Axons regulate Schwann cell expression of the major myelin and NGF receptor genes

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Summary

The elaboration of myelin by Schwann cells is triggered by contact with appropriate peripheral axons. Among the most prominent features of this interaction is the activation and high-level expression of the genes encoding the major myelin proteins P₀ and Myelin Basic Protein (MBP). Although the initial induction of these genes is thought to be dependent upon contact with axons, neither the inductive signal of the axon nor the receptor and associated second messenger system of the Schwann cell that transduces this signal has been identified. In this report, we demonstrate that expression of the P₀ and MBP genes in rapidly myelinating Schwann cells is sharply reduced upon withdrawal of axons, but that this expression can be substantially restored by agents that raise the intracellular concentration of cyclic AMP. We further show that Schwann cell expression of a third gene, i.e. that encoding the Nerve Growth Factor receptor, is strongly activated by the withdrawal of axons, and that this activation is largely independent of cAMP.

Key words: myelination, Schwann cells, gene expression, cyclic AMP, NGF, RNA.

Introduction

Many events in neural development are mediated through cell–cell interactions. Among the most dramatic of these interactions is one that occurs between neurones and glia, and results in the elaboration of the myelin sheath, the electrical insulation that surrounds all of the rapidly conducting axons of higher vertebrates (Ritchie, 1984). In the peripheral nervous system (PNS), this insulating sheath is produced by Schwann cells following an inductive, contact-dependent interaction with a subset of peripheral axons (Aguayo et al. 1977; Bray et al. 1981). This interaction has two notable metabolic consequences: an enormous increase in Schwann cell plasma membrane biosynthesis and surface area (Webster, 1971), and the induction of a set of proteins unique to myelin-forming glia (Lemke, 1986). The most abundant of these induced proteins are the structural proteins P₀ and Myelin Basic Protein (MBP), which together account for over 60% of the protein present in the peripheral myelin sheath (Greenfield et al. 1973). Neither of these proteins is expressed by Schwann cells that do not synthesize myelin (Brockes et al. 1980).

Previous studies have suggested that Schwann cell expression of the P₀ and MBP proteins remains dependent upon axonal contact throughout the period of myelination and beyond (Politis et al. 1982). We now show that this axon dependence is directly reflected in the steady-state levels of the mRNAs encoding these proteins. In addition to this positive effect on myelin mRNA levels, we demonstrate that axons are capable of exerting an equally striking negative effect on a third Schwann cell mRNA, that encoding the Nerve Growth Factor (NGF) receptor. We find that the positive effect of axons on Schwann cell mRNA levels is mimicked by elevation of intracellular cyclic AMP, while their negative effect is not.
Materials and methods

Materials

The cDNA clones used in this study have been described previously and are indicated individually in figure legends and the acknowledgements. Forskolin was purchased from Calbiochem; cholera toxin and 12-O-tetradecanoylphorbol 13-acetate (TPA) were purchased from Sigma; [35S]methionine was purchased from New England Nuclear. All other reagents were as described previously (Brockes et al. 1979; Lemke & Axel, 1985).

Schwann cell culture

Schwann cells were isolated from the sciatic nerves of neonatal (2–3 day) Sprague-Dawley rats and purified by immunoselection as described by Brockes et al. (1979). For some applications (see figure legends), these purified cells were expanded by the procedure of Porter et al. (1986) for 3–4 weeks prior to use.

RNA isolation and analysis

Total cellular RNA was isolated from neonatal nerves and cultured cells via lithium chloride precipitation of guanidine thiocyanate homogenates (Cathala et al. 1983). Isolation of poly(A)+ RNA and Northern blot analyses were carried out as described previously (Lemke & Axel, 1985).

Metabolic labelling and immunoprecipitation

Cultured Schwann cells (2×10^6) were labelled for 12 h with 600 μCi [35S]methionine in methionine-free medium containing 10% dialysed fetal calf serum. Labelled proteins were extracted and immunoprecipitated according to standard procedures using two different monospecific rabbit anti-P0 antisera (Brockes et al. 1980; Trapp et al. 1981).

Results and discussion

We first compared the levels of the major myelin and Nerve Growth Factor receptor (NGFR) mRNAs expressed by Schwann cells in neonatal rat sciatic nerve to those expressed by Schwann cells dissociated from this same nerve, purified by immunoselection and then cultured in vitro (Brockes et al. 1979). When grown in a conventional cell culture medium in the absence of axons, purified neonatal Schwann cells cease myelin membrane biosynthesis and markedly down-regulate several myelin components, most notably the myelin structural proteins P0 and MBP (Mirsky et al. 1980; Fryxell et al. 1983). Several laboratories have noted that this ‘dedifferentiation’ in vitro is similar to that observed in vivo when Schwann cell–axon interactions are disrupted through nerve crush or transection, in that the same cessation of myelination and down-regulation of MBP and P0 protein expression are observed (Politis et al. 1982; Poduslo et al. 1984). The in vitro behaviour of purified Schwann cells has been interpreted as primarily reflecting the absence of axons, in that upon re-introduction to axons via coculture with purified neurones, cultured Schwann cells re-express the major myelin proteins and proceed to myelinate neuronal processes (Bunge & Bunge, 1984; Ranscht et al. 1987). Our studies therefore involve comparing an actively myelinating population of Schwann cells developing in the presence of axons in vivo to a nonmyelinating population of Schwann cells cultured in the absence of axons in vitro.

Axonal effects are exerted at the level of mRNA expression

Down-regulation of the major myelin proteins results from a corresponding decrease in the steady-state level of their messenger RNAs (Fig. 1A,B). Schwann cells cultured in the absence of axons express the P0 and MBP mRNAs at a level approximately 40-fold lower than that of cells in the postnatal day-2 sciatic nerve (the age at which cells were dissociated for culture). The low basal level of expression of these RNAs is reached after 5 days in culture and is
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maintained indefinitely thereafter (>4 months). Parallel studies that we have performed in vivo demonstrate a similarly dramatic reduction in \( P_0 \) and MBP mRNA expression over a similar time course in Schwann cells distal to a sciatic nerve transection (Trapp et al. 1988). An inverse response is seen for the NGF receptor (NGFR) gene, however. In this case, the trace amounts of NGFR mRNA expressed by Schwann cells in the presence of axons are strikingly elevated in cells cultured in the absence of axons (Fig. 1C). This observation correlates well with the recent demonstration that NGF binding and NGFR molecules increase dramatically upon transection of peripheral nerves (Taniuchi et al. 1986). Thus, for both the major myelin and NGFR genes, the primary metabolic effect of axons is exerted at the level of mRNA expression and presumably reflects diminished or enhanced transcription.

Axonal effects are mimicked by elevations in cAMP

Down-regulation of \( P_0 \) gene expression in cultured neonatal Schwann cells is reversed by agents that raise intracellular levels of cAMP (Fig. 2A). Addition of low concentrations of forskolin (2 \( \mu \)M), a specific and reversible activator of adenylate cyclase, results in a marked increase in \( P_0 \) mRNA. This effect is seen at the protein level as well (Fig. 2B), and is fully reversed when forskolin is withdrawn, providing that the drug has not been present for more than three weeks (see below). The low concentration of forskolin used in these experiments is also sufficient to induce the cellular flattening and expansion previously observed when purified Schwann cells are treated with high concentrations of cAMP analogues (Sobue et al. 1986), but is well below the concentration required either to stimulate significantly Schwann cell division (Porter et al. 1986) or maximally activate adenylate cyclase (Seamon et al. 1981).

Although we observed that MBP mRNA levels were also elevated by addition of 2 \( \mu \)M-forskolin, the magnitude of this stimulation was well below that observed for \( P_0 \) mRNA. To investigate these responses further, we performed a dose-response titration, measuring the steady-state \( P_0 \) and MBP mRNA levels in Schwann cells 36 h after the addition of varying concentrations of forskolin. Fig. 3A gives the Northern blot profile for this titration. These data demonstrate that while both genes are cAMP-inducible, the threshold for the MBP response is at least tenfold higher than that for \( P_0 \). The differences in these two responses are quantified in Fig. 3E. When the same set of RNAs was analysed for NGFR mRNA (Fig. 3C), we observed that this message is highly expressed by Schwann cells grown in the absence of forskolin and diminishes only minimally upon addition of the drug for 36 h. Thus the positive effect of axons on major myelin mRNA expression is closely mimicked by elevated cAMP, while their negative effect on NGFR expression is not. The levels of \( P_0 \) and MBP mRNAs expressed by purified cells cultured with 100 \( \mu \)M-forskolin are 37% and 41%, respectively, of those expressed by Schwann cells in the neonatal nerve (data not shown). These values indicate that the 36 h forskolin treatments we have used are substantially, though not completely, capable of restoring \( P_0 \) and MBP mRNA expression. The induction of major myelin mRNAs by cAMP appears to be specific: the effect of forskolin is duplicated by cholera toxin (10 ng ml\(^{-1}\)), an irreversible stimulator of adenylate cyclase (Moss & Vaughan, 1979) (data not shown), but not by TPA, a tumour promoter that activates protein kinase C (Castagna et al. 1982) (Fig. 3D).

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**Fig. 2. Steady-state \( P_0 \) RNA and protein expression in the presence and absence of forskolin.** Purified rat Schwann cells were cultured for 3 weeks in the presence of a mitogenic mix of 2 \( \mu \)M-forskolin and 20 \( \mu \)g ml\(^{-1}\) crude Glial Growth Factor (Porter et al. 1986; Lemke & Brockes, 1984), then withdrawn from these mitogens for 4 days, and finally switched to medium containing 2 \( \mu \)M-forskolin (+F) or to medium alone (−F) for 36 h prior to harvesting. (A) Northern blot of \( P_0 \) RNA with and without forskolin. Each lane contains 3 \( \mu \)g total RNA. (B) SDS–polyacrylamide gel of immunoprecipitation of \( ^{35} \)S-labelled proteins synthesized by Schwann cells with and without forskolin. Each lane contains 3 \( \mu \)g total RNA. (B) SDS–polyacrylamide gel of immunoprecipitation of \( ^{35} \)S-labelled proteins synthesized by Schwann cells with and without forskolin. Labelled proteins were immunoprecipitated with anti-\( P_0 \) rabbit antisera (Lemke & Axel, 1985; Brockes et al. 1980; Trapp et al. 1981) (IP) or nonimmune sera (nIP). The position of \( ^{35} \)S-\( P_0 \) is arrowed.
NGF does not affect Schwann cell expression of the major myelin and NGFR genes

The function of the Schwann cell NGF receptors induced upon withdrawal of axons remains open to speculation (Heumann et al. 1987). We have examined the effect of added NGF on the expression of the major myelin and NGFR genes. The data presented in Fig. 4 demonstrate that NGF is without effect on the steady-state levels of mRNAs transcribed from either the \( P_0 \), MBP or NGFR genes. Studies of NGF binding to cultured Schwann cells have previously shown that the binding sites expressed by these cells are of the low affinity type (DiStefano & Johnson, 1988), for which a clear function has yet to be described.

Conclusions

The above observations indicate that axons are capable of both positive and negative regulation of Schwann cell gene expression. In addition, they implicate cAMP as a key second messenger through which the myelination signal of the axon is conveyed to the Schwann cell genome. Although our evidence in this regard is indirect, several observations are consistent with this interpretation. First and most importantly, the marked difference in sensitivity of the \( P_0 \) and MBP genes to cAMP induction is well-correlated with expression of the corresponding proteins by Schwann cells in the neonatal sciatic nerve (cells in the presence of axons). In all species thus far examined, \( P_0 \) levels are 5- to 10-fold higher than those of MBP (Greenfield et al. 1973). Thus the differential
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Axons. Second, we have observed that prolonged transcriptionally active when transfected into purified similar to the differential activation of these genes by activation of the major myelin genes by cAMP is very cAMP serves, through cAMP-dependent protein

et al. Third, Sobue et al. (1986) have shown that very high concentrations of cAMP analogs (1 mm) and forskolin (200 μm) are capable of inducing expression of galactocerebroside, a glycolipid characteristic of Schwann cells and myelin. Finally, we have found that the isolated promoter region of the cloned rat P0 gene is transcriptionally active when transfected into purified Schwann cells only if these cells are cultured in the presence of forskolin (Lemke et al. 1988).

Expression of the P0 and MBP genes is absolutely restricted to myelin-forming glial cells, whereas cAMP serves, through cAMP-dependent protein kinases, as a second messenger in the regulation of a diverse set of biological phenomena. If cAMP does indeed play a role in the regulation of Schwann cell expression of the major myelin genes, then the specificity of this expression must reside in molecules with which these genes and cAMP-dependent kinases interact.

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References


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