tical to those obtained with Lyn are also obtained with Fyn. Therefore, CD19 serves as a substrate for Src-family PTKs, but also amplifies Src-family PTK activity.

The region of the CD19 cytoplasmic domain involved in the amplification of Lyn kinase activity was defined using CD19 peptides and CD19 fusion proteins. Although the binding of phosphopeptides from some proteins can activate Src-family PTKs during SH2 domain interactions,[68,69] CD19 peptides containing individual phosphotyrosine residues of CD19 are unable to amplify Lyn activation. However, a CD19-exon 12–14 fusion protein amplifies Lyn to the same extent as the entire CD19-exon 7–14 cytoplasmic domain. Fusion proteins representing exon 7–13, exon 7–10 and exon 8–12 proteins are without activity as is a CD19 exon 14 fusion protein containing only CD19-Y^{513}. A fusion protein encoding the ~140 amino acid long cytoplasmic domain of CD22 is also unable to amplify Lyn kinase activity, despite the presence of six well-conserved tyrosine residues that include phosphorylation and binding sites for Lyn.[5,70] This suggests that Lyn amplification is unique to a region of CD19 including exons 12–14 rather than just individual phosphopeptide sequences.

Whether the two Lyn binding sites within CD19-exons 13 and 14 are essential elements for amplification of Lyn kinase activity was assessed using mutated CD19-exon 12–14 fusion proteins in which Y^{482} and/or Y^{513} were substituted with phenylalanine(s). Compared with an intact exon 12–14 fusion protein, these three mutated CD19-exon 12–14 fusion proteins were unable to amplify Lyn activity during *in vitro* kinase assays. In addition, Lyn autophosphorylation is only enhanced by CD19-GST fusion proteins containing intact Y^{482} and Y^{513} residues. Similarly, the simultaneous addition of both CD19-Y^{482} and CD19-Y^{513} peptides or both CD19-GST $Y^{482}F$ and CD19-GST $Y^{513}F$ fusion proteins to *in vitro* kinase assays does not affect Lyn activity. This establishes that intact Y^{482} and Y^{513} residues are both required for Lyn kinase activation by CD19 and that CD19-Y^{513} phosphorylation is required before subsequent CD19-Y^{482} phosphorylation on the same molecule.

Based on the above results, it is most likely that the increase in Lyn enzymatic activity observed in these assays results from enhanced conversion of Lyn to an activated state rather than enhancing the enzymatic activity of previously activated Lyn. Therefore, we propose that two Lyn molecules recruited to phosphorylated CD19-Y^{482} and CD19-Y^{513} residues through their SH2 domains are thereby brought into close proximity (Fig. 3C). The spatial proximity of the Lyn/CD19/Lyn complex results in transphosphorylation and activation of the Lyn molecules bound to CD19 (Fig. 3D). This explains why the linkage and spatial orientation of both CD19-Y^{482} and CD19-Y^{513} is indispensable for CD19 amplification of Lyn kinase activity.

CD19 binding amplifies Lyn phosphorylation of Vav

In addition to amplifying Src-family PTK activity, CD19 also facilitates molecular interactions that lead to Vav phosphorylation, which is decreased in CD19-deficient B cells.[65] Vav binds phosphorylated CD19 tyrosine residues directly via its SH2 domain. The primary binding site for Vav is Y^{391}, but Vav also interacts with Y^{421}, and weakly with Y^{403} and Y^{513} (Fig. 1). That CD19 provides phosphorylated docking sites for both Src-family PTKs and Vav is important since *in vitro* kinase assays have demonstrated that Lyn can phosphorylate Vav, and that CD19/Lyn interactions facilitate Vav phosphorylation.[21] Thus, the formation of Lyn/CD19 complexes is likely to enhance both the binding of Vav to CD19 and Lyn phosphorylation of Vav (Fig. 3E). The abolition of Vav tyrosine phosphorylation in Lyn-deficient mice supports this notion.[28] Therefore, phosphorylated CD19 provides distinct and specific SH2-domain recognition regions to which Lyn (and other Src-family PTKs) and Vav bind.

Constitutive CD19 phosphorylation by Lyn facilitates the assembly of Lyn/CD19/Vav ternary complexes that are found in resting splenic B cells.[21] Interactions between CD19, Lyn, and Vav increase after BCR crosslinking in parallel with increased CD19 phosphorylation. Thus, Src-family PTK binding to CD19 amplifies kinase activity that facilitates efficient Vav phosphorylation by the activated PTK. Vav may then attract other SH2-domain-containing signaling molecules to the CD19 complex, which leads to downstream activation of MAPK cascades.[71–73] The regulation of Src-family PTK activation and Vav phosphorylation by CD19 thereby provides a potent molecular mechanism for amplifying BCR signals.

CD19/PI 3-kinase complexes

CD19 also interacts with effector molecules downstream of BCR signaling such as phosphatidylinositol 3-kinase (PI 3-kinase).[61,64,65,74,75] The tandem SH2 domains of PI 3-kinase p85 subunit bind CD19 dually phosphorylated at Y^{482} and Y^{513}.[56,61] Dual phosphorylation of CD19-Y^{482} and CD19-Y^{513} by Lyn through processive phosphorylation thereby provides docking sites for PI 3-kinase.[56] PI 3-kinase also binds dually-phosphorylated CD19 in cell lines.[61–63,75,76] The kinetics of Lyn's interactions with CD19 suggest that Lyn phosphorylation and binding to CD19 precedes PI 3-kinase binding (Fig. 3E). Lyn constitutively associates with CD19 at relatively high levels in primary B cells