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Invited Review

Schwann cell extracellular matrix molecules and their receptors

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Summary. The major cellular constituents of the mammalian peripheral nervous system are neurons (axons) and Schwann cells. During peripheral nerve development Schwann cells actively deposit extracellular matrix (ECM), comprised of basal lamina sheets that surround individual axon-Schwann cell units and collagen fibrils. These ECM structures are formed from a diverse set of macromolecules, consisting of glyco-proteins, collagens and proteoglycans. To interact with ECM, Schwann cells express a number of integrin and non-integrin cell surface receptors. The expression of many Schwann cell ECM proteins and their receptors is developmentally regulated and, in some cases, dependent on axonal contact. Schwann cell ECM acts as an organizer of peripheral nerve tissue and strongly influences Schwann cell adhesion, growth and differentiation and regulates axonal growth during development and regeneration.

Key words: Schwann cells, Extracellular matrix, Peripheral nerve

Introduction

In addition to the major cellular constituents, neurons and their axons and Schwann cells, peripheral nerves are richly endowed with non-cellular structural elements referred to collectively as the extracellular matrix (ECM). Two major subdivisions of the ECM are the basal lamina and the fibrillar matrix. The former is a thin sheet of ECM comprised of a cross-linked network of collagens and other molecules that encases individual axon-Schwann cell units and is in direct contact with the outer Schwann cell membrane. The fibrillar matrix consists of a collection of collagen-based fibrils that in mature nerves lie external to the basal lamina. These two morphologically recognizable ECM structures contain distinct subsets of components from each of the 3 major classes of ECM macromolecules: collagens, glyco-proteins and proteoglycans. These ECM structures assemble spontaneously on the surfaces of cells and in the extracellular space. The major producer of peripheral nerve ECM is the Schwann cell, but at least some aspects of ECM production and/or assembly are regulated by neurons. The functions of ECM are diverse and manifold. Processes regulated by ECM include cell morphology and tissue organization, Schwann cell and axon migration, Schwann cell proliferation, and myelination. The structure and composition of the peripheral nerve ECM changes during development to promote the functional processes appropriate for each stage.

This review will summarize data on the molecular composition and function of Schwann cell ECM as well as ECM receptors expressed by Schwann cells. Molecular mechanisms of myelin formation and axon-Schwann cell interactions will not be discussed. For information on these topics the reader is referred to excellent reviews published elsewhere (Doyle and Colman, 1993; Lemke, 1993; Snipes and Suter, 1994; Scherer and Salzer, 1996; Scherer, 1997).

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Composition of Schwann cell ECM

The macromolecular composition of the Schwann cell ECM has been investigated by a combination of biochemical, immunohistochemical and molecular techniques. These studies have revealed a striking diversity of ECM molecules, which includes glyco-proteins, collagens, and proteoglycans. The principal molecules that make up the Schwann cell ECM are introduced below. In trying to understand the function of this ECM, however, it should be kept in mind that the component molecules do not operate as individual units, but as part of a highly organized macromolecular complex. Invoking the adage "the whole is greater than the parts", the assembled ECM is likely to have biochemical and physical properties that are not manifested by individual ECM molecules alone.

Glycoproteins

Laminin

Laminin is a large glycoprotein consisting of three disulfide linked polypeptide chains, α, ß and γ. Laminin is an essential structural component of basal lamina, where it interacts with other basal lamina proteins such as collagen type IV and perlecan (see Yurchenco and O'Rear, 1994 for review). Schwann cells secrete laminin and deposit it into ECM both in vivo and in vitro (Cornbrooks et al., 1983). The predominant isoform of laminin found in Schwann cell basal lamina is laminin-2 (merosin), with a chain composition of α2ß1γ1 (Leivo and Engvall, 1988; Sanes et al., 1990; Jaakkola et al., 1993).

Fibronectin

Fibronectin is a multi-domain glycoprotein of approximately 450 kDa, consisting of two very similar but not identical disulfide linked subunits. Schwann cells in vitro synthesize fibronectin (Cornbrooks et al., 1983) and deposit it into fibrillar ECM after treatment with ascorbate (see below). Fibronectin is also present in the endoneurium of peripheral nerves in the areas surrounding individual axon-Schwann units (Cornbrooks et al., 1983; Lorimier et al., 1992).

Tenascin

Tenascin is a large oligomeric glycoprotein, which is prominently expressed in the developing nervous system. In developing chick peripheral nerve Schwann cell precursors (satellite cells) express tenascin at a high level (Wehrle-Haller et al., 1991). In adult nerves endoneurial tenascin expression is confined to the node of Ranvier (Martini et al., 1990).

Collagens

Collagen types I and III

Schwann cells co-cultured with neurons deposit both basal lamina and thin collagenous fibrils. Under these conditions Schwann cells synthesize the fibril forming collagen types I and III (Bunge et al., 1980). These collagens are also present in the endoneurium in vivo (Lorimier et al., 1992). In situ hybridization studies of rat sciatic nerve reveal the presence of α1(I) collagen mRNA in Schwann cells, indicating that Schwann cells are a source of endoneurial collagen fibrils in vivo (Jaakkola et al., 1989b). There is also evidence suggesting that endoneurial fibroblasts also contribute to the deposition of these fibrils (Jaakkola et al., 1989a).

Collagen type IV

Collagen type IV is a principal structural component of basal lamina. In contrast to the fibril forming collagens, collagen type IV forms a covalently stabilized polymer network (Yurchenco and Schittny, 1990). Synthesis of collagen type IV by Schwann cells and the presence of this collagen in Schwann cell basal lamina are well documented (Carey et al., 1983; Eldridge et al., 1987; Lorimier et al., 1992). The α1 and α2, but not other collagen type IV chains are detected in peripheral nerve (Miner and Sanes, 1994), suggesting that Schwann cells secrete collagen type IV molecules with the composition $[\alpha1(IV)]_2\,[\alpha2(IV)]_1$.

Collagen type V/XI (p200)

Expression of type V collagen by Schwann cells was suggested previously, based on electrophoretic mobility of pepsin-resistant polypeptides present in Schwann cell conditioned medium (Carey et al., 1983). More recently, mRNAs coding for collagen α1(V) and the related gene product α1(XI) have been identified by RT-PCR in newborn rat sciatic nerve and in neonatal Schwann cells. Immunoblot analysis and immunofluorescent staining have revealed the presence of collagen α1(V) in newborn rat peripheral nerves and its synthesis by Schwann cells (Carey et al., unpublished data). p200 is a 200 kDa protein that was identified and purified from conditioned medium of Schwann cell cultures initially on the basis of its high affinity binding to syndecan-3, a transmembrane heparan sulfate proteoglycan expressed on the Schwann cell surface (see below). Biochemical characterization and partial sequence analysis of p200 revealed the presence of multiple collagen repeats (G-X-X) in the primary sequence (Chernousov et al., 1996). p200 assembles into non-covalent, pepsin resistant trimers in association with α1(V) collagen chains, and is incorporated into the ECM of Schwann cell cultures and neonatal rat peripheral nerves (Chernousov et al., 1999; Chernousov and Carey, unpublished). In contrast to collagen α1(V), which is widely expressed in developing and adult tissues (Wu et al., 1998), the p200 polypeptide is expressed in a restricted subset of cells and tissues, most prominently in developing (but not mature) peripheral nerve (Chernousov et al., 1996). These findings suggest that Schwann cells synthesize collagen type V with a cell type specific subunit composition comprised of heterotrimers of collagen α1(V) chain and p200. A distinguishing characteristic of this collagen its unusually high affinity for heparan sulfate, due to the presence of the p200 subunit.

ECM proteoglycans

Perlecan

Perlecan is a large heparan sulfate proteoglycan with a core protein of greater than 400 kDa. Immunohistochemical studies revealed the presence of perlecan in basement membranes of many different tissues including the Schwann cell basal lamina (Eldridge et al., 1986;

Couchman and Ljubimov, 1989). Schwann cells synthesize perlecan in vitro (Eldridge et al., 1986). It has been reported that the core protein of perlecan binds fibronectin (Heremans et al., 1990). Interestingly, perlecan is present in the fibrillar matrix deposited by Schwann cells in vitro, where it co-distributes with fibronectin (Chernousov et al., 1998). Perlecan can also bind ECM proteins, including fibronectin, laminin, and various collagens, via its covalently attached heparan sulfate chains (Fujiwara et al., 1984; Koda and Bernfield, 1984).

Agrin

Agrin was described originally as a glycoprotein component of the neuromuscular junction, where it causes clustering of acetylcholine receptors (Nitkin et al., 1987; Reist et al., 1987). Subsequently, agrin was identified in other tissues, including the endoneurium of the peripheral nerve (Gesemann et al., 1998; Reist et al., 1987; Yamada et al., 1996) where it co-localizes with and binds to α-dystroglycan, its putative receptor (see below). Agrin isolated from embryonic chick brain has been reported to occur as a heparan sulfate proteoglycan (Tsen et al., 1995).

Collagen type XVIII

Collagen type XVIII is a member of Multiplexin family of collagens, which consist of collagens with multiple triple-helical domains (Oh et al., 1994). Collagen XVIII was recently shown to be present in basal laminae of chick embryos, and is especially abundant in basal laminae of peripheral ganglia and nerves (Halfter et al., 1998). In situ hybridization experiments showed that Schwann cells are the source of this collagen. Biochemical studies indicated that this collagen exists almost exclusively as a heparan sulfate proteoglycan in chick embryonic tissue (Halfter et al., 1998).

Chondroitin sulfate proteoglycans

Two chondroitin sulfate proteoglycans with apparent molecular weights of 130 and 900 kDa were isolated from human sciatic nerve (Braunewell et al., 1995a). Immunocytochemical data revealed the presence of these proteoglycans in Schwann cell basal laminae in vivo and on the surface of cultured Schwann cells in vitro. These proteoglycans are immunologically related to the previously described chondroitin sulfate proteoglycans decorin and versican (Braunewell et al., 1995a). These proteoglycans bind to fibronectin in vitro, but not to laminin or collagens (Braunewell et al., 1995b). Another chondroitin sulfate proteoglycan of approximately 400 kDa, which is immunologically distinct from versican, was isolated from conditioned medium of cultured Schwann cells and from peripheral nerve extracts (Muir et al., 1989; Zuo et al., 1998). In contrast with decorin and versican-related proteoglycans, this proteoglycan does bind laminin in vitro (Muir et al., 1989).

Schwann cell ECM assembly

Fibrillar matrix

Schwann cells cultured in the absence of neurons in medium containing ascorbate assemble a fibrillar ECM. This matrix contains the typical fibrillar matrix components fibronectin, collagen type I and collagen type V (McGarvey et al., 1984; Baron-VanEvercooren et al., 1986; Chernousov et al., 1998; Carey and Stahl, unpublished). It also contains collagen type IV and perlecan, which are normally found in association with basal lamina-type structures (Baron-VanEvercooren et al., 1986; Chernousov et al., 1998). Despite the presence of these molecules, electron microscopic analysis fails to detect the assembly of morphologically recognizable basal lamina structures in these cultures (Baron-VanEvercooren et al., 1986). The lack of well-organized basal lamina is consistent with the absence of laminin from this ECM. Immunofluorescent staining of this matrix reveals two types of fibrils: an extended and highly branched network of thin fibrils that contain fibronectin and collagen type IV, and less abundant, thick fibrils that contain collagen type I and collagen type V. These two types of fibrils only partially overlap (Chernousov et al., 1998).

The dependence of fibronectin deposition into the Schwann cell ECM on collagens, suggested by the requirement for ascorbate, is quite unusual, and suggests a potentially novel mechanism for ECM assembly. Interestingly, Schwann cells can also efficiently incorporate exogenous fibronectin into this fibrillar matrix, by a mechanism that is also ascorbate-dependent (Chernousov et al., 1998). This suggests that Schwann cells possess the capacity to remodel existing ECM structures, and incorporate the structural components into novel nerve-specific structures.

It has been suggested that this fibrillar ECM that is assembled in Schwann cell cultures represents a "primary" nerve ECM, that is equivalent to the matrix deposited by Schwann cells during the pre-myelinating stages of peripheral nerve development (Chernousov et al., 1998). This is supported by the observation that these ECM proteins can be detected in embryonic peripheral nerves by immunofluorescence staining before the onset of myelination (Jaakkola et al., 1993; Chernousov et al., 1999). This embryonic matrix is postulated to promote the proliferation and migration of Schwann cells and to modulate the growth and organization of peripheral nerve fibers. Consistent with this, when Schwann cell proliferation and migration are halted and the process of terminal differentiation is initiated this matrix is replaced by basal lamina-type ECM.

Basal lamina assembly

Primary co-cultures of neurons and Schwann cells provide a useful model to study Schwann cell basal

lamina assembly and function. This model was used to demonstrate that Schwann cell basal lamina assembly does not occur in the absence of neurons (Bunge et al., 1982a; Fields and Raine, 1982). The biochemical mechanism underlying this effect of neurons is not understood, but it provides a striking example of the functional interdependence of Schwann cells and neurons. As described above, the molecular components that make up the basal lamina are thought to be synthesized by Schwann cells. There is no evidence for the production by neurons of ECM structural components. A plausible hypothesis to account for these findings is that neuronal contact up-regulates the expression by Schwann cells of one or more key structural components of the basal lamina, or the expression or activation of Schwann cell receptors that are needed for basal lamina assembly.

Other experiments have provided evidence for the cooperative nature of Schwann cell basal lamina assembly at the molecular level. For instance, Schwann cells do not assemble basal lamina or incorporate laminin into basal lamina structures if the culture medium lacks ascorbate (Eldridge et al., 1987). This effect has been attributed to an ascorbate-dependent post-translational modification of collagen type IV, and is consistent with the idea that collagen type IV provides a structural scaffold for basal lamina. Basal lamina assembly can also be disrupted by addition to the culture medium of derivative of ß-D-xyloside, an inhibitor of proteoglycan biosynthesis (Carey et al., 1987). It has been proposed that proteoglycans stabilize the basal lamina via non-covalent cross-linking of matrix components through binding of these proteins to the heparan sulfate chains of the proteoglycans. The ability to manipulate basal lamina assembly by depletion of ascorbate or addition of proteoglycan synthesis inhibitors has provided insight into the role of basal lamina in Schwann cell terminal differentiation (discussed below).

Schwann cell ECM receptors

Adhesive and signaling activities of ECM are mediated by cell surface proteins that bind one or more component molecules of the matrix. Several classes of such receptors have been identified. These display a range of specificity's vis-à-vis the ECM ligands they bind, from highly specific (e.g. dystroglycan and some integrins) to only moderately specific (e.g. heparan sulfate proteoglycans and some integrins). The relatively low affinities of some of these receptors for isolated ECM ligands is likely to be compensated by the high local concentrations of ECM molecules that result from their association into polymers and large macromolecular complexes. The three-dimensional organization of ECM macromolecular complexes also plays a role in organizing and clustering ECM receptors in the plasma membrane. Certain activities of ECM receptors are regulated by ligand mediated clustering. The ECM receptors that have been identified in Schwann cells are described below.

Integrins

Integrins are a family of heterodimeric receptors for ECM molecules that are important mediators of cell-ECM adhesion and ECM-dependent signaling. Integrins bind a number of cytoskeleton-associated and signaling proteins via their cytoplasmic domains (see Dedhar and Hannigan, 1996 for review). These cytoplasmic binding and signaling activities are regulated by ECM ligand binding and ligand-dependent integrin oligomerization. In addition, association of integrins with cytoplasmic proteins regulates integrin affinity for ECM ligands, a phenomenon that has been termed "inside-out" signaling.

Schwann cells display on their cell surface integrins that bind collagens (α1ß1, α2ß1), laminin (α2ß1, α6ß1, α6ß4) and fibronectin (α5ß1, αvß3) as their primary ligands, as well as the less characterized integrin αvß8 (Hsiao et al., 1991; Einheber et al., 1993; Niessen et al., 1994; Milner et al., 1997). As described above, Schwann cells also secrete these ECM proteins. Thus, the repertoire of Schwann cell integrins corresponds to the molecular composition of the nerve ECM. It should be noted that some of these integrins display cell-specific binding characteristics. For example, α2ß1 integrin binds to collagen and laminin or to collagen only, depending on the cell type being examined (Elices and Helmer, 1989; Kirchhofer et al., 1990). Experiments that determine the exact functional roles of Schwann cell integrins, for example using type-specific function-blocking antibodies, are needed.

Cell surface proteoglycans

Another class of molecule that has been suggested to function as ECM receptors is cell surface heparan sulfate proteoglycans. Two different types of proteoglycans have been described on the Schwann cell surface. One of them is the lipid-anchored proteoglycan glypican-1. Glypican-1 isolated from Schwann cells binds laminin in vitro and is localized in the outer Schwann cell membrane that contacts the laminin-rich basal lamina (Carey et al., 1990; Carey and Stahl, 1990). The other Schwann cell heparan sulfate proteoglycan is the transmembrane proteoglycan syndecan-3 (N-syndecan) (Carey et al., 1992). Syndecan-3 does not bind laminin, but does bind p200, the Schwann cell-specific collagen subunit (Chernousov and Carey, 1993; Chernousov et al., 1996). These data support a role for cell surface proteoglycans as co-receptors for ECM proteins, which act together with other ECM receptors to provide the full range of interactions of Schwann cells with the ECM. Syndecan-3 contains a short cytoplasmic tail that could potentially mediate interactions with cytoplasmic structural and signaling proteins (Carey et al., 1997).

Dystroglycan

Dystroglycan was originally identified in skeletal muscle as a component of the dystrophin-glycoprotein complex (Ibraghimov-Beskrovnaya et al., 1992). The dystroglycan gene product is cleaved into 2 proteins, α-dystroglycan and ß-dystroglycan, during post-translational processing (Ibraghimov-Beskrovnaya et al., 1992).

α-Dystroglycan has been identified in peripheral nerve (Matsumura et al., 1993). Schwann cell α-dystroglycan is a receptor for agrin and laminin-2 (Yamada et al., 1994, 1996).

Functions of Schwann cell ECM

The structures and biochemical compositions of developing and mature peripheral nerves are distinct. This difference presumably reflects the distinct functional activities in which the neurons and Schwann cells are engaged at these stages of nerve maturation. In the pre-myelinating stage nerve cells produce axons that grow, usually as distinct bundles or fascicles, towards their targets in the periphery. Undifferentiated Schwann cells migrate along these bundles and proliferate in response to mitogens expressed on axonal membranes. The mature stage, in contrast, is very stable and is characterized by the establishment and maintenance of the intimate relationship between axons and Schwann cells viz. myelination of large caliber axons and unmyelinated ensheathment of smaller axons. There is compelling evidence that interactions with ECM play a key role in regulating these functions.

Pre-myelinating nerve development

Schwann cells adhesion and migration

A number of ECM proteins secreted by Schwann cells promote adhesion of these cells in vitro. These include laminin, fibronectin, collagen types I and IV, p200 and collagen type XVIII (McGarvey et al., 1984; Chernousov et al., 1996; Milner et al., 1997; Halfter et al., 1998; Chernousov and Carey, unpublished). The Schwann cell adhesive activities of these proteins are not equal however. Laminin is considered the most active, followed by fibronectin and collagen type IV (McGarvey et al., 1984; Milner et al., 1997; Chernousov and Carey, unpublished). Collagen type XVIII displays only moderate adhesive activity, and is at least 10 times less effective than laminin (Halfter et al., 1998). Fibronectin and laminin also promote migration of Schwann cell and stimulate their growth in cell culture (Baron-VanEvercooren et al., 1982; McGarvey et al., 1984; Anton et al., 1994; Milner et al., 1997).

Not all ECM proteins promote adhesion or migration of Schwann cells. Tenascin, which is abundantly expressed in the embryonic peripheral nervous system, does not support migration of Schwann cell precursors. Moreover, tenascin actively inhibits migration of Schwann cells on fibronectin (Wehrle-Haller and Chiquet, 1993). Likewise, decorin- and versican-like proteoglycans inhibit adhesion of Schwann cells to fibronectin, but not to laminin or collagen (Braunewell et al., 1995b). This inhibitory effect is apparently specific for these proteoglycans and is not produced by perlecan (Braunewell et al., 1995b). The physiological functions of such inhibitory activities are not known.

Neurite outgrowth

Several Schwann cell ECM molecules including fibronectin, laminin, tenascin and collagen type IV promotes outgrowth of neurites from peripheral neurons in vitro (Rogers et al., 1983; Lein et al., 1991; Wehrle-Haller and Chiquet, 1993; Anton et al., 1994). Tenascin is of particular interest, because, as mentioned above, it does not support the migration of Schwann cell precursors. The relevance of these findings, which are based largely on experiments carried out in vitro, to regulation of neurite outgrowth in vivo remains to be established.

The Schwann cell ECM also contains molecule(s) with neurite-outgrowth-inhibiting activity. A 400 kDa chondroitin sulfate proteoglycan synthesized by Schwann cells was shown to inhibit the neurite-outgrowth-promoting activity of laminin (Zuo et al., 1998). The inhibitory activity was attributed mainly to the chondroitin sulfate chains of the proteoglycan, since it was abolished by treatment with chondroitinase.

Thus, the ECM that is deposited by Schwann cells during development appears to contain a complex mixture of permissive and nonpermissive substrates. A function of this ECM, therefore, might be to facilitate directed axon growth. It would do this by promoting the growth of axons along appropriate pathways and inhibiting inappropriate growth, such as collateral sprouting.

Myelination of axons by Schwann cells

Several lines of evidence, mostly obtained using primary co-cultures of embryonic sensory neurons and Schwann cells, strongly suggest that interactions between Schwann cells and basal lamina ECM are required for myelination of axons. Co-cultures grown in serum free medium without ascorbate fail to assemble ECM (Eldridge et al., 1987). The Schwann cells in these cultures proliferate but fail to myelinate axons. Myelination can be induced by addition of ascorbic acid, which stimulates assembly of ECM, or by addition of exogenous basal lamina-type ECM components (Carey et al., 1986; Eldridge et al., 1987, 1989). Similarly, inhibition of basal lamina assembly by addition of proteoglycan synthesis inhibitor blocks myelination (Carey et al., 1987).

The importance of basal lamina for Schwann cell terminal differentiation is also supported by the

phenotype of the *dy/dy* mouse. These mice have a mutation in the laminin-2 gene (Sunada et al., 1994; Xu et al., 1994). Schwann cells in spinal roots of these animals exhibit significant deficits in basal lamina assembly and ensheathment of axons. Interestingly, these defects are much less severe in distal parts of the peripheral nervous system (Bradley and Jenkison, 1973; Bunge et al., 1982b). This suggests that ECM proteins present in these tissues can compensate for the loss of laminin-2.

It is interesting to consider these findings with earlier studies of developing tadpole peripheral nerves, which revealed the lack of basal lamina structures around migrating but not myelinating Schwann cells (Billings-Gagliardi et al., 1974). Together, these findings suggest the possibility that appearance of basal lamina serves as a trigger to initiate the process of myelination.

The effect of basal lamina contact on myelination by Schwann cells is conceptually similar to the effect of basal lamina contact on epithelial cell polarization. The establishment and maintenance of epithelial polarization, which involves spatial segregation of membrane proteins and organelles, is dependent on and initiated by adhesion to the basal lamina sheet (Hall et al., 1982; Li et al., 1987). A myelinating Schwann cell is also functionally and spatially polarized, with one portion of the plasma membrane (topologically equivalent to the apical surface of an epithelial cell) specialized for adhesion to the membrane of the associated axon. The outer Schwann cell membrane (topologically equivalent to the basal surface of an epithelial cell) is in continuous contact with the sheet of ECM that comprises the Schwann cell basal lamina.

Regulation of Schwann cell ECM protein and receptor expression

As described above, the structure and composition of peripheral nerve ECM changes during nerve development. These changes parallel the distinct functional processes that are operative in embryonic, early postnatal and adult nerves. Some ECM molecules are expressed only transiently during peripheral nerve development. For instance, expression of p200 is detected in rat peripheral nerve beginning around embryonic day 16. Expression continues through the first 2-3 weeks of postnatal development and then declines dramatically so that the protein is not detected in adult nerves (Chernousov et al., 1996, 1999). In contrast to this pattern, fibronectin is present in embryonic nerve, and continues to be expressed in adult nerve, although at a reduced level compared to developing nerve (Lefcort et al., 1992). On the other hand laminin, which is detected in peripheral nerves during embryonic development, is very abundant in adult nerves, where it is a principal component of the basal lamina (Cornbrooks et al., 1983; Sanes et al., 1990; Jaakkola et al., 1993). It was reported, however, that laminin mRNA levels decline during late postnatal development (Edgar, 1991), suggesting that Schwann cells decrease production of laminin after the basal lamina is assembled.

The mechanisms that regulate ECM protein expression by Schwann cells are not known. Neuronal contact appears to play an important role in the process. As described above, assembly of basal lamina by cultured Schwann cells is dependent on co-culture with neurons. There is evidence that axonal contact up-regulates Schwann cell expression of certain ECM proteins, such as collagen type IV and laminin (Carey et al., 1983; Bunge et al., 1990).

The expression of Schwann cell ECM receptors is also developmentally regulated. The expression of α5ß1 is reduced with peripheral nerve maturation, which corresponds to reduced levels of its ligand, fibronectin (Lefcort et al., 1992). Undifferentiated Schwann cells express the laminin-binding integrin α6ß1, while myelinating Schwann cells switch to the laminin-binding integrin α6ß4 (Einheber et al., 1993). In agreement with this pattern, ß4 integrin subunit mRNA and protein levels increase in vivo during postnatal peripheral nerve development (Einheber et al., 1993; Feltri et al., 1994). The functional significance of this switch is not obvious, since both integrins bind the same ECM ligand. Presumably, the α6ß4 isoform possesses unique properties that are required by myelinating Schwann cells. The ß4 subunit contains an unusually long cytoplasmic tail (Hogervorst et al., 1990). Perhaps specific integrin-cytoskeletal interactions are needed to stabilize the complex structural organization of a myelinating Schwann cell.

In contrast to this pattern of expression, contact with neurons down-regulates Schwann cell expression of the collagen/laminin binding integrin α1ß1 (Fernandez-Valle et al., 1994). In vivo, this integrin is expressed during postnatal development and in adult sciatic nerve, mostly by non-myelin forming Schwann cells (Stewart et al., 1997).

Other Schwann cell ECM receptors also display developmentally regulated patterns of expression. The cell surface proteoglycan syndecan-3 is expressed by Schwann cells at high levels in the late embryonic and early postnatal periods of peripheral nerve development, but syndecan-3 is not expressed in adult rats (Carey et al., 1992, 1997). In contrast, expression of the lipid-anchored heparan sulfate proteoglycan glypican-1 persists in adult nerves (Carey and Stahl, 1990; Carey et al., 1993). Interestingly, this pattern of expression mirrors the pattern of expression of the proposed ECM ligands for syndecan-3 and glypican, namely p200 and laminin (Sanes et al., 1990; Jaakkola et al., 1993; Chernousov et al., 1996).

Expression of ECM proteins by Schwann cells has been reported to increase in response to peripheral nerve injury. It was suggested that the primary function of this ECM material is to provide a permissive environment for growth of regenerating axons. Indeed, several ECM proteins that are up-regulated in injured nerve have been

found to promote neurite outgrowth in vitro. Examples of such proteins include laminin, fibronectin and tenascin (Kuecherer-Ehret et al., 1990; Martini et al., 1990; Lefcort et al., 1992). Interestingly, other ECM molecules, such as chondroitin sulfate proteoglycans, which are also up-regulated after the nerve injury, have inhibitory effects on neurite outgrowth (Zuo et al., 1998). Expression of p200 is also induced following nerve injury (Chernousov et al., 1999). Thus, the ECM that is deposited during regeneration is similar in composition to the ECM that is produced during nerve development. This is not totally surprising, since many developmental functions (e.g. axonal migration, Schwann cell proliferation) are recapitulated during nerve regeneration.

Conclusion

Over the past several years, remarkable progress has been made in identifying molecular components of Schwann cell ECM and their receptors. It has become clear that ECM molecules not only provide structural support for cellular constituents of the peripheral nervous system, but also directly affect their behavior both during development and in mature nerves. One of the most important remaining challenges is to understand how the diverse ECM components act together to promote the orderly construction and functioning of peripheral nerves. It will be also very important to delineate the molecular mechanisms that regulate Schwann cell ECM protein expression during nerve development, which allow them to assemble distinct ECM structures appropriate for each developmental stage.

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