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ANNUAL REVIEW OF CELL BIOLOGY

PREFACE

The first two volumes of the *Annual Review of Cell Biology* were received with considerable interest by the scientific community, as judged by the number of copies acquired by individuals and libraries. Moreover, they proved useful, as indicated by the frequency with which their chapters were cited in the recent literature. These developments encourage the Editorial Committee to continue its current policies, which are based on the premise that cell biology is part of a continuous body of knowledge properly defined as cellular and molecular biology. These policies also proceed from the assumption that principles of cellular organization and function apply to all living organisms and represent—in our times—a common denominator for all biological sciences, basic or applied.

Over the last decades, few fields of scientific research have advanced as rapidly as cellular and molecular biology, and few have enjoyed the advantages inherent in acquiring, in a generation, broad vistas over apparently inexhaustible territories. But few have had to face the problems generated by onrushing, often spectacular advances achieved with sustained vigor in many directions. Broad vistas and vast territories imply great diversity, which in itself invites divergence and encourages fragmentation. The generation of researchers responsible for the opening of those vistas now has the problem of keeping the central areas of the field in focus. In-depth research in such areas will undoubtedly continue to generate new concepts and new technologies, which in time will animate and illuminate other areas. Hence the Editorial Committee proposes to continue to concentrate on central topics in cellular and molecular biology. Yet it also intends to cover significant, specific developments in broad fields of primordial importance, such as plant cell biology, or in active fields currently undergoing impressive and exciting developments, such as immunology and developmental biology.

Keeping all these desiderata in proper balance is not an easy task. Over the last three years, the Editorial Committee has done its best to achieve this goal and will continue to do so. In the process, it welcomes comments and suggestions from other members of the scientific research community, especially members of the American Society for Cell Biology.

The Editorial Committee believes that a vigorous Annual Review, judiciously selective in its coverage and demanding in the quality of its content, is particularly timely in cellular and molecular biology. It can become a unifying factor clearly needed in a large field undergoing rapid expansion in diverse directions. It can keep many of us well informed or reasonably educated in fields adjacent to our individual areas of interest. We trust that our readers will continue to benefit from this exercise in communication.

GEORGE E. PALADE
EDITOR

ANNUAL REVIEWS INC. is a nonprofit scientific publisher established to promote the advancement of the sciences. Beginning in 1932 with the *Annual Review of Biochemistry*, the Company has pursued as its principal function the publication of high quality, reasonably priced *Annual Review* volumes. The volumes are organized by Editors and Editorial Committees who invite qualified authors to contribute critical articles reviewing significant developments within each major discipline. The Editor-in-Chief invites those interested in serving as future Editorial Committee members to communicate directly with him. Annual Reviews Inc. is administered by a Board of Directors, whose members serve without compensation.

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UBIQUITIN-MEDIATED PATHWAYS FOR INTRACELLULAR PROTEOLYSIS

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INTRODUCTION

Proteolysis occurs in nearly all cellular compartments, where it serves a number of purposes ranging from removal of protein targeting sequences to complete hydrolysis of polypeptide chains. The cytosolic process is particularly fascinating because individual proteins are destroyed at vastly different rates. We are just beginning to understand the mechanisms

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involved in this process, particularly the importance of ubiquitin. This remarkable eucaryotic protein has scarcely changed during the 3 billion years of evolution that separate yeast and man. Besides its evolutionary conservation, ubiquitin is unusual in that it can be covalently attached to histones, to various cytoplasmic proteins, and to an external domain on the lymphocyte homing receptor. Although the physiological significance of histone and homing receptor ubiquitination remains unclear, there is abundant evidence that conjugation of ubiquitin to cytosolic proteins can mark those proteins for destruction. Ubiquitin also plays a key role in the heat-shock response.

This review concentrates on intracellular proteolysis and ubiquitin's function in that process. Space limitations require a selective review of the literature. Therefore, I have focused on experiments that use rabbit reticulocyte lysate or microinjection. For other perspectives see Hershko & Ciechanover (1986), Beynon & Bond (1986), Mayer & Doherty (1986), and *Ubiquitin*, a multiauthored book forthcoming from Plenum Press.

GENERAL PROPERTIES OF INTRACELLULAR PROTEOLYSIS

Rates

Metabolic labeling experiments have revealed two sets of proteins with stabilities that differ more than tenfold. When rat livers or cultured mammalian cells are exposed to radioactive amino acids for a few minutes, 20–40% of the newly synthesized proteins are degraded within the following hour (Poole & Wibo 1973). After cells are exposed to radioactive amino acids for 24 hr or longer, degradation rates are between 2 and 4% per hour, values similar to those measured by nonisotopic methods (Steinberg & Vaughan 1956). These two distinct classes of proteins are designated "short-lived" and "long-lived" proteins.

Turnover rates of individual proteins do not necessarily fall within the ranges just presented. In fact, half-lives of proteins vary from several minutes to weeks, irrespective of their cellular location. The term "half-life" implies that loss of an individual protein is a first-order process. As Schimke (1973) pointed out, this kinetic behavior has two important implications: (*a*) "aging" of a protein molecule does not lead to an increased probability of its being degraded; (*b*) all molecules of a specific protein are present in a common pool. Exponential loss has been clearly established for some enzymes. However, the degradation of other proteins, especially in muscle or nerve, does not conform to first-order kinetics. For example, the turnover of vimentin and actin displays biphasic kinetics in

astrocytes; about 40% of each protein is degraded with a half-life of 12–18 hr, whereas more than half of the remaining molecules are still present 8 days later (Chiu & Goldman 1984). The stabilization of a fraction of each protein population may reflect its assembly into macromolecular complexes (Siekevitz 1972). Alternatively, nerve processes may be proteolytically privileged sites since proteins exhibit remarkable stability during slow axonal transport (Brady & Lasek 1981).

Basal rates of proteolysis can double when mammalian cells are deprived of polypeptide hormones or essential nutrients. It is well established that most of the enhanced proteolysis results from autophagy, a process whereby portions of the cytoplasm are encapsulated in membrane vesicles that subsequently fuse with lysosomes. Several studies have shown that, except for more rapid degradation of mitochondria (Chandler & Ballard 1983), cytoplasmic components are randomly included within autophagic vacuoles during enhanced degradation (Amenta & Brocher 1981). Little is known about the cellular and molecular mechanisms responsible for autophagy, and there is no evidence for or against ubiquitin's participation in the process.

Nucleotide Dependence

Simpson (1953) demonstrated that ATP is required for the degradation of intracellular proteins. This observation has since been confirmed for individual enzymes (Hershko & Tomkins 1971), for proteins microinjected into cultured mammalian cells (Katznelson & Kulka 1983), and for both short- and long-lived proteins in general (Gronostajski et al 1985). Recently, however, two papers reported intracellular proteolysis in the absence of ATP. The degradation of chick reticulocyte β-spectrin was unaffected by ATP depletion, whereas breakdown of α-spectrin ceased under similar conditions (Woods & Lazarides 1985). Likewise, abnormal globin chains were degraded in the absence of high-energy compounds, albeit at an incredibly low rate (Fagan et al 1986). Since removal of ATP produces an abnormal metabolic condition, depletion may activate proteases normally inhibited by ATP-dependent processes, e.g. phosphorylation. For this reason, results from ATP-depletion experiments may not apply to normal physiological conditions.

Sites of Proteolysis

Proteolytic activities have been identified in virtually every cellular compartment. Although the physiological significance of some, e.g. leader peptidase, transit peptide protease, and hormone processing enzymes, seems apparent, we do not know the contribution of most cellular proteases to overall protein metabolism, nor do we know which enzymes participate

in the degradation of specific proteins. There is evidence that cytoskeletal proteins are the principal substrates for calcium-activated proteases (Vorgias & Traub 1986). Still, to my knowledge, we have yet to identify the protease(s) responsible for the degradation of any specific cytoplasmic protein. Clearly this is an important task for the future.

A general discussion of cellular proteases is outside the scope of this essay, and the recent review by Bond & Butler (1987) provides a guide to the literature. However, two issues relating to possible sites of proteolysis do warrant further consideration. It has been suggested that the degradation of transit peptides in mitochondria and leader peptides in the endoplasmic reticulum can account for the class of short-lived proteins (Kominami et al 1983; Hough & Rechsteiner 1984). Temperature studies support the notion that different pathways are responsible for the destruction of short-lived and long-lived proteins. Degradation of short-lived proteins exhibits a temperature dependence typical of many enzyme reactions; the Q_{10} is about two (Neff et al 1979). In contrast, the Q_{10} for turnover of long-lived proteins is often greater than four (Hough & Rechsteiner 1984). Nevertheless, several observations challenge the hypothesis that the existence of short-lived proteins reflects co- or post-translational proteolytic processing. Proteins containing transit or leader peptides do not account for 20–40% of newly synthesized polypeptide chains. Moreover, targeting sequences are removed rapidly after synthesis (Reid & Schatz 1982). Also, many proteins with short half-lives, e.g. *myc*, *fos*, *p53*, *e1a*, are found in the nucleus. Thus it appears that most short-lived proteins inhabit the nuclear and cytosolic compartments. Their instability surely has regulatory significance, and a common degradative pathway, characterized by a low activation energy, may be responsible for their rapid breakdown.

The second issue concerns the possible role of lysosomes in the selective degradation of cytosolic proteins. The diverse half-lives of cytosolic proteins would require differential stabilities within lysosomes or selective transfer into these organelles, and mechanisms to accomplish this have been proposed (Dean 1984). It has been suggested that lysosomes can directly take up proteins, a process known as microautophagy (Ahlberg & Glaumann 1985). However, recent studies indicate that lysosomes do not participate in the turnover of short-lived proteins, but rather they contribute to the degradation of long-lived proteins.

In 1973, Poole & Wibo demonstrated that cells contain two mechanistically distinct proteolytic pathways. They showed that while fresh serum inhibited the degradation of long-lived proteins in cultured rat fibroblasts, it had no effect on the degradation of short-lived proteins. A year later, Wibo & Poole demonstrated that the organic amine, chloro-