

Preface

The finding that cellular proteins are turning over—synthesized and degraded constantly—has traversed a torturous road from its discovery in the 1940s until it has reached its current central position as a major regulatory pathway. The dynamic state of the proteome was discovered by Rudolph Schoenheimer who used radiolabeled compounds to demonstrate that proteins are in a constant state of generation and destruction (1). Yet, the extent, the mechanisms and the physiological significance of roles of protein degradation have remained elusive for many years. Simpson reported that degradation of labeled proteins in liver slices requires metabolic energy (2). This—thermodynamically paradoxical finding—where investment of energy is still required for the degradation of energy-rich macromolecules—proteins—to low energy small molecules—amino acids—has been corroborated in many studies ever since in both eukaryotes and prokaryotes. Since proteolysis is an exergonic process, the requirement for energy had remained an enigma. Simpson tried to explain that *“The fact that a supply of energy seems to be necessary for both the incorporation and the release of amino acids from protein might well mean that the two processes are interrelated”*. He concluded however by saying that *“...the fact that protein hydrolysis as catalyzed by the familiar proteases and peptidases occurs exergonically, together with the consideration that autolysis in excised organs or tissue minces continues for weeks, long after phosphorylation or oxidation ceased, renders improbable the hypothesis of the direct energy dependence of the reactions leading to protein breakdown”* (2). The basic principle that cleavage of a peptide bond is exergonic, has not and could not have been challenged. Yet, the simple notion that proteases cannot exist in one compartment with their substrates without an energy barrier separating them, along with the high specificity of the process as we currently know it, makes energy investment in an

apparently exergonic process something we all accept now. Yet, the road to this acceptance has been long.

The discovery of the lysosome by Dr. Christian de Duve (reviewed in Ref. 3) has resolved some of the enigmas. Since the lysosome contains many acidic proteases, it was suggested that it must play a role in the degradation of intracellular proteins. Mortimore demonstrated a direct correlation between accelerated protein degradation that follows deprivation of nutrients to perfused liver, and increased lysosomal autophagy that is accompanied by a variety of structural alterations in the lysosomal system. Both the accelerated degradation and the structural changes could be reversed by re-supplementation of amino acids and hormones or serum (see for example Ref. 4). It was found that energy is required for activity of the lysosomal membrane proton pump that maintains the low intralysosomal pH necessary for optimal activity of the proteases (5). Different lines of experimental evidence along with the development of specific inhibitors strongly suggested that multiple pathways are involved in intracellular protein degradation, and the lysosome plays a role only in certain aspects of this process. Proteins were classified into short- and long-lived (reviewed in Refs. 6,7), but their different stability could not be explained based on the known mechanism of action of the lysosome that involves micro- and macroautophagy. During this process, entire droplets of cytosol and even subcellular organelles are engulfed with all the contained proteins digested at similar rates. While it was clear that lysosomal proteases are neither selective nor specific, certain studies still attempted to attribute specificity to lysosomal degradation. According to one model for example, all cellular proteins are engulfed into the lysosome, but only short-lived proteins that are sensitive to lysosomal proteases are degraded, whereas the more resistant, long-lived proteins escape back into the cytosol (8). The development of specific inhibitors of lysosomal proteases and of lysosomotropic agents—weak bases such as chloroquine or ammonium chloride—that are entrapped within the lysosome and increase the pH, thus inactivating lysosomal proteases—enabled researchers to examine in more detail the existence of distinct proteolytic pathways. Knowles and Ballard (9) and Neff and colleagues (10) demonstrated that leupeptin, antipain and chymostatin—specific lysosomal protease inhibitors—inhibit selectively degradation of long-lived but not of short-lived and abnormal proteins.