Hydroxylation of target EGF domain aspartate or asparagine residues is catalyzed by a β-hydroxylase located in the ER. EGF domains are ~45 amino acids long and contain one potential hydroxylation site. Hydroxylation is consensus sequence dependent and is usually partial, with only a fraction of target molecules being hydroxylated in practice. Full carboxylation and hydroxylation, on the other hand, is essential to maintaining biological activity of protein C (Grinnell et al., 1991, 2006; Liu et al., 2014; Yan et al., 1990). The native molecule displays nine carboxylation and one hydroxylation sites. Such stringent PTM requirements could not be met by CHO cells, forcing the developers of the recombinant version to develop a modified human cell line (HEK 293) in its manufacture (Liu et al., 2014).

4.10 SULFATION

Sulfation is a PTM required for a limited number of therapeutic proteins. *O*-Sulfation entails the attachment of a sulfate (SO_3^-) group to tyrosine residues by a sulfotransferases-mediated co/posttranslational process within the *trans* Golgi network and is predominantly associated with secretory and membrane proteins (Yang et al., 2015); it is sometimes encountered *O*-linked to serine or threonine (Medzihradszky et al., 2004). In the context of biopharmaceuticals, native hirudin (a leech-derived anticoagulant) and blood factors VIII and IX are usually sulfated. Neither of the approved recombinant forms of hirudin are sulfated, although it has been shown that sulfated hirudin (at Tyr63) displays 10-fold tighter affinity for thrombin than does unsulfated analogues (Costagliola et al., 2002). While over 90% of native factor IX molecules are sulfated, <15% of the approved recombinant form are; with apparently little if any difference in product efficacy (McGrath, 2006). Sulfation of factor VIII is required for optimal binding to its plasma carrier protein (von Willebrand's factor); interestingly, people inheriting a factor VIII Tyr1680 → Phe mutation often display mild hemophilia (Yang et al., 2015). Several hormone cell surface receptors are known to be tyrosine sulfated, and sulfation is required for high-affinity ligand binding and subsequent receptor activation (Choe et al., 2003; Ludeman and Stone, 2014).

4.11 GLYCOSYLATION

As referenced earlier, a recombinant form of the glycoprotein erythropoietin required a mammalian cell production platform since glycosylation and the precise glycoform were shown to be essential to *in vivo* activity (Macdougall et al., 2012). When first produced, in CHO cells, the product was shown to have an enhanced activity *in vitro*, compared to the natural form isolated from urine, but it was inactive *in vivo*. This was due to the absence of terminal sialic residues from the three *N*-linked oligosaccharide moieties; the consequence was exposure of terminal galactose residues, resulting in uptake by the asialo-glycoprotein receptor and rapid clearance through the liver. Process improvements led to a product having ~30% of sialylated EPO, and methods were developed to harvest/purify this fraction, the remainder being discarded. Since expiry of the patent, many biosimilar EPO products have been generated and approved by regulatory authorities; however, increased incidences of the development of ADA, with consequent pure red cell aplasia (PRCA), have been reported