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Signaling Axis in Schwann Cell Proliferation and Differentiation

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Abstract

Recent progress in molecular biology has markedly expanded our knowledge of the molecular mechanism behind the proliferation and differentiation processes of Schwann cells, the myelin-forming cells in peripheral nervous systems. Intracellular signaling molecules participate in integrating various stimuli from cytokines and other humoral factors and control the transcriptional activities of the genes that regulate mitosis or differentiation. This article provides an overview of the roles played by the intracellular pathways regulating Schwann cell functions. In Schwann cell proliferation, cyclic adenosine monophosphate signals and mitogen-activated protein kinase pathways play pivotal roles and may also interact with each other. Regarding differentiation, myelin formation is regulated by various cytokines and extracellular matrix molecules. Specifically, platelet-derived growth factor, neuregulin, and insulin-like growth factor-I all are classified as ligands for receptor-type tyrosine kinase and activate common intracellular signaling cascades, mitogen-activated protein kinase pathways, and phosphatidylinositol-3-kinase pathways. The balance of activities between these two pathways appears crucial in regulating Schwann cell differentiation, in which phosphatidylinositol-3-kinase pathways promote myelin formation. Analysis of these signaling molecules in Schwann cells will enable us not only to understand their physiological development but also to innovate new approaches to treat disorders related to myelination.

Index Entries: Signal transduction; MAG; Mek; Erk; Akt; PKA; GSK-3β; NF-κB; p75; laminin.

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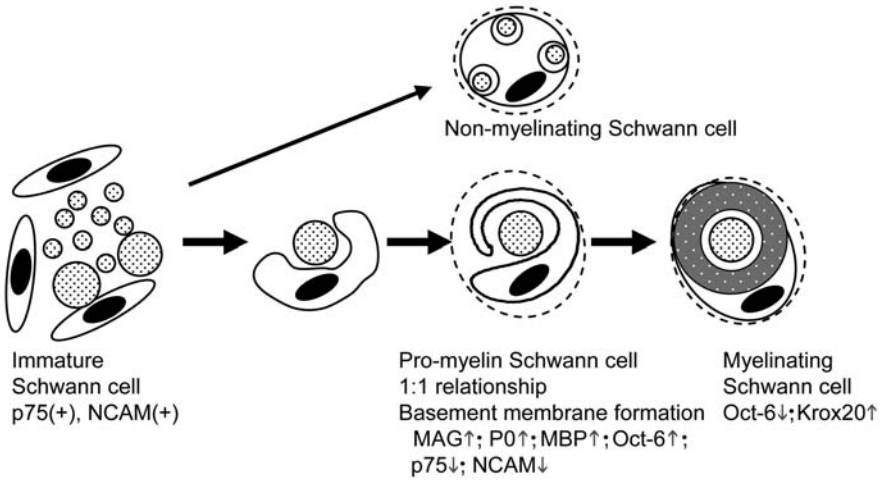

Fig. 1. Development of myelinating Schwann cells. Schwann cells arise from the neural crest and then proliferate and migrate along axons. Around birth, they segregate axons and form a 1:1 relationship, provided the axon has the proper diameter. Expression of transcriptional factors, such as Oct-6 and Krox20, and myelin-related protein, such as myelin-associated glycoprotein, P0, and myelin basic protein, are strictly regulated in accordance with progress toward myelin formation *(2).*

Introduction

Since Theodor Schwann first described the ensheathing structure surrounding peripheral nervous axons, Schwann cells have been recognized as playing an indispensable role in supporting peripheral axons. The course of their development has been intensively studied in the light of anatomical localization and specific markers *(1–3).* Briefly, Schwann cells arise from the neural crest and become Schwann cell precursors expressing p75 and growth-associated protein (GAP)-43 around E14-15 in rats. They proliferate and migrate along axons and become immature Schwann cells that express S-100 and neuronal cellular adhesion molecules (NCAMs) at E16-17. Immature Schwann cells can differentiate into either promyelinating or nonmyelinating Schwann cells, depending on the presence of extrinsic stimuli such as axonal membrane-bound molecules, which have not yet been identified (Fig. 1). At the promyelinating stage, Schwann cells reduce mitotic activities and create a 1:1 relationship with axons, increasing the expression of promyelinating proteins, such as myelin-associated glycoprotein (MAG) and protein zero (P0), and transcription factor Oct-6. They then form basement membranes and begin to extend their plasma membrane around axons to form a compact myelin structure that facilitates the propagation of axon potentials.

When Schwann cells start myelination, they express Krox20, a zinc finger transcription factor and, converesly, reduce expression of Oct-6. Because mutant mice lacking these genes display abnormal myelination—particularly the severe loss of myelination in Krox20 null mice—the shift in the expression level between Oct-6 and Krox20 plays a pivotal role for myelin formation *(4–6).* Together, toward the initiation of myelination, the temporal patterns and intensity of various regulatory genes should be strictly controlled so that Schwann cells form myelin in the proper place at the proper time.

Similarly to many other cells, differentiation and gene expression modification of Schwann cells are regulated by various extracellular signals, such as humoral cytokines, membrane-bound molecules (especially those on the surface of axons), and extracellular matrix molecules. Increasing knowledge from in vitro cul-

ture studies as well as recent genetically modified mice phenotypes has characterized the importance of these extracellular signal molecules in Schwann cell differentiation *(7,8).* However, much remains to be learned about how these signal molecules actually control the activities of transcription factors and gene expression. In other words, there are still unknown gaps between the events occurring around Schwann cell membranes induced by extracellular signals and the regulation of gene expression within nuclei. This article discusses the functions of several intracellular signaling pathways in Schwann cells that integrate extracellular signals and transmit them into nuclei to regulate proliferation and promyelinating differentiation.

Signal Transduction of Schwann Cell Proliferation

In the developmental processes, the number of Schwann cells begins to increase from the precursor stage through the early promyelinating stage. Because the number of myelin-forming Schwann cells is tightly regulated according to the number of axons to be myelinated, researchers believe that direct interaction with axons regulates Schwann cell proliferation *(9).* Initially, cholera toxin and dibutyryl cyclic adenosine monophosphate (db-cAMP), activators of adenyl cyclase, were found to induce Schwann cell proliferation in primary cultures from neonatal rat sciatic nerves *(10,11).* Adenyl cyclase is physiologically activated by stimulatory G protein upon ligands binding to G protein-coupled receptors and raises the intracellular concentration of cAMP *(12).* The wide variety of effectors of cAMP makes it difficult to identify the exact target downstream of cAMP that results in Schwann cell proliferation. Nevertheless, the importance of cAMP-dependent protein kinase A (PKA), one of the main targets of cAMP, has been noted based on the findings that either a specific chemical inhibitor (H-89) or the retroviral suppression of PKA inhibits cAMP-induced Schwann cell proliferation *(13,14).* However, these findings do not exclude the participation of PKA-independent signals such as a pathway toward the small guanosine triphosphatase Rap1 through cAMP-regulated guanine nucleotide exchange factors (cAMP-GEF/Epac; ref. *15*). Among the various PKA downstream molecules, cAMP response element binding protein (CREB) is a well-characterized molecule that activates the transcription of various genes by binding to the CRE sites in their promoter region. In cocultures of Schwann cells with purified dorsal root ganglion (DRG) neurons, CREB phosphorylation is observed when the Schwann cells make contact with axonal membrane and proliferate, indicating the physiological role of CREB in Schwann cell proliferation *(16–18).* The target genes of CREB remain elusive, and it is not clear yet whether a PKA–CREB signal is indispensable for Schwann cell proliferation. Notably, in some other cell types, the activation of cAMP signals correlates with a reduction of cell mitosis and a progression of cell differentiation *(19).*

Despite our knowledge of the importance of cAMP, the physiological regulatory mechanisms of cAMP at the intracellular level in Schwann cells in vivo remain unclear. Intracellular cAMP elevation is achieved either by a ligand binding to G protein-coupled receptors to activate adenyl cyclase or by a reduction in phosphodiesterase (PDE) activity, which hydrolyzes and causes cAMP degradation. Regarding possible ligands in the case of Schwann cells, calcitonin-gene-related peptide and adenosine have been reported, both of which are released from axons *(20,21).* Regarding PDE activity, PDE4 messenger RNA levels increase in the distal part of injured peripheral nerves, where cAMP concentrations remain at a low level until remyelination occurs *(22).*

Various kinds of growth factors also participate in Schwann cell proliferation. Similarly to other cell types, platelet-derived growth factor (PDGF) and fibroblast growth factor are mitotic for Schwann cells *(23),* and both bind to their specific receptors and induce dimerization of those receptors to transmit signals to

downstream effectors. These receptors are categorized as receptor-type tyrosine kinase (RTK) and include many other cytokine receptors, such as receptors for nerve growth factor, epidermal growth factor (EGF), and insulin-like growth factor (IGF)-I. Neuregulin is one of the EGF family member cytokines and was originally reported as glial growth factor, isolated from bovine brain and pituitary to stimulate Schwann cell proliferation *(24–26).* The receptor of neuregulin is also a RTK composed of the heterodimer of ErbB2 and ErbB3. Mice with mutations in *neuregulin-1, erbB2,* or *erbB3* all show a reduced number of Schwann cell precursors, providing strong evidence that neuregulin plays an indispensable physiological role in Schwann cell precursor proliferation and survival *(7,27).*

These RTK family members, including ErbB heterodimer, recruit signaling molecules and activate several downstream cascades. Among them are two main cascades: mitogen-activated protein kinase (MAPK) pathways and phosphatidylinositol-3-kinase (PI-3K) pathways. MAPK pathways are activated through activation of the small guanosine triphosphatase Ras, and signals are then transmitted to Raf-Mek-Erk in descending order by phosphorylation of each molecule *(28).* In many cell types, MAPK pathways play central roles in cell cycle progression and/or cell differentiation *(29).* The importance of these pathways in Schwann cell proliferation has also been mentioned *(13).*

cAMP–PKA signals and Mek–Erk signals are both important in Schwann cell proliferation. However, whether these two pathways interact physiologically or work independently remains unclear. Because growth factors such as PDGF or fibroblast growth factor require db-cAMP for their mitotic function, elevation of intracellular cAMP concentration is a prerequisite for their effects *(23).* Recently, Monje *(30)* reported that an elevation of cAMP concentration in Schwann cells prolongs the duration of Erk phosphorylation after growth factor stimulation and that a certain length of sustained Erk phosphorylation is required for Schwann cell proliferation. These findings suggest that the cAMP–PKA signal modulates the intensity of Mek–Erk signals and that Mek–Erk pathways directly regulate cell cycle machineries to promote Schwann cell proliferation. Further studies related to ligands regulating intracellar cAMP level are needed to determine the mitotic process of Schwann cells in vivo.

Signal Transduction in Schwann Cell Differentiation

Schwann cell differentiation can be divided into two programs according to their final phenotypes: myelinating Schwann cells and nonmyelinating Schwann cells. Regardless of which phenotypes they may manifest in the future, they arise from the neural crest and differentiate in the same manner up to immature Schwann cells when they express p75 and NCAM on their cell surfaces. When they migrate and proliferate along axons that are supposed to be myelinated, they reduce mitotic activity and take a promyelinating phenotype, increasing their expression of P0 and MAG *(1).* To date, axon diameter is the sole established factor for determining whether Schwann cells reach the promyelinating state *(31).* Therefore, myelination-inducing factors are supposed to be expressed on the axonal membrane, and, therefore, large diameter axons may transmit enough signals to Schwann cells to start myelination around them.

In vitro culture studies and gene knockout studies have led to understanding of the molecular mechanism regulating Schwann cell myelination. In vitro culture studies have revealed that the progression toward myelinating Schwann cells is associated with the upregulation of P0, MAG, and Oct-6 or the downregulation of NCAM and p75 *(32,33).* PDGF, a mitogen for Schwann cells, is reported to suppress promyelinating gene expression, whereas IGF-I, also mitogenic for the cells, stimulates promyelinating gene expression *(23,34).* IGF-I is also able to enhance myelin

formation in coculture systems *(35).* We recently reported the effect of recombinant neuregulin (neuregulin EGF-domain) on cultured Schwann cells and revealed its inhibitory effects on promyelinating gene expressions *(36).* Interestingly, although all these cytokines (i.e., neuregulin, PDGF, and IGF-I) are ligands for RTKs and activate common intracellular, MAPK, and PI-3K pathways, their effects on the fate of Schwann cells are quite different. Neuregulin and PDGF strongly suppresses the expression of Schwann cell differentiation markers, whereas IGF-1 promotes it. We noticed differences in the magnitude of activation of each intracellular pathway among these cytokines. Neuregulin EGF-domain and PDGF strongly activated both MAPK pathways and PI-3K pathways. On the other hand, IGF-I activated MAPK pathways to a much lesser extent, whereas it activated PI-3K pathways as strongly as neuregulin and PDGF. Therefore, we assumed that the balance between MAPK and PI-3K activation might explain their different effects on Schwann cell differentiation. Selective activation of each pathway by adenoviral gene transfer enabled us to observe the function of these pathways separately and revealed that activation of MAPK pathways lead to downregulation of promyelinating markers; on the other hand, activation of PI-3K pathways upregulated them. Consistent with these findings, selective activation of Ras or Raf, upstream regulators of MAPK pathways, induced dedifferentiation of Schwann cells, even in the presence of axonal signals *(37).* These results led us to conclude that PI-3K pathways enhance progression toward myelinating Schwann cells, whereas MAPK pathways suppress it. The effect of each cytokine depends on the relative strength of these two pathways.

The activity of MAPK pathways is regulated not only by ligand binding to their receptors but also by interference of other intracellular molecules. Interestingly, as Schwann cells differentiate to myelinating form, they express neurofibromin coded by the *Nf1* gene, whose defect in humans causes neurofibromatosis type I. Neurofibromin has a Ras–GAP activity function that converts Ras to the inactivated form and eventually suppresses the downstream MAPK signals *(38).* It appears that expression of neurofibromin is a proper intracellular machinery to keep MAPK activity at low levels when Schwann cells myelinate around axons. MAPK phosphatase (MKP)-3, a protein phosphatase for the Erk, also acts as a possible suppressor of MAPK activity in Schwann cells *(39).*

Whereas MAPK pathways suppress Schwann cell differentiation, activation of PI-3K pathways enhances it. To confirm the function of these pathways, we used cocultures of Schwann cells and DRG neurons in which Schwann cells were forced to express either an active form of Akt or the catalytic subunit of PI-3K, p110, by adenoviral vectors *(16,40).* The number of myelin-forming cells was significantly increased in the PI-3K-activated Schwann cell group compared to the control gene-delivered group *(36).* In this system, DRG neurons were not subjected to viral infection, so the difference in final myelination quantity can be referred solely to the function of the Schwann cells. Interestingly, other coculture studies using LY294002, a specific inhibitor for PI-3K, revealed that inhibition of PI-3K pathways is critical for myelin formation during the first 3 d from the induction with myelin-forming medium *(41).* The finding that LY294002 fails to inhibit myelin formation in later periods suggests that PI-3K pathways in Schwann cells play pivotal roles in the initial step of myelin formation.

Numerous signals are considered as candidates for downstream effectors of PI-3K–Akt pathways in various cell types. Glycogen synthase kinase-3β is one of these candidates, but the precise function of this molecule in Schwann cell differentiation is unknown *(36,42).* As for activators of PI-3K pathways, IGF-I appears to be the only RTK ligand to convey strong PI-3K activation and weak MAPK activation thus far, and it also enhances Schwann cell differentiation. Therefore, one can expect to use IGF-I to resolve

clinical problems in which Schwann cell maturation is required, such as remyelination after peripheral nerve grafts *(43).* An investigation further downstream of Akt or Glycogen synthase kinase 3β would provide more specific therapeutic targets to control Schwann cell differentiation and myelination.

We mentioned the suppressive effects of MAPK pathways on Schwann cell differentiation and that neuregulin EGF-domain reduces promyelinating gene expression through these pathways. However, a recent study using transgenic mice provided strong evidence that neuregulin—particularly neuregulin 1 type III expressed on axonal membrane—promotes Schwann cell myelination *(44).* In these mice, neuregulin 1 type III is overexpressed in axons, and a thicker myelin formation can be observed in peripheral nerves compared to wild-type mice. Even axons of a smaller diameter, which usually maturate as unmyelinated fibers, can be myelinated when the transgene is overexpressed in these axons *(45).* Although the intracellular signals—particularly activation of MAPK pathways—of Schwann cells within the developing peripheral nervous system of these transgenic mice remain unknown, membrane-bound neuregulin 1 type III and soluble recombinant neuregulin EGF-domain may exert different effects on MAPK activation. Alternatively, Schwann cells in vivo may use some machinery to reduce the activation of MAPK pathways while they maintain their neuregulin-triggered PI-3K activation to proceed toward promyelinating differentiation.

Other Signals in Schwann Cell Differentiation

cAMP Signals

cAMP signals are regarded to be important both in proliferation and differentiation of Schwann cell development. In culture studies, elevation of cAMP by db-cAMP or forskolin leads to upregulation of promyelinating genes such as P0, MAG, and transcriptional factor Oct-6 *(2,46).* Because these pathways also play a pivotal role in proliferation, it has been suggested that cAMP acts as a mitogen in the proliferating condition of Schwann cells, and when the cells cannot proliferate because of either contact inhibition or low serum concentration, it induces promyelinating differentiation *(47).* However, although forskolin increases the transcriptional activities of MAG promoter, no evidence exists that any one of the cAMP signal downstream effectors, such as CREB, binds to a promoter of *mag* gene as an enhancer *(48).* Therefore, cAMP signals may regulate differentiation in an indirect fashion. Additionally, the method by which cAMP signals exert distinct functions in proliferation and differentiation remains unknown. There are two possible mechanisms to explain this issue: (a) the downstream of cAMP can be modified by other signals to function as a differentiating factor or (b) cAMP signals exert a more general function to increase transcriptional activities in a less specific manner, and other signals determine which groups of genes are to be activated. In either scenario, cAMP signals do not appear to be a main component in deciding cell fate in Schwann cell differentiation. Nevertheless, the finding that IGF-I (a differentiating cytokine mentioned earlier) requires the elevation of intracellular cAMP level to some extent to induce promyelinating gene expression suggests that cAMP signals are necessary in this context *(34).* To elucidate the precise function of cAMP in Schwann cells, we need to identify specific target molecules of this signal in the future.

Nuclear Factor-κB Signals

Nuclear factor-κB (NF-κB) is a transcription factor that regulates gene expression related to survival and differentiation. In the resting state, inhibitory κB (IκB) forms a complex with NF-κB to repress its function. Upon cytokine stimulation, IκB is phosphorylated by IκB kinase complex, which induces ubiquitination and proteasomal degradation of IκB, releasing

NF-κB to translocate to the nucleus and thereby effecting changes in transcription *(49).* NF-κB has been shown to be necessary for Schwann cell myelination from coculture studies of Schwann cells and DRG neurons. Schwann cells with attenuated NF-κB activation by introducing a mutated form of IκB failed to form myelin. Additionally, Nickols et al. *(50)* recently reported that NF-κB was highly upregulated in promyelinating Schwann cells, and DRG cultures taken from mutant mice lacking NF-κB subunit p65 failed to form myelin. The specific activator or downstream effectors of this signal are not yet clear.

The use of coculture experiments has also revealed the importance of brain-derived growth factor (BDNF) and neurotrophin-3 (NT3) in the myelination program. BDNF promotes myelination via p75 neurotrophin receptor ($p75^{NTR}$), whereas NT3 inhibits myelination via NT3 receptor TrkC *(51,52).* Interestingly, $p75^{NTR}$ potently activates NF-κB putatively via TRAF6, a member of the tumor necrosis factor receptor-associated factor family *(53).* Therefore, BDNF may enhance myelination by activating NF-κB as well as other downstream molecules of $p75^{NTR}$.

Signals From Extracellular Matrix

Schwann cells receive signals for differentiation not only from humoral factors but also from extracellular matrix—particularly that in the basement membrane. In coculture experiments, ascorbic acid is necessary for Schwann cells to form basement membrane and begin myelination, suggesting that the progression toward myelin formation requires signals from the basement membrane *(16).* Laminin-2 is a major component of the basement membrane, and mutation in the gene encoding α2 laminin chain causes dysmyelinating peripheral neuropathy in patients with congenital muscular dystrophy *(54).* β1 integrin—mainly α6β1 integrin—is one of the putative laminin receptors and Schwann cells lacking β1 integrin fail to establish a 1:1 relationship with axons in the myelination process *(8).* Upon binding to laminin-2, integrins form clusters and recruit signaling molecules such as facial adhesion kinase, paxillin, and Fyn *(55).* Although the downstream effectors of this complex are not yet clear, integrins may interact with cytoskeletal structure or regulate myelin-related gene expressions. Dystroglycan–dystrophin complex also transmits signals from laminin, and a mutation in periaxin (one of the components of the signaling structure) has been reported in Charcot-Marie-Tooth disease, which manifests dysmyelination *(56).* Periaxin-null mice can form myelinated axons initially, but they develop late-onset demyelinating neuropathy, suggesting that signals from the dystroglycan–dystrophin complex contribute to the stabilization of myelinated fibers *(57,58).*

Comparison With Oligodendrocytes

Whereas Schwann cells participate in forming the myelin structure in peripheral nerves, in central nervous systems (CNS), oligodendrocytes form myelin. The major difference between their functions is that a single Schwann cell basically myelinates one segment of a single axon, whereas a single oligodendrocyte forms myelin around several axons simultaneously. Unlike Schwann cells, oligodendrocytes arise from neuroepithelium, similarly to neurons and astrocytes. Therefore, besides their common function in myelination, Schwann cells and oligodendrocytes are distinct in origin.

Although intracellular signals in oligodendrocytes are not yet clear, similar cytokines to those found in Schwann cells such as PDGF, neuregulin, and IGF-I are known to regulate the proliferation and differentiation of these cells *(59).* In terms of differentiation and myelin formation, IGF-I exerts its effects similarly to Schwann cells. Transgenic mice overexpressing IGF-I manifest hypermyelination, and the deletion mutant of IGF-I leads to hypomyelination in the CNS of neonatal mice *(60,61).* Moreover, transgenic mice expressing the active form of Akt, an effector of PI-3K,

under control of the proteolipid protein promoter showed increased myelination *(62)*. Therefore, PI-3K–Akt pathways located downstream of RTKs promote differentiation and myelination in oligodendrocytes. To date, no reports have been found regarding the downstream effectors of Akt or any suppressive effects of MAPK on oligodendrocyte differentiation.

Perspectives for Therapeutic Application of Myelinating Signals

Among the various pathological conditions in the peripheral nervous system and the CNS, several disorders are related to the dysfunction of myelination. Probably the most characterized myelin disorder is multiple sclerosis, in which failure of remyelination by endogenous oligodendrocyte precursors has been raised as one of the key obstacles preventing a cure for the disease. To date, the subcutaneous injection of IGF-I has been clinically evaluated for these patients *(63)*. Schwann cell transplantation is also under clinical trial in several patients *(64)*; thus far, however, no evidence exists that implanted cells can form myelin in once-demyelinated lesions.

Transplantation of Schwann cells is considered not only in degenerative disorders but also in traumatic disorders such as spinal cord injury *(65)*. In this context, Schwann cells are supposed to function first as a path on which regenerating axons grow, and then the transplanted cells are expected to form myelin around new axons for proper axonal signal transmission. Because there are demyelinated lesions present in traumatic insults to the CNS, transplanted cells are also expected to remyelinate surviving axons in such cases, which would otherwise undergo axonal degeneration later. We believe the proper modification of Schwann cell or oligodendrocyte differentiation and myelin formation by either gene transfer or humoral factors would improve the outcome of these therapeutic approaches to cure demyelinated lesions. To achieve such goals, future studies need to specify the intracellular signals for therapeutic target.

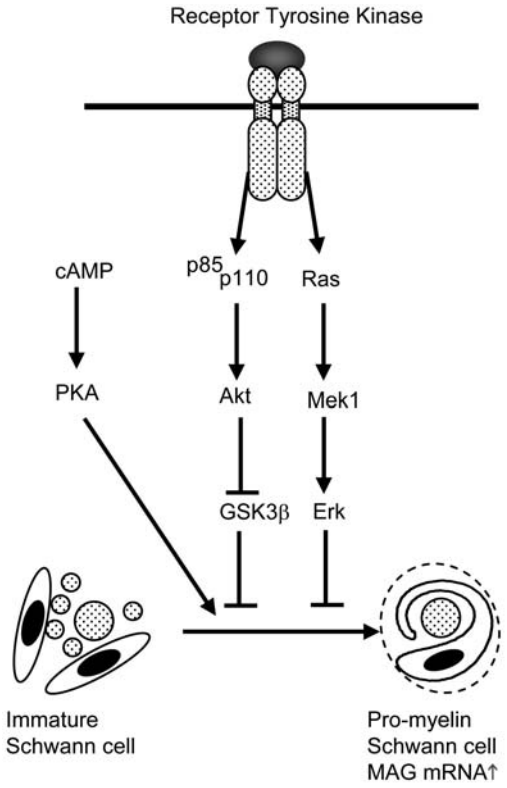

Fig. 2. Schematic view of intracellular signal cascades triggered by receptor tyrosine kinase in Schwann cell differentiation. Upregulation of myelin-associated glycoprotein (MAG) messenger RNA implies a transition from immature Schwann cells to a promyelinating state, and subsequently, myelination occurs. Activation of both cAMP and Akt (also GSK-3β) signals and simultaneous downregulation of MAPK pathways are required for MAG expression. Presently, the targets of these three pathways are not clear. (Reproduced with permission from ref. *36*.)

Conclusions

This article discussed signal transduction in Schwann cell proliferation and differentiation toward myelin forming cells. The determination of individual cell fate appears to depend not on a single molecule or signaling pathway but, rather, on a mutual relationship between multiple signaling pathways. Signals from RTK, MAPK, and PI-3K pathways play piv-

otal roles in both proliferation and differentiation, and the balance between activations of these two pathways determines resulting cell functions (Fig. 2). The elevation of intracellular cAMP concentration also promotes various cell functions, at least partly, by modulating other signaling pathways. Therefore, elucidating the relationships and crosstalk mechanisms between the different signaling pathways may convey a new understanding of Schwann cell biology and may also enable researchers to solve clinical disorders related to myelination.

References

1. Mirsky R. and Jessen K. R. (1996) Schwann cell development, differentiation and myelination. *Curr. Opin. Neurobiol.* **6,** 89–96.
2. Mirsky R. and Jessen K. R. (2001) Embryonic and early postnatal development of Schwann cells. In: Glial Cell Development, 2nd ed., Jessen K. R. and Richardson W. D., eds. New York: Oxford University Press, pp. 1–20.
3. Jessen K. R. and Mirsky R. (2002) Signals that determine Schwann cell identity. *J. Anat.* **200,** 367–376.
4. Topilko P., Schneider-Maunoury S., Levi G., et al. (1994) Krox-20 controls myelination in the peripheral nervous system. *Nature* **371,** 796–799.
5. Jaegle M., Mandemakers W., Broos L., et al. (1996) The POU factor Oct-6 and Schwann cell differentiation. *Science* **273,** 507–510.
6. Zorick T. S., Syroid D. E., Brown A., Gridley T., and Lemke G. (1999) Krox-20 controls SCIP expression, cell cycle exit and susceptibility to apoptosis in developing myelinating Schwann cells. *Development* **126,** 1397–1406.
7. Garratt A. N., Voiculescu O., Topilko P., Charnay P., and Birchmeier C. (2000) A dual role of erbB2 in myelination and in expansion of the schwann cell precursor pool. *J. Cell Biol.* **148,** 1035–1046.
8. Feltri M. L., Graus Porta D., Previtali S. C., et al. (2002) Conditional disruption of beta 1 integrin in Schwann cells impedes interactions with axons. *J. Cell Biol.* **156,** 199–209.
9. Wood P. M. and Bunge R. P. (1975) Evidence that sensory axons are mitogenic for Schwann cells. *Nature* **256,** 662–664.
10. Raff M. C., Hornby-Smith A., and Brockes J. P. (1978) Cyclic AMP as a mitogenic signal for cultured rat Schwann cells. *Nature* **273,** 672,673.
11. Brockes J. P., Fields K. L., and Raff M. C. (1979) Studies on cultured rat Schwann cells. I. Establishment of purified populations from cultures of peripheral nerve. *Brain Res.* **165,** 105–118.
12. Chin K. V., Yang W. L., Ravatn R., et al. (2002) Reinventing the wheel of cyclic AMP: novel mechanisms of cAMP signaling. *Ann. NY Acad. Sci.* **968,** 49–64.
13. Kim H. A., DeClue J. E., and Ratner N. (1997) cAMP-dependent protein kinase A is required for Schwann cell growth: interactions between the cAMP and neuregulin/tyrosine kinase pathways. *J. Neurosci. Res.* **49,** 236–247.
14. Howe D. G. and McCarthy K. D. (2000) Retroviral inhibition of cAMP-dependent protein kinase inhibits myelination but not Schwann cell mitosis stimulated by interaction with neurons. *J. Neurosci.* **20,** 3513–3521.
15. Zwartkruis F. J. and Bos J. L. (1999) Ras and Rap1: two highly related small GTPases with distinct function. *Exp. Cell Res.* **253,** 157–165.
16. Eldridge C. F., Bunge M. B., Bunge R. P., and Wood P. M. (1987) Differentiation of axon-related Schwann cells in vitro. I. Ascorbic acid regulates basal lamina assembly and myelin formation. *J. Cell Biol.* **105,** 1023–1034.
17. Rahmatullah M., Schroering A., Rothblum K., Stahl R. C., Urban B., and Carey D. J. (1998) Synergistic regulation of Schwann cell proliferation by heregulin and forskolin. *Mol. Cell Biol.* **18,** 6245–6252.
18. Lee M. M., Badache A., and DeVries G. H. (1999) Phosphorylation of CREB in axon-induced Schwann cell proliferation. *J. Neurosci. Res.* **55,** 702–712.
19. Cho-Chung Y. S., Clair T., Tortora G., and Yokozaki H. (1991) Role of site-selective cAMP analogs in the control and reversal of malignancy. *Pharmacol. Ther.* **50,** 1–33.
20. Cheng L., Khan M., and Mudge A. W. (1995) Calcitonin gene-related peptide promotes Schwann cell proliferation. *J. Cell Biol.* **129,** 789–796.
21. Stevens B., Ishibashi T., and Fields R. D. (2004) Effects of adenosine and growth factors on Schwann cell proliferation are context—dependent. Program No. 494.5, *2004 Abstract Viewer/Itinerary Planner.* Washington, DC: Society for Neuroscience.
22. Walikonis R. S. and Poduslo J. F. (1998) Activity of cyclic AMP phosphodiesterases and adenylyl cyclase in peripheral nerve after crush and permanent transection injuries. *J. Biol. Chem.* **273,** 9070–9077.

23. Davis J. B. and Stroobant P. (1990) Platelet-derived growth factors and fibroblast growth factors are mitogens for rat Schwann cells. *J. Cell Biol.* **110,** 1353–1360.
24. Lemke G. E. and Brockes J. P. (1984) Identification and purification of glial growth factor. *J. Neurosci.* **4,** 75–83.
25. Marchionni M. A., Goodearl A. D., Chen M. S., et al. (1993) Glial growth factors are alternatively spliced erbB2 ligands expressed in the nervous system. *Nature* **362,** 312–318.
26. Garratt A. N., Britsch S., and Birchmeier C. (2000) Neuregulin, a factor with many functions in the life of a schwann cell. *Bioessays* **22,** 987–996.
27. Morris J. K., Lin W., Hauser C., Marchuk Y., Getman D., and Lee K. F. (1999) Rescue of the cardiac defect in ErbB2 mutant mice reveals essential roles of ErbB2 in peripheral nervous system development. *Neuron* **23,** 273–283.
28. Hunter T. (1997) Oncoprotein networks. *Cell* **88,** 333–346.
29. Zhang W. and Liu H. T. (2002) MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Res.* **12,** 9–18.
30. Monje P. V., Bunge M. B., and Wood P. M. (2004) Cyclic AMP controls cell cycle progression by modulating the duration of MEK-ERK activation in Schwann cells. Program No. 148.10, *2004 Abstract Viewer/Itinerary Planner.* Washington, DC: Society for Neuroscience.
31. Voyvodic J. T. (1989) Target size regulates calibre and myelination of sympathetic axons. *Nature* **342,** 430–433.
32. Einheber S., Hannocks M. J., Metz C. N., Rifkin D. B., and Salzer J. L. (1995) Transforming growth factor-beta 1 regulates axon/Schwann cell interactions. *J. Cell Biol.* **129,** 443–458.
33. Arroyo E. J., Bermingham J. R. Jr., Rosenfeld M. G., and Scherer S. S. (1998) Promyelinating Schwann cells express Tst-1/SCIP/Oct-6. *J. Neurosci.* **18,** 7891–7902.
34. Stewart H. J., Bradke F., Tabernero A., Morrell D., Jessen K. R., and Mirsky R. (1996) Regulation of rat Schwann cell Po expression and DNA synthesis by insulin-like growth factors in vitro. *Eur. J. Neurosci.* **8,** 553–564.
35. Russell J. W., Cheng H. L., and Golovoy D. (2000) Insulin-like growth factor-I promotes myelination of peripheral sensory axons. *J. Neuropathol. Exp. Neurol.* **59,** 575–584.
36. Ogata T., Iijima S., Hoshikawa S., et al. (2004) Opposing extracellular signal-regulated kinase and Akt pathways control schwann cell myelination. *J. Neurosci.* **24,** 6724–6732.
37. Harrisingh M. C., Perez-Nadales E., Parkinson D. B., Malcolm D. S., Mudge A. W., and Lloyd A. C. (2004) The Ras/Raf/ERK signalling pathway drives Schwann cell dedifferentiation. *EMBO J.* **23,** 3061–3071.
38. Basu T. N., Gutmann D. H., Fletcher J. A., Glover T. W., Collins F. S., and Downward J. (1992) Aberrant regulation of ras proteins in malignant tumour cells from type 1 neurofibromatosis patients. *Nature* **356,** 713–715.
39. Bermingham J. R. Jr., Shumas S., Whisenhunt T., Rosenfeld M. G., and Scherer S. S. (2001) Modification of representational difference analysis applied to the isolation of forskolin-regulated genes from Schwann cells. *J. Neurosci. Res.* **63,** 516–524.
40. Katagiri H., Asano T., Ishihara H., et al. (1996) Overexpression of catalytic subunit p110alpha of phosphatidylinositol 3-kinase increases glucose transport activity with translocation of glucose transporters in 3T3-L1 adipocytes. *J. Biol. Chem.* **271,** 16,987–16,990.
41. Maurel P. and Salzer J. L. (2000) Axonal regulation of Schwann cell proliferation and survival and the initial events of myelination requires PI 3-kinase activity. *J. Neurosci.* **20,** 4635–4645.
42. Cohen P. and Frame S. (2001) The renaissance of GSK3. *Nat. Rev. Mol. Cell Biol.* **2,** 769–776.
43. Fansa H., Schneider W., Wolf G., and Keilhoff G. (2002) Influence of insulin-like growth factor-I (IGF-I) on nerve autografts and tissue-engineered nerve grafts. *Muscle Nerve* **26,** 87–93.
44. Michailov G. V., Sereda M. W., Brinkmann B. G., et al. (2004) Axonal neuregulin-1 regulates myelin sheath thickness. *Science* **304,** 700–703.
45. Schwab M. H., Michailov G. V., Brinkmann B. G., et al. (2004) A threshold level of Neuregulin-1 induces myelination. Program No. 494.11, *2004 Abstract Viewer/Itinerary Planner.* Washington, DC: Society for Neuroscience.
46. Monuki E. S., Weinmaster G., Kuhn R., and Lemke G. (1989) SCIP: a glial POU domain gene regulated by cyclic AMP. *Neuron* **3,** 783–793.
47. Morgan L., Jessen K. R., and Mirsky R. (1991) The effects of cAMP on differentiation of cultured Schwann cells: progression from an early phenotype (04+) to a myelin phenotype (P0+, GFAP-, N-CAM-, NGF-receptor-) depends on growth inhibition. *J. Cell Biol.* **112,** 457–467.
48. Ye P., Laszkiewicz I., Wiggins R. C., and Konat G. W. (1994) Transcriptional regulation of myelin associated glycoprotein gene expression by cyclic AMP. *J. Neurosci. Res.* **37,** 683–690.

49. Gilmore T. D. (1999) The Rel/NF-kappaB signal transduction pathway: introduction. *Oncogene* **18,** 6842–6844.
50. Nickols J. C., Valentine W., Kanwal S., and Carter B. D. (2003) Activation of the transcription factor NF-kappaB in Schwann cells is required for peripheral myelin formation. *Nat. Neurosci.* **6,** 161–167.
51. Chan J. R., Cosgaya J. M., Wu Y. J. and Shooter E. M. (2001) Neurotrophins are key mediators of the myelination program in the peripheral nervous system. *Proc. Natl. Acad. Sci. USA* **98,** 14,661–14,668.
52. Cosgaya J. M., Chan J. R., and Shooter E. M. (2002) The neurotrophin receptor p75NTR as a positive modulator of myelination. *Science* **298,** 1245–1248.
53. Ye X., Mehlen P., Rabizadeh S., VanArsdale T., et al. (1999) TRAF family proteins interact with the common neurotrophin receptor and modulate apoptosis induction. *J. Biol. Chem.* **274,** 30,202–30,208.
54. Helbling-Leclerc A., Zhang X., Topaloglu H., et al. (1995) Mutations in the laminin alpha 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. *Nat. Genet.* **11,** 216–218.
55. Chen L. M., Bailey D., and Fernandez-Valle C. (2000) Association of beta 1 integrin with focal adhesion kinase and paxillin in differentiating Schwann cells. *J. Neurosci.* **20,** 3776–3784.
56. Guilbot A., Williams A., Ravise N., et al. (2001) A mutation in periaxin is responsible for CMT4F, an autosomal recessive form of Charcot-Marie-Tooth disease. *Hum. Mol. Genet.* **10,** 415–421.
57. Gillespie C. S., Sherman D. L., Fleetwood-Walker S. M., et al. (2000) Peripheral demyelination and neuropathic pain behavior in periaxin-deficient mice. *Neuron* **26,** 523–531.
58. Sherman D. L., Fabrizi C., Gillespie C. S., and Brophy P. J. (2001) Specific disruption of a schwann cell dystrophin-related protein complex in a demyelinating neuropathy. *Neuron* **30,** 677–687.
59. McMorris F. A. and McKinnon R. D. (1996) Regulation of oligodendrocyte development and CNS myelination by growth factors: prospects for therapy of demyelinating disease. *Brain Pathol.* **6,** 313–329.
60. Ye P., Carson J., and D'Ercole A. J. (1995) In vivo actions of insulin-like growth factor-I (IGF-I) on brain myelination: studies of IGF-I and IGF binding protein-1 (IGFBP-1) transgenic mice. *J. Neurosci.* **15,** 7344–7356.
61. Ye P., Li L., Richards R. G., DiAugustine R. P., and D'Ercole A. J. (2002) Myelination is altered in insulin-like growth factor-I null mutant mice. *J. Neurosci.* **22,** 6041–6051.
62. Priyadarshini S., Flores A. I., and Macklin W. B. (2004) Overexpression of Akt1 in oligodendrocytes enhances myelination and regulates neuronal differentiation *in vivo.* Program No. 722.16, *2004 Abstract Viewer/Itinerary Planner.* Washington, DC: Society for Neuroscience.
63. Frank J. A., Richert N., Lewis B., et al. (2002) A pilot study of recombinant insulin-like growth factor-1 in seven multiple sderosis patients. *Mult. Scler.* **8,** 24–29.
64. Halfpenny C., Benn T., and Scolding N. (2002) Cell transplantation, myelin repair, and multiple sclerosis. *Lancet Neurol.* **1,** 31–40.
65. Bunge M. B. (2002) Bridging the transected or contused adult rat spinal cord with Schwann cell and olfactory ensheathing glia transplants. *Prog. Brain Res.* **137,** 275–282.