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Elucidating the molecular mechanisms that underlie the target control of motoneuron death

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ABSTRACT Approximately half of the motoneurons generated during normal embryonic development undergo programmed cell death. Most of this death occurs during the time when synaptic connections are being formed between motoneurons and their target, skeletal muscle. Subsequent muscle activity stemming from this connection helps determine the final number of surviving motoneurons. These observations have given rise to the idea that motoneuron survival is dependent upon access to muscle derived trophic factors, presumably through intact neuromuscular synapses. However, it is not yet understood how the muscle regulates the supply of such trophic factors, or if there are additional mechanisms operating to control the fate of the innervating motoneuron. Recent observations have highlighted target independent mechanisms that also operate to support the survival of motoneurons, such as early trophic-independent periods of motoneuron death, trophic factors derived from Schwann cells and selection of motoneurons during pathfinding. Here we review recent investigations into motoneuron cell death when the molecular signalling between motoneurons and muscle has been genetically disrupted. From these studies, we suggest that in addition to trophic factors from muscle and/or Schwann cells, specific adhesive interactions between motoneurons and muscle are needed to regulate motoneuron survival. Such interactions, along with intact synaptic basal lamina, may help to regulate the supply and presentation of trophic factors to motoneurons.

KEY WORDS: *Motor neuron survival, basal lamina, agrin, anoikis, trophic factor, branching, programmed cell death*

Introduction

A large percentage of motoneurons undergo apoptosis or programmed cell death (PCD) during embryonic development in vertebrates. Historically, most work elucidating the molecular mechanisms that control motoneuron death stem from target ablation and pharmacological experiments in the chick. These experiments show that both trophic factors and activity of motor units (α-motoneurons and the skeletal muscle fibres they innervate) are important in regulating the number of surviving motoneurons during embryonic development. More recently, molecular genetic studies show specific cellular interactions are required for the survival of motor units. This review provides a brief background on how the motor unit controls motoneuron survival during embryonic development. We then propose a novel mechanism by which motoneurons are selected to live or die through cell-matrix interactions (anoikis) at the neuromuscular synapse. We then describe ways in which our laboratory is testing this theory with specific reference to agrin, rapsyn and MuSK-deficient mice.

The Spatio-Temporal Sequence of Motoneuron Death

The spatio-temporal succession of motoneuron death in the spinal cord is an ordered morphological event (Yamamoto and Henderson, 1999). Apoptotic nuclei are evident in the cervical region of the spinal cord followed by thoracic, brachial, lumbar and sacral motoneurons in mice and rats (Yamamoto and Henderson, 1999). The chick has a somewhat different sequence of motoneuron death with cervical motoneurons dying first, followed by lumbar, thoracic, brachial and sacral (Yamamoto and Henderson, 1999). This stereotyped sequence indicates that motoneuron death during normal embryonic development is a highly controlled process.

Abbreviations used in this paper: AChR, Acetylcholine receptor; BDNF, Brain-derived neurotrophic factor; CNS, Central nervous system; CNTF, Ciliary neurotrophic factor; Endo N, endoneuraminidase; FLIP, FLICE inhibitory protein; GDNF, Glial-derived neurotrophic factor; IGF, Insulin-like growth factor; NCAM, Neural cell adhesion molecule; PCD, Programmed cell death; TrkB, Tyrosine kinase B receptor.

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The first signs of cell death are in proliferating neural precursor cells and young postmitotic neuroblasts (de la Rosa and de Pablo, 2000). This period of cell death begins in neurons that are projecting toward their target and is named projecting-neuron cell death. This form of cell death is unresponsive to trophic factors and is also independent of interactions with the target, skeletal muscle (Yaginuma *et al.*, 1996). The authors suggest that this period of cell death is cell autonomous or is dependent on other interactions within the central nervous system (CNS) (Yaginuma *et al.*, 1996).

Projecting motoneuron death may also be explained by motoneuron-motoneuron, or motoneuron-target interactions during the "waiting period" of axonal outgrowth (Tosney and Landmesser, 1985; Wang and Scott, 2000; Eisen and Melancon, 2001). In the chick, motoneurons extend axons to their primordial limb bud and wait for approximately 24 hours until their target muscle differentiates and proliferates before innervating the muscle (Tosney and Landmesser, 1985; Wang and Scott, 2000). This waiting period also exists in the zebrafish and is known to be a selection point between two identified motoneurons where only one motoneuron may grow towards its final innervation site and the other dies (Eisen and Melancon, 2001). The selection process is thought to depend on interactions between the growing axons and its target muscle (Eisen and Melancon, 2001). Selection of motoneurons to live or die at the waiting period in mammals is yet to be studied. The study of early PCD is in its infancy making its significance in moulding the CNS unclear (reviewed in de la Rosa and de Pablo, 2000).

Although motoneurons may undergo projecting cell death early in embryonic development, most motoneurons that undergo PCD die during the period when skeletal muscle becomes functionally innervated (Hamburger, 1975; Chu-Wang and Oppenheim, 1978; Dahm and Landmesser, 1991; Hamburger and Hamilton, 1992) as described in the subsequent sections.

Motoneurons Require the Presence of the Target Skeletal Muscle and the Schwann Cell to Survive PCD

Shorey (1909) first documented decreases in motoneuron number after amputating the target limb, however she thought that the decrease was due to changes in early motoneuron differentiation (Shorey, 1909). It was only later that Victor Hamburger and Levi Montalcini recognised that the decrease in motoneuron number after limb ablation was an exacerbation of the normal PCD of motoneurons and concluded that the target limb may regulate motoneuron survival during embryonic development (Hamburger, 1934; reviewed in Oppenheim, 1991). As the limb is a heterogeneous mixture of many cell types it was not known what target cells promoted the survival of motoneurons. Surgical removal of somites, the developmental precursors of muscle cells, increased motoneuron death like that in limb bud ablation providing evidence that motoneuron survival is dependent on their target skeletal muscle fibres (Phelan and Hollyday, 1991). Further evidence that motoneurons depend on their target skeletal muscle to survive stems from transgenic mice that lack skeletal muscle which retain only 10% of the normal number of surviving motoneurons (Grieshammer *et al.*, 1998). Thus, motoneurons depend on the presence of the skeletal muscle fibre to survive.

Motoneurons may also require Schwann cells to survive the period of naturally occurring PCD. Deletion of Erb-b3 gene leads to the absence of Schwann cell precursors and the formation of Schwann cells surrounding both motor and sensory neurons (Riethmacher *et al.*, 1997). This resulted in the death of 79% of the peak number of motoneurons during the period of PCD (Riethmacher *et al.*, 1997). The large increase in motoneuron death occurred between E15.5 and E18.5 indicating that the Schwann cells are not required for neurite growth to their target, but are required during the period when muscles become innervated and functional (Riethmacher *et al.*, 1997). Thus, other contributing factors to motoneuron death may occur in these mice including changed electrical activity of the motoneuron or an involvement of Erb-b3 in regulating the expression of trophic factors (Riethmacher *et al.*, 1997). To test these possibilities, the generation of Cre-conditional transgenic mice that lack specifically Schwann cells is required (Grieshammer *et al.*, 1998). Taken together, it appears that Schwann cells are insufficient to rescue motoneurons in the absence of skeletal muscle and the muscle fibre is insufficient to rescue motoneurons without Schwann cells.

Results from the target ablation studies led to the proposal that skeletal muscle contains trophic factors that promote motoneuron survival during embryonic development (Hamburger and Levi-Montalcini 1949). Indeed trophic factors from the muscle and Schwann cell are retrogradely transported to the motoneuron cell body (Bartlett *et al.*, 1998; Reynolds *et al.*, 2000). Comprehensive reviews of trophic factors that promote motoneuron survival are presented elsewhere (Henderson *et al.*, 1998; Sendtner *et al.*, 2000). Although muscle derived trophic factors promote motoneuron survival after limb ablation, only a small proportion of motoneurons that normally die after limb ablation survive with the addition of muscle extract, which presumably contains all muscle derived trophic factors (Caldero *et al.*, 1998). Therefore, it is likely that structural and functional aspects of muscle differentiation are also required to support motoneurons during embryonic development.

Muscle Activity, Motoneuron Survival and Intramuscular Branching are intricately related during Embryonic Development

Blocking neuromuscular activity with acetylcholine receptor (AChR) antagonists such as *d*-tubocurare, α-cobratoxin and α-bungaratoxin during embryonic development promotes motoneuron survival in the chick (Pittman and Oppenheim, 1978; Pittman and Oppenheim, 1979). In fact nearly all motoneurons survived the period of motoneuron death when muscle activity is absent (Pittman and Oppenheim, 1978; Pittman and Oppenheim, 1979). Similar responses are found in the mouse (Houenou *et al.*, 1990) and rat (Harris and McCaig, 1984). Conversely, activating muscle and nerve during the period of PCD increased the number of dying motoneurons (Oppenheim and Nunez, 1982). These studies indicate that motoneuron survival is not only dependent on the presence of skeletal muscle but also dependent on its activity.

Blocking neuromuscular activity not only stops motoneuron death, but also increases motor neurite branching within the muscle and the number of neuromuscular synapses (Pittman and Oppenhiem, 1979; Harris and McCaig 1984; Dahm and Landmesser, 1988, 1991; Houenou, *et al.*, 1990; reviewed in Landmesser, 1992). Both paralytic and sub-paralytic doses of *d*-tubocurare promote the survival of motoneurons, increase branching and the number of neuromuscular synapses during embryonic development (Oppenheim *et al.*, 2000). The promotion of moto-

neuron survival, branching and neuromuscular synapse formation is significantly greater with paralytic versus sub-paralytic doses of *d*-tubocurare indicating that changes in motoneuron survival results from the precise regulation of muscle activity (Oppenheim *et al.*, 2000).

Furthermore, a close relationship between motoneuron survival and innervation exists when the extent of branching is decreased with endoneuraminidase (endo N; Tang and Landmesser, 1993; Usiak and Landmesser, 1999). Administration of *d*-tubocurare increases the level of polysialic acid and neuron cell adhesion molecule (NCAM) which can subsequently be removed by endo N (Tang and Landmesser, 1993; Usiak and Landmesser, 1999). Endo N decreases branching and innervation and also decreases the number of surviving motoneurons which would otherwise be increased by *d*-tubocurare treatment (Tang and Landmesser, 1993; Usiak and Landmesser, 1999). Interestingly, this reduction in survival and branching may be due to a change in transmitter release from the motor neurite terminals (Rafuse *et al.*, 2000), however this needs to be directly tested. Together these studies indicate a precise relationship between motoneuron survival, intramuscular axonal branching and neuromuscular synapse formation that are regulated by muscle activity.

Breaking the Relationship between Muscle Activity, Motoneuron Survival and Branching when comparing Agrin, Rapsyn and MuSK-Deficient Mice

The neuromuscular synapse is a highly specialised connection between the motor nerve terminal and the skeletal muscle fibre (Sanes and Lichtman, 1999). The motor nerve terminal occupies only 0.1% of the muscle membrane and is able to transduce signals from the motor nerve terminal to the skeletal muscle fibre to induce muscle contraction. The specialisations include active zones in the pre-synaptic terminal that align adjacent to AChR clusters on muscle fibre junctional folds (Fig. 1). Acetylcholine receptors concentrate to 10,000/μm^2 in the synapse compared to 10/μm^2 in non-synaptic areas (Colledge and Froehner, 1998). During development of the neuromuscular synapse, agrin is thought to be secreted by the nerve terminal to activate its muscle specific kinase receptor (MuSK) to bring about AChR clustering through a receptor associated protein of the synapse, rapsyn (Apel and Merlie, 1995; Apel *et al.*, 1997; Fuhrer *et al.*, 1999; Gautam *et al.*, 1999). Recently, the genes that encode these proteins were knocked out in mice. These mice have few or no AChR clusters underneath motor nerve terminals and show varying degrees of disruption of innervation and transcription of AChRs (Gautam *et al.*, 1995; DeChiara *et al.*, 1996; Gautam *et al.*, 1996).

Rapsyn-deficient mice live for approximately six hours after birth and are thought to have the least synapse disruption of the three mice generated (Gautam *et al.*, 1995). AChR clusters are not found in rapsyn-deficient mice, however membrane bound AChRs are found in the centre of the muscle (Gautam *et al.*, 1995). The restricted AChR expression is brought about by centrally located muscle nuclei (synaptic nuclei) that retain high levels of gene transcription for the AChR subunits in rapsyn-deficient mice similar to that in wild-type mice (Gautam *et al.*, 1995).

Agrin-deficient mice die at birth and are thought to have a disrupted synapse that is more severe than rapsyn-deficient, but not as disrupted as MuSK-deficient mice (Gautam *et al.*, 1996). Agrin is not required for the initial formation of AChR clusters (Lin *et al.*, 2001), however it is required for their maintenance and maturation (Gautam *et al.*, 1996). Thus, agrin-deficient mice have AChR clusters at neurite terminals at the initial stages of neuromuscular synaptogenesis but these receptor clusters soon dissipate (Gautam *et al.*, 1996). Moreover, transcriptional specialisation of AChR in the centre of muscle is lost in agrin-deficient mice so that membrane bound AChRs are found throughout the muscle (Gautam *et al.*, 1996).

MuSK-deficient mice also die at birth and are thought to have the

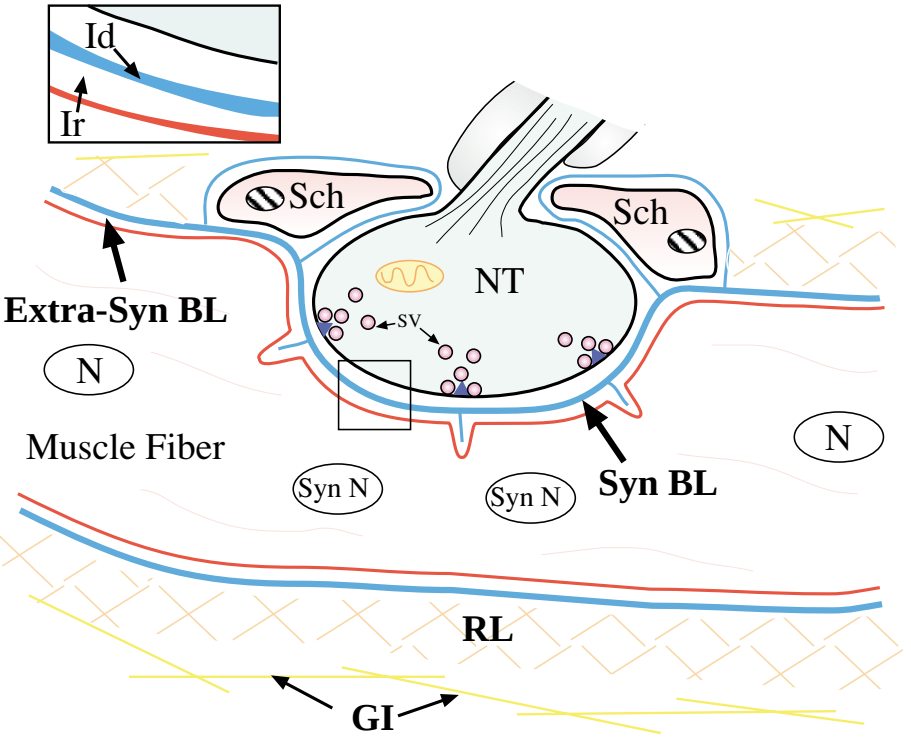

Fig. 1. Structure of the neuromuscular junction and the organisation of the extracellular matrix at synaptic and extra synaptic regions of the muscle. *Shown is a drawing of an adult neuromuscular synapse, where the pre-synaptic component [motor nerve terminal (NT) and capping Schwann cells (Sch)] have made contact with a skeletal muscle fiber. In between the pre-synaptic component and the muscle fiber is synaptic basal lamina (Syn BL) consisting of a lamina rara (lr) and lamina densa (ld), detailed in the boxed insert. This basal lamina extends over the entire surface of the muscle fiber (extra synaptic BL). Overlying the extra synaptic basal lamina is the reticular lamina (RL-Gl). Muscle synaptic nuclei (Syn N) are located beneath the postsynaptic junctional folds. Within the nerve terminal are active zones (blue triangles) with synaptic vesicles (sv) associated with them. Extrasynaptic muscle nuclei (N).*

most disrupted synapse of the three lines of mutant mice (DeChiara *et al.*, 1996). MuSK-deficient mice lack AChR clusters and transcriptional specialisation of AChRs by sub-synaptic nuclei in the centre of the muscle (DeChiara *et al.*, 1996). Thus, synapse disruption (and presumably disruption of muscle contraction) is greatest in MuSK followed by agrin followed by rapsyn (Gautam *et al.*, 1999).

As the level of muscle activity regulates motoneuron survival, branching and synapse formation we tested whether the same applies when comparing rapsyn and agrin-deficient mice (Banks *et al.*, 2001; Noakes *et al.*, 2001). We found that the force of muscle contraction reflected the biochemical disruption with rapsyn-deficient mice having a significantly greater muscle contraction compared to agrin-deficient mice (Noakes *et al.*, 2001). The decreased muscle contraction induced greater branching in agrin-deficient mice (Banks *et al.*, 2001; Noakes *et al.*, 2001), but not motoneuron survival compared to rapsyn-deficient mice at the end of the motoneuron death period in accordance with previous results (Banks *et al.*, 2001; Noakes *et al.*, 2001; Terrado *et al.*, 2001). By quantifying motoneuron numbers throughout the period of naturally occurring PCD we found that the number of motoneurons significantly decreased at the same time as the skeletal muscle basal lamina became disorganised in agrin-deficient mice at E16.5 (Noakes *et al.*, 2001). This does not occur in rapsyn-deficient mice (Gautam *et al.*, 1995; Banks *et al.*, 2001; Noakes *et al.*, 2001). Thus, it appears that the basal lamina is important for motoneuron survival during embryonic development. Basal lamina components are known to enhance the motoneuron survival properties of trophic factors (especially CNTF) *in vitro* (reviewed in Sendtner *et al.*, 2000). Recently CNTF receptor α has been found to associate with the extracellular matrix (Gould *et al.*, 2001) and is important in regulating motoneuron survival *in vivo* (DeChiara *et al.*, 1995; Bartlett *et al.*, 2001; however see Terrado *et al.*, 2001). The disruption of the basal lamina and subsequent inappropriate contact between the growing motor neurite terminal with muscle is likely to allow motoneuron death in agrin-deficient mice.

Motoneurons Attach to the Muscle Fibre through the Synaptic Basal Lamina

As motoneurons require the presence of the skeletal muscle to survive, it is interesting to note that motoneurons do not directly attach to the muscle fibre. Sandwiched between the motor nerve terminal and the postsynaptic membrane of the muscle is basal lamina, known as synaptic basal lamina (Fig. 1). Experiments where the muscle has first been denervated, followed by removal of both muscle and Schwann cells but leaving an intact synaptic basal lamina is enough to direct re-innervating motor neurons to stop at synaptic basal lamina and differentiate into a functional nerve terminal (Glicksman and Sanes, 1983). Likewise, if the muscle is allowed to regenerate within basal lamina tubes but the motor nerves or Schwann cells are not allowed to enter, the portion of the muscle membrane under the original synaptic basal lamina will form postsynaptic specialisations (Sanes *et al.*, 1978). Thus, the synaptic basal lamina can mediate both pre and postsynatic differentiation. This idea lead investigators to identify and clone molecules such as β2-laminin and neural agrin that are unique to the synaptic basal lamina and which induce pre- and postsynaptic differentiation respectively (Hall and Sanes, 1993; Noakes *et al.*, 1995; Gautam *et al.*, 1996; Burgess *et al.*, 1999; Burgess *et al.*, 2000). In this section we give a brief overview of the organisation of the muscle basal lamina before we propose how the basal lamina may function to regulate motoneuron survival.

Muscle fibres are surrounded by the sarcolemma, a highly organised structure consisting of the muscles plasma membrane, overlying basement membrane and glycocalyx (Fig 1). The basement membrane is further divided into reticular lamina and basal lamina. The basal lamina comprises an electron dense lamina densa and an electron lucent lamina rara (Sanes, 1994). At synaptic sites, only the basal lamina is present between the motor nerve terminal and underlying muscle fiber (see Fig. 1). The molecular composition of the sarcolemma consists of some of the following molecules: integrins and proteins of the dystrophin associated complex in the plasma membrane; fibronectin, laminins, collagens (I, III, IV and V), entactin, and agrin in the basement membrane and glycocalyx (Sanes, 1994).

The basal lamina functions in providing a strong adhesive connection between the motor nerve terminal and the muscle fibre in an environment where fibres are continually changing length in response to motor nerve stimulation. It is also vital for the differentiation and formation of skeletal muscle as shown in many muscular dystrophy diseases. Agrin is a vital contingent of the muscle basal lamina (Bezakova and Lomo, 2001; Moll *et al.*, 2001; reviewed in; Hagiwara and Fallon, 2001). Muscular dystrophy models that lack α2-laminin show a disrupted basal lamina that is somewhat rescued by agrin (Moll *et al.*, 2001). This process may involve the reorganising of castomeres in the muscle fibre membrane by agrin (Bezakova and Lomo, 2001). Thus, agrin is important for basal lamina formation in non-synaptic regions of the basal lamina.

So far the role of agrin at the neuromuscular junction is best known by its ability to cluster postsynaptic AChRs, thereby promoting maturation of the synapse which provides efficient transmission from the nerve terminal to the muscle (Gautam *et al.*, 1996; Sanes and Lichtman, 1999). We propose here that the synaptic basal lamina induced by neural agrin may also function in governing motoneuron survival during embryonic development. Agrin deposited into the synaptic basal lamina binds to laminin trimers through an interaction with the γ1 chain, a subunit present in all synaptic laminin trimers (Kammerer *et al.*, 1999). Stabilisation and concentration of laminins, in particular laminins 9 and 11 (α4,β2,γ1 and α5,β2,γ1 respectively), by agrin could be critical for increased synaptic efficacy during development, and in part, determine motoneuron fate. In our proposed model (see Fig. 2), agrin is needed to present laminin 9 and 11 to its pre-synaptic receptors that associate with the mechanics of transmitter release from the motor nerve terminal. The pre-synaptic receptor for the α5 subunit of laminin 11 appears to be the transmembrane proteoglycan SV2 (Son *et al.*, 2000), which is present on synaptic vesicles and becomes exposed on the pre-synaptic membrane upon fusion of these vesicles. It is proposed that the SV2-α5 interaction promotes pre-synaptic adhesion to the synaptic basal lamina (Son *et al.*, 2000). The pre-synaptic receptor for the α4 subunit of laminin 9 is not known, but is thought to be closely associated with the pre-synaptic calcium channels suggesting that α4 interaction with such a receptor could promote increases in transmitter release (Sunderland *et al.*, 2000). Finally, laminins 4, 9 and 11 could also interact with pre-synaptic integrin receptors to promote transmitter release (Wong *et al.*, 1999). Together, these observations suggest that the stabilisation and presentation of synaptic laminins through agrin play a key role in survival of the motoneuron.

Cell-Matrix Interactions regulate Cell Survival: Anoikis

Anoikis is "the subset of apoptosis triggered by inadequate or inappropriate cell-matrix contacts" (Frisch and Screaton, 2001). Anoikis was first found to occur in epithelial cells so correct cell numbers are maintained in an environment where constant turnover of cells is required. Anoikis is currently a major area of research due to its role in the spread of cancer. Prevention of anoikis is required for neoplasia as a cell must detach from the matrix, translocate to a different site within the body and then re-attach to spread tumours (Ruoslahti, 1999). From this recent literature, many similarities between matrix molecules that regulate anoikis of epithelial cells and those matrix molecules in the synaptic basal lamina that regulate motor neurite terminal adhesion to their target muscle fibres are becoming apparent.

Cell-matrix adhesion dependent death is thought to be primarily dependent on integrins via activation of integrin-linked kinase (Delcommenne *et al.*, 1998; Attwell *et al.*, 2000). Integrins are transmembrane heterodimers that function as low affinity receptors for fibronectin and laminin to attach a cell to the surrounding extracellular matrix. They also directly associate with receptor tyrosine kinases (Sundberg and Rubin, 1996; Falcioni *et al.*, 1997; Schneller *et al.*, 1997; Moro *et al.*, 1998) and activate signalling cascades that regulate gene expression (Clark and Brugge, 1995; Giancotti and Ruoslahti, 1999). The laminins and integrins that are implicated in anoikis of epithelial cells are those that are also found at the neuromuscular junction. By drawing correlations between these laminins and integrins we can begin to test whether motoneuron survival during embryonic development is dependent on its interactions with the skeletal muscle basal lamina.

The α1, α3-7, αv and β1 integrins are found at the presynaptic terminal and α1, α3, α7, α9, αv and β1 are localised to the postsynaptic membrane (Martin *et al.*, 1996). Integrins containing αv and β1 subunits are involved in agrin-induced AChR clusters as blockade of αv, β1 integrins inhibit agrin induced AChR clusters (Martin and Sanes, 1997). Blocking antibodies to β1 integrin increase the number of dying motoneurons after nerve crush injury (Wong *et al.*, 1999). Thus, integrin dimers containing αv and β1 subunits may have an altered distribution in agrin-deficient mice but not in rapsyn-deficient mice and may account for the sudden drop in motoneuron death when the basal lamina becomes patchy.

Integrin dimers containing αv and β1 play major roles in anoikis. Anoikis of human colonic crypt cells and differentiated human neuroblastoma cells is dependent on β1 integrin/matrix interactions (Strater *et al.*, 1996; Bonfoco *et al.*, 2000). Human carcinoma intestinal cells (Caco-2) are resistant to anoikis due to a low expression of αv integrin (Kozlova *et al.*, 2001). Expression of αvβ3 integrin in Caco-2 cells makes them much more susceptible to anoikis (Kozlova *et al.*, 2001). The αv integrin regulates angiogenesis of endothelial cells in carcinomas (Ruoslahti, 1999).

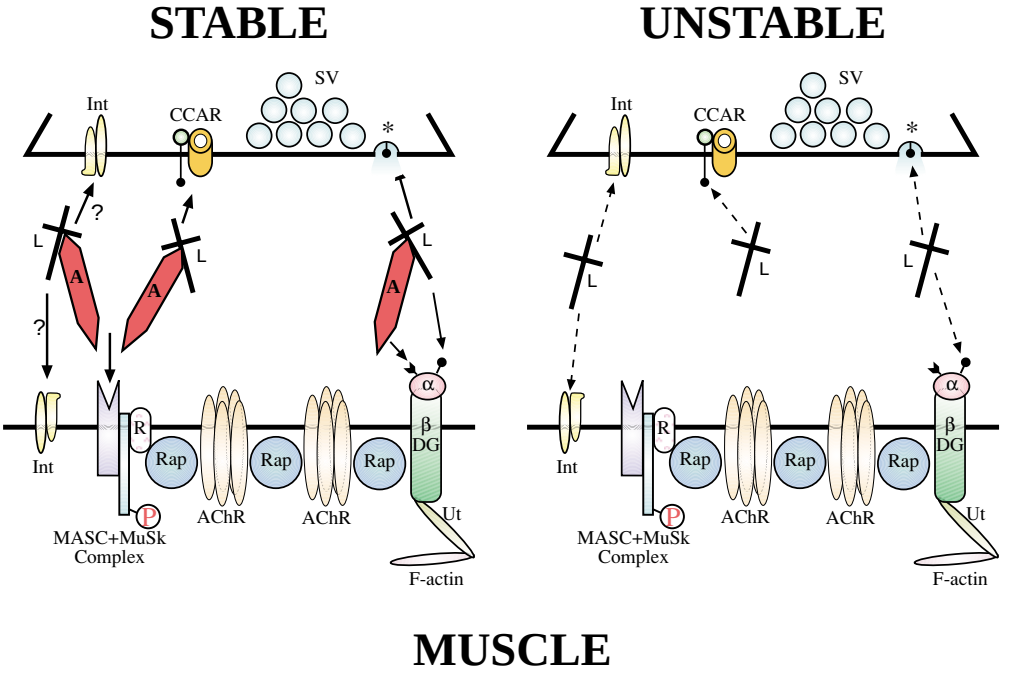

Fig. 2. A proposed model of the synaptic domains at stable and unstable neuromuscular junctions. *At stable synapses, agrin (A, red) is deposited into the synaptic cleft where it can bind to synaptic laminins 4, 9 and/or 11 (L). This allows for laminins to interact with pre-synaptic receptors at the nerve terminal. These receptor interactions include α5 laminin binding to a receptor complex associated with calcium channels (CCAR), α4 laminin binding to SV receptor at sites of synaptic vesicle (SV) release at active zones (*), and possible (**?**) interactions of all three laminins with pre-synaptic integrin receptors (Int, yellow). In addition, these laminins and agrin can bind to postsynaptic receptor complexes as well. These include the following: laminin and agrin binding to α-dystroglycan (α), part of the dystophin associated transmembrane complex (α+βDG) that links to the muscle's cytoskeleton through utrophin (Ut) and F-actin; agrin binding to the muscle specific kinase (MuSK) complex that is composed of a muscle associated specific component (MASC) and transmembrane linker (R) to rapsyn (Rap), which upon agrin binding induces phosphorylation of MuSk (red P) which causes acetylcholine receptor (AChR) clustering under nerve terminals via rapsyn; and potential (**?**) interactions of laminins with integrin receptors in the postsynaptic membrane. Together these molecular interactions bring about stable adhesion of pre- and postsynaptic elements to promote motoneuron viability. At unstable synapses, when molecules such as agrin are missing the presentation of laminins to both pre- and postsynaptic receptors are lost and/or are less stable (dashed arrows). In the case of missing agrin, induction of postsynaptic specialisations are not maintained. Together, this could result in unstable adhesion interactions between pre and postsynaptic elements that result in loss of these nerve terminals, and death of the motoneuron. This could be triggered by a number of mechanisms such as: a drop in transmitter release through a loss of laminin pre-synaptic receptor interaction; a loss of laminin pre-synaptic integrin interaction that could trigger anoikis;a failure to induce postsynaptic specialisations due to a loss agrin-MuSK interactions; and/or by a loss of laminin and agrin interacting with α-dystroglycan.*

However, the induction of cell death using αvβ3 antagonists may occur just prior to detachment (Brassard *et al.*, 1999). Integrins αv and β1 are likely candidates to function in regulating motoneuron survival during embryonic development through anoikis. Besides integrin αv and β1, localising laminins and integrins to the neuromuscular synapse, and finding laminins and integrins that function in anoikis is still in its infancy leaving open many other possibilities, the most promising include integrin α7 and laminins α5 and β2.

Trophic Factors which Promote Motoneuron Survival and Synaptic Plasticity may Inhibit Anoikis

The proposal that motoneurons undergo anoikis implicates that adhesion between the motor nerve terminal and the synaptic basal lamina is required for cell survival. However, neuromuscular synapse formation is highly plastic during the coincident period of motoneuron PCD leaving open the question as to how motor units are able to survive while they are establishing synaptic connections.

Trophic factors and integrins that promote motor neurite terminal plasticity *in vivo* include glial derived neurotrophic factor (GDNF) and insulin-like growth factor (IGF) (D'Costa *et al.*, 1998; Ivankovic-Dikic *et al.*, 2000; Keller-Peck *et al.*, 2001). Administration of IGF into developing chick embryos promotes motor terminal branching and synapse formation and ultimately motoneuron survival (D'Costa *et al.*, 1998). It is thought that through increased terminal endings, motoneurons have greater access to muscle derived trophic factors allowing more motoneurons to survive (D'Costa *et al.*, 1998). IGF may also function to inhibit anoikis by activating the Akt pathway when cells are detached from matrix molecules (Prisco *et al.*, 1999; Yu *et al.*, 2001). It is not yet known whether GDNF functions to inhibit anoikis. Trophic factors such as IGF and GDNF promote motor neurite terminal plasticity and may function to inhibit motoneurons form undergoing anoikis while final innervation patterns are decided.

The hypothesis that a motoneurons fate is dependent on its survival through increased terminal endings indicate that trophic factors and their receptors are involved in synapse formation (Oppenheim, 1991). Neurotrophin 4 potentiates pre-synaptic activity at the neuromuscular junction (Wang *et al.*, 1998). Neurotrophin 4 and brain-derived neurotrophic factor (BDNF) destabilise AChR clusters at the neuromuscular synapse by activating their tyrosine kinase B receptor (TrkB; Wells *et al.*, 1999). Moreover, disruption of TrkB-mediated signalling disrupts the formation of the neuromuscular synapse (Gonzalez *et al.*, 1999). Conversely, neutralisation of the TrkB receptor results in increases in AChR clustering (Wells *et al.*, 1999). These studies indicate that trophic factors have local actions at the neuromuscular synapse supporting the hypothesis that neuromuscular synapse formation is required for motoneurons to access muscle derived trophic factors.

We therefore propose a model that trophic factors inhibit anoikis while motoneurons are finding their innervation patterns and once neuromuscular synapses become established, other trophic factors act to potentiate the presynaptic activity and make a stronger connection through deposition of matrix molecules. In this light, motoneurons that do not have a local expression of IGF or GDNF and are unable to compete effectively for muscle derived trophic factors at neuromuscular synapses detach from the muscle fibre and undergo anoikis.

Links between Apoptosis Pathways in Motoneurons and those involved in Anoikis

A key problem in the study of anoikis lies in characterising the initiating death pathway (Frisch and Screaton, 2001). Recently it was found that the Fas pathway is induced by detachment from matrix (Aoudjit and Vuori, 2001). Fas is activated by its ligand FasL (Fas ligand) to induce apoptosis through caspase 8. FLIP, a negative regulator of caspase 8 can inhibit this pathway (Irmler *et al.*, 1997).

A recent study showed that embryonic motoneurons express both Fas and its ligand FasL at the beginning of PCD and is induced by trophic factor deprivation (Raoul *et al.*, 1999). Blocking the Fas activation by FasL saves motoneurons which would otherwise die in culture (Raoul *et al.*, 1999). Furthermore, trophic factors BDNF, GDNF and cardiotrophin 1 are able to inhibit the Fas induced death by inducing the expression of FLIP (Raoul *et al.*, 1999). In addition, trophic factor deprivation increases the FasL expression (Raoul *et al.*, 1999). As motoneurons and epithelial cells that undergo anoikis use the same Fas/FasL pathway, we postulate that without proper nerve-muscle connections, motoneurons do not obtain the correct trophic support and are induced to die by anoikis.

Conclusion

In this review we provide correlative evidence to suggest that a motoneuron's dependence on its muscle fibre during the period of PCD may be regulated by its interactions with the muscle's basal lamina. The most convincing evidence stems from a large drop in motoneuron survival in agrin-deficient mice at the same time the basal lamina becomes less stable (Noakes *et al.*, 2001). Given these correlations, we propose that motoneuron survival during embryonic development is in part regulated by anoikis. During the early period of synapse formation, trophic expression in muscle fibres is low (Griesbeck *et al.*, 1995; D'Costa *et al.*, 1998), making it necessary for motoneurons to make connections with the muscle to survive. Agrin deposited by the developing motor nerve terminal into the synaptic basal lamina may act to promote motoneuron survival by concentrating target secreted trophic factors to the neuromuscular junction, as well as promoting the structural development of basal lamina and the anchoring of it to the muscle's cytoskeleton. These concentrated trophic factors in turn promote the functional and molecular maturation of the neuromuscular synapse. This reinforces the specialised connection, and the ultimate survival of the motoneuron.

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References

AOUDJIT, F. and VUORI, K. (2001) Matrix attachment regulates Fas-induced apoptosis in endothelial cells: a role for c-flip and implications for anoikis. *J Cell Biol* 152(3): 633-43.

APEL, E.D., GLASS, D.J., MOSqCOSO, L.M., YANCOPOULOS, G.D. and SANES, J.R. (1997) Rapsyn is required for MuSK signaling and recruits synaptic components to a MuSK-containing scaffold. *Neuron* 18(4): 623-35.

APEL, E.D. and MERLIE, J.P. (1995) Assembly of the postsynaptic apparatus. *Curr Opin Neurobiol* 5(1): 62-7.

ATTWELL, S., ROSKELLEY, C. and DEDHAR, S. (2000) The integrin-linked kinase (ILK) suppresses anoikis. *Oncogene* 19(33): 3811-5.

BANKS, G.B., CHAU, T.N., BARTLETT, S.E. and NOAKES, P.G. (2001) Promotion of motoneuron survival and branching in rapsyn-deficient mice. *J Comp Neurol* 429(1): 156-65.

BARTLETT, S.E., BANKS, G.B., REYNOLDS, A.J., WATERS, M.J., HENDRY, I.A. and NOAKES, P.G. (2001) Alterations in ciliary neurotrophic factor signaling in rapsyn deficient mice. *J Neurosci Res* 64(6): 575-81.

BARTLETT, S.E., REYNOLDS, A.J. and HENDRY, I.A. (1998) Retrograde axonal transport of neurotrophins: differences between neuronal populations and implications for motor neuron disease. *Immunol Cell Biol* 76(5): 419-23.

BEZAKOVA, G. and LOMO, T. (2001) Muscle activity and muscle agrin regulate the organization of cytoskeletal proteins and attached acetylcholine receptor (AchR) aggregates in skeletal muscle fibers. *J Cell Biol* 153(7): 1453-63.

BONFOCO, E., CHEN, W., PAUL, R., CHERESH, D. A. and COOPER, N. R. (2000) beta1 integrin antagonism on adherent, differentiated human neuroblastoma cells triggers an apoptotic signaling pathway. *Neuroscience* 101(4): 1145-52.

BRASSARD, D.L., MAXWELL, E., MALKOWSKI, M., NAGABYUSHAN, T.L., KUMAR, C.C. and ARMSTRONG, L. (1999) Integrin alpha(v)beta(3)-mediated activation of apoptosis. *Exp Cell Res* 251(1): 33-45.

BURGESS, R.W., NGUYEN, Q.T., SON, Y.J., LICHTMAN, J.W. and SANES, J.R. (1999) Alternatively spliced isoforms of nerve- and muscle-derived agrin: their roles at the neuromuscular junction. *Neuron* 23(1): 33-44.

BURGESS, R.W., SKARNES, W.C. and SANES, J.R. (2000) Agrin isoforms with distinct amino termini. Differential expression, localization, and function. *J Cell Biol* 151(1): 41-52.

CALDERO, J., PREVETTE, D., MEI, X., OAKLEY, R.A., LI, L., MILLIGAN, C., Houenou, L., Burek, M. and Oppenheim, R. W. (1998) Peripheral target regulation of the development and survival of spinal sensory and motor neurons in the chick embryo. *J Neurosci* 18(1): 356-70.

CHU-WANG, I. W. and OPPENHEIM, R.W. (1978) Cell death of motoneurons in the chick embryo spinal cord. II. A quantitative and qualitative analysis of degeneration in the ventral root, including evidence for axon outgrowth and limb innervation prior to cell death. *J Comp Neurol* 177(1): 59-85.

CLARK, E.A. and BRUGGE, J.S. (1995) Integrins and signal transduction pathways: the road taken. *Science* 268(5208): 233-9.

COLLEDGE, M. and FROEHNER, S.C. (1998) Signals mediating ion channel clustering at the neuromuscular junction. *Curr Opin Neurobiol* 8(3): 357-63.

DAHM, L.M. and LANDMESSER, L.T. (1988) The regulation of intramuscular nerve branching during normal development and following activity blockage. *Dev. Biol.* 130: 621-644.

DAHM, L.M. and LANDMESSER, L.T. (1991) The regulation of synaptogenesis during normal development and following activity blockade. *J Neurosci* 11(1): 238-55.

D'COSTA, A.P., PREVETTE, D.M., HOUENOU, L.J., WANG, S., ZACKENFELS, K., ROHRER, H., ZAPF, J., CARONI, P. and OPPENHEIM, R.W. (1998) Mechanisms of insulin-like growth factor regulation of programmed cell death of developing avian motoneurons. *J Neurobiol* 36(3): 379-94.

DE LA ROSA, E.J. and DE PABLO, F. (2000) Cell death in early neural development: beyond the neurotrophic theory. *Trends Neurosci* 23(10): 454-8.

DECHIARA, T.M., BOWEN, D.C., VALENZUELA, D.M., SIMMONS, M.V., POUEYMIROU, W.T., THOMAS, S., KINETZ, E., COMPTON, D.L., ROJAS, E., PARK, J.S., SMITH, C., DISTEFANO, P.S., GLASS, D.J., BURDEN, S.J. and YANCOPOULOS, G.D. (1996) The receptor tyrosine kinase MuSK is required for neuromuscular junction formation in vivo. *Cell* 85(4): 501-12.

DECHIARA, T.M., VEJSADA, R., POUEYMIROU, W.T., ACHESON, A., SURI, C., CONOVER, J.C., FRIEMAN, B., MCCLAIN, J., PAN, L., STAHL, N. and et al. (1995) Mice lacking the CNTF receptor, unlike mice lacking CNTF, exhibit profound motor neuron deficits at birth. *Cell* 83(2): 313-22.

DELCOMMENNE, M., TAN, C., GRAY, V., RUE, L., WOODGETT, J. and DEDHAR, S. (1998) Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. *Proc Natl Acad Sci U S A* 95(19): 11211-6.

EISEN, J.S. and MELANCON, E. (2001) Interactions with identified muscle cells break motoneuron equivalence in embryonic zebrafish. *Nat Neurosci* 15: 15.

FALCIONI, R., ANTONNI, A., NISTICO, P., DI STEFANO, S., CRESCENZI, M., NATALI, P.G. and SACCHI, A. (1997) Alpha 6 beta 4 and alpha 6 beta 1 integrins associate with ErbB-2 in human carcinoma cell lines. *Exp Cell Res* 236(1): 76-85.

FRISCH, S.M. and SCREATON, R.A. (2001) Anoikis mechanisms. *Curr Opin Cell Biol* 13(5): 555-62.

FUHRER, C., GAUTAM, M., SUGIYAMA, J.E. and HALL, Z.W. (1999) Roles of Rapsyn and Agrin in Interaction of Postsynaptic Proteins with Acetylcholine Receptors. *J Neurosci* 19(15): 6405-6416.

GAUTAM, M., DECHIARA, T.M., GLASS, D.J., YANCOPOULOS, G.D. and SANES, J.R. (1999) Distinct phenotypes of mutant mice lacking agrin, MuSK, or rapsyn. *Brain Res Dev Brain Res* 114(2): 171-178.

GAUTAM, M., NOAKES, P.G., MOSCOSO, L., RUPP, F., SCHELLER, R.H., MERLIE, J.P. and SANES, J.R. (1996) Defective neuromuscular synaptogenesis in agrin-deficient mutant mice. *Cell* 85(4): 525-35.

GAUTAM, M., NOAKES, P.G., MUDD, J., NICHOL, M., CHU, G.C., SANES, J.R. and MERLIE, J.P. (1995) Failure of postsynaptic specialization to develop at neuromuscular junctions of rapsyn-deficient mice. *Nature* 377(6546): 232-6.

GIANCOTTI, F.G. and RUOSLAHTI, E. (1999) Integrin signaling. *Science* 285(5430): 1028-32.

GLICKSMAN, M.A. and SANES, J.R. (1983) Differentiation of motor nerve terminals formed in the absence of muscle fibres. *J Neurocytol* 12(4): 661-71.

GONZALEZ, M., RUGGIERO, F.P., CHANG, Q., SHI, Y. J., RICH, M.M., KRANER, S. and BALICE-GORDON, R.J. (1999) Disruption of Trkb-mediated signaling induces disassembly of postsynaptic receptor clusters at neuromuscular junctions. *Neuron* 24(3): 567-83.

GOULD, T.W., SUN, W., VINSANT, S.L. and OPPENHEIM, R.W. (2001) Motor pool-specific patterns of neurotrophic receptor expression in the chick lumbar spinal cord: Development and function. *Society For Neuroscience* 27(136.2).

GRIESBECK, O., PARSADANIAN, A.S., SENDTNER, M. and THOENEN, H. (1995) Expression of neurotrophins in skeletal muscle: quantitative comparison and significance for motoneuron survival and maintenance of function. *J Neurosci Res* 42(1): 21-33.

GRIESHAMMER, U., LEWANDOSKI, M., PREVETTE, D., OPPENHEIM, R.W. and Martin, G.R. (1998) Muscle-specific cell ablation conditional upon Cre-mediated DNA recombination in transgenic mice leads to massive spinal and cranial motoneuron loss. *Dev Biol* 197(2): 234-47.

HAGIWARA, H. and FALLON, J.R. (2001) Shaping membrane architecture: agrins in and out of the synapse. *J. Cell. Biol.* 153: F39-42.

HALL, Z.W. and SANES, J.R. (1993) Synaptic structure and development: the neuromuscular junction. *Cell* 72 Suppl: 99-121.

HAMBURGER, V. (1934) The effects of wing bud extirpation in chick embruyos on the development of the central nervous system. *J. Exp. Zool.* 68: 449-494.

HAMBURGER, V. and LEVI-MONTALCINI, R. (1949) Proliferation, differentiation and degeneration in the spinal ganglia of the chick embryo under normal and experimental conditions. *J. Exp. Zool.* 111: 457-501.

HAMBURGER, V. (1975) Cell death in the development of the lateral motor column of the chick embryo. *J-Comp-Neurol* 160(4): 535-46.

HAMBURGER, V. and HAMILTON, H.L. (1992) A series of normal stages in the development of the chick embryo. 1951. *Dev Dyn* 195(4): 231-72.

HARRIS, A.J. and MCCAIG, C.D. (1984) Motoneuron death and motor unit size during embryonic development of the rat. *J Neurosci* 4(1): 13-24.

HENDERSON, C.E., YAMAMOTO, Y., LIVET, J., ARCE, V., GARCES, A. and DELAPEYRIERE, O. (1998) Role of neurotrophic factors in motoneuron development. *J Physiol Paris* 92(3-4): 279-81.

HOUENOU, L.J., PINCON-RAYMOND, M., GARCIA, L., HARRIS, A.J. and RIEGER, F. (1990) Neuromuscular development following tetrodotoxin-induced inactivity in mouse embryos. *J Neurobiol* 21(8): 1249-61.

IRMLER, M., THOME, M., HAHNE, M., SCHNEIDER, P., HOFMANN, K., STEINER, V., BODMER, J.L., SCHROTER, M., BURNS, K., MATTMANN, C., RIMOLDI, D., FRENCH, L.E. and TSCHOPP, J. (1997) Inhibition of death receptor signals by cellular FLIP. *Nature* 388(6638): 190-5.

IVANKOVIC-DIKIC, I., GRONROOS, E., BLAUKAT, A., BARTH, B.U. and DIKIC, I. (2000) Pyk2 and FAK regulate neurite outgrowth induced by growth factors and integrins. *Nat Cell Biol* 2(9): 574-81.

KAMMERER, R.A., SCHULTHESS, T., LANDWEHR, R., SCHUMACHER, B., LUSTIG, A., YURCHENCO, P.D., RUEGG, M.A., ENGEL, J. and DENZER, A.J. (1999) Interaction of agrin with laminin requires a coiled-coil conformation of the agrin-binding site within the laminin gamma1 chain. *Embo J* 18(23): 6762-70.

KELLER-PECK, C.R., FENG, G., SANES, J.R., YAN, Q., LICHTMAN, J.W. and SNIDER, W.D. (2001) Glial cell line-derived neurotrophic factor administration in postnatal life results in motor unit enlargement and continuous synaptic remodeling at the neuromuscular junction. *J Neurosci* 21(16): 6136-46.

KOZLOVA, N. I., MOROZEVICH, G.E., CHUBUKINA, A.N. and BERMAN, A.E. (2001) Integrin alphavbeta3 promotes anchorage-dependent apoptosis in human intestinal carcinoma cells. *Oncogene* 20(34): 4710-7.

LANDMESSER, L. (1992) The relationship of intramuscular nerve branching and synaptogenesis to motoneuron survival. *J Neurobiol* 23(9): 1131-9.

LIN, W., BURGESS, R.W., DOMINGUEZ, B., PFAFF, S.L., SANES, J.R. and LEE, K.F. (2001) Distinct roles of nerve and muscle in postsynaptic differentiation of the neuromuscular synapse. *Nature* 410(6832): 1057-64.

MARTIN, P.T., KAUFMAN, S.J., KRAMER, R.H. and SANES, J.R. (1996) Synaptic integrins in developing, adult, and mutant muscle: selective association of alpha1, alpha7A, and alpha7B integrins with the neuromuscular junction. *Dev Biol* 174(1): 125-39.

MARTIN, P.T. and SANES, J.R. (1997) Integrins mediate adhesion to agrin and modulate agrin signaling. *Development* 124(19): 3909-17.

MOLL, J., BARZAGHI, P., LIN, S., BEZAKOVA, G., LOCHMULLER, H., ENGVALL, E., MULLER, U. and RUEGG, M.A. (2001) An agrin minigene rescues dystrophic symptoms in a mouse model for congenital muscular dystrophy. *Nature* 413(6853): 302-7.

MORO, L., VENTURINO, M., BOZZO, C., SILENGO, L., ALTRUDA, F., BEGUINOT, L., TARONE, G. and DEFILIPPI, P. (1998) Integrins induce activation of EGF receptor: role in MAP kinase induction and adhesion-dependent cell survival. *Embo J* 17(22): 6622-32.

NOAKES, P.G., BANKS, G.B., CHOY, P.T., LAVIDIS, N.A. and BELLINGHAM, M.C. (2001) Motoneuron death, branching and muscle activity in agrin-deficient mice. *Society For Neuroscience* 27(suppl); 905.16 (Abstr.).

NOAKES, P.G., GAUTAM, M., MUDD, J., SANES, J.R. and MERLIE, J.P. (1995) Aberrant differentiation of neuromuscular junctions in mice lacking s- laminin/laminin beta 2. *Nature* 374(6519): 258-62.

OPPENHEIM, R.W. (1991) Cell death during development of the nervous system. *Annu Rev Neurosci* 14: 453-501.

OPPENHEIM, R.W. and NUNEZ, R. (1982) Electrical stimulation of hindlimb increases neuronal cell death in chick embryo. *Nature* 295(5844): 57-9.

OPPENHEIM, R.W., PREVETTE, D., D'COSTA, A., WANG, S., HOUENOU, L.J. and MCINTOSH, J.M. (2000) Reduction of neuromuscular activity is required for the rescue of motoneurons from naturally occurring cell death by nicotinic-blocking agents. *J Neurosci* 20(16): 6117-24.

PHELAN, K.A. and HOLLYDAY, M. (1991) Embryonic development and survival of brachial motoneurons projecting to muscleless chick wings. *J Comp Neurol* 311(3): 313-20.

PITTMAN, R. and OPPENHEIM, R.W. (1979) Cell death of motoneurons in the chick embryo spinal cord. IV. Evidence that a functional neuromuscular interaction is involved in the regulation of naturally occurring cell death and the stabilization of synapses. *J Comp Neurol* 187(2): 425-46.

PITTMAN, R.H. and OPPENHEIM, R.W. (1978) Neuromuscular blockade increases motoneurone survival during normal cell death in the chick embryo. Nature 271(5643): 364-6.

PRISCO, M., ROMANO, G., PERUZZI, F., VALENTINIS, B. and BASERGA, R. (1999) Insulin and IGF-I receptors signaling in protection from apoptosis. *Horm Metab Res* 31(2-3): 80-9.

RAFUSE, V.F., POLO-PARADA, L. and LANDMESSER, L.T. (2000) Structural and functional alterations of neuromuscular junctions in NCAM-deficient mice. *J Neurosci* 20(17): 6529-39.

RAOUL, C., HENDERSON, C.E. and PETTMANN, B. (1999) Programmed cell death of embryonic motoneurons triggered through the Fas death receptor. *J Cell Biol* 147(5): 1049-62.

REYNOLDS, A.J., BARTLETT, S.E. and HENDRY, I.A. (2000) Molecular mechanisms regulating the retrograde axonal transport of neurotrophins. *Brain Res Brain Res Rev* 33(2-3): 169-78.

RIETHMACHER, D., SONNENBERG-RIETHMACHER, E., BRINKMANN, V., YAMAAI, T., LEWIN, G.R. and BIRCHMEIER, C. (1997) Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. *Nature* 389(6652): 725-30.

RUOSLAHTI, E. (1999) Fibronectin and its integrin receptors in cancer. *Adv Cancer Res* 76: 1-20.

SANES, J. R. (1994). The extracellular matrix. *Myology*. A. G. Engel and C. Franzini-Armstrong. New York, McGraw-Hill**:** 242-260.

SANES, J.R. and LICHTMAN, J.W. (1999) Development of the vertebrate neuromuscular junction. *Annu Rev Neurosci* 22: 389-442.

SANES, J.R., MARSHALL, L.M. and MCMAHAN, U.J. (1978) Reinnervation of muscle fiber basal lamina after removal of myofibers. Differentiation of regenerating axons at original synaptic sites. *J Cell Biol* 78(1): 176-98.

SCHNELLER, M., VUORI, K. and RUOSLAHTI, E. (1997) Alphavbeta3 integrin associates with activated insulin and PDGFbeta receptors and potentiates the biological activity of PDGF. *Embo J* 16(18): 5600-7.

SENDTNER, M., PEI, G., BECK, M., SCHWEIZER, U. and WIESE, S. (2000) Developmental motoneuron cell death and neurotrophic factors. *Cell Tissue Res* 301(1): 71-84.

SHOREY, M. (1909) The effect of the destruction of peripheral areas on the differentiation of the neuroblasts. *J. Exp. Zool* 7(25-64).

SON, Y.J., SCRANTON, T.W., SUNDERLAND, W.J., BAEK, S.J., MINER, J.H., SANES, J.R. and CARLSON, S.S. (2000) The synaptic vesicle protein SV2 is complexed with an alpha5-containing laminin on the nerve terminal surface. *J Biol Chem* 275(1): 451-60.

STRATER, J., WEDDING, U., BARTH, T.F., KORETZ, K., ELSING, C. and MOLLER, P. (1996) Rapid onset of apoptosis in vitro follows disruption of beta 1- integrin/matrix interactions in human colonic crypt cells. *Gastroenterology* 110(6): 1776-84.

SUNDBERG, C. and RUBIN, K. (1996) Stimulation of beta1 integrins on fibroblasts induces PDGF independent tyrosine phosphorylation of PDGF beta-receptors. *J Cell Biol* 132(4): 741-52.

SUNDERLAND, W.J., SON, Y.J., MINER, J.H., SANES, J.R. and CARLSON, S.S. (2000) The presynaptic calcium channel is part of a transmembrane complex linking a synaptic laminin (alpha4beta2gamma1) with non-erythroid spectrin. *J Neurosci* 20(3): 1009-19.

TANG, J. and LANDMESSER, L. (1993) Reduction of intramuscular nerve branching and synaptogenesis is correlated with decreased motoneuron survival. *J Neurosci* 13(7): 3095-103.

TERRADO, J., BURGESS, R.W., DECHIARA, T., YANCOPOULOS, G., SANES, J.R. and KATO, A.C. (2001) Motoneuron Survival Is Enhanced in the Absence of Neuromuscular Junction Formation in Embryos. *J Neurosci* 21(9): 3144-3150.

TOSNEY, K.W. and LANDMESSER, L.T. (1985) Development of the major pathways for neurite outgrowth in the chick hindlimb. *Dev Biol* 109(1): 193-214.

USIAK, M.F. and LANDMESSER, L.T. (1999) Neuromuscular activity blockade induced by muscimol and d-tubocurarine differentially affects the survival of embryonic chick motoneurons. *J Neurosci* 19(18): 7925-39.

WANG, G. and SCOTT, S.A. (2000) The "waiting period" of sensory and motor axons in early chick hindlimb: its role in axon pathfinding and neuronal maturation. *J Neurosci* 20(14): 5358-66.

WANG, X., BERNINGER, B. and POO, M. (1998) Localized synaptic actions of neurotrophin-4. *J Neurosci* 18(13): 4985-92.

WELLS, D.G., MCKECHNIE, B.A., KELKAR, S. and FALLON, J.R. (1999) Neurotrophins regulate agrin-induced postsynaptic differentiation. *Proc Natl Acad Sci U S A* 96(3): 1112-1117.

WONG, K.C., MEYER, T., HARDING, D.I., DICK, J.R., VRBOVA, G. and GREENSMITH, L. (1999) Integrins at the neuromuscular junction are important for motoneuron survival. *Eur J Neurosci* 11(9): 3287-92.

YAGINUMA, H., TOMITA, M., TAKASHITA, N., MCKAY, S.E., CARDWELL, C., YIN, Q.W. and OPPENHEIM, R.W. (1996) A novel type of programmed neuronal death in the cervical spinal cord of the chick embryo. *J Neurosci* 16(11): 3685-703.

YAMAMOTO, Y. and HENDERSON, C.E. (1999) Patterns of programmed cell death in populations of developing spinal motoneurons in chicken, mouse, and rat. *Dev Biol* 214(1): 60-71.

YU, J.T., FOSTER, R.G. and DEAN, D.C. (2001) Transcriptional repression by RB-E2F and regulation of anchorage- independent survival. *Mol Cell Biol* 21(10): 3325-35.