Structural basis for the recognition of citrate synthase by the SCF^{Ucc1} ubiquitin ligase complex

Organisms capable of conducting the glyoxylate cycle can utilize acetate or fatty acids as a sole carbon source, producing glucose from acetyl-CoA through a sequence of reactions in the glyoxylate cycle, the TCA (tricarboxylic acid) cycle, and the gluconeogenic pathway. In the glyoxylate cycle, acetyl-CoA, derived from acetate or fatty acids, undergoes condensation with oxaloacetate (OAA) by citrate synthase to generate citrate. Our previous study demonstrated that Cit2, a citrate synthase in the glyoxylate cycle of *Saccharomyces cerevisiae*, is targeted for proteasomal degradation by the ubiquitin ligase complex SCF^{Ucc1} (Skp1–Cdc53–F-box protein Ucc1). Biochemical analyses revealed that OAA induces a conformational change in Cit2, inhibiting its recognition and ubiquitination by SCF^{Ucc1}. These findings suggested the existence of a positive feedback loop wherein higher amounts of gluconeogenic metabolites (e.g., acetyl-CoA and OAA) stabilize Cit2, further activating the glyoxylate cycle. In conjunction with cell biological analyses, we propose that SCF^{Ucc1}-mediated regulation of Cit2 acts as a metabolic switch for the glyoxylate cycle in response to changes in carbon source. However, the structural basis of how Ucc1, an F-box protein serving as a substrate recognition factor, discriminates the metabolite-dependent conformational change of Cit2 remained unclear.

To address this, we conducted an X-ray crystal structure analysis. Crystallization was performed for the Ucc1-Skp1 complex with Cit2 (Ucc1-Skp1-Cit2), Apo-Cit2, Cit2 in complex with OAA and CoA (Cit2-OAA-CoA), and Apo-Cit1. This study employs CoA instead of acetyl-CoA to prevent enzymatic reactions during crystallization. X-ray diffraction data were collected at SPring-8 **BL44XU**. Despite the absence of a homology structure for Ucc1 through sequence search, we successfully determined the initial phases of Ucc1-Skp1-Cit2 only using a Cit2 homology model for molecular replacement. Ultimately, we resolved the crystal structures of Ucc1-Skp1-Cit2, Apo-Cit2, Cit2-OAA-CoA, and Apo-Cit1 at resolutions of 2.30 Å, 2.39 Å, 1.48 Å, and 1.42 Å, respectively [1] (Fig. 1).

The Ucc1-Skp1-Cit2 structure comprises a Ucc1-Skp1 complex and a Cit2 dimer (Fig. 1(a)). Ucc1 features a typical F-box domain at its N terminus, while its substrate binding domain (SBD) adopts a unique structure. The interaction surface between Ucc1 and Cit2 involves complementary charged residues (Fig. 1(b)). The Apo-Cit2 structure consists of two Cit2 subunits, each containing large and small domains, with the ligand binding site situated between these domains (Fig. 1(c)). Apo-Cit2 lacks substrates OAA and acetyl-CoA in its open form, exposing its ligand

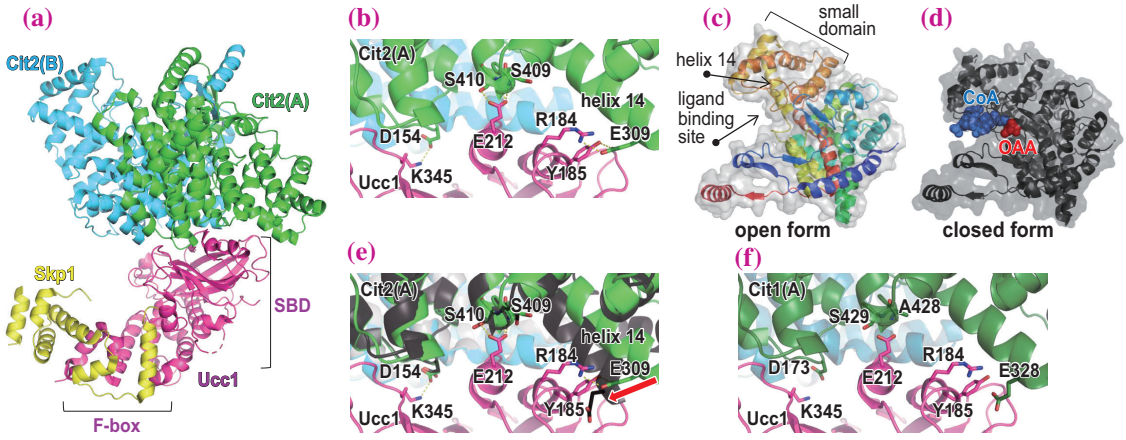

Fig. 1. Structural basis for the recognition mechanisms of citrate synthase by the Ucc1-Skp1 complex. **(a)** Overall structure of the Ucc1-Skp1-Cit2 complex. **(b)** Close-up view of the interaction between the Cit2 and Ucc1 in the Ucc1-Skp1-Cit2 complex. Critical hydrogen bonding residues are depicted as sticks. **(c)** Structure of subunit A in the ligand-free Cit2 (open form). Ribbon color-coded from blue to red (N- to C-terminus). **(d)** Structure of subunit A in Cit2 with OAA and CoA (closed form). **(e)** Superimposition of Cit2 closed form (black) on Cit2 in the Ucc1-Skp1-Cit2 complex. The red arrow indicates the movements of E309 in helix 14. **(f)** Predicted intermolecular contacts between Apo-Cit1 and Ucc1. Apo-Cit1 superimposed on Cit2 in the Ucc1-Skp1-Cit2 complex. Conserved amino acids predicted to form hydrogen bonds are shown as stick models.

binding sites to the solvent region. The structure of Apo-Cit2 closely resembles that of Cit2 in the Ucc1-Skp1-Cit2 complex. Additionally, we determined the crystal structure of Cit2-OAA-CoA, revealing a ligand-induced conformational change when OAA and CoA are present (Fig. 1(d)). This change involves the movement of the small domain to close the ligand binding site (closed form). The small domain of Cit2 contains E309, which interacts with R184 and Y185 in Ucc1. Therefore, such conformational changes may destruct these interactions. Indeed, mutant Cit2 E309A or Ucc1 R184A/Y185A substitutions result in reduced binding between them [1]. When the closed form of the Cit2 structure is superposed onto the Cit2 structure in the Ucc1-Skp1-Cit2 complex, helix 14, including E309 on Cit2, causes steric hindrance with Ucc1, (Fig. 1(e)). These results indicate that Ucc1 preferentially recognizes the open form, not the closed form of Cit2. Furthermore, these findings provide a structural basis for the previously proposed positive feedback loop in which a higher amount of gluconeogenic metabolites stabilizes Cit2 to activate the glyoxylate cycle [2] (Fig. 2(a)).

Recent advances in cell biology have unveiled factors hindering mitochondrial import, including the accumulation of reactive oxygen species associated with aging, mutations in mitochondrial DNA, and aggregated proteins linked to neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. The primary sequence of Cit2 shares over 75% identity with that of Cit1, a mitochondrial citrate synthase; indeed, the Apo-Cit2 structure was almost identical to the Apo-Cit1 structure (Fig. 1(f)). Additionally, the amino acid residues critical for Ucc1 binding in Cit2 are mostly conserved in Cit1. We hypothesized that SCF^{Ucc1} could also target non-imported Cit1, generated during mitochondrial import defects, for proteasomal degradation. Upon inhibiting mitochondrial import, non-imported Cit1 is recognized, ubiquitinated by SCF^{Ucc1}, and degraded by the proteasome [1]. Excessive accumulation of non-imported Cit1 induces ectopic citrate synthesis, causing an imbalance in sugar carbon flux, depletion in amino acid and nucleotide pools, and growth defects. All these results highlight SCF^{Ucc1} as a critical ubiquitin ligase controlling citrate metabolism (Fig. 2(b)).

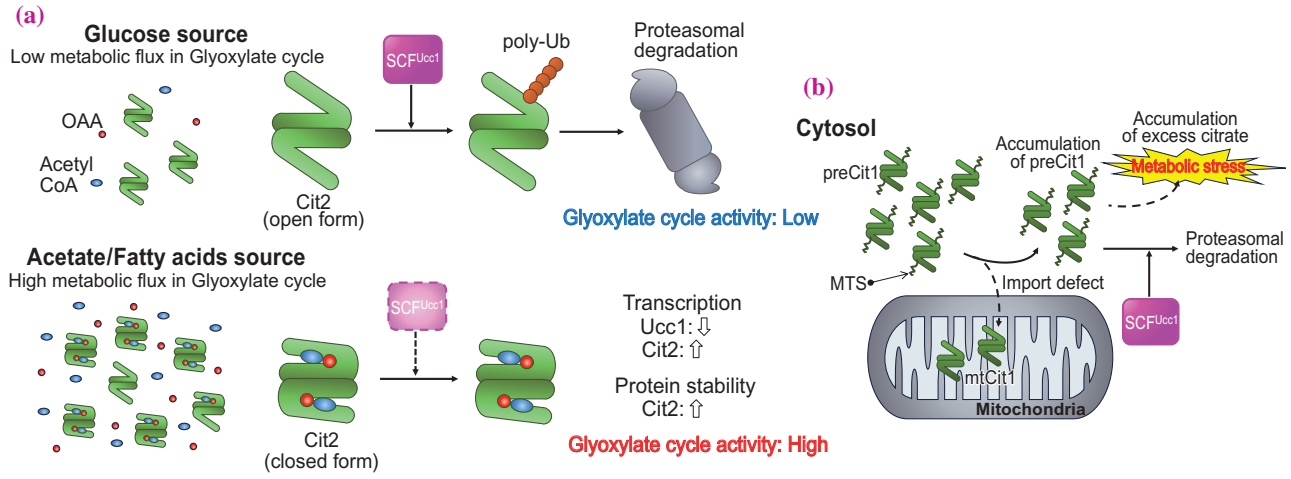

Fig. 2. Models illustrating the role of SCF^{Ucc1}. **(a)** SCF^{Ucc1}-mediated regulation of Cit2 in the modulation of metabolic flux. In acetate-grown cells, metabolites inhibit SCF^{Ucc1}-mediated ubiquitination of Cit2, activating the glyoxylate cycle to produce glucose through gluconeogenesis. **(b)** SCF^{Ucc1}-mediated degradation of mislocated Cit1 in the ectopic metabolic stress response.

Kazuya Nishio[a], Tsunehiro Mizushima[a] and Kunio Nakatsukasa[b,*]

[a] Department of Life Science, University of Hyogo
[b] Graduate School of Science, Nagoya City University

*Email: nakatsukasa@nsc.nagoya-cu.ac.jp

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