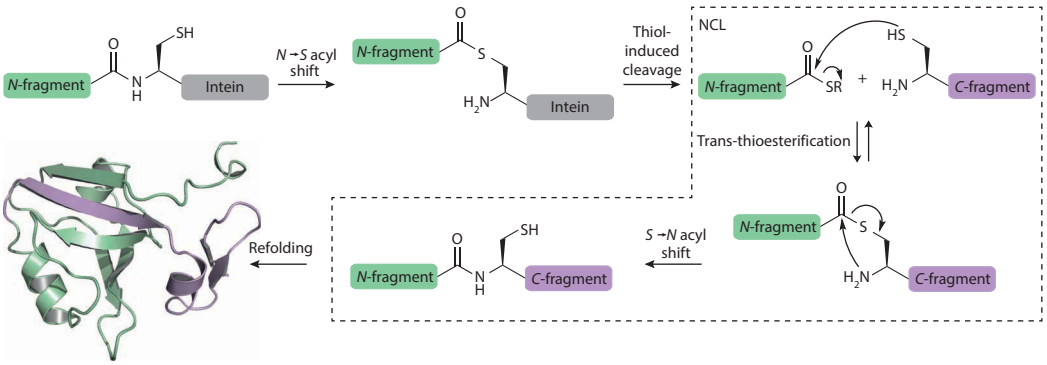

FIGURE 6.8 Principles of expressed protein ligation (EPL) and native chemical ligation (NCL). In NCL, an *N*-terminal peptide thioester fragment undergoes trans-thioesterification with a *C*-terminal peptide fragment containing an *N*-terminal cysteine. This is followed by a spontaneous *S*-to-*N* acyl shift, resulting in the formation of a native amide bond and a cysteine at the ligation site. In EPL, an *N*-terminal protein thioester is generated from a recombinant intein fusion construct by reaction with an exogenous thiol. The protein thioester is then reacted in an NCL reaction with a synthetic peptide, enabling semi-synthesis of larger proteins. After both NCL and EPL, the protein has to be refolded into its native structure.

with monodisperse polymer moieties in order to improve duration of action in vivo. The 166-residue protein was prepared by ligation of four peptide fragments, two of which was modified with the polymer and the EPO analog displayed improved properties in vivo compared to EPO.

In 1998, an extension of the NCL principles was introduced, called expressed protein ligation (EPL). The technology applies the same reaction as in NCL, but in contrast to NCL, one of the components is a recombinantly expressed protein, rather than a peptide (Figure 6.8). Protein thioesters is prepared by expressing the protein as a so-called intein fusion construct which can be converted into the thioester and subsequently reacted with a peptide with an *N*-terminal cysteine generating a full-length protein (Figure 6.8). Thus, the EPL methodology combines the advantages of molecular biology with chemical peptide synthesis and enables the addition of unnatural functionalities to a recombinant protein framework.

EPL has, similar to NCL, been applied in studies of a plethora of proteins. A prominent example is studies of histones which are important for storage of DNA and have flexible *N*-terminal tails that are heavily modified by PTMs. EPL has been applied to prepare full length, ubiquitylated histones which were used to demonstrate a direct cross-talk between PTMs on different histones. EPL has also been applied to integral membrane proteins, specifically the potassium channel KcsA. EPL was used to prepare truncated KcsA subunits (122-residues) which were then refolded and reconstituted into lipid membranes. This allowed incorporation of, for example, D-alanine and an amide-to-ester mutation (Figure 6.5) in the selectivity filter of the channel which has revealed important information of the mechanism behind ion conductance. In subsequent studies, a modular EPL approach, involving three fragments and two consecutive ligation steps, was used to generate full-length KcsA subunits (160-residues) that were refolded into the native channel. EPL has also been used to address the importance of backbone hydrogen bonds in intracellular signaling processes. Specifically, protein domains, the so-called PDZ domains, were generated by EPL, and amide-to-ester mutations (Figure 6.5) were introduced in the central recognition site to demonstrate the general importance of specific hydrogen bonds. Clearly, such backbone interactions cannot be addressed by conventional mutagenesis.

The EPL technology also holds commercial prospective and is exploited to generate recombinantly derived protein *C*-terminal thioesters that can be converted into the corresponding hydrazide or oxyamine derivatives, enabling subsequent chemical modification and thus, allowing a wide range of *C*-terminal modifications of proteins.