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Molecular Mechanisms Regulating Motor Neuron Development and Degeneration

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Abstract

Motor neurons are a well-defined, although heterogeneous group of cells responsible for transmitting information from the central nervous system to the locomotor system. Spinal motor neurons are specified by soluble factors produced by structures adjacent to the primordial spinal cord, signaling through homeodomain proteins. Axonal pathfinding is regulated by cell-surface receptors that interact with extracellular ligands and once synaptic connections have formed, the survival of the somatic motor neuron is dependent on the provision of target-derived growth factors, although nontarget-derived factors, produced by either astrocytes or Schwann cells, are also potentially implicated. Somatic motor neuron degeneration leads to profound disability, and multiple pathogenetic mechanisms including aberrant growth factor signaling, abnormal neurofilament accumulation, excitotoxicity, and autoimmunity have been postulated to be responsible. Even when specific deficits have been identified, for example, mutations of the superoxide dismutase-1 gene in familial amyotrophic sclerosis and polyglutamine expansion of the androgen receptor in spinal and bulbar muscular atrophy, the mechanisms by which somatic motor neuronal degeneration occurs remain unclear. In order to treat motor system degeneration effectively, we will need to understand these mechanisms more thoroughly.

Index Entries: Motor neuron; amyotrophic lateral sclerosis; development; degeneration; excitotoxicity; growth factors.

Introduction

Motor neurons are a heterogeneous population that comprise two major groups of cells: upper and lower motor neurons. Upper motor neurons originate in the brain, in particular, the motor cortex, and they synapse either directly or indirectly onto lower motor neu-

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rons. Upper motor neurons are more accurately referred to as premotor neurons, and they are responsible for coveying descending commands for movement. Lower motor neurons are divisable into two categories: visceral and somatic motor neurons. Visceral motor neurons are autonomic preganglionic neurons that regulate the activity of ganglionic neurons, which innervate glands, blood vessels, and smooth muscle. Somatic motor neurons innervate skeletal muscle and include first, anterior horn cells, which as the name implies, are located in the anterior horn of the spinal cord, and second, lower motor neurons located in the cranial nerve nuclei. The cell body and axon of the somatic motor neuron, together with the neuromuscular junction and the extrafusal muscle fibers innervated by that neuron are collectively known as a motor unit. α-Motor neurons are the subset of somatic motor neurons that innervate extrafusal skeletal muscle, and they are directly responsible for locomotor activity. In contrast, γ-motor neurons innervate intrafusal muscle fibers within muscle spindles and regulate the firing rate of sensory nerve endings, thus providing the central nervous system (CNS) with proprioceptive information.

This article presents an analysis of current knowledge concerning the developmental biology and pathophysiology of the somatic motor neuron. This focus is appropriate, since the somatic motor neuron is the most intensively studied of the motor neuron subtypes and its dysfunction directly accounts for most of the disability that occurs in the majority of motor system degenerations. In this article, the term motor neuron will apply to the somatic motor neuronal pool, unless otherwise indicated.

Motor Neuron Development

Motor neurons are generated from neuroepithelial cells, which are multipotential precursors of the entire repertoire of neurons, oligodendrocytes, and astrocytes in the CNS. Distinct classes of neurons are formed at defined positions in the neural axis, and this selective restriction of fate is controlled by local signals. Much work has concentrated on identifying the source and nature of these signals, the nature of the cell intrinsic factors responsible for commitment of cells to specific neuronal fates, and the mechanisms by which specification is translated into specific phenotype.

Motor neuron specification from the precursor population is determined by a primordial structure known as the notochord, which sits ventral to the spinal cord during development (Yamada et al., 1993). This ventralizing activity induces specialized nonneuronal floor plate cells at the ventral midline and motor neurons, together with ventral interneurons, more laterally. Whereas induction of the floor plate requires cell contact with the notochord, motor neurons can be induced by diffusable activity (Yamada et al., 1993). Floor plate cells subsequently develop inductive properties to perpetuate the ventralization process at a time when the notochord becomes physically separated from the neural tube (Placzek et al., 1993). Both the notochord and floor plate express the molecule sonic hedgehog (Shh) (Krauss et al., 1993; Echelard et al., 1993), which has the capacity to act as a ventralizing inducing factor, able to induce motor neuron specification at low concentrations and the floor plate at a higher concentration (Roelink et al., 1995). Sonic hedgehog is synthesized as a precursor cleaved autoproteolytically to an active N-terminal and an inactive C-terminal fragment (Roelink et al., 1995). The N-terminal protein becomes preferentially tethered to the cell surface under the influence of the C-terminal fragment, possibly accounting for the fact that floor plate induction is dependent on cell contact. Signaling downstream of Shh is mediated via the multiple-pass transmembrane protein patched with Shh acting to repress the activity of this cell-surface receptor. Patched forms a heteromeric signaling complex with the protein smoothened, the working hypothesis being that unoccupied Patched inactivates

smoothened, whereas binding of Shh to Patched removes this inhibition (Nusse, 1996; Stone et al., 1996; Marigo et al., 1996). Floor plate and motor neuron induction have been confirmed to be dependent on Shh given that inhibitory antibodies against this molecule compromise the ventralizing activity of notochord in explant cultures (Ericson et al., 1996). Recent evidence has, however, suggested that Shh by itself may not be sufficient, given that other soluble factors, notably neurotrophin-3, potentiate the motor neuron inductive activity of Shh in the mammalian system (Dutton et al., 1999). The ventralising activity ultimately results in the induction of members of the Gli family of proteins (i Altaba, 1998), which are transcription factors that bind to DNA, thereby regulating the expression of downstream proteins. Recent work has also indicated that the Shh-triggered differentiation of progenitor cells into somatic motor neurons is directed via the induced expression of the homeodomain protein MNR2, which also acts as a transcription factor. It has also emerged that the activity initiated by MNR2 is maintained by the related homeodomain protein HB9 after MNR2 expression has been extinguished (Tanabe et al., 1998).

Somatic motor neurons are a heterogeneous population of cells. They are divisible into discrete motor columns, each of which has a restricted distribution within the neuraxis (Fig. 1). There are two major columns, the medial motor column (MMC) and the lateral motor column (LMC) (Landmesser 1978a,b). The LMC is restricted to the cervical and lumbar expansions of the spinal cord and neurons within this column innervate the limb musculature. The LMC is further subdivided into the LMC_m (m = medial), which supplies ventral limb musculature, and the LMC_l (l = lateral), which supplies the dorsal limb musculature. Motor neurons within the MMC supply axial musculature and are also separable into anatomically discrete medial and lateral subdivisions within the spinal cord, the latter being restricted to the thoracic segment; these cells innervate dorsal axial and ventral body wall

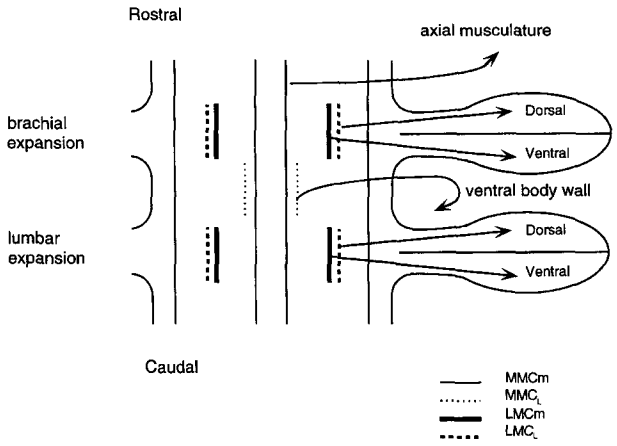

Fig. 1. Schematic representation of the organization of motor columns and the peripheral targets of motor neurons adapted from Ensini et al. (1998). The diagram shows the position of individual motor columns along the rostro-caudal axis of the spinal cord of a stage 35 chick embryo.

musculature, respectively. The rostro-caudal pattern of motor columns is probably established as a consequence of signaling from the paraxial and lateral plate mesoderm, which are also responsible for the generation of the limb buds (Ensini et al., 1998). Evidence for such epigenetic influence comes from grafting experiments in aves, in which brachial segments transposed to the thoracic region lose motor neurons with the molecular signature of the LMC. Elegant work has shown that there is a family of four proteins (comprising Isl-1, Isl-2, Lim-1, and Lim-3), known as the LIM homeodomain proteins, characterized by the presence of an amino-terminal pair of zinc binding LIM domains, that are implicated in establishing the regional specific identity of motor neurons within the different motor columns (Tsuchida et al., 1994; Kunst et al., 1997). Indirect evidence for this influence comes from the observation that different motor columns express different combinatorial patterns of the LIM homeodomain proteins. It has recently been suggested that the expression profile of these proteins is modifiable by retinoid-mediated signals produced in a

regionally restricted fashion by early born, medially located motor neurons; these signals influence the specification of later-born laterally located motor neurons as they migrate through the medial population, before reaching their final position (Sockanathan and Jessell, 1998).

Targeted deletion in mice of the LIM homeodomain protein, Isl-l, which is initially expressed by all lower motor neurons, results in an embryonic lethal phenotype characterized by apoptosis of motor neurons soon after their final mitotic division (Pfaff et al., 1996). This suggests that Isl-l is not involved in motor neuron specification *per se*, but more likely that it is implicated in potentiating the differentiation of previously committed cells. In contrast, in *Drosophila*, mutation of the *isl* gene does not compromise motor neuron viability, but causes defects in motor neuron pathfinding (Thor and Thomas, 1997). In combination, these results suggest a dual function for LIM homeodomain proteins with the data obtained from *Drosophila* certainly suggesting that this family of transcription factors also has the capacity to regulate motor neuron axonal guidance. It will thus be important to determine the phenotype of mice deficient for other members of the LIM homeodomain family, in particular, to establish whether they exhibit axonal pathfinding deficits.

It is possible that the LIM homeodomain proteins influence axonal pathfinding by regulating the expression of cell-surface receptors. At the grossest level, this influence is most likely mediated by expediting differential pathfinding by axons emanating from different motor columns. For example, at the level of the brachial and lumbar expansions, axons from the MMCm are stimulated to grow dorsally, whereas those from the LMC are directed laterally to the limb buds, before dividing into ventral and dorsal divisions. It is thus of particular note that several cell-surface molecules that belong to the Eph family of tyrosine kinase receptors, namely EphA3, EphA4, and EphB2, are expressed by motor neurons (Henkemeyer et al., 1994; Soans et al., 1994; Kilpatrick et al., 1996). In the case of EphA3, this expression is restricted to the MMC, suggesting that differential receptor expression might influence the disparate axonal pathfinding of motor neurons from the various motor columns (Kilpatrick et al., 1996). The Eph receptors are subdivided into two groups according to the types of ligands that they bind; EphA receptors bind to ligands that are bound to the cell surface by glycosylphosphatidylinositol (GPI) linkage (ephrin-A ligands), whereas EphB receptors bind to transmembrane ligands (ephrin-B ligands). The exception to the rule is EphA4, which has the capacity to bind to both ephrin-A and ephrin-B ligands. It is of considerable interest that two ephrin-B ligands (ephrin-B1 and ephrin-B2) have been identified as potentially important regulators of motor axonogenesis (Wang and Anderson, 1997). The relevant ligands are expressed in a segmental distribution in the caudal half of the somitic compartment, a segment that is avoided by motor axons. These ligands can also induce motor neuron growth cone collapse in tissue culture. Collectively, these findings suggest that the Eph-ephrin system could be important in generating the segmental patterning of the motor system, probably acting via repulsive interaction. In addition, expression of EphA4 is critical in regulating upper motor neuron axonal growth (Dottori et al., 1998). When this gene is deleted in mice, the corticospinal tract fails to decussate appropriately in the medulla, and it also fails to innervate spinal interneurons appropriately within the dorsal horn. Instead, the axons spread diffusely to the ventral horn, and they even decussate to the contralateral side of the spinal cord.

Two other receptor families are known to regulate axonogenesis: the neuropilins, which are stimulated by ligands known as the semaphorins (Kolodkin and Ginty, 1997), and a group of receptors whose prototypic member is the molecule deleted in colon cancer (DCC), which bind to a ligand family known as the netrins (Keino-Masu et al., 1996; Drescher, 1996). In each of these systems, the ligands are potentially bifunctional able to elicit attractive

or repulsive signals, depending on the relative expression profile of the various receptors. Furthermore, at particular "choice" points, the decision of a particular axon to fasciculate or to defasciculate, and hence to diverge or to remain attached to the parent axonal tract, almost certainly depends not on the expression of any particular receptor–ligand pair, but on the net balance of attractive and repulsive signals that are operative. The fasciculation of motor neurons in *Drosophila* appears particularly dependent on fasciculin II (Lin and Goodman, 1994), which acts as a cell-adhesion molecule, probably via homophilic interaction. Motor neuron axon guidance also appears to be influenced at key choice points by expression of the secreted molecule, Beat (Fambrough and Goodman, 1996), which probably interferes with cellular adhesive interactions, promoting alternative pathfinding by a subset of motor axons.

In summary, it is now established that there are discrete molecules that induce motor neuron differentiation. Furthermore, each motor neuron has a discrete molecular signature that influences its pathfinding and, ultimately, its innervation pattern. This knowledge is likely to assist in the understanding of the molecular mechanisms that influence the differential susceptibility of different motor neuron pools to motor system degeneration and to provide clues regarding how to promote targeted regeneration of motor axons in the context of either die-back neuropathy or axotomy.

Growth Factor Support of Motor Neurons

Once lower motor neurons have formed synaptic connections, they are subject to a selection process that results in the culling of supernumerary neurons. This process is regulated by target-derived neurotrophic survival factors that are produced in limiting concentrations by muscle, such that those neurons that have made the most capricious or ineffectual synaptic contacts die owing to inadequate growth factor support (Hamburger and Levi-Montalcini, 1949; Purves, 1980). In addition, there is now a large body of evidence to indicate that Schwann cells and astrocytes can also provide trophic support for motor neurons (Arce et al., 1998).

There are several families of growth factors that are potentially implicated in maintaining the survival of motor neurons (Table 1). These families include the neurotrophins whose effects are predominantly exerted within the nervous system (Leibrock et al., 1989; Hallbook et al., 1991; Drago et al., 1994), members of the interleukin-6 (IL-6) family of cytokines (Gimenez-Gallego et al., 1985; Esch et al., Hilton et al., 1988; Stockli et al., 1989; Bartlett et al., 1992), insulin-like growth factor (IGF), and also members of the fibroblast growth factor (FGF) and transforming growth factor (TGF) β families (Oppenheim, 1996). When these growth growth factors are added in combination, they often exert additive survival-promoting effects on motor neurons, and it is of note that these activities are potentiated when cAMP is upregulated (Hanson et al., 1998).

Exogenous growth factors not only inhibit the naturally occurring motor neuron death that occurs at the time of target innervation, but they can also inhibit motor neuron death after axotomy. They can also partially inhibit motor neuron degeneration in several animal models of amyotrophic lateral sclerosis (ALS). However, a number of the animal models of motor system degeneration equate more with die-back neuropathy than with primary motor neuronopathy, and thus, it is unclear whether information concerning therapeutic benefit of growth factors in these models is also relevant to the treatment of ALS. The Cu^{2+}/Zn^{2+} superoxide dismutase-1 (SOD1) mutant mouse is probably a more accurate model of ALS *(see below)*, but even in this model, the neuropathology is dependent on the specific mutation generated (Wong and Borchelt, 1995).

Three growth factors have been subject to clinical trials in ALS. The trials of systemi-

Table 1
Some of the Growth Factors That Promote Motor Neuron Survival

Growth factor	Receptor
Brain-derived neurotrophic factor (BDNF)	trkB
Neurotrophin-3 (NT-3)	trkC
Neurotrophin-4 (NT-4) (Ip et al., 1993)	trkB
Ciliary neurotrophic factor (CNTF)	gp130 LIFRβ CNTFRα
Leukemia-inhibitory factor (LIF)	gp130 LIFRβ
Interleukin 6 (IL-6)	gp130 IL-6R
Cardiotrophin-1 (CT-1) (Grotzinger et al., 1997)	gp130 LIFRβ
Fibroblast growth factor-1 (FGF-1)	All FGF receptors[a]
Fibroblast growth factor-2 (FGF-2)	FGFR1b, 1c, 2c, 3c
Fibroblast growth factor-5 (FGF-5)	FGFR1c, 2c
Fibroblast growth factor-9 (FGF-9) (Ornitz et al., 1996)	FGFR2c/3b/3c
Insulin	Insulin receptor
Insulin-like growth factor 1 (IGF-1)	IGF-1R
Insulin-like growth factor 2 (IGF-2) (Heidenreich, 1993; Konishi et al., 1994)	IGF-2R or -1R or insulin receptor
Glial cell-line-derived neurotrophic factor (GDNF)	Ret GFRα1
Neurturin	Ret GFRα2
Persephin (Rosenthal, 1999)	Ret GFRα4
Hepatocyte growth factor (Yamamoto et al., 1997)	c-Met

[a] Of the FGFR family members, FGFR1 has been shown to be widely expressed by motor neurons and FGF-R3 by a subset of spinal motor neurons (Philippe et al., 1998).

cally administered ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF) have yielded negative results (Anonymous, 1996; Bradley et al., 1997). In contrast, a trial using insulin-like growth factor 1 (IGF-1) has reported efficacy using a composite functional scale known as the Appel score (Appel et al., 1987), although it remains unclear whether IGF-l exerts a significant effect in terms of altering mortality (Leigh et al., 1997). One caveat with respect to the IGF-1 studies is that a second multicenter study conducted in Europe has failed to confirm clinical efficacy (Borasio et al., 1998).

Of the relevant growth factors, the neurotrophins are the best characterized. There are four neurotrophins: nerve growth factor (NGF) (Cohen, 1960; Angeletti and Bradshaw, 1991), BDNF (Barde et al., 1982; Hofer and Barde, 1988, Leibrock et al., 1989), neurotrophin-3, (NT-3) (Hohn et al., 1990), and neurotrophin-4 (NT-4) (Hallbook et al., 1991). These molecules signal transduce through three high-affinity receptors, namely trkA, which selectively binds NGF, trkB, which binds both BDNF and NT-4, and trkC, which binds NT-3 (Ip et al., 1993). The affinity of binding is increased by the association of the high-affinity receptors with a low-affinity receptor, known as p75 (Hantzopoulos et al., 1994). Of the four neurotrophins, BDNF, NT-3, and NT-4 have been shown to exert effects on the motor neuron pool (Oppenheim, 1996). Gene targeting stud-

ies support a role for NT-3 in the development of γ-motor neuron fibers in the spinal roots (Kucera et al., 1995). Correspondingly, mice lacking a functional trkC receptor show a 30% loss of motor neuron fibers in the ventral root of the spinal cord, although the major deficits in both trkC and NT-3 knockout mice are in the dorsal columns (Klein et al., 1994). Of the three neurotrophins that potentiate motor neuron survival, the BDNF-induced effect is the best characterized (Koliatsos et al., 1993; Henderson et al., 1993). BDNF synthesized by the target tissue (Henderson et al., 1993), is retrogradely transported to the motor neuron perikaryon (DiStefano et al., 1992) and clearly reduces the axotomy-induced death of motor neurons in neonatal rodents (Sendtner et al., 1992, Yan et al., 1992; Koliatsos et al., 1993). However, in mutant mice lacking BDNF (Ernfors et al., 1994), there is no demonstrable motor neuron loss, suggesting that there is redundancy in the system, or in other words, that other growth factors can compensate for BDNF deficiency. Systemic BDNF ameliorates disease in the Wobbler mouse (Ikeda et al., 1995a), but not in ALS (Bradley et al., 1997). The efficacy of intrathecally administered BDNF in the context of ALS is currently being assessed (Ochs et al., 1998).

Several members of the IL-6 family of cytokines, namely CNTF, leukemia-inhibitory factor (LIF), cardiotrophin-1 (CT-1), and IL-6 itself, promote motor neuron survival when administered exogenously either in vitro or in vivo (Oppenheim, 1996; Pennica et al., 1996). These molecules all signal through the gp130 receptor (Grotzinger et al., 1997) and as a consequence, it has been difficult to determine which of the group might have a physiological role during development. The level of expression of CNTF is extremely low before birth (Stockli et al., 1991). Unlike the neurotrophins, it does not have a signal sequence (Negro et al., 1991), and therefore, it is not normally secreted from the accessory cells, such as astrocytes, that synthesize it (Stockli et al., 1991). This has led to the hypothesis that CNTF acts predominantly as a trauma factor released into the extracellular space after accessory cell death. There have been conflicting reports concerning the ability of CNTF to rescue axotomized motor neurons from death (Sendtner et al., 1991; Clatterbuck et al., 1994). Nevertheless, most mice homozygous null for the CNTF gene do develop motor neuron loss and weakness as adults. In addition, mice deficient for the CNTFα receptor show clear evidence of severe motor neuron deficiency in postnatal life, suggesting that a second molecule related to CNTF, also acting through this receptor, is essential for motor neuron development (DeChiara et al., 1995). A phase-three study to assess the efficacy of CNTF in ALS has been conducted and yielded negative results. In addition, many patients in the treated group developed systemic side effects, including weight loss (Anonymous, 1996).

LIF (Hall and Rao, 1992) also signals via gp 130, but unlike CNTF, LIF does not require the CNTFα receptor to bind to the cell (Wong et al., 1995; Robledo et al., 1996). There is thus the possibility that LIF will exert more potent biological activity than CNTF. LIF potentiates the survival of motor neurons in vitro (Martinou et al., 1992; Richards et al., 1992) and in axotomy models (Hughes et al, 1993; Cheema et al., 1994), and it partially alleviates motor neuron degeneration in the Wobbler mouse (Ikeda et al., 1995b). Although LIF knockout mice do not show overt motor neuron loss, neonatal mice deficient for the low-affinity LIF receptor (LIFR) subunit, which die shortly after birth, exhibit a greater than 35% reduction in the number of facial lower motor neurons and a 40% reduction in spinal motor neurons. These findings suggest that one or more LIF-related ligands is critical for the normal development of motor neurons (Li et al., 1995).

Treatment with IL-6 rescues developing spinal motor neurons from axotomy-induced cell death (Ikeda et al., 1996a), and coadministration of IL-6 and soluble IL-6 receptor complex has been reported to delay the progression of disease in the Wobbler mouse

(Ikeda et al., 1996b). Cardiotrophin-1 was originally identified for its hypertrophic effects on cardiac myocytes (Pennica et al., 1995). It supports the survival of a similar proportion of motor neurons to that which is lost in the CNTF and LIFR knockout mice, and it also reduces motor neuron death after neonatal axotomy (Pennica et al., 1996).

Several members of the FGF family, including the prototypical FGFs, namely acidic and basic FGF (FGF-1 and FGF-2, respectively) as well as FGF-5 and FGF-9 support the survival of motor neurons in vitro, either alone or in combination with other growth factors (Hughes et al., 1993; Oppenheim, 1996; Nakamura et al., 1997). FGF-1 also prevents axotomy-induced facial motor neuron degeneration in vivo (Cuevas et al., 1995), and FGF-2 has been reported to have a small but significant effect in ameliorating disease in the Wobbler mouse (Ikeda et al., 1995c).

IGF-1 also promotes motor neuron survival (Hughes et al., 1993). In addition, IGF-1 induces lower motor neuron axonal sprouting; as a consequence, there is also interest in determining whether IGF-1 might be of benefit in treating the postpolio syndrome (Shetty et al., 1995).

Glial cell-line-derived neurotrophic factor (GDNF), a member of the transforming growth factor-β superfamily (Lin et al., 1993), is a potent survival factor for motor neurons. It has been shown that GDNF is retrogradely transported (Henderson et al., 1994; Yan et al., 1995; Oppenheim et al., 1995) by neonatal rat motor neurons in a receptor-dependent manner, and that it is expressed both in the embryonic limb bud and by Schwann cells (Henderson et al., 1994), indicating that it has the capacity to act both as a target-derived factor and in a nontarget-derived manner. Local administration of GDNF prevents axotomy-induced death and atrophy of lower motor neurons in the neonatal rat (Henderson et al., 1994; Zurn et al., 1994; Yan et al., 1995). It is the most potent trophic factor yet identified in this model, and its ability to inhibit neuronal atrophy is relatively specific, having not been previously observed with either BDNF, NT-3, or NT-4. Furthermore, either local or systemic administration of GDNF can markedly reduce the lesion-induced decrease in choline acetyl transferase reactivity in the facial nucleus of adult rats. It has also been reported that GDNF can abrogate motor neuron loss after lesion of the ventral root in adult mice (Li et al., 1995). Furthermore, mutations in the murine GDNF gene are associated with the loss of between 20 and 30% of motor neurons, indicating that GDNF exerts a physiological role in motor neuron development (Henderson et al., 1994). However, when pmn mice, which exhibit a dying-back "motoneuronopathy," were treated with encapsulated GDNF-secreting cells, there was no decrease in mortality in comparison with control animals, in contradistinction to the survival advantage observed when these animals were treated with CNTF. In the GDNF-treated mice, there was a reduction in the loss of motor neurons in the facial nucleus by approx 50% in comparison with control animals, similar to the reduction observed with CNTF. However, inhibition of axonal degeneration only occurred with CNTF and not with GDNF (Sagot et al., 1996), indicating that different growth factors can exert disparate effects on a given population of neurons. It has been established that GDNF mediates its actions through a multicomponent receptor system composed of a GPI-linked component (GDNFα) and also a transmembrane protein tyrosine kinase, Ret. Other GDNF family members have now been identified, namely neurturin and persephin, which can also support the survival of motor neurons (Kotzbauer et al., 1996; Milbrandt et al., 1998). Neurturin, persephin, and GDNF all signal via Ret, but bind to different family members of the GPI-linked receptor component, thus accounting for ligand specificity (Buj-Bello et al., 1997).

In addition, there are isolated reports suggesting that other growth factors can also exert activity on the motor neuron population. From this array of molecules, a multifunctional

growth factor, hepatocyte growth factor/scatter factor (HGF), is of particular interest. HGF was originally identified as a mitogen for hepatocytes and as a motogenic factor for epithelial cells (Stoker et al., 1987; Nakamura et al., 1989). Subsequently HGF has been shown to function as a muscle-derived chemoattractant for developing spinal motor axons and as a potent survival factor for motor neurons in vitro (Ebens et al., 1996; Yamamoto et al., 1997).

At first glance, it would appear that there is a plethora of growth factors that can potentiate motor neuron survival (Table 1). It is of note, however, that different pools of motor neurons exhibit different patterns of expression of cell-surface receptors (Fukushima et al., 1996). Thus, it will be of fundamental importance to define the regional expression profile of growth factor receptors by motor neurons in the human if we are to understand the growth factor responsiveness of different subsets of lower motor neurons that supply different groups of muscles. This will be an important step in defining which growth factors might be of maximal utility in treating ALS, given that most patients die from paralysis of either respiratory or bulbar musculature.

The Molecular Regulation of Motor Neuron Death

Growth factor receptor activation can also result in a paradoxical effect, that is, the potentiation of cell death rather than the promotion of survival. For example, the low-affinity neurotrophin receptor p75 is homologous to the Fas and tumor necrosis factor (TNF) receptors that act as death effectors (Carter And Lewin, 1997). A role for p75 in signalling the death of neural cells has now been established (Rabizadeh et al., 1993; Barrett and Bartlett, 1994), and it has recently been shown that NGF can signal through p75, in the absence of high-affinity trk receptor expression, to induce neural cell death (Frade et al., 1996; Yoon et al., 1998). It is thus of relevance that degenerating motor neurons exhibit upregulated expression of p75 (Rende et al., 1995). Nevertheless, motor neurons in Onuf's nucleus, which innervate pelvic striated muscle and which are selectively spared in ALS, also express high levels of p75 in a constitutive manner throughout life. This apparent paradox could be explained if the responsiveness of a cell to NGF stimulation is dependent on the relative levels of expression of p75 and trk. In this scenario, if p75 is expressed without trk receptors, neurotrophins could signal cell death, but if p75 were coexpressed with trk, p75 would potentiate neurotrophin binding to the high-affinity receptor and thus enhance neurotrophin-induced trophic support. Preliminary studies have indicated that downregulation of the p75 gene induced by therapeutic strategies, such as administration of antisense oligonucleotides, can abrogate motor neuron death after axotomy (Cheema, personal communication), and it will be of particular interest to determine if downregulation of p75 also abrogates cell death in animal models of motor neuron degeneration.

Growth factors also indirectly interact with a heterogeneous group of intracellular molecules known as the Bcl-2 family, some of whose members act as death agonists and others as death antagonists (Merry and Korsmeyer, 1997; Pettmann and Henderson, 1998). These molecules are active as either homodimers or heterodimers. Apoptotic death of motor neurons after axotomy is inhibited in transgenic mice exhibiting upregulated Bcl-2 expression (Farlie et al., 1995), an effect possibly mediated by inhibition of cytochrome-*c* loss from mitochondria and subsequently by inhibition of caspase activation (Pettmann and Henderson, 1998). On the other hand, the molecule Bax has been implicated in upregulating the death of motor neurons after growth factor depletion (Deckwerth et al., 1996). It is of particular note that there are reciprocal expression profiles of Bcl-2 and Bax in the motor neurons of ALS patients as opposed to controls, with an

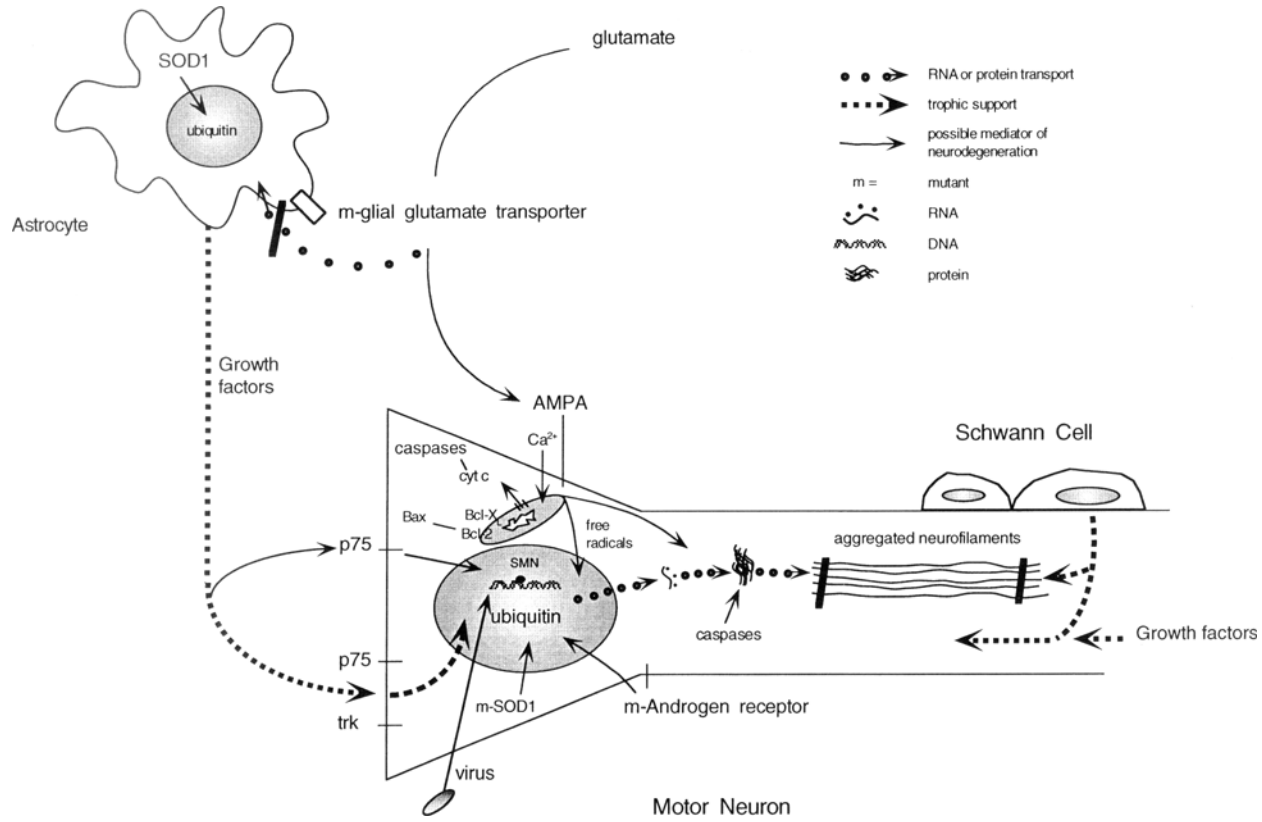

Fig. 2. Multiple molecular mechanisms contribute to trophic support and death of motor neurons. Trophic support is provided by growth factors either produced by astrocytes within the spinal cord or retrogradely transported from Schwann cells and muscle in the periphery. However, activation of the low-affinity neurotrophin receptor (p75) can also promote neuronal death when the high-affinity neurotrophin receptor (trk) is downregulated and activity of the transcription factor NF-κB is inhibited. Members of the Bcl-2 protein family also modulate neuronal cell death; some Bcl-2 family members regulate the activation of key members of proteolytic enzymes called caspases, probably by influencing release of cytochrome-*c* from mitochondria. Opening of the mitochondrial permeability transition pore as a consequence of excessive Ca^{2+} uptake, increased exposure to free radicals, and/or decline in energetic capacity could also initiate cytochrome-*c* release and caspase activation. Increased extracellular glutamate, for example, owing to mutation in the glial glutamate transporter, may also trigger Ca^{2+} influx by potentiated AMPA receptor activation. Inappropriate accumulation and/or aggregation of neurofilaments may result in impaired axonal transport. Other causes of motor neuron toxicity include viral (e.g., polio) infection or inappropriate protein sequestration by other mutant SOD1 or mutated androgen receptor. Alternatively, mutation in the SMN gene can inhibit pre-mRNA splicing and, therefore, interfere with protein synthesis.

increased ratio of Bax/Bcl-2 in the disease state (Mu et al., 1996). There is obviously considerable interest in determining how growth factors and members of the Bcl-2 family interact, and how expression profiles of these proteins can be modulated to influence motor neuron survival (Fig. 2) (Sagot et al., 1997).

Pathogenetic Mechanisms of Motor Neuron Degeneration

There are several pathogenetic mechanisms hypothesized to be implicated in mediating motor neuron degeneration in ALS (Fig. 2). These include:

1. Environmental-induced excitotoxicity leading to calcium-mediated neurodegeneration.
2. Abnormal neurofilament accumulation.
3. Autoimmunity.
4. Aberrant growth factor signaling.
5. Haploinsufficiency of the SOD1 gene leading to accumulation of free radicals and/or aberrant activity of mutant SOD1 causing a toxic gain of function.
6. Splicing abnormalities in the glial glutamate transporter.

As will be discussed, these mechanisms are not necessarily mutually exclusive.

Environmental-Induced Excitotoxicity

At one time, it appeared that the excitatory amino acid β-methyl-amino-*l*-alanine (BMAA), a constituent of flour from the false sago palm *Cycas circinalis*, might be the cause of the ALS parkinsonism dementia complex identified on the island of Guam (Duncan, 1991). It was postulated that BMAA could act as an environmental glutamate receptor agonist. Although a cycad toxin could be implicated in the condition, the candidature of BMAA now looks uncertain (Leigh, 1994). Nevertheless, motor neurons are selectively vulnerable to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor-mediated injury in vitro, which is calcium-dependent (Carriedo et al., 1996). It is also of note that patients with ALS have increased cerebrospinal fluid (CSF) glutamate (Rothstein et al., 1990). Until recently, there was no convincing evidence that such disturbances were primary, as opposed to epiphenomena. However, as will be discussed below, there is now emerging evidence that excitotoxicity may be the final mediator of neurodegeneration, both in familial ALS caused by mutation of the SOD1 gene and in sporadic ALS.

Abnormal Neurofilament Accumulation

Aberrant neurofilament accumulation in the cell bodies and axons of motor neurons is a prominent pathological feature of ALS. Neurofilaments are important determinants of the axonal diameter (Lee and Cleveland, 1994), which in turn influences conduction velocity and the axonal transport of vesicles and cytosolic proteins (Williamson et al., 1996). There are three neurofilament peptides, NF-L, NF-M, and NF-H, all of which are obligate heteropolymers (Lee and Cleveland, 1994). An essential question has been whether the abnormal accumulation of neurofilaments is a biproduct of the pathogenetic process or if it is an active participant in the genesis of ALS. This issue has been studied in transgenic mouse models, and indeed, high expression of NF-L (the core neurofilament peptide) leads to accumulation of neurofilaments in motor neurons and also causes proximal axonal swellings, degeneration of motor axons, and death of the animals by 3–4 wk of age (Xu et al., 1993). In addition, forced overexpression of human NF-H in transgenic mice results in a slow-onset, progressive neuronopathy (Cote et al., 1993). However, neither of these animals is a very accurate model of ALS, since significant motor neuron death does not occur, even in end-stage disease. However, transgenic mice with a point mutation in the central rod domain of NF-L, akin to the mutation identified in the keratin filament that occurs in bullous pemphigoid, do indeed exhibit chromatolysis of motor neurons (Lee et al., 1994). These findings have stimulated a search for mutations in neurofilament-encoding genes in patients with ALS (Vechio et al., 1996). Polymorphisms are seen, but in only one study has a deletion in NF-H been shown to cosegregate preferentially with ALS and then only in 5 of 356 patients assessed (Figlewicz et al., 1994). Thus, if mutations in the coding region of neurofilament genes are causative for ALS, they are a rare cause. Nevertheless, it remains theoretically possible that either dysregulated transcription of neurofilament genes (in particular of NF-L) or, alternatively, abnormality in neurofilament phosphorylation could be implicated in the pathogenesis of the disease.

Autoimmunity

It has been suggested that ALS could be an example of an autoimmune disease mediated by antibodies directed against the α-subunit of L-type Ca^{2+} channels (Kimura et al., 1994). These antibodies, when transferred passively to recipient mice, can enhance neurotransmitter release at neuromuscular junctions (Appel et al., 1991) and in vitro can induce the death of motor neuron/neuroblastoma hybrid cell lines (Smith et al., 1994). Antibodies detected against the GM1 ganglioside have also been detected in a subset of patients with ALS (Pestronk et al., 1989). However, it has been subsequently demonstrated that these antibodies are present in higher titers in patients with multifocal motor neuropathy (Salazar-Grueso et al., 1990). Multifocal motor neuropathy shares many of the clinical features of progressive muscular atrophy (PMA), although multifocal motor neuropathy is typically associated with more distal, asymmetric weakness and progresses more slowly than PMA. Furthermore, multifocal motor neuropathy is not a primary motor neuron degeneration, but rather a local demyelinating condition of peripheral nerves with secondary axonal degeneration. Multifocal motor neuropathy is also distinguishable from PMA by the presence of electrophysiologic multifocal motor conduction block, indicating segmental demyelination.

It is of note that immunomodulation by a variety of methods has not proven to be effective therapy for ALS, whereas multifocal motor neuropathy responds to the administration of either pooled immunoglobulin or intravenous cyclophosphamide (Van den Berg et al., 1998; Pestronk, 1998). This suggests a fundamental difference in the pathogenesis of sporadic ALS and multifocal motor neuropathy, and provides evidence, although indirect, that antibodies detected in ALS are likely to represent epiphenomena.

Aberrant Growth Factor Signaling

It is now established that adult motor neurons have the capacity to respond to growth factors. However, it is unclear whether a deficit in growth factor signaling is important in the pathogenesis of ALS. Such a deficit could potentially be generated by growth factor deficiency, downregulation of growth factor receptor expression, or aberrant intracellular signaling. Mutations in genetic sequences encoding either growth factors or their receptors could also be implicated, although it is of note that, to date, no such mutations or polymorphisms have been shown to cosegregate with familial ALS (Takahashi et al., 1994, Orrell et al., 1995). Immunohistochemical staining of control and ALS spinal cords for BDNF revealed no evidence of overt BDNF deficiency in surviving motor neurons in ALS, although it is obviously unknown whether degenerated neurons exhibited such deficiency (Kawamoto et al., 1998). Increased IGF-1 and IGF-2 binding sites have been identified in the ventral horns of patients with ALS (Dore et al., 1996), whereas significant increases of IGF binding protein and reduced IGF-1 and insulin levels occur in the sera of ALS patients (Torres-Aleman et al., 1998). Collectively, these findings suggest a homeostatic mechanism to allow degenerating motor neurons optimal access to IGF-1 in spite of reduced serum levels of this molecule. Levels of GDNF mRNA have been reported to be significantly increased in the lumbar spinal cord, but reduced in skeletal muscle of ALS patients (Yamamoto et al., 1996), whereas a marked reduction of CNTF expression in the anterior horn has been identified (Anand et al., 1995). It is uncertain whether these changes are epiphenomena or whether they are implicated in the pathogenesis of ALS.

SOD1 Mutations

Missense mutations in the SOD1 enzyme are causative for approx 10–15% of familial ALS (Rosen et al., 1993). SOD1 is ubiquitously expressed and functions to scavenge superoxide anions, suggesting the attractive hypothesis that mutant SOD1 causes elevated free radical toxicity in ALS (Bowling et al., 1993; Deng et al., 1993). However, there appears to

be no direct correlation between specific activity of the mutant enzyme and disease activity (Borchelt et al., 1994). Disease-associated mutations of SOD1 are located in both loop and β-strand domains throughout the protein, whereas only loops IV and VII form the active site of the enzyme, corroborating the view that loss of SOD1 activity is unlikely to be the cause of ALS. Furthermore, in animal models, deletion of the SOD1 gene does not result in motor neuron death (Reaume et al., 1996), and overexpression of mutant SOD1, but not of wild-type SOD1, results in motor neuron degeneration, suggesting a toxic gain of function (Wong and Borchelt, 1995).

Detailed analysis of transgenic animals genetically engineered to express mutated forms of SOD1 that are found in familial ALS has proven most interesting. The pathology delineated in these mice is similar to that of ALS, although there tends to be more vacuolation and less neurofilament accumulation in some of the animal models (Gurney et al., 1994; Wong and Borchelt, 1995). When arginine is substituted for glycine at position 85, the animals exhibit rapid progression of disease without changes in SOD1 specific activity (Bruijn et al., 1997). Pathological analysis of these mice reveals that the first exhibitable abnormality is the accumulation of astrocytic inclusions that contain both SOD1 and ubiquitin. This is followed by development of inclusions in a subset of spinal motor neurons and subsequently by rapid, nearly synchronous, death of large motor neurons around the time of onset of clinical features. Further evidence for a primary astrocytic abnormality in these mice was gained from the observation that there was a 50% decrease in the level of the glial glutamate transporter in four of five mice examined at 3 mo of age, prior to the age at which clinical signs were characteristically first observed. This raises the possibility of a sequential pathogenetic process beginning with conformational change of SOD1 in astrocytes. This would result in inappropriate sequestration of proteins, possibly including the glial glutamate transporter, resulting in the inability of astrocytes to sequester extracellular glutamate. This would ultimately result in motor neuron degeneration mediated by glutamate-induced excitotoxicity via AMPA receptor activation and intracellular calcium accumulation. This, of course, is highly speculative, but it is of note that mice administered antisense oligonucleotides against mRNA encoding the astrocytic glutamate transporter develop motor deficits (Rothstein et al., 1996). In addition, the anti-glutaminergic agent riluzole lengthens the survival of patients with ALS (Bensimon et al., 1994; Lacomblez et al., 1996).

An analysis of the biochemistry of mutant SOD1 suggests alternative and specific mechanisms by which it might induce disease. Misfolding of the mutated SOD1 protein can lead to an increased access of peroxynitrite (which is formed from chemical reaction between nitric oxide and superoxide anions) to the central copper cation and result in catalytic reactions that nitrate tyrosine residues on proteins, such as the neurofilaments and receptor tyrosine kinases (Beckman et al., 1993; Brown, 1998). This could ultimately result in alteration of either axonal transport or of signal transduction. Mutant forms of SOD1, but not the wild-type protein also interact with a number of proteins, including transporter proteins and proteins implicated in ribosomal assembly, suggesting additional mechanisms by which cellular metabolism could be disrupted (Kunst et al., 1997). Coaggregation of unidentified essential cellular components by misfolded proteins could be one of these mechanisms (Bruijn et al., 1998).

Splicing Abnormalities in the Glial Glutamate Transporter

Recent evidence has emerged to suggest that a large subset (60–70%) of patients with sporadic ALS exhibit aberrant splicing of RNA encoding the glial glutamate transporter protein, also known as the excitatory amino acid transporter 2 (EAAT2), within astrocytes located adjacent to motor cortex and spinal cord (Bai and Lipton, 1998; Lin et al., 1998). The

defect in EAAT2 processing does not appear to arise from either mutation in the gene encoding this protein or from alterations in the overall level of EAAT2 mRNA, nor does it result from a generic abnormality in the splicing apparatus, since other transcripts are not affected. However, extremely low levels of EAAT2 protein have been documented in the motor cortex of a subset of ALS patients, suggesting that the aberrantly spliced proteins exert a dominant negative effect. The aberrantly spliced EAAT2 mRNAs are of two major types, comprising either a partial intron 7-retention or an exon 9-deleted transcript, although many other internal deletions, occurring at lower frequencies, have also been identified. It is of note that the aberrant mRNA species have been detected in the CSF of patients with ALS and this could have diagnostic utility. Overall, these findings support the hypothesis that excitotoxicity contributes to the pathogenesis of ALS, since a deficit in the glial glutamate transporter would be expected to lead to an increase in the concentration of extracellular glutamate.

RNA splicing is expedited by the spliceosome, a macromolecular complex containing multiple proteins and small nuclear RNAs. The spliceosome recognizes 5′ and 3′ splice sites composed of specific consensus dinucleotides. In the case of sporadic ALS, the abnormality appears not to be in the splice site of the EAAT2 gene, but in unidentified factors that appear specific for the regulation of splicing of the EAAT2 transcript. The reason for the specificity of effect, both in terms of the gene involved and its spatial restriction to astrocytes in the motor cortex and anterior horn of spinal cord, remains unclear. It could be postulated that the aberrant splicing is secondary to neuronal degeneration, but the spliced variants have been detected in the CSF of patients up to 4 yr prior to death. In addition, patients with other genetic conditions associated with loss of motor neurons do not exhibit aberrantly spliced variants of EAAT2. It is possible that the aberrant EAAT2 transcripts are consequent to either a relaxation in splicing fidelity or an abnormality in the pathway for degradation of mutant mRNA, secondary to a somatic gene mutation. This could establish a positive feedback loop whereby loss of EAAT2 causes excitotoxicity in adjacent neurons, leading to generation of free radicals, which could, in turn, cause further damage to DNA in the surrounding glia. Although this explains how a self-perpetuating pathogenetic mechanism could be propagated, it fails to explain how the sequence of events is initiated. Certainly, somatic gene mutation could occur in embryogenesis, but it would be expected that the relevant mutation would be restricted to segments of the CNS generated from a single affected precursor cell rather than occurring throughout the neuraxis in regions adjacent to lower motor neurons.

Associated Conditions

Recent advances in the understanding of the molecular genetics of two conditions related to ALS have further enhanced our knowledge of the pathophysiology of the motor neuron. Spinal muscular atrophy (SMA) is an autosomal-recessive disorder characterized by degeneration of motor neurons in the spinal cord. A gene directly linked to SMA known as survival of lower motor neurons (SMN) has recently been identified (Lefebvre et al., 1995). Two copies of the SMN gene are located in a 500-kb inverted repeat of chromosome 5 at 5q 13.1. In over 98% of SMA patients, the telomeric copy of SMN is deleted or mutated on both chromosomes, whereas the centromeric copy is unaffected. The SMN protein is found in the cytoplasm and nucleus of cells, and is expressed at extremely high concentrations in motor neurons. Normally, this protein forms a complex with several spliceosomal small nuclear ribonuclear proteins (snRNP) that are responsible for catalyzing pre-mRNA splicing. Spinal muscular atrophy may therefore be the result of a genetic defect impairing spliceosomal snRNP assembly in motor neurons (Fischer et al., 1997; Liu et al., 1997) resulting in a

deficit of pre-mRNA splicing (Pellizzoni et al., 1998). It will thus be of great interest to compare and contrast this deficit with the molecular mechanism that leads to the selective deficiency of splicing of EAAT2 identified in sporadic ALS. A second gene that encodes the protein neuronal apoptosis inhibitory protein (NAIP), also located in the inverted repeat region of chromosome 5, is mutated in a subset of patients with SMA (Roy et al., 1995). The NAIP protein is homologous to two proteins well characterized to inhibit apoptosis in insect cells (Liston et al., 1996). Mutations in the NAIP protein probably potentiate, by unknown mechanisms, the phenotypic expression of disease established by deletions/mutations in the SMN gene (Chen et al., 1998).

The second condition of interest is spinal and bulbar muscular atrophy (Kennedy's disease). This is a slowly progressive form of motor neuron degeneration that selectively affects the lower motor neuron causing progressive proximal limb and bulbar muscle weakness, together with signs of androgen insufficiency, including testicular atrophy and gynecomastia. It is an X-linked condition caused by trinucleotide (CAG) repeat expansion of the androgen receptor, such that patients with Kennedy's disease have CAG repeats ranging in number from 40 to 62, whereas normal individuals have 10–36 repeats (La Spada et al., 1991). It is not known how mutation of the androgen receptor gene causes Kennedy's disease. Nevertheless, in the last few years, much has been learned about the neuropathology of both Kennedy's disease and other neurodegenerative disorders, such as Huntington's disease and the dominantly inherited spinocerebellar ataxias (types 1, 2, 6, and 7), that are also associated with trinucleotide CAG repeat expansions of specific genes. It has been postulated that a common pathogenetic mechanism applies to all of these conditions, including Kennedy's disease. In all cases, the CAG repeat is located within the coding region and it is thus translated into a stretch of polyglutamine residues. This expansion appears to confer on the "disease" protein a toxic gain of function through induction of molecular misfolding that results in altered protein interactions, possibly secondary to the expanded glutamine repeats acting as polar zippers (Perutz et al., 1994).

Evidence for the involvement of the polyglutamine stretch in the induction of disease comes from the observation that each of the relevant proteins is dissimilar outside of the polyglutamine tract. Furthermore, in each disease, the severity of the phenotype increases with increasing size of the glutamine repeats (anticipation), which is concordant with in vitro data indicating that insoluble high-mole-wt protein aggregates are only generated when the number of glutamine residues is expanded into the pathogenetic range. All of the protein aggregates that have been detected in vivo are located in the nucleus of the affected cells, even though the wild-type huntingtin protein (mutated in Huntington's disease) and ataxin-3 (mutated in spinocerebellar ataxia type-3) are predominantly cytoplasmic proteins. It has been suggested that the latter proteins may be subject to proteolysis, such that the N-terminal segment containing the glutamine expansion can then migrate to the nucleus; alternatively, the polyglutamine expansion may unmask a functional nuclear localization signal. In Kennedy's disease, as well as in Huntington's disease and Machado-Joseph disease (spinocerebellar ataxia type-3), there is localized neuronal degeneration that specifically accounts for the clinical phenotype. In the case of Huntington's disease, the nuclear aggregates or inclusions are restricted to neurons located in these regions (Li et al., 1998). The presence of ubiquitin in the neuronal inclusions suggests that these lesions might sequester degradation products, although it has been postulated that they might also sequester transcription factors. It is of note that nuclear inclusions that stain for antibodies against the N-terminal of the androgen receptor have also been identified in the nuclei of motor neurons of patients with Kennedy's disease (Li et al., 1998).

Most of the involved proteins, including the androgen receptor, are expressed widely in the

central nervous system (CNS), independent of the number of glutamine residues (Li et al., 1998), and there is, as yet, no satisfactory explanation regarding why only selective brain regions are affected in each of these diseases. It has been hypothesized that there might be region-specific transport mechanisms that direct expression of the relevant proteins to the nucleus. Alternatively, there may be region-specific expression of a second group of proteins that interact with the disease proteins to alter their folding, distribution, and/or degradation (Paulson et al., 1997). In this regard, it is of interest that only antibodies generated against the N-terminal portion of the androgen receptor recognize the nuclear inclusions identified in Kennedy's disease, suggesting either masking of the carboxy-terminus or proteolytic cleavage. Caspase cleavage sites have been identified in each of the relevant proteins, and caspase activity in vitro can lead to the generation of truncated polyglutamine-containing proteins, although mutant proteins are no more susceptible to this cleavage than their wild-type counterparts. This has suggested that it is not the generation of truncated products *per se*, but the propensity of these products to aggregate that is the responsible factor in determining whether neurodegeneration occurs (Wellington et al., 1998). This, of course, leaves unanswered how activation of effector caspases occurs, in order to initiate the cell death program. It is nevertheless possible that chronic low-grade caspase activity leads to the gradual accumulation of toxic polyglutamine-containing protein fragments over many years.

Recent data suggest that the nuclear inclusions may not be the direct cause of the phenotype of the trinucleotide repeat disorders (Sisodia, 1998; Klement et al., 1998; Saudou et al., 1998). It has been possible to generate mutant mice that overexpress mutant forms of ataxin-1 (Klement et al., 1998) with expanded polyglutamine repeats, which do not express a self-association domain. This latter domain is necessary to allow the polypeptide to self-aggregate, although the deficient form of the protein retains the ability to enter the nucleus. Somewhat surprisingly, transgenic mice carrying these constructs still developed neuropathology, almost indistinguishable from that of mice expressing human ataxin-1 with glutamine expansion, but with preservation of the self-association domain. Saudou et al. (1998) have suggested that nuclear inclusions occur as a consequence of ubiquitination, indicative of a scavenging pathway. In fact, in vitro work studying the toxic effects of the huntingtin protein mutated with expanded polyglutamine repeats suggests that inhibition of ubiquitin-conjugating enzymes results in a decrease in the number of nuclear inclusions, but also in an acceleration of cell death. This would indicate that pathogenesis is induced by upstream events possibly mediated by direct binding of the expanded polyglutamine residues to specific as yet unidentified proteins or, alternatively, the polyglutamine residues could potentiate these binding events by inducing a change in protein conformation. If this scenario is true, it will be of particular interest to determine the nature of the binding proteins, since these molecules could exert essential functions in the maintenance of motor neuron viability. As a consequence, modification of the level of expression of these molecules and, therefore, of their activity could serve to ameliorate disease.

Conclusions

In the last five years, there has been a revolution in our understanding of the biology of the motor neuron. Unfortunately, these advances have not, as yet, resulted in significant improvement in the management of patients with motor system degenerations. Nevertheless, our best chance of developing successful therapeutic strategies will most likely come from further advances in our understanding of the basic cellular and molecular neurobiology of these diseases. Hopefully, in the next five to ten years, this knowledge will be translated into the therapeutic advances that are so desperately required.

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