most proteins are glycosylated, and controlling glycosylation patterns of proteins is a key challenge. Glycosyltransferases catalyze the transfer of a monosaccharide to a protein and through directed evolution it was possible to modify the transferases, so that monosaccharides of interest could be selectively added to a protein framework. Another example is using transglutaminase (TGase) to obtain selective PEGylation. TGase catalyzes transfer reactions between the γ-carboxamide group of glutamine residues and primary amines, resulting in the formation of γ-amides of glutamic acid and ammonia. Thus, by using an amino-derivative of PEG (PEG-NH_2) as substrate for the enzymatic reaction it is possible to covalently bind the PEG polymer to a therapeutic protein.

6.4 EXPLORING NUCLEIC ACIDS

The central dogma of molecular biology, as proposed by Francis Crick, describes the process of converting DNA to proteins via RNA. Crick also hypothesized that RNA has more functions than just being a passive carrier of information. This was confirmed by the discovery of the first RNA enzymes (ribozymes), whereas the tremendous functional versatility of RNA we know today came much later. It is now evident that of all our genes, only 1.5% are coding for proteins. Hence the remaining 98.5% of genes must have other functions, and a large number of noncoding RNAs (ncRNAs) have been discovered. These are emerging as key regulators of gene expression at the transcriptional and post-transcriptional level and have been divided into two groups: small ncRNAs (<200 nucleotides) and large ncRNA (>200 nucleotides). Although, the majority of ncRNA transcripts belong to the latter group, very little is known about their biological function. In contrast, studies of small ncRNAs have revealed a wealth of novel properties of RNA. Small ncRNAs are typically involved in processing or regulation of other RNAs and are classified into a continuously increasing number of subgroups. The best characterized classes are micro RNA (miRNA), short interfering RNA (siRNA), and piwi-interacting RNA (piRNA) which all regulate gene expression through base-pairing to target nucleic acids. Probably the best known example is gene silencing through RNA interference (RNAi), where protein translation is inhibited after binding of the ncRNA to a specific mRNA (see Section 6.4.2).

The realization that the functions of RNA are much more diverse than first anticipated has inspired the application of in vitro directed evolution methods to further expand the properties of nucleic acids. In these studies, large libraries of RNA or DNA are used to screen for novel functionalities which has resulted in notable breakthroughs such as the discovery of oligonucleotides with high-affinity ligand binding abilities (aptamers) and the generation of the first DNA enzyme (DNAzyme) that can catalyze the cleavage of RNA phosphodiesters. The properties of ribozymes and DNAzymes are constantly being expanded, for example, to catalyze chemical transformations such as Diels–Alder cycloadditions, Michael addition, aldol condensation, as well as hydrolysis, ligation, and deglycosylation of nucleic acid substrates. At the same time extensive efforts have been invested into developing nucleic acid analogs, in which the tripartite chemical structure is modified. Recently, this led to the discovery of the unnatural polymer with catalytic activity (see Section 6.3.1). Thus, the study of nucleic acids is a core area in chemical biology and in the following, chemical biology studies of nucleic acids and RNAi as a novel therapeutic strategy will be discussed in more detail.

6.4.1 Modification of Nucleic Acids

The motivation for modification of nucleic acids is primarily related to increasing the stability and also exploring novel chemical properties of DNA and RNA. This is achieved by modifying the three principal components of nucleic acids: nucleobases, sugar moieties, and the phosphodiester backbone.

In particular, modifications of the backbone, leading to the so-called xeno nucleic acids (XNAs), have provided important tools and compounds with therapeutic perspectives. A fundamental characteristic