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The Interleukin-1β Converting Enzyme Family of Cysteine Proteases

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Abstract Interleukin-1β converting enzyme (ICE) is the first enzyme of a new family of cysteine endoproteinases to be isolated and characterized. An overview of the structure and activity of ICE is outlined together with highlights of salient features common to members of each of the family members. J. Cell. Biochem. 64:2–10. © 1997 Wiley-Liss, Inc.

Key words: ICE; cysteine proteases; inflammation; apoptosis; Ced3; secretion; cell activation

INTRODUCTION

The discovery of ICE and its rapidly increasing family of aspartate-specific homologous proteases has produced an intense interest by many investigators in the role of these proteases in apoptosis and in cellular regulation as well as inflammation. To review the biology and inhibition of members of this protease class, a one-day conference cosponsored by the New York Academy of Science and The Rockefeller University was devoted to this subject. Specific talks as shown by the subsequent papers in this issue address the mechanisms of interleukin-1β converting enzyme (ICE) activation and hydrolysis (David Giegel), the development and effects of ICE inhibitors (David Livingston), effects on inflammation and apotosis in ICE knock-out mice (Ping Li), the cloning and activity of new mammalian ICE homologs (Emad Alnemri), and the role of ICE and ICE homologs in cleaving a number of protein substrates during various models of apoptosis (Sten Orrenius and Antony Rosen).

DISCOVERY OF ICE

ICE was discovered in response to a search to inhibit the action of interleukin-1β (IL-1β) [Schmidt and Tocci, 1990]. IL-1β is a "master cytokine" that is normally present in undetectable levels, but is upregulated by proinflammatory stimuli such as bacterial lipopolysaccharides (LPS, Fig. 1). As such, it upregulates the synthesis and release of acute phase proteins such as interleukin-6 as well as matrix metalloproteinases, cyclooxygenase-2, the inducible form of nitric oxide synthetase, and the surface expression of leukocyte adhesion molecules [Dinarello, 1994]. IL-1β is synthesized as a cytosolic 31-kDa precursor protein that must be processed to a 17.5-kDa mature form in order to be active on its receptor [Mosley et al., 1987]. This protein is secreted in an unknown manner through the plasma membrane in a process that is independent from the cleavage itself [Thornberry et al., 1992].

The proIL-1β cleavage occurs following Asp^{116} by the cytoplasmic cysteine protease ICE [Schmidt and Tocci, 1990]. The ICE protein itself is present in unstimulated monocytic cells as a 45-kDa protein (p45) that lacks functional proIL-1β cleavage activity [Ayala et al., 1994]. Following cell stimulation, ICE becomes functionally activated permitting the cleavage of proIL-1β. Active ICE when purified from extracts of these cells is a 1:1 mixture of two subunits of 20- and 10-kDa (p20 and p10) that are processed from the precursor (see Fig. 2 [Thornberry, et al., 1992]).The details of the process of activation of p45 to p20/p10 are unclear, but presumably involves the aggregation

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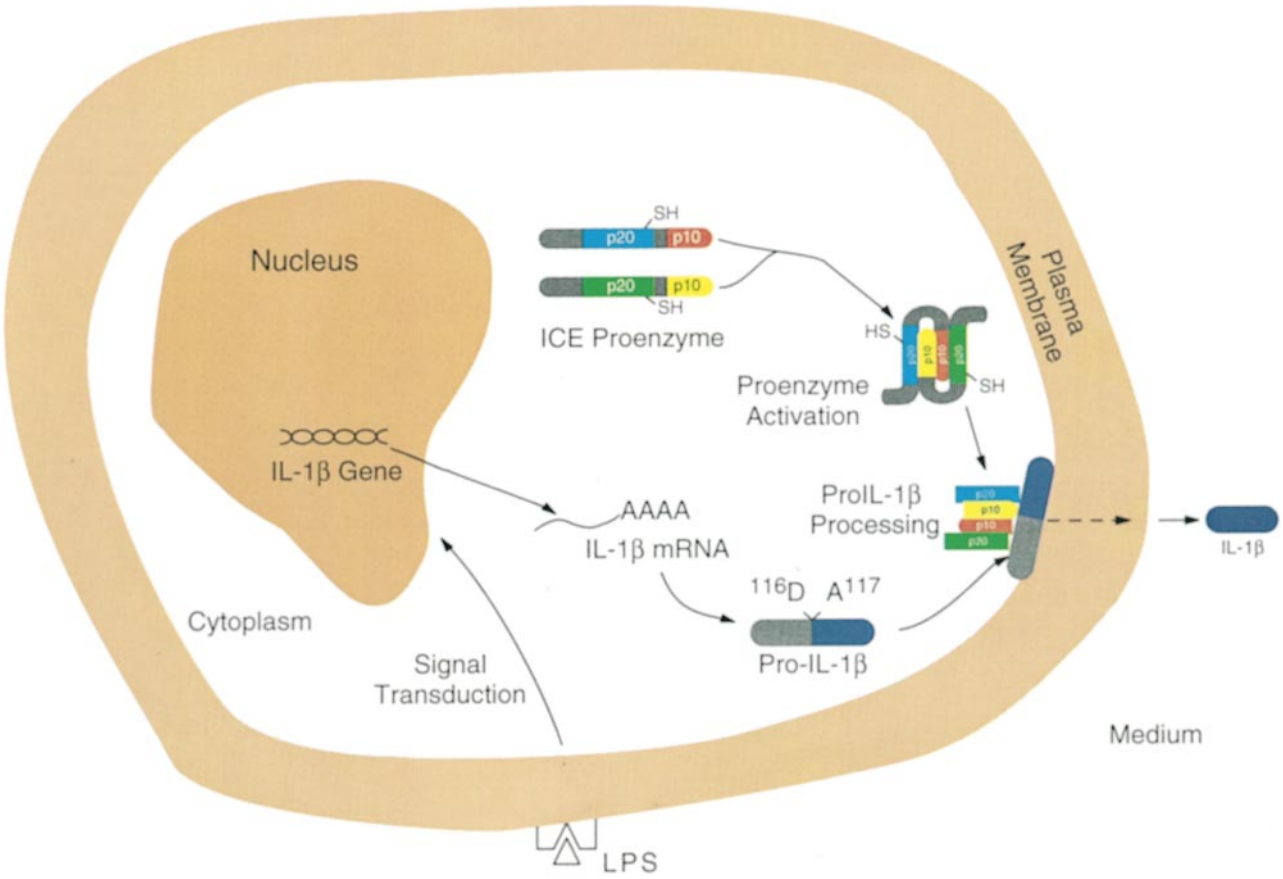


Fig. 1. Model for secretion of IL-1β from monocytes. The IL-1β precursor is synthesized as a 31-kDa ProIL-1β in response to specific cell activators such as LPS. ICE proenzyme present in cells is autocatalytically activated, and the resultant active complex at the plasma membrane cleaves ProIL-1β at Asp^{116} to generate mature 17-kDa IL-1β. In an unknown fashion the IL-1β is simultaneously secreted from the cells into the media.

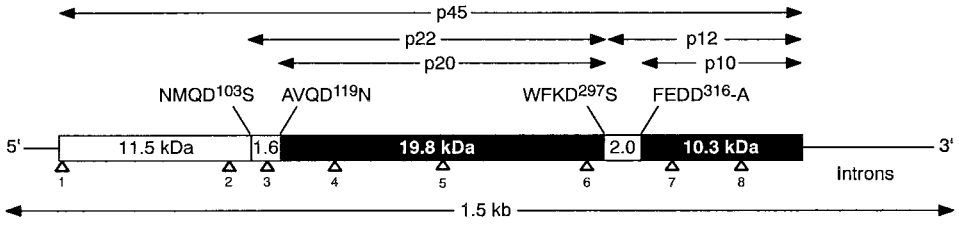


Fig. 2. Human ICE structure. The 45-kDa ICE precursor protein (p45) is translated from a 1.5 kb message formed from a gene containing eight introns. Activated ICE consists of equal amounts of p20 and p10 cleaved at the indicated Asp residues on p45. In vitro-activated ICE shows the presence of an additional cleavage site producing p22 which is processed down to p20.

of the precursors (Fig. 1) followed by cleavage in an ill-defined autocatalytic process [Gu et al., 1995]. The precursor domain of ICE with its Leu-rich regions is presumably important in the regulation of the activation of ICE. The X-ray crystal structure of the active enzyme when crystallized with an inhibitor is tetrameric with two p20 and p10 polypeptides [Walker et al., 1994; Wilson et al., 1994], substantiating earlier observations that ICE is multimeric [Thornberry et al., 1992]. While undetectable amounts of active ICE can be found at a biochemical level in activated monocytic cells [Ayala et al., 1994], immuno-electron microscopic evaluation indicates that active ICE is found associated at the plasma membrane with

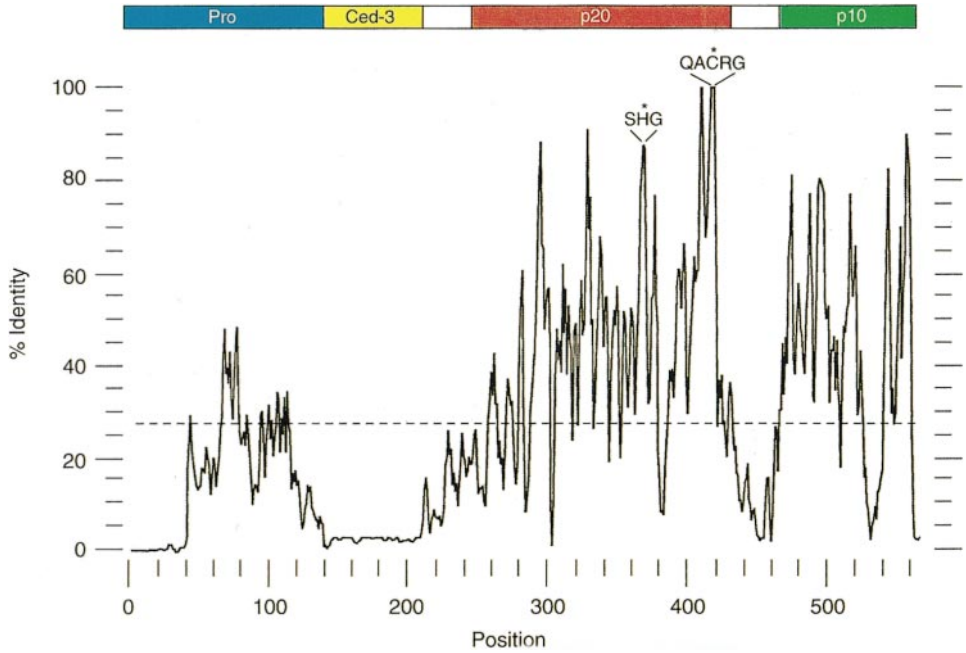


Fig. 3. Relative identity of all the ICE homologs across the sequence. Based on the protein sequence alignment (GCG Pileup program) of all the homologs, a GCG Plotsimilarity analysis (window of 3 [Devereux et al., 1984]) plotted the percent identity across the sequences. The active site His and Cys are indicated. The dashed line refers to the overall average identity of the sequences. The bar on the top denotes a generalized precursor structure found in the homologs. All contain the larger catalytic subunit (p20) and the smaller subunit (p10), and many of the homologs contain a prodomain (Pro). Ced-3 refers to an extra region found only in the different *Caenorhabditis* forms of Ced-3, but not in the mammalian homologs.

proIL-1β [Singer et al., 1995]. These proteins are presumably complexed with other proteins that permit the secretion of IL-1β at this site. The addition of specific ICE inhibitors prevents the processing of IL-1β [Thornberry et al., 1992] and inhibits the subsequent appearance of mature IL-1β in cell culture and in plasma of animal models of inflammation ([Fletcher et al., 1995]; see accompanying article by Livingston).

Because of its structure and activity, ICE represents the first member of a unique class of cysteine proteases. The active site Cys^{285} is found near the C-terminus of p20, while the major determinants of the substrate binding specificity are found on p10 (see below). Proteolysis by ICE minimally requires a tetrapeptide substrate in which Asp is essential in P_1. The p20 and p10 polypeptides are themselves cleaved from the p45 following specific Asp residues (see Fig. 2). A large hydrophobic group such as Tyr is preferred in P_4 of ICE substrates, while much more widespread amino acid substitution is permitted in P_2 and P_3 [Thornberry et al., 1992]. The interchangeability of the sidechains of these two residues suggested that they were surface exposed and not bound to the enzyme, a conclusion confirmed later by the X-ray structure of ICE [Walker et al., 1994; Wilson et al., 1994]. The accessibility of these residues on ICE permitted the preparation of affinity columns in which a peptide aldehyde inhibitor was linked via a P_2 Lys to agarose [Thornberry et al., 1992; Wilson et al., 1994].

SUGGESTED ROLE OF ICE IN APOPTOSIS

In 1993 Yuan and Horvitz reported that the *Caenorhadbditis* protein Ced-3 was highly homologous to ICE [Yuan et al., 1993]. Ced-3 is an essential protein for the apoptosis of specific cells during the embryonic development of *C. elegans* [Yuan and Horvitz, 1990]. Alignment of the sequence of Ced-3 with ICE indicated that it shared amino acids essential for catalytic activity of ICE, including the active site Cys and His. Transfection of ICE into cells can replace Ced-3 and induce apoptosis, and mutation of key amino acids prevents the ensuing apoptosis [Miura et al., 1993; Yuan et al., 1993]. The potential role of ICE in mammalian apoptosis was also suggested by the observation that the potent ICE inhibitor crmA [Komiyama et

al., 1994] when cotransfected with ICE or Ced-3 inhibits apoptosis [Miura et al., 1993]. Furthermore, addition of the antiapoptotic baculovirus protein p35 could both bind and inactivate ICE [Bump et al., 1995; Xue and Horvitz, 1995].

DISCOVERY OF ICE HOMOLOGS

It has become clear, however, that ICE is not the only mammalian protease homologous to Ced-3. A number of other homologs have been discovered and characterized over the past couple of years, including Nedd2/ICH1, $ICE_{rel}II$ (ICE2, TX, ICH2, MIH1), $ICE_{rel}III$, apopain (CPP32, YAMA), MCH2, MCH3 (ICE-LAP3, CMH1), and ICH3 (see accompanying papers in this issue). Transfection of the active forms of these proteases can also induce apoptosis [see, e.g., Kumar et al., 1994; Wang et al., 1994; Nicholson et al., 1995]. Alignment of these sequences indicates that, like Ced-3, all of them share a number of the key amino acids necessary for activity of ICE. Those regions of identity are shown graphically in Figure 3 by GCG Plotsimilarity analysis [Devereux et al., 1984). The regions of highest identity are in p20 and p10, particularly around the active site Cys and His. A lower region of similarity is found in the pro-domain. There is a loop found only in the three species of Ced-3 proteins [Yuan et al., 1993] and not in the mammalian homologs (see generalized precursor bar, Fig. 3). The N-terminal extended region of p20, observed as the p22 form [Miller et al., 1993], as well as the peptide region separating the p20 and p10, are regions of low homology; both are absent in the active enzyme.

ICE SUBFAMILIES

Phylogenetic analysis of the known ICE homologs indicates that the sequences are distributed in two subfamilies, exemplified by Ced-3 and ICE, respectively (Fig. 4). The placement in either subfamily correlates not only with regions of sequence identity unique to each subfamily, but also with the presence or absence of minor loops. In Figure 5 the residues highly conserved in all ICE homologs are shown in dark yellow. They are found in two patches within the molecule: around the active site region (top, containing the bound peptide inhibitor in green) as well as at the ends of the β-sheet region at the bottom of the molecule (Fig. 5A). The additional residues identical

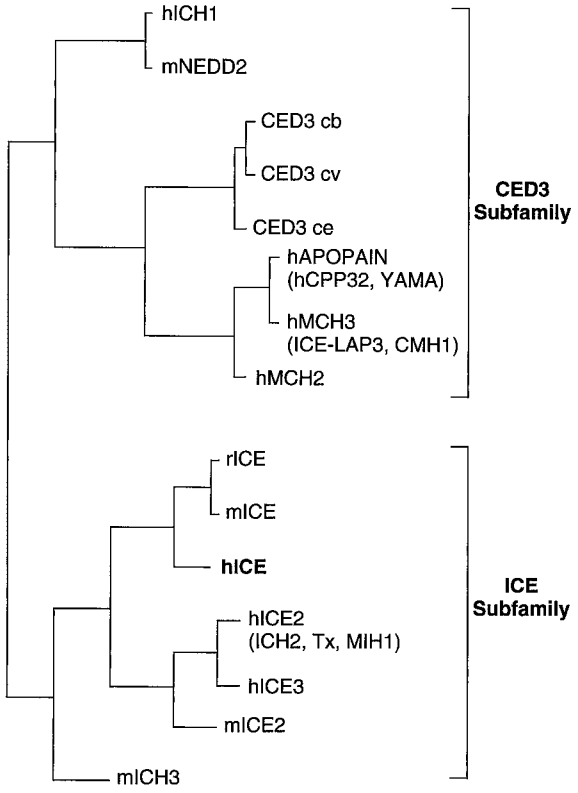


Fig. 4. Phylogenetic tree of ICE family of proteases. An alignment of published sequences of the different ICE homologs was based on a protein alignment using the GCG Pileup program followed by the Jukes Cantor Distances and UPGMA Growtree programs (Programs from Genetics Computer Group, Inc., Madison, WI).

within the members of the ICE subfamily (Fig. 4) are shown in red in Figure 5. These residues are found primarily on the β-strands (Fig. 5A). The CPK surface projection of these residues shown in Figure 5B indicates that almost all of the totally conserved residues outside of the active site are internal as are a large number of the subfamily-specific residues. As such, their role probably is to maintain the overall structure of the protein. Many of the surface exposed subfamily residues face the other heterodimer present in the complete tetramer (positioned on the right side of the view in Fig. 5B). These conserved residues may play an essential role in the assembly of the active tetramer.

ACTIVE SITE BINDING

The inhibitor peptide is bound at the top of the active ICE molecule (Fig. 5), and the exposed ICE residues surrounding the bound Ac-Tyr-Val-Ala-Asp peptide are indicated in the

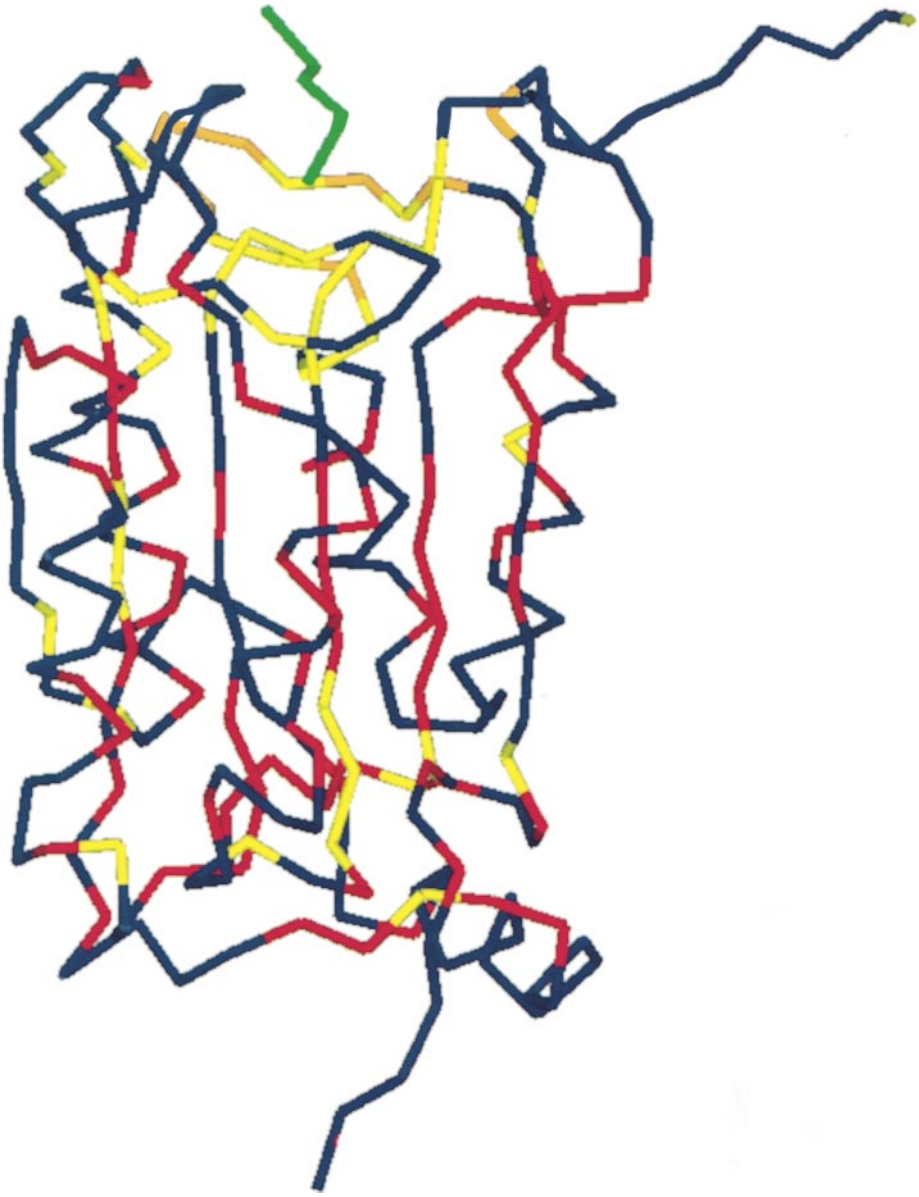


Fig. 5. Homologous residues within the ICE family. A view of the p20/p10 heterodimer structure (Wilson, et al., 1994) is shown with peptide inhibitor highlighted in green (top of molecule), the residues highly conserved in all of the homologs in yellow, the residues identical in the ICE subfamily in red, and the residues flanking the inhibitor peptide in orange. Above: Peptide backbone tracing. Right: CPK surface image of the residues of A. The program C_View (©Dr. J. C. Culberson, Merck & Co, Inc) was used to display the data.

space-filling model view of Figure 6A. Those residues necessary for catalytic activity include the essential Cys^{285} and His^{237} as well as the Gly^{238} and Ser^{339} necessary for this orientation. The carboxylate binding pocket of the P_1 Asp includes Arg^{179} and Arg^{341} as well as Glu^{283} and Ser^{347} [Walker et al., 1994; Wilson et al., 1994]—all the homologs share these residues (Fig. 6B). Of greater divergence between the homolog members are the residues associated with the bound P_2-P_4 residues of the peptide. The P_4 substrate residue, in particular, is bound in a pocket demarcated by the residues Pro^{343} and His^{342} which sit just upon the Ac-Tyr in ICE as

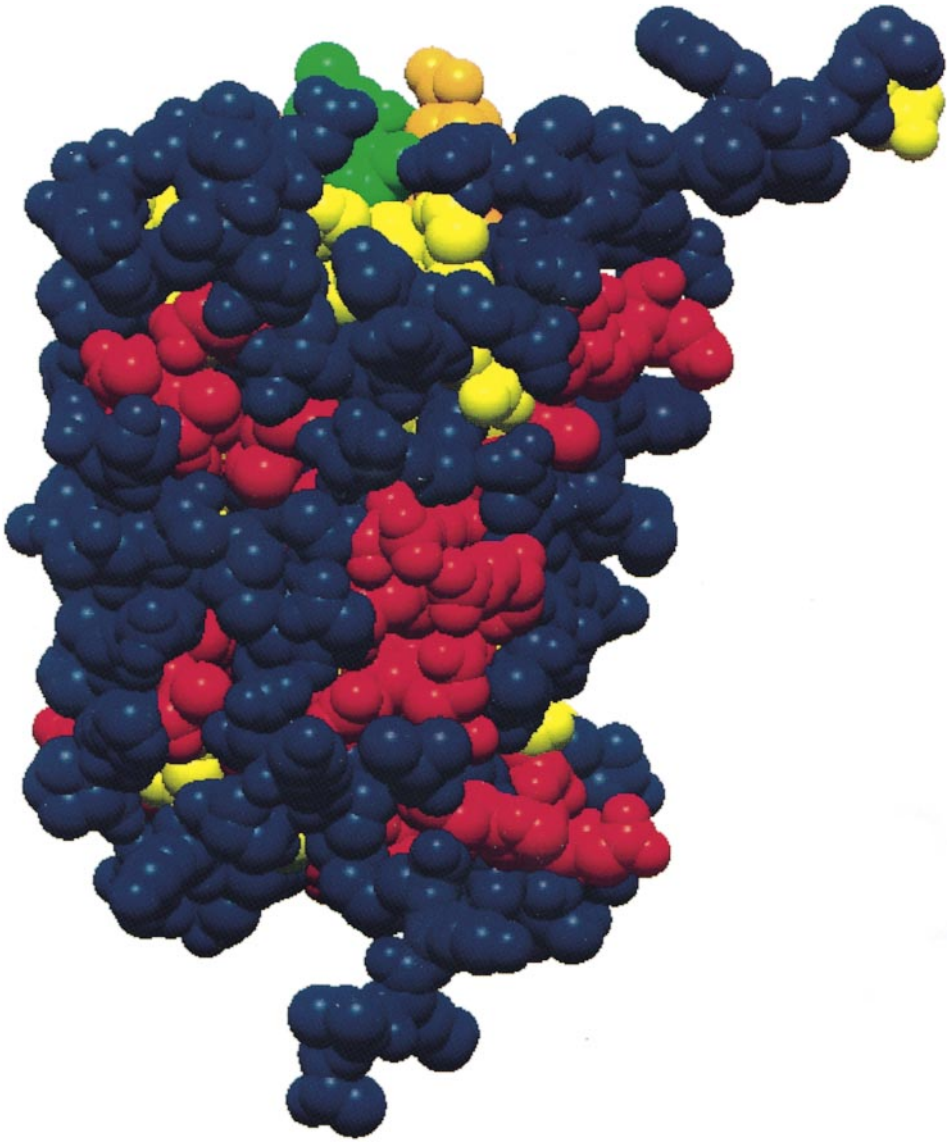
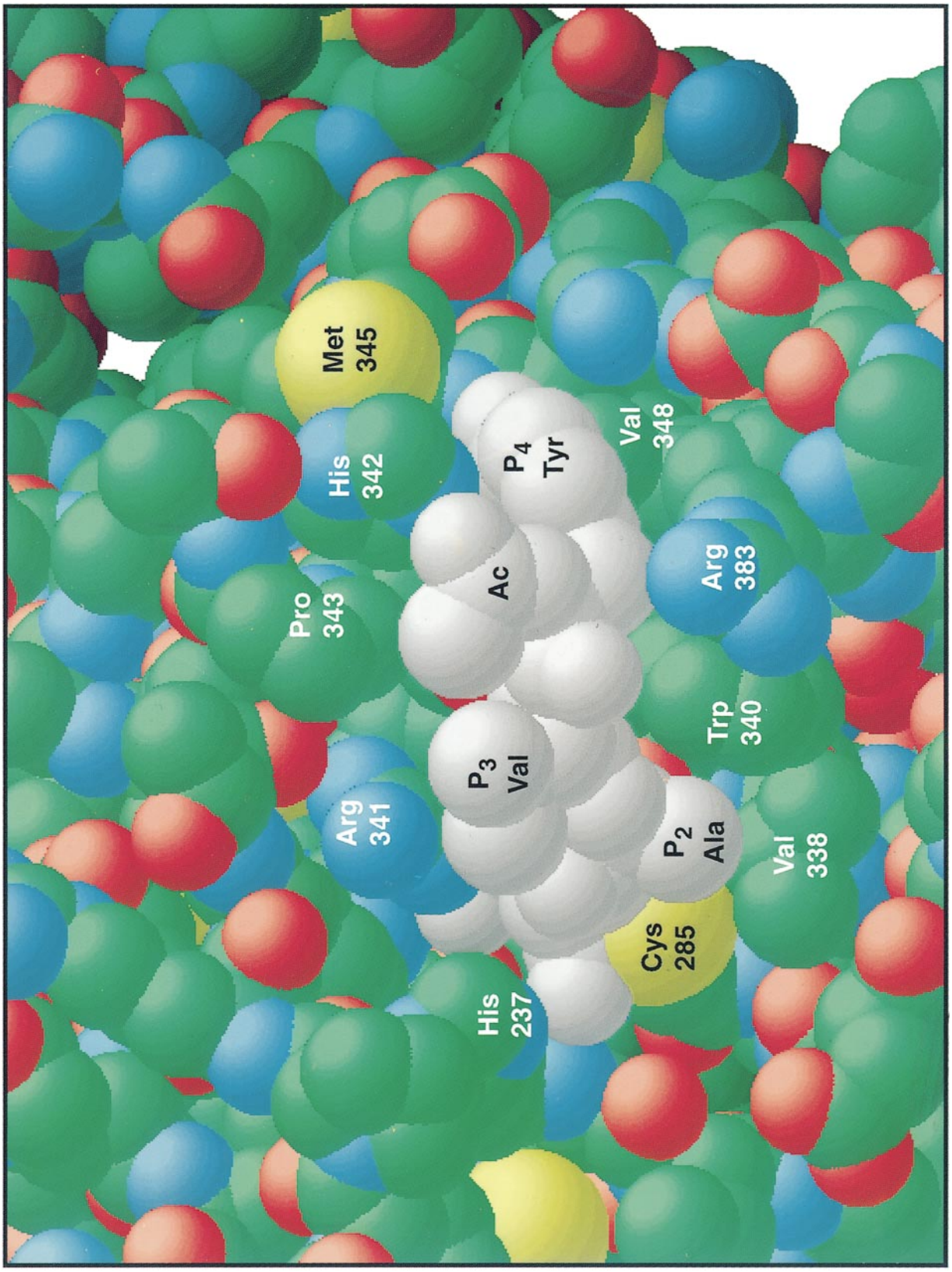


Figure 5. (Continued.)

well as Met[345], Val[348] and Arg[383] which define the boundary closest to the bound inhibitor. These P_4 residues form a pocket unique to each of the homologs. This provides a structural basis for the observed importance of the P_4 substrate residue for homolog activity (see papers by Alnemri and Rosen].

CONCLUSIONS

What we have learned about the structure and activity of ICE provides a basis for understanding data that is now just being generated about the other homolog members and is discussed in the accompanying papers. These shared features include a cysteine protease present as an inactive precursor from which two subunits, equivalent to p20 and p10, are rapidly generated upon activation by proteolysis at unique Asp residues. These proteases are characterized by having common residues for the binding of Asp at P_1 but divergent residues that define the P_4 specificity pocket. Critical issues to be addressed over the next several years include the specific cellular and tissue distribution of the various homologs, determination of the cellular protein substrates unique to each


Met
345
His
342
Pro
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Arg
341
His
237
Cys
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P3
Val
Ac
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Tyr
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Arg
383
Trp
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P2
Ala
Val
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Figure 6.

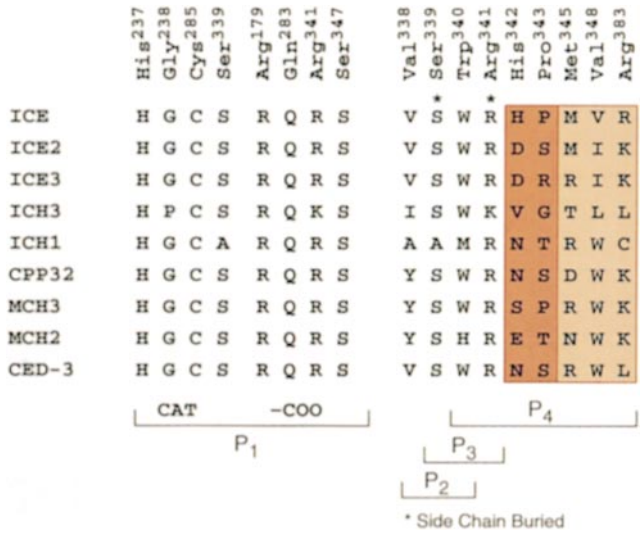

Fig. 6. (Continued.) ICE family residues important for inhibitor binding. Left: Structure of the Ac-Tyr-Val-Ala-Asp-CHO inhibitor of ICE (Wilson et al., 1994) visualized with the program Mol Vue (©Dr. M. Miller, Merck & Co, Inc). Above: Comparison of residues of the different ICE homologs involved in inhibititor binding and activity as determined from the inhibited ICE structure (A). CAT, residues involved in substrate catalysis; —COO, residues involved in the carboxylate binding pocket of the P_1 Asp. Residues closely associated with the P_2, P_3, and P_4 residues are indicated. Those residues most important for P_4 binding are shaded in red with the two residues most closely with the P_4 Tyr of ICE indicated in darker red.

homolog, and perhaps most importantly the signalling mechanisms responsible for activating and deactivating the proteases within those cells.

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