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Structural biology of the separase–securin complex with crucial roles in chromosome segregation

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The cysteine protease separase opens the cohesin ring by cleaving its kleisin subunit and is a pivotal cell cycle factor for the transition from metaphase to anaphase. It is inhibited by forming a complex with the chaperone securin, and in vertebrates, also by the Cdk1–cyclin B1 complex. Separase is activated upon the destruction of securin or cyclin B1 by the proteasome, after ubiquitination by the anaphase-promoting complex/cyclosome (APC/C). Here we review recent structures of the active protease segment of *Chaetomium thermophilum* separase in complex with a substrate-mimic inhibitor and full-length *Saccharomyces cerevisiae* and *Caenorhabditis elegans* separase in complex with securin. These structures define the mechanism for substrate recognition and catalysis by separase, and show that securin has extensive contacts with separase, consistent with its chaperone function. They confirm that securin inhibits separase by binding as a pseudo substrate.

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Introduction

Separase is a large (140–250 kDa) eukaryotic endopeptidase belonging to the CD clan of cysteine proteases, which also includes caspases and gingipain [1], reviewed in [2–4,5••]. It cleaves the kleisin subunit (Scc1/Rad21/Mcd1 for mitosis and Rec8 for meiosis) of the cohesin complex that entraps sister chromatids during cell division, and therefore it has essential roles in chromosome segregation [1,6–11]. While most of the cohesins located on the chromosome arms are removed through a phosphorylation-dependent 'prophase pathway' [6,9], centromeric cohesins are protected by shugoshin and are subjected to separase cleavage for chromosome segregation during the transition from metaphase to anaphase [1,10,12,13]. Over-expression of separase is linked to aneuploidy and tumorigenesis, making it a potential target for anti-cancer drug discovery [14,15•].

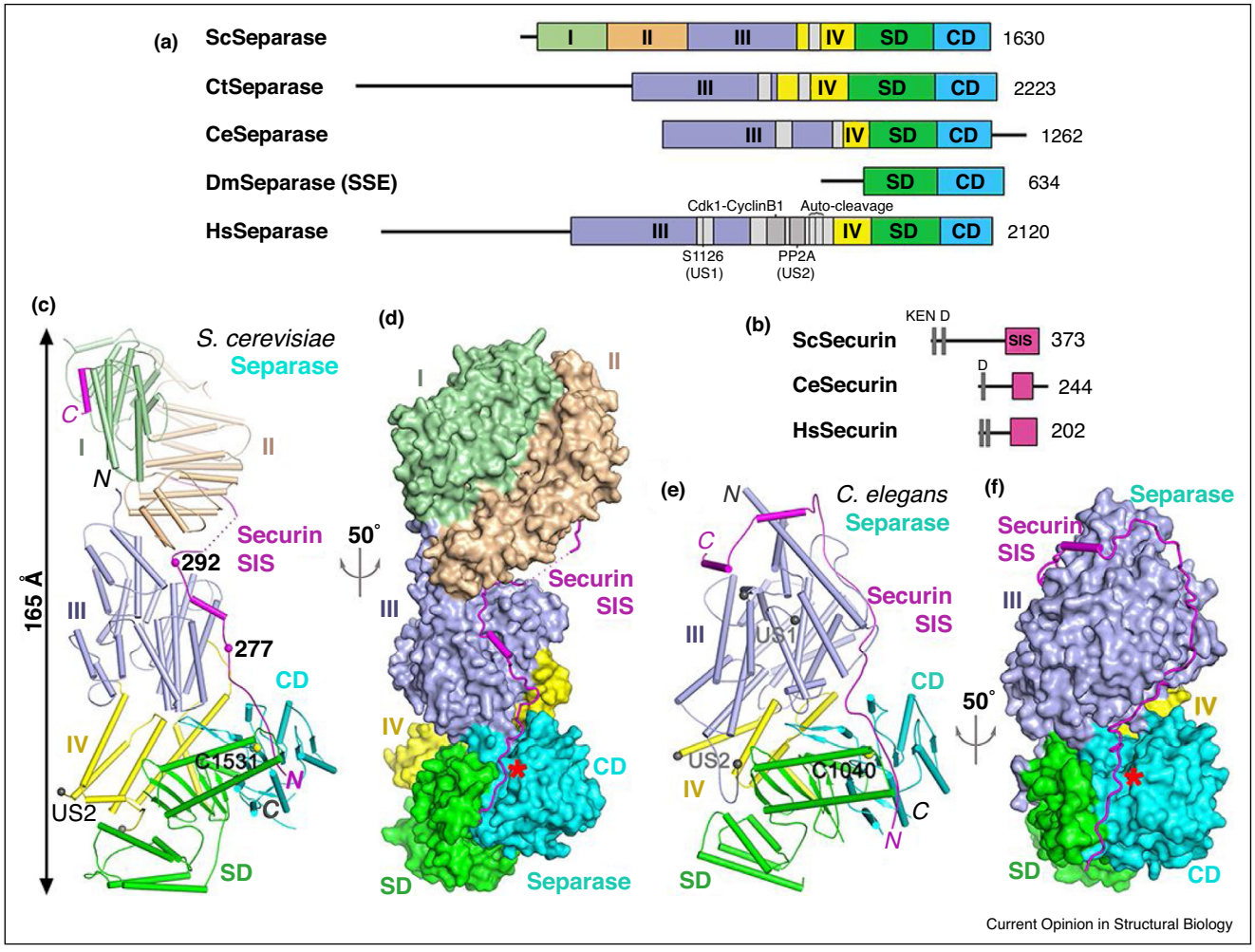
Besides its roles in chromosome segregation, separase also has important functions in other cellular events, such as stabilizing the anaphase spindle by cleaving and localizing the kinetochore-associated protein Slk19 [16], regulating centriole disengagement in mammals by cleaving pericentrin/kendrin [17–19], DNA damage repair [20], membrane trafficking [21], telomere protection [22], and Cdk1 inhibition [23].

Consistent with its crucial cellular functions, the activity of separase is tightly regulated. Securin, a natively unfolded protein in solution [24,25], is the first reported regulator of separase and acts as both a chaperone and an inhibitor [26–33]. Securin binds to nascent separase protein co-translationally to help its proper folding and forms a stable complex with separase until the onset of anaphase. In vertebrates, the Cdk1–cyclin B1 complex is another regulator of separase activity [23,33–35]. Cdk1 phosphorylates separase and then forms a stable complex with it through interactions between cyclin B1 and a Cdc6-like sequence in the N-terminal regulatory region of separase, and this process is dependent on the isomerization of separase by Pin1 [36•].

Separase is activated by the destruction of securin [37–39] and cyclin B1 [23] via the proteasome pathway upon ubiquitination in their N-terminal region by the anaphase-promoting complex/cyclosome (APC/C) [40,41]. APC/C-mediated reduction in securin level in aged female mice is linked to premature chromosome segregation in meiosis II [42]. Besides the two key mechanisms mentioned above, other regulatory processes have also been reported. For example, auto-cleavage of separase in higher eukaryotes occurs upon activation, which affects mitosis progression but not the protease activity of separase [31,43–46]. Protein phosphatase 2A (PP2A) binds to a region of separase adjacent to the auto-cleavage sites [47], which stabilizes separase-associated securin through dephosphorylation [48]. Phosphorylation of securin in yeast enhances its interaction with separase and promotes the nuclear localization of separase [49].

The primary structure of separase contains a C-terminal caspase-like catalytic domain (CD) of ~200 residues and an N-terminal α-helical regulatory region (Figure 1a). An

Figure 1



Structures of separase–securin complexes. **(a)** Domain organization of separase. The helical region of yeast separase is divided into four domains (I–IV) and given different colors, which is followed by SD (substrate-binding domain) and CD (catalytic domain). The domain boundaries for the N-terminal region of *C. thermophilum* and human separase are not known and therefore are not indicated. The unstructured segments (US) in the helical region are shown in gray. For *Drosophila* separase, only the subunit containing the SD-CD (known as SSE) is shown. Sc: *S. cerevisiae* (yeast), Ct: *Chaetomium thermophilum*, Ce: *C. elegans*, Dm: *D. melanogaster*, Hs: *Homo sapiens*. **(b)** Domain organization of securin. The separase interaction segment (SIS) is shown in magenta. The N-terminal KEN and D-boxes are indicated. **(c)** Schematic drawing of the structure of the yeast separase–securin complex. The domains of separase are colored as in Figure 1a, and the securin SIS is in magenta. The catalytic Cys1531 is shown as a sphere model. Two of the phosphorylation sites in securin are indicated with spheres. The ends of the unstructured segment (US2) are indicated by the two gray spheres. **(d)** Structure of the yeast separase–securin complex, with separase shown as a molecular surface, viewed after 50° rotation around the vertical axis from panel c. The active site of separase is indicated with the red star. **(e)** Schematic drawing of the structure of the *C. elegans* separase–securin complex. The catalytic Cys1040 is shown as a sphere model. **(f)** Structure of the *C. elegans* separase–securin complex, with separase shown as a molecular surface. All structure figures were produced with PyMOL (www.pymol.org).

additional domain is located between the helical region and CD, and this domain has been named the substrate-binding domain (SD) [50••] or the pseudo-protease domain (PPD) [51•]. The CD is conserved among eukaryotes, with 34% sequence identity between yeast and human separase. The conservation of the SD is weaker, with 24% identity between yeast and human separase. In contrast, the α helical region is poorly conserved, both in sequence and in length, contributing to the extensive size variations among these enzymes (Figure 1a). Some separases also contain C-terminal extensions beyond the CD, further increasing the size variation. *Drosophila* separase is distinct in being composed of two separate subunits [52,53] (Figure 1a).

Securin has a KEN-box and a D-box in its N-terminal region which are crucial for ubiquitination by APC/C, while its C-terminal region mediates the binding and inhibition of separase (Figure 1b). This region has been named the separase interaction segment (SIS) [50••] or the separase-binding motif (SBM) [54•].