18.5 'Radical SAM' Proteins with Iron–Sulfur (FeS) Clusters

18.5.1 Discovery of Radical SAM Enzymes

SAM-dependent 'radical SAM' enzymes with FeS clusters were recognized as a superfamily as recent as 15 years ago.[18,89] These enzymes use a novel common mechanism of catalysis in all kingdoms in many metabolic pathways: a reduced $[4Fe–4S]^{+}$ cluster transfers an electron to SAM to generate methionine and a 5′-deoxyadenosyl radical,[221,222] (see ref. 20 for details). Radical SAM proteins share a CX_3CX_2C motif, or variations thereof, which forms the FeS cluster (Figure 18.2). Three cysteines bind three of the four irons of [4Fe–4S] at the active site of the enzyme, while the cluster requires the sulfur moiety of SAM at the fourth iron for the generation of a 5′-deoxyadenosyl radical derived from SAM. The lack of a fourth cysteine makes these proteins hard to work with, as the cluster is oxygen-sensitive resulting in inactive [3Fe–4S] (which can be reverted back to [4Fe–4S] by reducing agents in an anaerobic atmosphere).[20]

The mechanism of radical SAM enzymes is similar to that of B12 enzymes.[20,223] The radical produced can be the end product or an intermediate in a complex chain of reactions, while SAM itself can be used up, or regenerated to SAM *via* methionine.[20] The review by Broderick *et al.*[20] gives extensive coverage of common features of these enzymes, as well as biochemical, spectroscopic, structural, and mechanistic details. Some examples are presented in Table 18.1 and in the next section, in view of their relevance to the role of SAM in central metabolism and aging. By the end of May, 2016, ~114 000 enzymes had been found, eight of these in humans.[224] Most of these enzymes have been identified in bacteria, and hence may be relevant in microbiota that colonize our gut (see Section 18.6.5).

18.5.2 The Radical SAM-Binding Domain

In 2008, the first structure of a radical SAM enzyme was reported.[225] By 2014, the crystal structures of 14 radical SAM enzymes were known and supported the notion of a common fold composed of a full or partial TIM barrel (Section 18.2.2). A full barrel consists of eight alternating alpha helices and beta strands with the beta strands on the inside. The size of the barrel varies depending on the size of the substrate, which binds within the TIM barrel.[20,226] SAM associates with the fourth iron of the [4Fe–4S] cluster in the same way in these 14 crystal structures.[20]

Many parallels exist between radical SAM and B12 enzymes, but also striking contrasts: the cofactor for B12 enzymes binds outside the barrel, and the dozen or so known B12 enzymes are mainly bacterial, while radical SAM enzymes occur in all kingdoms with an astounding rise in numbers from 600 in 2001 to ~48 000 in 2014, with the latest figure >110 000.[20,224,227] Broderick *et al.*[20] state that "ultimately, the use of the TIM barrel fold by B12 and radical