論文内容の要旨

論文題目

Analysis of mechanisms regulating ubiquitination activity of SCF complexes

（SCF 複合体のユビキチン化活性の制御機構に関する解析）

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Ubiquitin is an evolutionarily conserved small protein and its attachment to specific proteins, known as ubiquitination, regulates not only the stability but the localization and functions of substrate proteins involved in a plethora of cellular processes. Ubiquitination is mediated by the concerted actions of three classes of enzymes, namely, ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin protein ligase (E3). E3 recruits E2 to specific proteins to define the substrate specificity. One of the major E3 families is SCF complex, composed of a Skp1 adaptor protein, a Cullin scaffold protein, a RING finger protein, and an F-box protein. While the first three subunits are common subunits shared among all the complexes, an F-box protein is variable component and responsible for substrate recognition to define specific functions of each complex. Despite the remarkable progress in this field, it is not understood whether the SCF complex-dependent ubiquitination system is rather static or dynamically remodeled. To address this issue, the amount of each F-box protein incorporated in the complexes has to be compared between different conditions.

In this thesis, I exploited stable isotope labeling by amino acids in cell culture (SILAC) followed by mass spectrometry (MS) for quantitative profiling of F-box proteins incorporated in SCF complexes of the budding yeast *Saccharomyces cerevisiae* in Part I. SCF complexes were purified *en masse* using a genomically encoded affinity-tag attached to either of the invariant components Skp1, Cdc53 (cullin), or Hrt1 (RING finger protein). Among the 17 known F-box proteins of the yeast, 16 were successfully identified by MS. At first, I used SILAC to compare SCF complexes under different cultivation conditions. The abundance of Saf1, an F-box protein involved in entry into quiescence, in the complexes was increased during transition from log to post-diauxic phase. Next, I examined whether overproduction of a substrate affects the level of the complex responsible for its ubiquitination. The

abundance of Met30, but not other F-box proteins, in the complexes was increased in cells where Met4, a substrate of the Met30-containing complex, was overexpressed.

These results illustrate a cellular response to environmental and genetic perturbations, wherein the cell remodels the SCF complex-dependent ubiquitination system by altering the composition of incorporated F-box proteins, presumably to redirect the activity of the system toward appropriate substrates to be ubiquitinated under individual conditions.

In Part II, I exploited another mechanism regulating ubiquitination activity of SCF complexes. Neddylation is a post-translational modification of covalent attachment of the ubiquitin-like protein Nedd8/Rub1 to a cullin. The SCF complex including the neddylated cullin has more ubiquitination activity than the complex including the non-neddylated form *in vitro*, suggesting a direct role of neddylation in ubiquitin ligase activity. The structural study provided an evidence that neddylation induced a drastic conformational change. Although this study offered a plausible explanation about its enhancing effect on SCF complexes, the effect of neddylation on the N-terminal region of a cullin remains to be cleared because the authors used the C-terminal truncated cullin. Since F-box proteins interact with the N-terminus of a cullin via Skp1 adaptor protein, any change in this region would induce compositional alteration of F-box proteins incorporating SCF complexes, leading to remodeling activity and selectivity of ubiquitination activity.

To examine the overall effect of neddylation, I investigated the interaction stability of F-box proteins with SCF complex components, using mass spectrometry-based strategy. Interactions of neddylated Cdc53 with Skp1 and F-box proteins were stable. By contrast, in the non-neddylated form, interactions of Cdc53 with Skp1 were partially dynamic and each F-box protein showed a different stability of interaction with Cdc53, suggesting that modulation of interaction stability of F-box proteins with SCF complex components is a plausible mechanism by which neddylation regulates the ubiquitination activity