of a complex of neurotrophin bound to Trk receptors. The retrograde transport of this complex occurs in a class of endocytotic vesicles called signaling endosomes. The transport of these vesicles brings activated Trk receptors into cellular compartments able to activate signaling pathways and transcriptional programs essential for neuronal survival, maturation, and synaptic differentiation.

The picture is more complex for neurons in the central nervous system. The survival of motor neurons, for example, is not dependent on a single neurotrophic factor. Instead, different classes of motor neurons require neurotrophins, GDNF, and interleukin-6–like proteins expressed by muscles or peripheral glial cells. The survival of these neuronal classes depends on the exposure of axons to local neurotrophic factors.

Neurotrophic Factors Suppress a Latent Cell Death Program

Neurotrophic factors were once believed to promote the survival of neural cells by stimulating their metabolism in beneficial ways, hence their name. It is now evident, however, that neurotrophic factors suppress a latent death program present in all cells of the body, including neurons.

This biochemical pathway can be considered a suicide program. Once it is activated, cells die by apoptosis (Greek, falling away): They round up, form blebs, condense their chromatin, and fragment their nuclei. Apoptotic cell deaths are distinguishable from necrosis, which typically results from acute traumatic injury and involves rapid lysis of cell membranes without activation of the cell death program.

The first clue that deprivation of neurotrophic factors kills neurons by unleashing an active biochemical program emerged from studies that assessed neuronal survival after inhibition of RNA and protein synthesis. Exposure of sympathetic neurons to protein synthesis inhibitors was found to prevent the death of sympathetic neurons triggered by removal of NGF. These results sparked the idea that neurons have the ability to synthesize proteins that are lethal and that NGF prevents their synthesis, thereby suppressing an endogenous cell death program.

Key insights into the biochemical nature of the endogenous cell death program emerged from genetic studies of the nematode *Caenorhabditis elegans*. During the development of *C. elegans*, a precise number of cells is generated and a fixed number of these cells die—the same number from embryo to embryo. The findings prompted a screen for genes that block or enhance cell death, which led to the identification of the cell death (*ced*) genes. Two of these genes, *ced-3* and *ced-4*, are needed for the death of neurons; in their absence, every one of the cells destined to die instead survives. A third gene, *ced-9*, is needed for survival and works by antagonizing the activities of *ced-3* and *ced-4* (Figure 46–19). Thus, in the absence of *ced-9*, many additional cells die, even though these deaths still depend on *ced-3* and *ced-4* activity.

The cell death pathway in *C. elegans* has been conserved in mammals. Similar proteins and pathways control the apoptotic death of central and peripheral neurons, indeed of all developing cells. The worm *ced-9* gene encodes a protein that is related to members of the mammalian Bcl-2 family, which protect

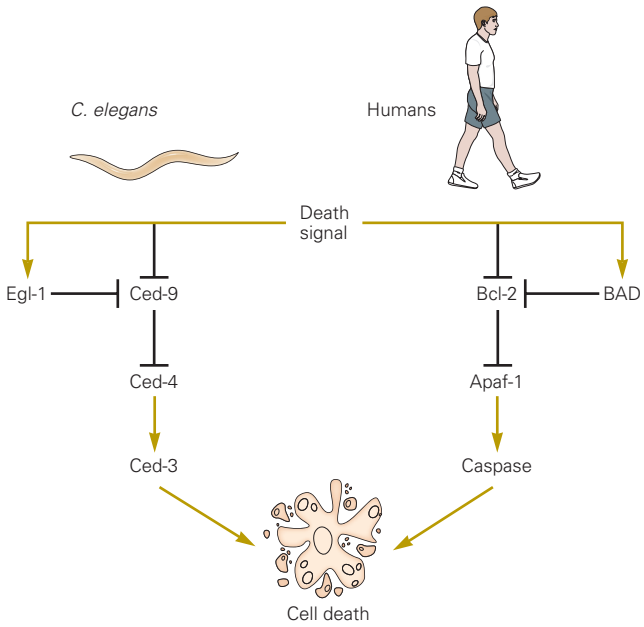

Figure 46–19 Neurons and other cells express a conserved death program. Different cellular insults trigger a genetic cascade that involves a series of death effector genes. These death genes and pathways have been conserved in the evolution of species from worms to humans. The core death pathway activates a set of proteolytic enzymes, the caspases. Caspases cleave many downstream and essential protein substrates (see Figure 46–20), resulting in the death of cells by a process termed *apoptosis*. Genetic analysis of the worm *Caenorhabditis elegans* indicates that the Ced-9 protein acts upstream and inhibits the activity of Ced-4 and Ced-3, two proteins that promote cell death. Many vertebrate homologs of Ced-9, the Bcl-2 family of proteins, have been identified. Some of these proteins, such as Bcl-2 itself, inhibit cell death, but others promote cell death by antagonizing the actions of Bcl-2. The Bcl-2 class proteins act upstream of Apaf-1 (a vertebrate homolog of Ced-4) and the caspases (vertebrate homologs of Ced-3).

lymphocytes and other cells from apoptotic death. The worm *ced-3* gene encodes a protein closely related to a class of mammalian cysteine proteases called caspases. The worm *ced-4* gene encodes a protein that is functionally related to a mammalian protein called apoptosis activating factor-1 (Apaf-1).

The mammalian apoptotic cell death pathway works in a way that resembles the worm pathway (Figure 46–20). The morphological and histochemical changes that accompany the apoptosis of mammalian cells result from the activation of caspases, which cleave specific aspartic acid residues within cellular

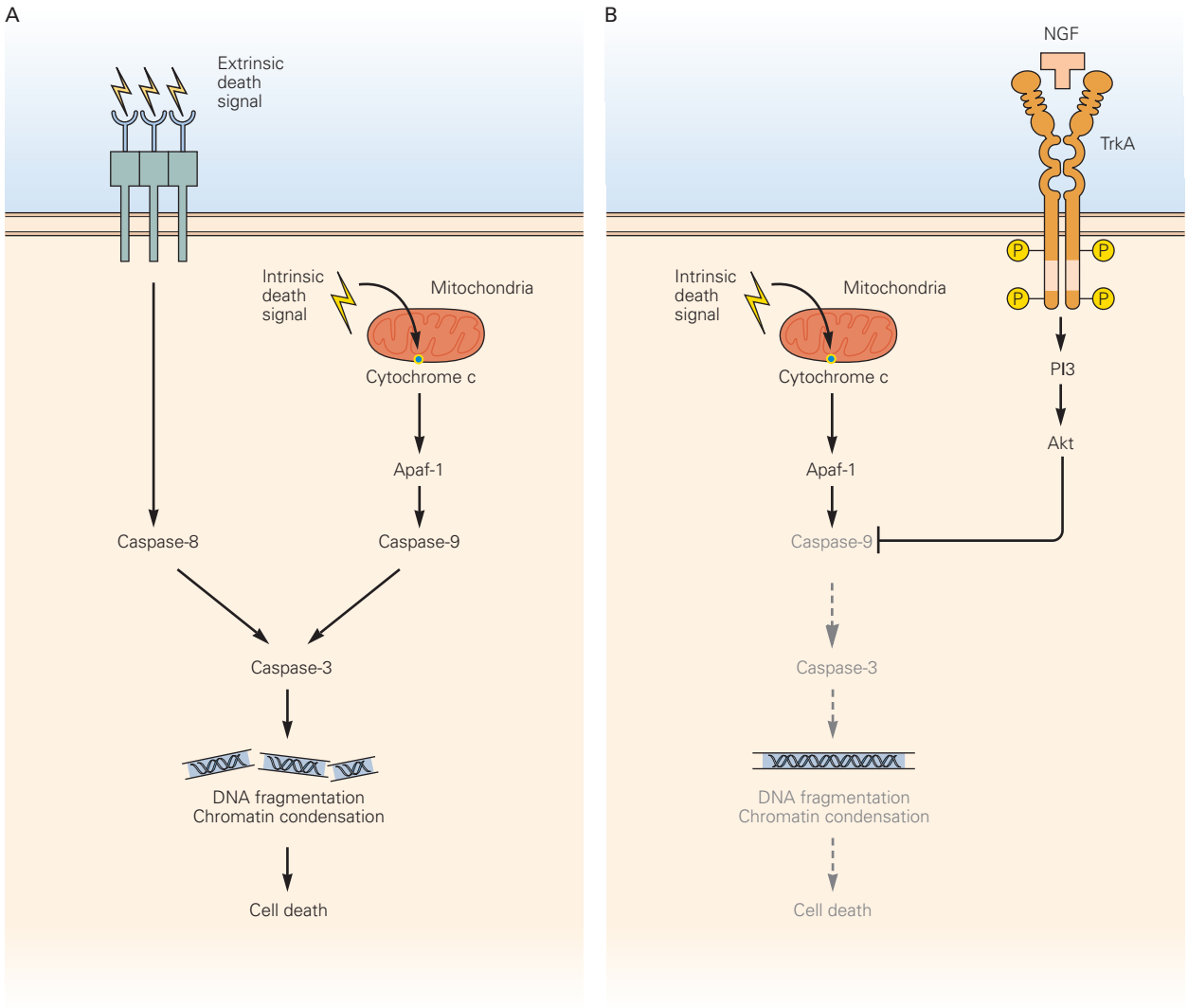

Figure 46–20 Neurotrophic factors suppress caspase activation and cell death. (Adapted from Jesenberger and Jentsch 2002.)

A. Two types of pathways trigger cell death: extrinsic activation of surface membrane death receptors and intrinsic activation of a mitochondrial pathway. Both pathways result in activation of caspases such as caspase-8 and caspase-9, which initiate a proteolytic cleavage cascade that converges at the level of caspase-3 activation. Cleavage of the caspase precursor removes the caspase prodomain and produces a proteolytically active enzyme conformation.

The extrinsic pathway involves activation of death receptors by ligands such as tumor necrosis factor receptor 1 or Fas/CD95. The intrinsic pathway involves stress-induced signals such as DNA damage that initiate the release of cytochrome c from the mitochondrial intermembrane space. Cytochrome c binds to Apaf-1 and recruits and activates caspase-9.

B. Binding of neurotrophins to Trk receptors recruits the PI3 kinase pathway and Akt and suppresses the cell death pathway by inhibiting caspase-9. This pathway is inhibited in developing neurons by neurotrophic factors, explaining why withdrawal of these factors leads to apoptosis. (Abbreviations: **NGF**, nerve growth factor; **P**, phosphate.)

proteins. Two classes of caspases regulate apoptotic death: the initiator and effector caspases. Initiator caspases (caspase-8, -9, and -10) cleave and activate effector caspases. Effector caspases (caspase-3 and -7) cleave other protein substrates, thus triggering the apoptotic process. Perhaps 1% of all proteins in the cell serve as substrates for effector caspases. Their cleavage contributes to neuronal apoptosis through many pathways: by activation of proteolytic cascades, inactivation of repair, DNA cleavage, mitochondrial permeabilization, and initiation of phagocytosis.

The survival of mammalian neurons depends on the balance between antiapoptotic and proapoptotic members of the Bcl-2 family of proteins. Some Bcl-2 proteins such as BAX and BAK increase the permeability of the mitochondrial outer membranes, causing the release of proapoptotic proteins such as cytochrome *c* into the cytosol. The release of cytochrome *c* induces Apaf-1 to bind and activate caspase-9, leading to the cleavage and activation of effector caspases. The binding of neurotrophic factors to their tyrosine kinase receptors is thought to lead to the phosphorylation of protein substrates that promote Bcl-2–like activities (Figure 46–20B). Thus, withdrawal of neurotrophic factors from neurons changes the balance from antiapoptotic to proapoptotic members of the Bcl-2 family, which triggers the neuron's demise.

The caspase cell death program can also be activated by many cellular insults, including DNA damage and anoxia. The activation of cell-surface death receptors such as Fas by extracellular ligands results in the activation of caspase-8 or -10 as well as the recruitment of death effector proteins such as FADD. Recruitment of an initiator caspase to the Fas-FADD complex then leads to activation of effector caspases. Because many neurodegenerative disorders result in apoptotic death, pharmacological strategies to inhibit caspases are under investigation.

Highlights

1. Stem cells near the ventricular surface of the neural tube divide to expand the neuroepithelium. Further divisions then generate the neurons and glia of the central nervous system as well as radial glia.
2. Processes of radial glia extend from the ventricular to the pial surface. Radial glial cells continue dividing to form neurons and astrocytes. In the cortex, they also serve as a scaffold on which newborn excitatory neurons migrate to appropriate layers.
3. The choice between neuronal and glial fate is determined by signals from ligands of the Delta family to receptors of the Notch family on neighboring cells. Initially, cells express both Notch and Delta. Activation of Notch leads to a glial fate, downregulating Delta, which in turn attenuates Notch activity on the neighbors, promoting their differentiation into neurons.
4. As cortical principal (excitatory) neurons migrate along radial glia, they form cortical layers in an inside-out sequence (layer 6 forms before layer 5, and so on). Disruptions of migration are among the causes of intellectual disability and epilepsy.
5. Unlike excitatory neurons, forebrain interneurons arise subcortically in ganglionic eminences and then migrate tangentially into the cortex, basal ganglia, and other forebrain structures.
6. Neural crest cells migrate from their source at the dorsal tip of the neural tube through somites and mesenchyme to form sensory and autonomic neurons and glia, as well as several nonneural cell types.
7. For principal neurons, interneurons, and peripheral neurons, intrinsic differences and cues encountered along the migratory path interact to induce expression of distinct combinations of transcription factors. The transcriptional programs then lead to diversification of the developing neurons into multiple classes and types.
8. The greater complexity of the primate, and particularly the human brain compared to those of lower mammals is due in part to a larger pool of neuronal progenitors, including a second type of radial glial cell.
9. A recent advance in the ability to study the human brain is the discovery that complex neuronal ensembles called cerebral organoids can be generated from stem cells. Although they fail to acquire characteristics of the mature cortex, they enable analysis of some aspects of early brain development and its disorders and may be useful in testing possible therapeutics.
10. The neurotransmitters that neurons use are determined as part of the transcriptional program that endows each neuronal type with its defining characteristics. However, extrinsic factors, including patterns of electric activity and hormonal milieu, can lead to transmitter switching in some cases.
11. The nervous system generates up to twice as many neurons as survive in adulthood. The excess is eliminated by a cell death program that is conserved from invertebrates to humans.

12. Trophic factors play a crucial role in determining which neurons within a population live or die. They control survival by holding the cell death program in check. In some cases, neurons appear to compete for a limited supply of neurotrophic factors; the cell death program is activated in those that lose the competition.
13. Multiple trophic factors are produced in the body, with each controlling the fate of only some neuronal types. The best-studied, called neurotrophins (nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4), bind to and activate kinases called Trk receptors.

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The Growth and Guidance of Axons

In the two preceding chapters, we saw how neurons are generated in appropriate numbers, at correct times, and in the right places. These early developmental steps set the stage for later events that direct neurons to form functional connections with target cells. To form connections, neurons have to extend long processes—axons and dendrites—which permit connectivity with postsynaptic cells and synaptic input from other neurons. In this chapter, we examine how neurons elaborate axons and dendrites and how axons are guided to their targets.

We begin the chapter by discussing how certain neuronal processes become axons and others dendrites. We then describe the growing axon, which may have to travel a long distance and ignore many inappropriate neuronal partners before terminating in just the right region and recognizing its correct synaptic targets. We consider the strategies by which the axon overcomes these challenges. Finally, we illustrate general features of axonal guidance by describing the development of two well-studied axonal pathways: one that conveys visual information from the retina to the brain and another that conveys cutaneous sensory information from the spinal cord to the brain.

Differences Between Axons and Dendrites Emerge Early in Development

The processes of neurons vary enormously in their length, thickness, branching pattern, and molecular architecture. Nonetheless, most neuronal processes fit into one of two functional categories: axons and dendrites. More than a century ago, Santiago Ramón y Cajal hypothesized that this distinction underlies the ability of neurons to transmit information in a particular direction, an idea he formalized as the law of dynamic polarization. Cajal wrote that "the transmission of the nerve impulse is always from the dendritic branches and the cell body to the axon." In the decades before electrophysiological methods were up to the task, this law provided a means of analyzing neural circuits histologically. Although exceptions have been found, Ramón y Cajal's law remains a basic principle