Abstract

Selective protein degradation by the ubiquitin-proteasome system is essential for cellular homeostasis and the regulation of diverse biological processes. The selectivity of this system is imparted by hundreds of ubiquitin ligases that specifically recognise substrates and catalyse their ubiquitination, thereby targeting them for degradation. Among ubiquitin ligases, multisubunit cullin-RING ubiquitin ligases constitute the largest group. However, despite significant advances in understanding their assembly, regulation, and molecular architecture, the substrates and functions of most of them remain unknown. This thesis focuses on two ubiquitin ligases from the cullin-RING ubiquitin ligase 4 (CRL4) subfamily: $CRL4^{DCAF4}$ and $CRL4^{DCAF12}$. To identify their candidate substrates and to address their biological roles, several different approaches have been employed. First, proteomic screening revealed a wide range of candidate substrates. Next, detailed characterisation of the identified interactions and exploration of the condition under which candidate substrates undergo degradation was performed. Finally, knockout human cell lines and mice with a targeted disruption of genes encoding DCAF4 and DCAF12 were generated to explore the physiological roles of $CRL4^{DCAF4}$ and $CRL4^{DCAF12}$. In summary, the herein presented identification and validation of novel substrates of $CRL4^{DCAF4}$ and $CRL4^{DCAF12}$ followed by the exploration of their biological roles provides an important insight into the function of these two understudied ubiquitin ligases.