Developmental Abnormalities of Myelin Basic Protein Expression in \textit{fyn} Knock-out Brain Reveal a Role of Fyn in Posttranscriptional Regulation*

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Fyn protein-tyrosine kinase (PTK), a member of the Src-PTK family, is essential for myelin development in the central nervous system (CNS). The absence of Fyn activity results in defects in the morphogenesis of oligodendrocyte precursors (OPCs) and CNS hypomyelination. However, molecular mechanisms for Fyn to control CNS myelinogenesis remain elusive. Here we show that Fyn-PTK is significantly up-regulated in early OPC differentiation, concentrated in the compact myelin, and declines during myelin development. Despite the high levels of Fyn-PTK expression during early OPC differentiation, Fyn deficiency does not affect the expression of mRNAs that encode myelin structural proteins, including that for the myelin basic protein (MBP), until postnatal day 13 (P13). However, the accumulation rate of MBP mRNA is significantly attenuated during the most active period of myelinogenesis (P13 and P20). Interestingly, the absence of Fyn causes a preferential reduction of the exon-2 containing MBP mRNA isoforms derived from alternative splicing, providing the first evidence that Fyn is required for posttranscriptional regulation of MBP. Consistent with this idea, Fyn phosphorylates the selective RNA-binding protein QKI, which likely modulates the activity of QKI in binding and stabilizing the MBP mRNA. Furthermore, Fyn deficiency exerts an opposing influence on MBP isoform patterning in comparison to that by QKI deficiency. These observations collectively suggest that Fyn plays critical roles in promoting accelerated MBP expression during myelinogenesis in a MBP isoform-preferential manner, and QKI may act in the same pathway downstream of Fyn for MBP mRNA homeostasis.

In the central nervous system (CNS), oligodendrocytes are responsible for myelinating neuronal axons with specialized membrane lamellae (1). Sufficient myelin production under accurate developmental control is essential for CNS function (1), since deficiency in myelination results in various neurological disorders (2, 3). The process of myelination depends on coordinated expression of myelin structural genes governed by a variety of developmental signals (4–7). Fyn protein-tyrosine kinase (PTK), a member of the Src-PTK family, is a critical player in CNS myelination (8–13). Fyn is up-regulated in differentiating oligodendrocyte progenitors (OPCs) in primary cultures (10, 14), followed by a down-regulation during active myelinogenesis (13, 15). Hence, Fyn has been proposed to function as a critical mediator of developmental signals to control CNS myelinogenesis (8, 10, 12, 13, 16). Indeed, the absence of Fyn activity results in aberrant OPC morphogenesis (8, 10) and hypomyelination in the forebrain, characterized by a decrease in total myelin content and a reduced number of myelin lamellae on neuronal axons (11, 12, 17). Furthermore, reduced expression of genes encoding myelin structural proteins in the \textit{fyn} knock-out (ko) brain has been reported in several studies (11, 13, 18). However, molecular mechanisms underlying hypomyelination in the \textit{fyn} ko brain remain unknown. No comprehensive model has been proposed to describe the role of Fyn signaling in regulating myelin protein expression and myelin development.

In this study, we observed a marked up-regulation of Fyn expression upon induced differentiation of the CG4 oligodendrocyte cell line, consistent with the hypothesis that Fyn signaling promotes early differentiation of OPCs. During normal brain development, Fyn was enriched in the compact myelin and gradually declined with age. In the \textit{fyn} ko brain, myelin structural gene mRNAs, represented by the myelin basic protein (MBP) transcripts, were expressed at normal levels until postnatal day 13 (P13) but failed to accumulate rapidly thereafter, indicating a functional requirement of Fyn in accelerating myelin synthesis. Interestingly, an isoform-preferential reduction of MBP mRNA was observed in the \textit{fyn} ko brain, suggesting the involvement of Fyn in posttranscriptional regulation of MBP during myelin development. Furthermore, Fyn can phosphorylate the selective RNA-binding protein QKI at the C-terminal tyrosines that controls the activity for QKI to bind MBP mRNA, suggesting the possibility for QKI to act downstream of Fyn in CNS myelination.

**MATERIALS AND METHODS**

\textit{Animals and RNA Preparation—}Animals were treated according to National Institutes of Health regulations under the approval of the Emory University Institutional Animal Care and Use Committee. The \textit{fyn} ko colony (generously provided by Dr. P. Stein, University of Pennsylvania) and the quakingviable (qkv) colony (purchased from Jackson Laboratory) were maintained at the Emory animal facility. Brain regions were dissected at ages indicated in the corresponding figure for total RNA extraction using TRIzol™ according to manufacturer’s protocol (Invitrogen). The quantity of RNA from each sample was determined by \(A_{260}\) reading and further confirmed by ethidium bromide-stained agarose gel electrophoresis.

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The abbreviations used are: CNS, central nervous system; PTK, protein-tyrosine kinase; OPC, oligodendrocyte precursor; ko, knock-out; MBP, myelin basic protein; NSE, neuron-specific enolase; CNP, 2',3'-cyclic nucleotide 3'-phosphodiesterase; GFAP, glial fibrillary acidic protein; RPA, RNase protection analysis; GAPDH, glyceraldehyde phosphate dehydrogenase; wt, wild type; PLP, proteolipid protein.
Cell Culture, Transfection, and Phosphorylation—CG4 cells were maintained for proliferation and induced for differentiation by established procedures (19). Cells were harvested for preparation of whole cell lysate in 1× Laemmli buffer. HEK293T cells were maintained at standard conditions and plasmid transfection was performed using Lipofectamine 2000 following manufacturer’s instructions (Invitrogen). Immunoprecipitation was carried out using anti FLAG-M2 antibody (Sigma). For the detection of phosphotyrosine, immunoblot analysis was performed with phosphatase inhibitors as described previously (20). Recombinant His-QKI-7 was used for the in vitro phosphorylation assay by purified Fyn-PTK (Upstate Biotechnology) in the presence of [γ-32P]ATP using our previously published conditions (20).

Immunoblot Analysis and Antibodies—Compact myelin was isolated from the forebrains by homogenizing the tissue in 10% w/v sucrose followed by sucrose gradient fractionation as described previously (20, 21). Whole tissue lysates were prepared by sonicating minced tissue in 1× laemmli buffer. The quantity of total protein in each sample was estimated by the Bradford assay (Bio-Rad) before subjected to SDS-PAGE. Hybridization to the primary antibody was performed in PBS containing 0.2% Tween 20 and 10% milk. All antibodies used in immunoblot analysis are commercially available: anti-neuron-specific enolase (NSE), anti-MBP, and anti-2’,3’-cyclic nucleotide 3’-phosphodiesterase (CNP) (Chemicon); anti-FY20 (Transduction Laboratories), anti-glia fibrillary acidic protein (GFAP), anti-Src, anti-Src pY416 (Sigma), and anti-Fyn (Santa Cruz Biotechnology).

RNAse Protection Analysis (RPA) and RPA Probes— Antisense riboprobes were generated by in vitro transcription following manufacturer’s instruction (Stratagene) in the presence of [32P]UTP (Amersham Biosciences) using linearized plasmids as DNA templates. The synthesis of the MBP probe and CNP probe were described previously (22). The cDNA template for the PLP-DM20 probe was generated from reverse transcription-PCR and the primer sequence will be available upon request. The glyceraldehyde phosphate dehydrogenase (GAPDH) RPA probe and β-actin probe were generated using DNA templates purchased from Ambion. RPA was carried out according to previously published procedures (22).

RESULTS

Expression of Fyn in Differentiating OPCs and in the Developing Myelin—To understand the role of Fyn in oligodendroglia development, we first examined the expression profile of Fyn upon induced differentiation of the CG4 oligodendrocyte cell line. As shown in Fig. 1A, CG4 cells underwent vigorous morphogenesis accompanied by a marked up-regulation of myelin structural proteins represented by the CNP, recapitulating differentiation of primary cultured oligodendrocytes. Fyn expression level was significantly elevated when cells extended multiple branched processes (Fig. 1B). In contrast, Src levels gradually declined, indicating distinct regulation of Src-PTK members during the early stage of OPC differentiation.

Both Fyn and Src were enriched in the fraction containing compact myelin isolated from normal brain at the peak of myelinogenesis (~P20) as well as in young adulthood (Fig. 2A). The purity of isolated myelin was indicated by the absence of neuronal and astrocytic proteins, represented by the NSE and the GFAP respectively. Interestingly, Fyn levels gradually declined in the compact myelin isolated from the wt brain during active myelinogenesis (P20, Fig. 2B). As expected, Fyn was undetectable in the fyn ko brain (Fig. 2B). Consequently, the absence of Fyn resulted in a significant reduction of the total activity of Src-PTKs in the isolated myelin (Fig. 2C), as measured by the levels of phosphotyrosine at position 416 (Tyr(P)-416) that is present in all the activated Src-PTK members.

Fyn Deficiency Causes an Abnormally Slow Accumulation of MBP mRNA during Myelin Development—To identify the molecular defects that lead to hypomyelination in the fyn ko brain, we analyzed the expression levels of several myelin structural protein mRNAs during myelin development by quantitative reverse transcription-PCR. The purity of isolated myelin was indicated by the absence of neuronal and astrocytic proteins, represented by the NSE and the GFAP respectively. Interestingly, Fyn levels gradually declined in the compact myelin isolated from the wt brain during active myelinogenesis (P20, Fig. 2B). As expected, Fyn was undetectable in the fyn ko brain (Fig. 2B). Consequently, the absence of Fyn resulted in a significant reduction of the total activity of Src-PTKs in the isolated myelin (Fig. 2C), as measured by the levels of phosphotyrosine at position 416 (Tyr(P)-416) that is present in all the activated Src-PTK members.

FIG. 1. Up-regulation of Fyn expression upon induced OPC differentiation. A, morphological changes during the early differentiation of the CG4 oligodendrocyte cell line. The day for differentiation is marked in each panel. B, immunoblot demonstrating Fyn up-regulation. Whole cell lysate was prepared for the SDS-PAGE and subjected to sequential probing by the antibodies marked on the left. eIF5-a, translation initiation factor 5, was used as a housekeeping loading control.

FIG. 2. Src-PTKs in compact myelin isolated from the developing forebrain. A, immunoblot demonstrates that both Fyn and Src are enriched in purified compact myelin. Ten micrograms of total protein was loaded in each lane. The detected proteins by specific antibodies are labeled on the left. Actin was used as a loading control, NSE as a neuronal marker, and GFAP as an astracyte marker. B, Fyn abundance in the wt compact myelin declines during myelinogenesis. As expected, no Fyn signal is detected in the fyn ko myelin. (C) The absence of Fyn causes significant reduction of total Src-PTK activity in the compact myelin. Tyr(P)-416 is detected by a specific antibody that recognizes all the activated Src-PTK members.
RPA. Fig. 3 shows the expression profile of MBP mRNA isoforms in the forebrain of *fyn* ko mice and age-matched wt controls. Four major MBP mRNA isoforms derived from alternative splicing, which encode the 21.5, 18.5, 17.2, and 14 kDa MBP protein isoforms (M21.5, M18.5, M17.2, and M14, respectively), were detected at comparable levels in wt and *fyn* ko forebrain up to P13 (Fig. 3, A and B). A rapid accumulation of MBP mRNAs was detected in the wt forebrain between P13 and P20 (Fig. 3C). However, all the MBP mRNA isoforms were at lower levels in the *fyn* ko forebrain as compared with that in the wt controls after P13, resulting in a significantly reduced accumulation rate of MBP mRNAs during the most active period of myelination between P13 and P20 (Fig. 3C). In contrast, expression of MBP mRNAs in the *fyn* ko brain stem was normal throughout the entire profile of myelin development (data not shown). This result suggests that Fyn is required for accelerated MBP expression only in a subclass of oligodendrocytes in the CNS during the most active myelogenesis.

**Fig. 3. Developmental profile of MBP mRNA isoform expression in wt and *fyn* ko forebrain.** A, representative RPA detecting all four major MBP mRNA isoforms. The age of the animals is marked on top of the corresponding lanes. The housekeeping GAPDH mRNA was used as a loading control. B, phosphorimager analysis of MBP mRNA isoforms. Phosphorimager readings of MBP isoforms were normalized to that of GAPDH. The level of each MBP mRNA isoform in the wt control was set at 100%. The numbers of independent experiments are indicated at the bottom in each age group. Standard *t* test was performed. * indicates *p* < 0.05, ** indicates *p* < 0.001 for all isoforms when compared with that in wt. C, accumulation speed of MBP mRNAs during active myelination. Phosphorimager readings of RPA signals were plotted against age as indicated at the bottom.

**Fyn Deficiency Results in an Isoform-preferential Reduction of Alternatively Spliced Oligodendrocyte mRNAs**—A more detailed analysis revealed that the M21.5 and M17.2 MBP mRNA isoforms (both contain exon 2) appeared to be more severely reduced than the M18.5 and M14 MBP mRNA isoforms (both lack exon 2) in the *fyn* ko brain (Fig. 3B). To reaffirm this phenomenon, we employed a semiquantitative reverse transcription-PCR assay that amplified the exon 2(+) and exon 2(-) MBP transcripts in the same reaction. As shown in Fig. 4, the ratio of exon 2(+) MBP mRNAs to the exon 2(-) MBP mRNAs was reduced in the *fyn* ko brain, confirming the preferential reduction of the exon 2(+)-MBP mRNA isoforms caused by Fyn deficiency. The isoform-preferential deficits caused by Fyn deficiency cannot be explained by transcriptional abnormality and thus provides the first evidence that Fyn is required for posttranscriptional regulation in oligodendrocytes.

Besides the reduced MBP mRNA expression during active myelination in the *fyn* ko forebrain, the mRNAs encoding CNPs and DM20 were also significantly reduced whereas the PLP mRNA, an alternatively spliced mRNA derived from the same transcript encoding the DM20 mRNA, was at normal level (Fig. 4). Hence, Fyn deficiency causes a broad range of abnormalities in the expression of myelin structural genes, which is conceivable to result in deficient myelin synthesis and the hypomyelination phenotype.

To test whether Fyn deficiency may cause additional post-transcriptional abnormalities, we performed linear sucrose gradient fractionation experiments to evaluate MBP translation in the absence of Fyn. As shown in Fig. 5, forebrain cytoplasmic extracts derived from *fyn* ko mice and wt controls were fractionated on parallel linear sucrose gradients and the distribution of each MBP mRNA isoform was determined by RPA. A nearly identical polyribosome association profile of MBP mRNA was observed in *fyn* ko and wt brain, with the majority of the MBP mRNAs associated with polyribosomes engaged in active translation, demonstrating that MBP translation is not affected by Fyn deficiency.

We next tested whether the absence of Fyn may affect the efficiency for the transport of MBP mRNAs to the compact myelin, which is essential for localized MBP production and
QKI May Act Downstream of Fyn in MBP mRNA Homeostasis—During normal development, expression of MBP in myelination is under the control of both transcriptional and posttranscriptional regulations. Previous studies demonstrated that the selective RNA-binding protein QKI is a critical factor required to maintain the stability of MBP mRNAs in an isoform-preferential manner (21). Since Src family kinases negatively modulate the RNA-binding activity of QKI (20), we next tested whether QKI can be phosphorylated by Fyn, which possibly influences the homeostasis of MBP mRNAs. As shown in Fig. 8A, incubation with Fyn-PTK resulted in phosphorylation of purified recombinant QKI in vitro. In addition, when co-expressed with Fyn in HEK293T cells, QKI was tyrosine-phosphorylated (Fig. 8B). In contrast, the mutant QKI, in which the C-terminal tyrosine cluster was replaced by phenylalanines thus lost the phosphorylation-dependant regulation in binding MBP mRNA (20), failed to be phosphorylated by Fyn in parallel experiments. These data demonstrate that the tyrosine cluster that controls the RNA binding activity of QKI is a target for Fyn-PTK.

To further explore the potential coordination between Fyn and QKI in the pathway of regulating MBP expression during myelination, we compared the abnormalities in MBP isoform patterning in the fyn ko brain with that in the homozygous qkv’ brain in which QKI is diminished in oligodendrocytes (25, 26). As shown in Fig. 8C, Fyn deficiency led to a preferential reduction of the 21.5-kDa MBP isoform (juvenile isoform). In contrast, QKI deficiency preferentially diminished the expression of the 14-kDa MBP isoform (adult isoform). In addition, the ratio of the M21.5 MBP mRNA to that of the M14 MBP mRNA was reduced in the fyn ko brain but abnormally increased in the qkv’ brain as compared with that in wt controls. These opposing patterns of MBP isoforms resulting from Fyn deficiency as compared with that caused by QKI deficiency are consistent with our previous study that tyrosine phosphorylation of QKI at the C-terminal tyrosines by Src-PTKs negatively modulates the activity of QKI in binding MBP mRNA (20), which is expected to influence MBP mRNA homeostasis during myelination.

**DISCUSSION**

In this study, we show that Fyn-PTK is a critical factor for the rapid up-regulation of myelin structural gene expression during the most active period of myelogenesis in brain development. The isoform-preferential reduction of MBP and DM20 mRNAs in the fyn ko brain indicates that Fyn signaling is involved in posttranscriptional regulation of a subclass of oligodendrocyte-specific genes, perhaps via phosphorylation of RNA-binding proteins that control the homeostasis of these mRNA isoforms, possibly including QKI.

We observed elevated Fyn expression upon induced differentiation of the CG4 oligodendrocyte cell line (Fig. 1B) and a gradual decline of Fyn in myelin fractions enriched of oligodendrocyte membrane (Fig. 2B). Consistent with the previous reports indicating high levels of Fyn expression during early differentiation of primary OPCs (10) and reduced Fyn activity during myelin development (15, 27), these results collectively demonstrate a bi-directional regulation of Fyn signaling in myelogenesis.

Fyn activity is essential for OPC development (10, 28), which is associated with the initial up-regulation of myelin protein expression (Fig. 1B). A potential role of Fyn in transcriptional up-regulation of MBP in early OPC differentiation was proposed previously (27). In addition, the reported reduction of mature oligodendrocytes in the fyn ko corpus callosum (8, 11) suggests a role of Fyn in OPC maturation. Although these hypotheses are consistent with the end result of CNS hypomyelination caused by Fyn deficiency, they do not explain the normal MBP mRNA levels observed in fyn ko forebrain before the age of P13 (Fig. 3). Rather, the function of Fyn in MBP expression appears to be required since the start of accelerated myelogenesis. Furthermore, the rapid accumulation of MBP mRNA during active myelination (P10 and P20) is inversely correlated with Fyn activity (Fig. 2B and Ref. 15). One possible explanation for this phenomenon is that Fyn may negatively regulate a factor that promotes MBP mRNA production in early OPC development before myelination, and the decline of Fyn-PTK activity later on during active myelination accelerates MBP accumulation. Consistent with this notion, Fyn phosphorylates QKI, a selective RNA-binding protein essential for MBP expression (20), at the C-terminal tyrosine cluster (Fig. 8) that is known to attenuate the ability of QKI in binding MBP mRNA once phosphorylated (20). In addition, the quantitative levels of phosphorylation at these tyrosines in QKI decline along with the acceleration of myelogenesis (20), consistent...
with the hypothesis that the developmentally programmed regulation of QKI tyrosine phosphorylation enhances the ability of QKI in stabilizing MBP mRNA, which conceivably contributes to the accelerated MBP mRNA accumulation (20). Fyn deficiency conceivably attenuates the activity of this pathway, which could be one of the underlying mechanisms for the slow accumulation of MBP mRNAs in the \textit{fyn} \textit{ko} brain (Fig. 3). Unfortunately, it is difficult to demonstrate reduced phosphorylation of endogenous QKI in the \textit{fyn} \textit{ko} oligodendrocytes, due to the lack of antibodies that recognize the C-terminal phosphotyrosines. This issue is further complicated by the compensatory up-regulation of other Src-PTYs caused by Fyn deficiency (29). Nonetheless, the opposing effect of Fyn and QKI on MBP isoform patterning (Fig. 8) is consistent with the hypothesis that Fyn and QKI may work in the same pathway.

The involvement of Fyn in posttranscriptional regulation during CNS myelin development is further supported by the preferential reduction of the exon 2(+) MBP isoforms (Figs. 3,
The levels of MBP isoforms were determined by phosphorimager readings of RPA signals and normalized to that of the GAPDH housekeeping.

Src or Fyn in the presence of QKI.

Whether Fyn may compensate for Fyn deficiency to certain extent in the forebrain of fyn ko mice, we detected normal expression levels, perhaps more effectively in PNS and the hind brain/spinal cord, as well as in neonatal forebrain, which may partly explain the spatial and temporal features of hypomyelination in the developing nervous system of the fyn ko mice. However, such compensatory regulation obviously could not completely replace the roles of Fyn in myelogenesis. The distinct regulation of Src and Fyn in oligodendrocyte development (Fig. 1B) further indicates that among the closely related Src-PTK members, Fyn plays unique roles in mediating developmental signals to control myelin development. In this regard, fyn ko oligodendrocytes may provide a useful model system for the identification of specific targets for Fyn signaling in future studies.

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| 4, and 8 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
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| DM20 mRNA | mRNAs encoding MBP, CNP, PLP, and DM20 in the brain stem derived from the same animals (data not shown). | since maturation defects in OPC usually results in a preferential loss of mature MBP-expressing oligodendrocytes, | and the DM20 mRNA derived from the brain. The MBP protein isoforms are indicated on the left. B. Fyn phosphorylates the C-terminal tyrosine cluster in QKI when co-expressed in HEK293T cells. Plasmids encoding FLAG-tagged wt QKI or mutant QKI in which the C-terminal five tyrosines were replaced by phenylalanines (Y-F mt) was co-transfected with a plasmid encoding hemagglutinin-tagged Fyn into HEK293T cells. Immunoprecipitation (IP) of wt and mutant QKI was performed using anti-FLAG antibody, and the presence of phosphotyrosine was determined on immunoblot (IB) by the anti-PY20 antibody. The expression levels of hemagglutinin-Fyn and FLAG-QKI were shown on immunoblot in the bottom panels. C, immunoblot detects opposing abnormal MBP isoform patterns in fyn ko and qkv brain. The MBP protein isoforms are indicated on the left. D, opposing effect of Fyn and QKI on the ratio of M21.5 to M14 MBP mRNA isoforms. The levels of MBP isoforms were determined by phosphorimager readings of RPA signals and normalized to that of the GAPDH housekeeping mRNA as described in Fig. 3. ** indicates p < 0.01 by standard t test (n = 3). | Fyn-PTK phosphorylates QKI and exerts opposing influence on MBP isoform patterning as compared with that caused by QKI. A, phosphorylation of purified His-tagged QKI by Fyn in vitro. Recombinant His-QKI purified from bacterium was incubated with purified Src or Fyn in the presence of [γ-32P]ATP as indicated on top before subjected to SDS-PAGE. The migration of phosphorylated His-QKI was marked on the left. B, Fyn phosphorylates the C-terminal tyrosine cluster in QKI when co-expressed in HEK293T cells. Plasmids encoding FLAG-tagged wt QKI or mutant QKI in which the C-terminal five tyrosines were replaced by phenylalanines (Y-F mt) was co-transfected with a plasmid encoding hemagglutinin-tagged Fyn into HEK293T cells. Immunoprecipitation (IP) of wt and mutant QKI was performed using anti-FLAG antibody, and the presence of phosphotyrosine was determined on immunoblot (IB) by the anti-PY20 antibody. The expression levels of hemagglutinin-Fyn and FLAG-QKI were shown on immunoblot in the bottom panels. C, immunoblot detects opposing abnormal MBP isoform patterns in fyn ko and qkv brain. The MBP protein isoforms are indicated on the left. D, opposing effect of Fyn and QKI on the ratio of M21.5 to M14 MBP mRNA isoforms. The levels of MBP isoforms were determined by phosphorimager readings of RPA signals and normalized to that of the GAPDH housekeeping mRNA as described in Fig. 3. ** indicates p < 0.01 by standard t test (n = 3). | Interestingly, these preferentially reduced MBP isoforms are more abundant in immature oligodendrocytes (1). Hence, the hypomyelination in fyn ko brain is unlikely explained simply by the loss of mature MBP-expressing oligodendrocytes, since maturation defects in OPC usually results in a preferential reduction of the adult isoforms of MBP, as shown in the qkv and jp smad hypomyelination mutants (21, 30). Whether Fyn may regulate alternative splicing of the MBP and PLP primary transcripts remains to be determined by future studies. On the other hand, the preferential interaction of QKI with the adult MBP mRNA isoform over the juvenile MBP mRNA isoform (21) raises a question whether Fyn may modulate the differential stability of these mRNA isoforms via RNA-binding proteins, including QKI. Taken together, the major role of Fyn in MBP expression is to govern the rapid accumulation of the MBP mRNA during myelination, most likely via multiple mechanisms, including facilitated OPC differentiation, the maintenance of sufficient mature oligodendrocytes, as well as post-transcriptional regulation of MBP.

Interestingly, myelination in the spinal cord and the peripheral nervous system is not affected by the absence of Fyn (11). Consistent with this view, despite the abnormal MBP expression in the forebrain of fyn ko mice, we detected normal expression of mRNAs encoding MBP, CNP, PLP, and DM20 in the brain stem derived from the same animals (data not shown). Therefore, the function of Fyn in controlling myelin protein mRNA expression appears to be required only in a subpopulation of oligodendrocytes localized in the forebrain. The enhanced activity of other Src-PTK members, due to the absence of Fyn (29), may compensate for Fyn deficiency to certain levels, perhaps more effectively in PNS and the hind brain/spinal cord, as well as in neonatal forebrain, which may partly explain the spatial and temporal features of hypomyelination in the developing nervous system of the fyn ko mice. However, such compensatory regulation obviously could not completely replace the roles of Fyn in myelogenesis. The distinct regulation of Src and Fyn in oligodendrocyte development (Fig. 1B) further indicates that among the closely related Src-PTK members, Fyn plays unique roles in mediating developmental signals to control myelin development. In this regard, fyn ko oligodendrocytes may provide a useful model system for the identification of specific targets for Fyn signaling in future studies.

FIG. 8. Fyn-PTK phosphorylates QKI and exerts opposing influence on MBP isoform patterning as compared with that caused by QKI. A, phosphorylation of purified His-tagged QKI by Fyn in vitro. Recombinant His-QKI purified from bacterium was incubated with purified Src or Fyn in the presence of [γ-32P]ATP as indicated on top before subjected to SDS-PAGE. The migration of phosphorylated His-QKI was marked on the left. B, Fyn phosphorylates the C-terminal tyrosine cluster in QKI when co-expressed in HEK293T cells. Plasmids encoding FLAG-tagged wt QKI or mutant QKI in which the C-terminal five tyrosines were replaced by phenylalanines (Y-F mt) was co-transfected with a plasmid encoding hemagglutinin-tagged Fyn into HEK293T cells. Immunoprecipitation (IP) of wt and mutant QKI was performed using anti-FLAG antibody, and the presence of phosphotyrosine was determined on immunoblot (IB) by the anti-PY20 antibody. The expression levels of hemagglutinin-Fyn and FLAG-QKI were shown on immunoblot in the bottom panels. C, immunoblot detects opposing abnormal MBP isoform patterns in fyn ko and qkv brain. The MBP protein isoforms are indicated on the left. D, opposing effect of Fyn and QKI on the ratio of M21.5 to M14 MBP mRNA isoforms. The levels of MBP isoforms were determined by phosphorimager readings of RPA signals and normalized to that of the GAPDH housekeeping mRNA as described in Fig. 3. ** indicates p < 0.01 by standard t test (n = 3).
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