Circular proteins have now been discovered in all kingdoms of life and are characterized by their exceptional stability and the diversity of their biological activities, primarily in the realm of host defense functions. This thematic minireview series provides an overview of the distribution, evolution, activities, and biological synthesis of circular proteins. It also reviews approaches that biological chemists are taking to develop synthetic methods for making circular proteins in the laboratory. These approaches include solid-phase peptide synthesis based on an adaption of native chemical ligation technology and recombinant DNA approaches that are amenable to the in-cell production of cyclic peptide libraries. The thioester-mediated native chemical ligation approach mimics, to some extent, elements of the natural biosynthetic reaction, which, for disulfide-rich cyclic peptides, appears to involve asparaginyl endopeptidase-mediated processing from larger precursor proteins.

Conventional proteins are synthesized on the ribosome as topologically linear chains of amino acids that typically then fold into complex three-dimensional shapes that are determined by the linear sequence of amino acids. The shapes of folded proteins have a major role in function because shape determines the nature of specific interactions with target molecules, whether they be small molecules, other proteins, nucleic acids, or membranes.

Circular proteins (1–3) differ from linear proteins in that their backbones have one additional peptide bond that joins the N and C termini to make a topologically circular backbone. This simple addition typically does not change the global protein shape but gives the circular protein some significant advantages over its linear counterpart. The most significant advantage is enhanced stability, including stability against unfolding at high temperatures, in the presence of chemical chaotropes, or, most notably, against breakdown by proteolytic enzymes. Other advantages include the enhanced potency that can arise from locking the protein rigidly into its bioactive form, thereby reducing entropic penalties associated with the binding of flexible protein ligands. These biochemical advantages benefit the organism that produces the circular protein in its native environment and are also advantageous when circular proteins are used in agricultural, diagnostic, or pharmaceutical applications, where stability, potency, and target selectivity are important attributes.

This series of minireviews describes the discovery, biological activities, biosynthesis, and applications of circular proteins in bacteria and higher organisms and the approaches that biological chemists are taking to synthesize head-to-tail cyclic peptide chains. Nonribosomal cyclic peptides (such as cyclosporin) that have revolutionized organ transplant therapy because of their immunosuppressive properties fall outside the scope of this series. Like many previously known cyclic peptides, cyclosporin is biosynthesized by complex peptide synthetases that typically incorporate non-proteogenic amino acids. By contrast, our focus here is on ribosomally synthesized proteins that are enhanced by post-translational cyclization. We use the term circular proteins to emphasize their topology and proteinaceous nature, even though many are not much larger than cyclosporin. Like cyclosporin, the ribosomally synthesized circular proteins have great potential as drugs.

In the first minireview in the series, Göransson and colleagues provide an overview of circular proteins in fungi and plants, which appear to be particularly profligate users of native peptide cyclization. The authors focus on three circular protein families: cyclotides and sunflower trypsin inhibitors from plants and Amanita toxins from fungi. They place a particular emphasis on the biological origin, structure, and activity of these peptides, which range from 12 to 40 amino acids in size. Cyclotides are the largest class of circular proteins known and are particularly stable because they have both a cystine knot and a cyclic backbone. Membranes appear to be the common target of cyclotides, which are able to disrupt liposomes, bacterial membranes, and membranes of enveloped viruses. Because of this activity, cyclotides are able to disrupt the midgut membranes of caterpillars that have ingested cyclotides, accounting for their insecticidal activity. The biosynthetic pathways of cyclotides and other plant cyclic peptides are not yet fully understood, but it is clear that they are processed from precursor proteins and that asparaginyl endopeptidases, which are cysteine proteases, have an important role in the processing and cyclization (4, 5).

In the second minireview, Maqueda and colleagues focus on bacterial circular proteins, including pilins, bacteriocins, and cyanobactins. Here, there is a much greater understanding of the biosynthetic pathways that lead to the cyclic proteins than there is in higher organisms. This knowledge extends to extensive characterization of the gene clusters involved in producing not only the precursors but also the auxiliary proteins involved in processing, maturation pathways, and export from the producing organism. These circular proteins have a range of potential applications. For example, the bacteriocins are highly toxic to bacteria other than the producing strain; some are used as food preservatives, and others show potential in the medical or veterinary treatment of bacterial infections.

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In the penultimate minireview, Tam and Wong describe the importance of entropy-mediated cyclization in the solid-phase peptide synthesis of cyclic peptides to overcome the entropy barrier of coupling the N- and C-terminal ends of large peptide chains for head-to-tail cyclization. A common theme linking man-made approaches for circular protein production and those of nature is the use of thioester chemistry via native chemical ligation in reaction vessels or intein-based approaches in recombinant cells or via cysteine protease-mediated cyclization in nature. The supernucleophilicity of a thiol side chain at the N terminus of a peptide chain combined with a C-terminal thioester is the key element in the approach described by Tam and Wong, based on native chemical ligation (7). This approach is particularly effective for disulfide-rich peptides, where the reaction is effectively accelerated by the presence of multiple intermediate cysteine thios in a “thia-zip” process.

In the last minireview of the series, Aboye and Camarero describe how cyclization reactions can be achieved using recombinant DNA expression techniques. Several approaches have been successfully used, including “expressed protein ligation,” intein-mediated protein trans-splicing, protease-catalyzed transpeptidation, and genetic code reprogramming. Several of these approaches can conveniently be used to make genetically encoded libraries of cyclic peptides within cells that can be screened for their ability to modulate intracellular processes.

We hope that the articles in this series will raise awareness of circular proteins and stimulate readers to discover new examples, explore the repertoire of biosynthetic mechanisms available, and develop new applications that exploit the stability advantages of circular proteins. One of us (D.J. C.) has somewhat flippantly described conventional proteins as nature’s unfinished business because they are one peptide bond short of a full complement of bonds between all amino acids in the peptide chain. With increased knowledge of natural circular proteins and with an array of chemical and biological methods for making artificial examples, biological chemists can now assist in completing this unfinished business by chemically reengineering interesting proteins to join their ends and make them more stable.

Remaining challenges in the field include the need to develop assays that will rapidly and specifically detect the presence of cyclic proteins in biological specimens (because their lack of termini confound conventional proteomic sequencing methodologies) and the need to delineate their biosynthetic mechanisms. Nevertheless, it is clear that the recent advances in the ability to synthesize cyclic peptides will provide many opportunities to develop novel peptide-based therapeutic and diagnostic reagents.

REFERENCES
