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Answer

ATP-dependent proteolysis, a fine system or a meaningless one? A reply

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The letter of Rapoport [1] demonstrates a welcome interest in the problem of the validity of ATP-dependent proteolysis in mammalian cells.

Rapoport suggested that lipoxygenase (LOX) is not directly involved in protein degradation of exogenously added substrates in reticulocyte extracts. This suggestion was based on their observations that the LOX inhibitor SHAM does not inhibit the degradation of serum albumin [2] and that phenylhydrazine-induced reticulocytes are qualitatively different from those induced by anemia [3].

We incidentally found that the LOX inhibitor, NDGA, also inhibited the newly found protease, ingensin [4–10]. It is possible, therefore, that the inhibitory effect of SHAM found by Rapoport et al. is mediated not by the inhibition of LOX but by that of protease itself. Consequently, we consider it important to furnish additional evidence that not all of the LOX inhibitors also inhibit ATP-dependent proteolysis. Secondly, there is no evidence that reticulocyte extracts contain two independent proteolytic pathways stimulated by ATP, one for endogenous proteins and the other for exogenously added proteins. ATP-dependent proteolysis can be demonstrated using phenylhydrazine-induced reticulocytes and exogenously added substrates as shown by the authors who first discovered ATP-dependent proteolysis [11] and the ubiquitin-dependent pathway [12]. Thirdly, there is no evidence that almost all of the hemoglobin molecules are degraded by an ATP-dependent proteolytic system.

Finally, it is worth emphasizing that the experiments regarding the purification of protease from reticulocyte extracts [13,14] cited by Rapoport were conducted in crude extracts and involved partially purified enzyme. We, therefore, regard any data obtained from these experiments as less than preliminary.

ATP-dependent proteolytic activity is generally undetectable in crude mammalian cell homogenates but the workers in the field of protein degradation place too much emphasis on the role of ATP or ubiquitin as the effectors of protein degradation. The questions as to why ATP-dependent proteolysis is evident only in reticulocyte extracts in spite of the undetectable difference in the content of ubiquitin and how this system works for eliminating so-called 'abnormal proteins' in the presence of constant levels of the activator, ATP, have not yet been answered. In the absence of the purification of these proteolytic enzymes and other components, the conclusion and implication seem premature. A more descriptive, less speculative work might be of interest to workers in the field of protein degradation. We are

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now trying to purify all of the neutral proteolytic enzymes from reticulocyte extracts ([10]; in preparation) of which five have been already isolated. We hope these studies may ultimately clarify the ATP requirement for proteolysis in the near future.

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