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ICE Viewpoint

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The following series of articles summarizes the rapid growth in the knowledge of the activity and inhibition of the ICE family of aspartate specific cysteinyl proteases. The articles originated as talks given in December, 1995 at Rockefeller University at the New York Academy of Science–sponsored symposium, "The Biology and Inhibition of Interleukin-1β Converting Enzyme (ICE) and Related Cysteine Proteases." ICE, the prototype enzyme in this family, was characterized in 1992 as the enzyme responsible for the activation of interleukin-1β. Within a year, it had been established that the sequence of the pro-apoptotic Caenorhabditis protein Ced-3 shared key structural elements with ICE and that ICE could itself induce apoptosis when expressed in cells. Within the past two years, nine more mammalian homologs have been identified, many of which have been shown to induce apoptosis in cells.

The first article by Miller et al. provides a brief introduction to the activity and discovery of ICE itself. ICE is present in cells as an inactive precursor protein that becomes active by removing a precursor domain and processing to 20- and 10-kDa fragments which associate to form an active $(p20)_2(p10)_2$ tetramer. The crystal structure of ICE bound with a tetrapeptide inhibitor has identified the p20 and p10 amino acids necessary for activity. While those residues necessary for catalytic activity are conserved among the homologs, those most closely associated with the inhibitor residues are unique, a pattern that provides a structural basis for homolog substrate specificity.

In the article by David Giegel, the kinetics of ICE activity are examined in detail. ICE is a thiol protease characterized by a relatively slow rate of hydrolysis relative to papain, perhaps because of the relative orientations of the active site His and Cys. The ICE prodomain affects the rate of precursor activation and proteolysis and may target its location in different sites within the cell. David Livingston describes the molecular interactions of ICE with the different structural elements of ICE inhibitors. The activity of both reversible aldehyde and ketone inhibitors as well as irreversible acyloxymethyl ketone inhibitors of cysteine proteases are discussed in vitro for ICE and various homologs. Such inhibitors are less potent in cells, but they do reduce inflammation in various animal models.

Ping Li summarizes the effects of transgenic mice lacking ICE. These animals show greater than a 98% reduction in IL-1β as well as an 80% reduction in IL-1α levels. Inflammation as measured by LPS-induced lethality is prevented in the knockouts, but few effects on apoptosis induced by different stimuli are observed, suggesting that ICE itself is unlikely to be an effector of apoptosis.

Emad Alnemri describes the identification and activity of several mammalian homologs of Ced-3. Using a combination of degenerate nucleotide primers and together with cDNAs identified in the database of human expressed sequence tags, homologs such as the closely related CPP32 and Mch-3 were identified. Both proteases have similar DXXD substrate specificities that enable them to cleave substrates such as the DNA repair enzyme poly (ADP-ribose) polymerase (PARP). The homolog Mch-2 is more dissimilar, has a different peptide substrate selectivity, and can cleave lamins as well as PARP. All of these proteases have very short precursor domains and are processed to p20- and p10-like subunits.

Sten Orrenius analyzes the apoptotic cleavage events in Fas-mediated apoptosis ranging from the early cleavage of the plasma membrane-associated fodrin and nuclear PARP to the later cleavage of lamins. The partially purified ICE homolog associated with the apoptotic cleavage was shown to be CPP32, and within minutes after stimulation of Fas, CPP32 was found activated. The addition of a DEVD-H inhibitor of CPP32 could prevent the apoptotic cleavages.

Antony Rosen and Livia Casciola-Rosen show that apoptosis causes the cleavage of a number of cellular protein substrates that can become autoantigens in patients with systemic lupus erythematosus (SLE). In addition to PARP, proteins such as the U1-70-kDa ribonuclear protein and the catalytic subunit of DNA-dependent protein kinase contain unique sites of apoptotic cleavage similar to those found in SLE patients. Using both specific SLE antisera and peptide inhibitors of ICE homologs, the apoptotic cleavage of all these substrates was attributable to simultaneous cleavage by CPP32.

Since the publication of the homology between ICE and Ced-3, determination of the biological role of each member of the ICE family has been the focus of intense research. Based on structural homology, the ICE family can be broken into two subfamilies: those proteins that are most closely related to ICE (Ich-2 and ICE(relIII)) and those proteins that are most closely related to CPP32 and Ced-3 (Mch-2, Mch-3 and Mch-4). Most of the evidence generated to date appears to implicate members of the CPP32 subfamily as being more directly involved with apoptotic events than the ICE subfamily. The proapoptotic cleavages occur following the activation of unique ICE homologs or alternatively subsequent to a protease cascade whereby certain members of the ICE family are activated by other members (similar to the serine protease coagulation cascade).

Although a strong relationship between the ICE family and apoptosis is beginning to emerge, there are other possibilities for the role of this protease family in normal cell functioning. In addition to the IL-1β activation function of ICE itself, one such possibility is the activation of the sterol regulatory binding protein by an unknown ICE homolog (see Wang et al, EMBO J, (1996) 15:1012). In all probability, other roles for members of the ICE family in vivo will be uncovered as the field matures.

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