phosphopeptides or intact protein at detectable levels, suggesting that phosphorylated CD19 interacts specifically with Src-family PTKs.

While Lyn is required for CD19 phosphorylation, the reverse is also true. Overall tyrosine phosphorylation of cellular proteins is dramatically decreased in CD19-deficient B cells before and after BCR ligation.[21] Lyn, Fyn, Blk, and Lck phosphorylation increase modestly in CD19-deficient B cells following BCR crosslinking, although CD19-deficient B cells express these Src-family PTKs at wild type levels. Altered maturation does not explain this observation since CD19-deficient B cells develop normally and express wild type levels of Fgr which is predominantly expressed by mature B cells.[55] B cells from mice that overexpress CD19 mature normally and express Lyn protein at wild type levels.[32,46] However, Lyn kinase activity is increased in B cells from transgenic mice that overexpress CD19.[56] Analogously, CD19 expression by a plasmacytoma cell line enhances Lyn tyrosine phosphorylation when compared with parental CD19-negative cells.[57] These studies collectively demonstrate that CD19 expression regulates Src-family PTK activity.

Consistent with decreased tyrosine phosphorylation of the Src-family PTKs, CD79a/CD79b phosphorylation is decreased in CD19-deficient B cells after BCR ligation. Nonetheless, Syk phosphorylation and kinase activity is normal in CD19-deficient B cells before and after BCR crosslinking. Tyrosine phosphorylation of PLC-γ2, which is downstream of Syk, is also intact. Thus, low level Src-family PTK activation in the absence of CD19 induces sufficient CD79a/CD79b phosphorylation for the recruitment of Syk, which subsequently becomes phosphorylated.[1] Therefore, a critical function for CD19 is the amplification of Src-family PTK-dependent signaling cascades, rather than Syk-dependent pathways. Consistent with this, wild type levels of Src-family PTK activation are not necessary for optimal Syk activation since Syk can be activated in the absence of Lyn.[58] In addition, Syk self-amplification through autophosphorylation is an efficient process.[59]

Tyrosine phosphorylation sites within CD19

Although CD19 has nine tyrosine residues, the location of functional tyrosine phosphorylation sites within CD19 has become controversial. Cell lines transfected with CD19 cDNAs encoding mutations of CD19-Y^{482} and CD19-Y^{513} do not phosphorylate the remaining seven CD19 tyrosines at detectable levels.[60,61] Therefore, CD19-Y^{482} and CD19-Y^{513} have been proposed to be the only phosphorylated tyrosines within CD19. However, CD19 functions as a transmembrane adapter protein for the recruitment of Vav to phosphorylated CD19-Y^{391}, a primary binding site for Vav.[62–64] Vav also binds other phosphotyrosines within the CD19 cytoplasmic domain at lower levels.[56] CD19-Y^{391} phosphorylation has physiological relevance since CD19 expression is required for optimal Vav phosphorylation,[65] and mutation of CD19-Y^{391} results in impaired activation of MAPKs.[63] Therefore, CD19-Y^{482} and CD19-Y^{513} are unlikely to be the only phosphorylated tyrosines in CD19.

In vitro studies examining tyrosine phosphorylation sites within recombinant CD19 have offered an explanation for the paradox outlined above.[56] These studies demonstrate that CD19 has two preferential Lyn phosphorylation sites when presented as peptides: Y^{391} and Y^{513}, although other residues are phosphorylated at lower levels. However, the phosphorylation pattern is different for an intact CD19 cytoplasmic domain. We therefore propose that the CD19 cytoplasmic domain is highly folded due to its multiple highly-charged regions.[66] In its folded state, CD19-Y^{513} is likely to be the preferential Lyn phosphorylation site following BCR engagement and Lyn activation (Fig. 3A,B), and then phosphorylated Y^{513} acts as a nucleation site to retain Lyn through its SH2 domain (Fig. 3B). Phosphorylated CD19-Y^{513} is the preferred Lyn binding site among CD19 phosphotyrosine motifs. CD19-Y^{513} phosphorylation may "unfold" the CD19 cytoplasmic domain and thereby allow other tyrosines to be phosphorylated (Fig. 3C). The C-terminal location of CD19-Y^{513} as a preferred Lyn phosphorylation and binding site is consistent with a model of "processive phosphorylation". Processive phosphorylation is where a C-terminal SH2 domain binding site in target substrates enhances Src-family PTK phosphorylation of N-terminal tyrosines.[67] A CD19 fusion protein lacking an intact Y^{513} residue is not phosphorylated by Lyn during *in vitro* assays, demonstrating a requirement for CD19-Y^{513} phosphorylation for subsequent phosphorylation of other residues. Phosphorylation of a CD19-Y^{482}F mutant of CD19 exon 12–14 fusion protein is markedly reduced during *in vitro* kinase assays compared with an intact exon 12–14 protein, further verifying that CD19-Y^{482} is a Lyn phosphorylation site after phosphorylation and binding to Y^{513}. Thus, Lyn may phosphorylate more proximal N-terminal CD19 tyrosines, including Y^{482} and Y^{391}, through processive phosphorylation after CD19-Y^{513} phosphorylation and binding.

CD19 amplifies Lyn activity by "processive amplification"

The recent finding that Lyn interactions with CD19 directly amplify Lyn activation provides a new understanding of CD19 function and explains many of the