Structural Basis for the Inactivation of Stress Sensor Keap1 by Selective Autophagy Substrate p62

Keap1 (Kelch-like ECH-associated protein 1) is a sensor protein for oxidative stress and an adaptor protein of cullin-RING ubiquitin ligase complex. In the normal condition Keap1 represses the cytoprotective transcription factor Nrf2, which is ubiquitylated by the Cul3–Rbx1 Keap1 complex and degraded by the proteasome. Under oxidative stress Keap1 is inactivated and derepression of Nrf2 results in activation of many cytoprotective genes. A recent report has shown that accumulation of p62 in autophagy deficient mice also activates Nrf2 target genes. Here we describe the crystal structure of Keap1 in complex with p62. The data reveals how impaired selective autophagy activates the cytoprotective gene expression.

Keap1 is a substrate adaptor of a Cullin-3-based E3 ubiquitin ligase complex that recognizes Nrf2 (NF-E2-related Factor 2), and also acts as a cellular sensor for xenobiotics and oxidative stresses. Nrf2 is a transcriptional factor regulating the expression of cytoprotective genes in response to such stresses. Under unstressed conditions Keap1 binds Nrf2 and results in rapid degradation of Nrf2 through the proteasome pathway. In contrast, upon exposure to oxidative stress, reactive cysteine residues in IVR (Intervening region) and BTB (Broad complex, Tramtrack and Bric-à-Brac) domains of Keap1 [Fig. 1(a)] are modified by electrophiles. This modification prevents Nrf2 from rapid degradation and induces Nrf2 activity by repression of Keap1.

Previous mouse genetic studies on the autophagy-essential protein Atg7 showed that impaired autophagy caused marked accumulation of p62 along with induction of antioxidant proteins. These antioxidant genes are regulated by transcription factor Nrf2. Nuclear accumulation of Nrf2 was observed in livers deficient in Atg7, but was recovered by the additional loss of p62. To understand cellular regulation by p62, proteins that interact with p62 were screened by a proteomic approach using HEK293T cells expressing tagged p62 protein. To our surprise Keap1 was identified as a p62-interacting protein [1].

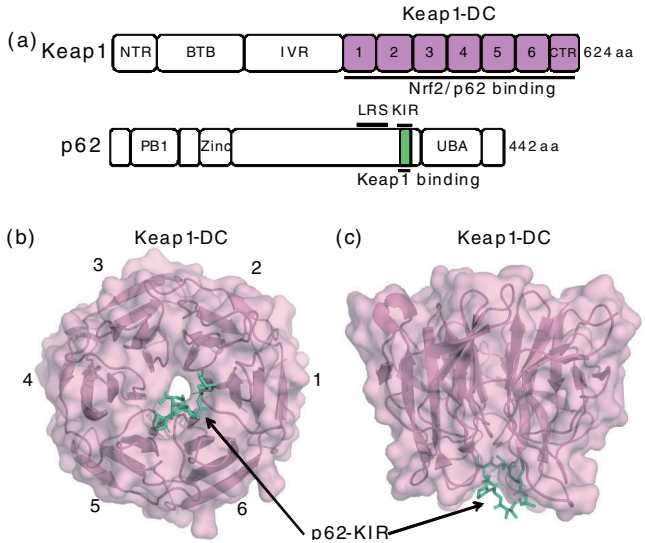

Figure 1
Structure of Keap1-DC in complex with p62-KIR. (a) Diagrams of the domain organization of Keap1 and p62. (b) Bottom view and (c) side view of the complex structure. The ribbon model represents Keap1-DC and the stick model shows p62-KIR. Each β-propeller blade is numbered from 1 to 6.

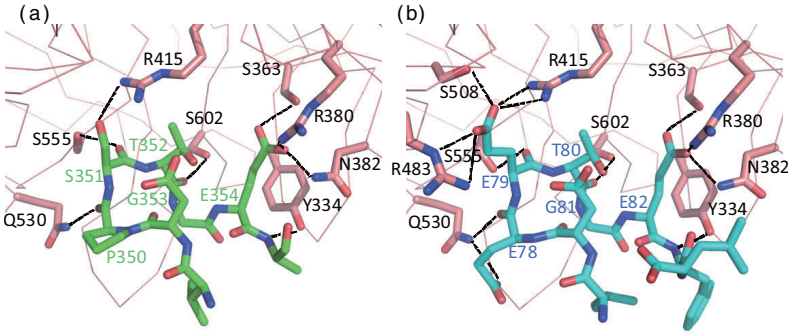

Figure 2
Inter-molecular hydrogen bonds of Keap1-DC in complex with p62-KIR (a) and in complex with the Nrf2-ETGE region [(b); PDB ID; 1x2r].

p62 consists of an N-terminal region that includes Phox and Bem1p (PB1) and a Zinc finger (Zinc), a central region containing an LC3-recognition sequence (LRS), and a C-terminal region with a ubiquitin-associated domain (UBA) [Fig. 1(a)]. To determine which region of p62 is required for its interaction with Keap1, truncated p62 and Keap1 fragments were prepared. The pull-down assays with Keap1 DC (double glycine repeat, and C-terminal) domain revealed that the region of p62 (amino acids 345-359) is essential and sufficient for the interaction between p62 and Keap1-DC. Therefore, amino acids 345-359 in p62 were named KIR (the Keap1 interacting region).

To understand how p62-KIR interacts with Keap1-DC, we determined the crystal structure of Keap1-DC (aa 309–624) in complex with the p62-KIR (aa 346–359; KEVDPSTGELQSLQ) at a resolution of 2.8 Å. Keap1-DC forms a 6-bladed β-propeller structure with pseudo 6-fold symmetry [Fig. 1(b)]. The KIR peptide binds to the bottom side of the β-propeller structure [Figs. 1(b) and (c)]. A simulated-annealing F_o–F_c omit map clearly revealed the electron density in the peptide region. Eight residues (V348 to L355) out of 14 of p62-KIR were visible, except for the L355 side chain.

Recent single-particle electron microscopy analysis revealed that Keap1 is a forked-stem dimer structure with two large spheres including the Keap1-DC domain [2]. The DLG and ETGE binding motifs present in the Neh2 domain of Nrf2 bind individually to the same binding pocket located at the bottom surface of Keap1 [3]. Homodimeric Keap1 binds one Nrf2 molecule. Since the two-site binding facilitates the ubiquitination of lysine residues located between the DLG and ETGE motifs, the two-site substrate recognition mechanism is crucial for the rapid ubiquitination of Nrf2.

The structure of the Keap1-DC domain in complex with p62-KIR [Fig. 2(a)] revealed an overlap with the interactions between the Keap1-DC domain and Nrf2-ETGE [Fig. 2(b)]. Eight amino acid residues of Keap1-DC (Y334, S363, R380, N382, R415, Q530, S555, and S602) form hydrogen bonds with p62-KIR [Fig. 2(a)]. These eight residues and two additional Keap1 residues (R483 and S508) are involved in Keap1 recognition of the Nrf2-ETGE [Fig. 2(b)]. These data suggested that p62-KIR binds to Keap1 in a manner very similar to the Nrf2-ETGE and Nrf2-DLG motifs.

Structural and kinetics analyses have strongly argued that p62 competitively inhibits the Keap1-Nrf2 interaction, leading to stabilization of Nrf2 and the expression of cytoprotective genes [1].

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