Domain structure of separase and its binding to securin as determined by EM

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After the degradation of its inhibitor securin, separase initiates chromosome segregation during the metaphase-to-anaphase transition by cleaving cohesin. Here we present a density map at a resolution of 25 Å of negatively stained separase–securin complex. Based on labeling data and sequence analysis, we propose a model for the structure of separase, consisting of 26 ARM repeats, an unstructured region of 280 residues and two caspase-like domains, with securin binding to the ARM repeats.

The separation of sister chromatids and their segregation to opposite poles of a dividing cell are crucial events in cell proliferation. In metaphase, the replicated sister chromatids are held together by the multisubunit complex cohesin[1]. During anaphase, when the anaphase-promoting complex targets the inhibitor securin for ubiquitin-mediated degradation by the proteasome, the cysteine-protease separase cleaves the cohesin subunit SCC1 and initiates segregation of the sister chromatids[2]. Separases (~1,600–2,100 residues) exist in all eukaryotes but only the last 600 residues show conservation among all separases. Sequence similarity among securins (~200–300 residues), the family of separase inhibitors, is found only between closely related species.

Endogenous human separase (2,120 residues) in complex with securin (202 residues) was purified from nocodazole-arrested HeLa-S3 cells (**Supplementary Fig. 1** online) Based on hydrodynamic data from gel filtration and rate zonal centrifugation experiments, we calculated a molecular mass of 220 kDa, in good agreement with the expected molecular mass of 255 kDa for a one-to-one separase-securin heterodimer (**Supplementary Fig. 1**). A large frictional ratio of 1.75 and projection averages from negatively stained particles (**Supplementary Fig. 1**) revealed that purified separase–securin complex has a distinct elongated shape.

We coexpressed human separase and securin in insect cells and isolated the recombinant complex. Images taken of negatively stained samples showed the particles to be monodisperse (**Supplementary Fig. 2** online). Averages calculated after classification of the particle images resulted in the same whale-shaped projections observed for the endogenous complex (**Fig. 1a**). The projection averages show particles with the body of the whale always well represented, whereas the tail is often blurred, suggesting that the tail domain has a variable structure and/or that the tail domain has a variable orientation with respect to the body domain owing to a flexible linker region. The projection averages also show a stain distribution inside the whale's body that separates this domain into two interlocking crescent-shaped densities, indicating the presence of two subdomains, plus a head domain delineated by the accumulation of stain in a crevice.

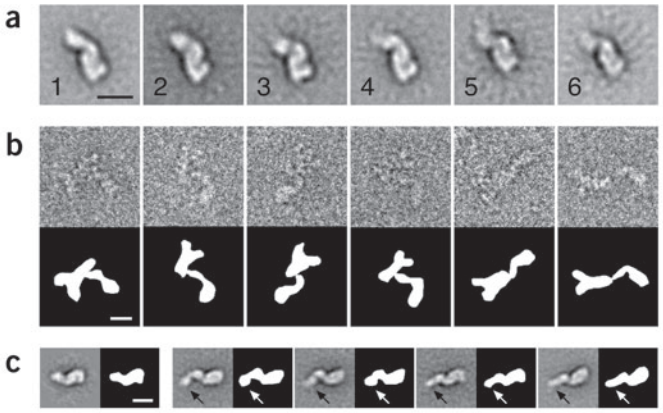

Figure 1 EM of human separase–securin complex. (**a**) Representative class averages. (**b**) Gallery of separase–securin complexes with a ZZ domain in the separase N terminus interacting with rabbit antibodies. Top panels are raw images and panels below are schematic representations. (**c**) Gallery of class averages of separase–securin complexes with an MBP tag at the securin C terminus. The arrows point to the extra MBP density. The first class average shows the separase–securin complex without a tag. Scale bars, 10 nm.

We used the random conical tilt approach to determine the three-dimensional structure of separase-securin[3]. We only combined class averages in which the tail domain was well resolved (**Fig. 1a**, panels 1 and 2). The final map at a resolution of 25 Å confirmed the whale-shaped appearance of the complex with a length of 180 Å, width of 60 Å and height of 50 Å (**Supplementary Fig. 2**). There is a clear separation between the body and tail domains. Whereas the tail consists of a single elongated density, the body has a crevice that separates the body of the whale into two subdomains, which we termed the trunk and head domains (**Supplementary Fig. 2**).

To understand the organization of the complex, we analyzed modified forms that carried tags on either separase or securin. Fusion of the IgG-binding domain of protein A (ZZ domain) to the separase N terminus and incubation of the corresponding ZZ-separase–securin complex with an antibody reproducibly showed the antibody bound to the tail of the whale-shaped particles (**Fig. 1b**). To localize securin, we expressed complexes that contained maltose-binding protein (MBP) at

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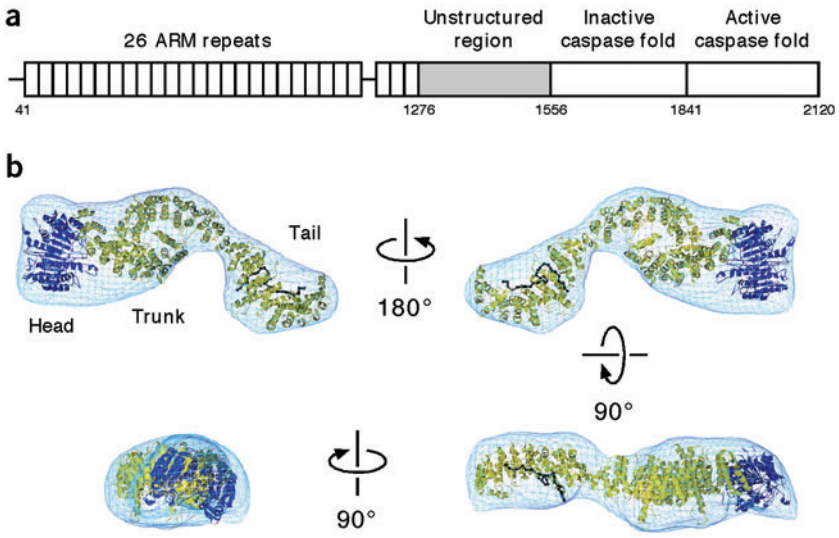

Figure 2 Structure of the separase–securin complex. (**a**) Domain structure of human separase. (**b**) Surface-rendered 25-Å density map and speculative model for the securin–separase complex approximated using the structures of importin-β (yellow) and caspase-3 (blue). As a suggested partial model for securin binding to separase, the first nine repeats show 27 residues of the nonclassical NLS of PTHrP (black) bound to importin-β.

the securin C terminus. Projection averages showed separase–securin-MBP complexes with an enlarged tail as compared with native complexes (**Fig. 1c**), indicating that the securin C terminus colocalizes with the separase N terminus.

Detailed bioinformatic analysis (secondary structure, polypeptide disorder and loop predictions) of the aligned sequences of human, mouse and rat separases (**Supplementary Figs. 3–5** online) indicated that human separase consists of an unstructured central stretch of 280 residues that separates a superhelical N-terminal half comprising 26 ARM or HEAT repeats from two C-terminal caspase domains, of which only the second one seems to be active (**Fig. 2a**). We could thus generate a speculative model for separase by manually placing crystal structures into our density map; three copies of the crystal structure of importin-β[4] were used to represent the 26 ARM repeats of the separase N-terminal domain and a crystal structure of the caspase-3 homodimer[5] to represent the two C-terminal caspase domains of separase (**Fig. 2b**). The tail domain of the complex, containing the separase N terminus, accommodates nicely one of the importin-β crystal structures, which would thus represent the N-terminal nine ARM repeats of separase. The two importin-β crystal structures representing the remaining 17 separase ARM repeats fit into the trunk domain if placed in an interlocking fashion as suggested by the projection averages, whereas the caspase-3 homodimer can account for the head domain density. The crystal structures do not completely fill the density map, so that it could also accommodate the putatively disordered central domain of separase, as well as securin. We included the 27 residues of the nonclassical nuclear localization signal (NLS) of the parathyroid hormone-related protein (PTHrP) that binds to importin-β[6] as a probable model of how securin may bind separase.

The presented model provides a starting point to address some unanswered questions. A varying number of N-terminal ARM or HEAT repeats[7] can now explain the different lengths of separase genes. All the phosphorylation and self-cleavage sites of separase[8–10] are located either in the loop between ARM repeats 23 and 24 or in the central unstructured region, regions which might therefore have a regulatory function. The identification of two caspase folds in the separase C terminus suggests that upon activation of separase self-cleavage may result in conformational changes, as in the case for the homodimeric caspase-3 (ref. 11). Finally, by proposing that securin binds to the separase ARM repeats in an extended fashion, like axin binds to β-catenin[12], securin bound to the N-terminal ARM repeats of separase could extend to the C-terminal caspase folds.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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