

# F-box protein substrate recognition

## A new insight

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Ubiquitin E3 ligases represent an increasingly diverse group of proteins whose precise biologic role still remains enigmatic. Of the SCF (Skp1-Cullin1-F-box) E3 ligase family, for example, only very few subunits from over 60 family members are well-characterized. The SCF apparatus contains a multi-subunit catalytic core consisting of Skp1, Cullin1 and the E2 ubiquitin-conjugating (Ubc) enzyme and an F box receptor-like component that targets many substrates. The F-box proteins have two functional domains: an NH<sub>2</sub>-terminal F-box motif that binds Skp1, and a C-terminal leucine-rich repeat (LRR), WD motif or another signature that recognizes substrates.<sup>1</sup> SCF ligase complexes are critically involved in regulating DNA repair, cell cycle progression, inflammation, centrosome stability, mitotic fidelity and cellular proliferation.<sup>2</sup> Hence, it is not surprising that novel targeted therapies might involve modulating activities of SCF subunits in cells during neoplasia, cell growth and repair or inflammation.

Following its initial description, F-box protein Fbxl2 was shown to interact with hepatitis C virus.<sup>3</sup> However, until recently, the authentication of Fbxl2 as a ubiquitin E3 ligase component and its molecular behavior was not demonstrated. Recent studies show that Fbxl2 acts as the receptor component of a classical SCF ubiquitin E3 ligase, recognizing a calmodulin (CaM)-binding signature.<sup>4</sup> CaM is a highly conserved protein that binds to its targets in a calcium-dependent or calcium-independent manner by recognizing specific molecular signatures including an IQ motif (I/LQXXXRGXXXR), a 1-10 or 1-5-10-binding motif.<sup>5</sup>

Indeed, recognition of an IQ motif by Fbxl2 represents an unusual

characteristic of F box proteins that typically are recruited by phosphodegrons within target substrates.

The first authenticated substrate of Fbxl2 is CTP: phosphocholine cytidyltransferase (CCT $\alpha$ ), a key rate-regulatory enzyme that is indispensable for membrane phosphatidylcholine production. Fbxl2 is sufficient to mediate CCT $\alpha$  monoubiquitination in a calcium-dependent process through actions of the SCF<sup>Fbxl2</sup> complex. However, both CaM and Fbxl2 competitively interact to vie for occupancy within a canonical IQ motif within CCT $\alpha$ , where CaM acts as a stabilizing ligand for CCT $\alpha$  protein. This competition model reveals that CaM acts as an antagonist of the SCF subunit to oppose Fbxl2-mediated substrate ubiquitination.<sup>4</sup>

Fbxl2 also targets cell cycle proteins. The SCF<sup>Fbxl2</sup> complex targets cyclin D2 in B-lymphocytes and leukemic cells, resulting in G<sub>0</sub> arrest and apoptosis.<sup>6</sup> Cyclin D2 is the dominant D-type cyclin with leukemic cells and is essential for G<sub>0</sub>/G<sub>1</sub> phase progression, which translocates the cyclin-dependent kinase inhibitor, p27, out of the nucleus. Fbxl2 also ubiquitinates cyclin D3 and impairs G<sub>2</sub>/M transition.<sup>7</sup> Cyclin D3 is a multi-functional protein that directly interacts with and confers activity for CDK11p58 that controls centrosome maturation and bipolar spindle formation.<sup>8</sup>

Both cyclin D2 and cyclin D3 harbor canonical IQ motifs typical of calcium-independent CaM binding proteins. Within the IQ motif, it appears that the glutamine residue is essential for Fbxl2 targeting as cyclin D2 and cyclin D3 variants harboring glutamine substitutions within the IQ motif exhibit significantly extended half-lives in vivo. Interestingly,

threonine<sup>286</sup>, within cyclin D1, is recognized by the F-box proteins Fbxo4, Fbxw8 and Fbxo31 to facilitate its ubiquitination.<sup>9</sup> Although this threonine site is highly conserved among the D cyclins, Fbxl2 did not utilize this molecular signal for targeting, but rather docks within a consensus IQ signature within these cyclins to facilitate their ubiquitination.

The intermolecular competition between SCF<sup>Fbxl2</sup> complex and CaM regulate other proteins involved in mitotic progression. Recently, we identified that CaM, acting as a sensor of chromosome bridges, protects Aurora B at the midbody to stabilize the ingressed furrow for delayed abscission (unpublished data). However, these activities are opposed by Fbxl2, which again engages CaM for access to Aurora B within a shared IQ motif. Last, chemical inhibition of CaM was highly effective in reducing both Aurora B levels and tumor viability. These observations implicate a role for the F-box protein and CaM as diametric homeostatic sensors that regulate the cytokinesis program through modulation of Aurora B concentrations.

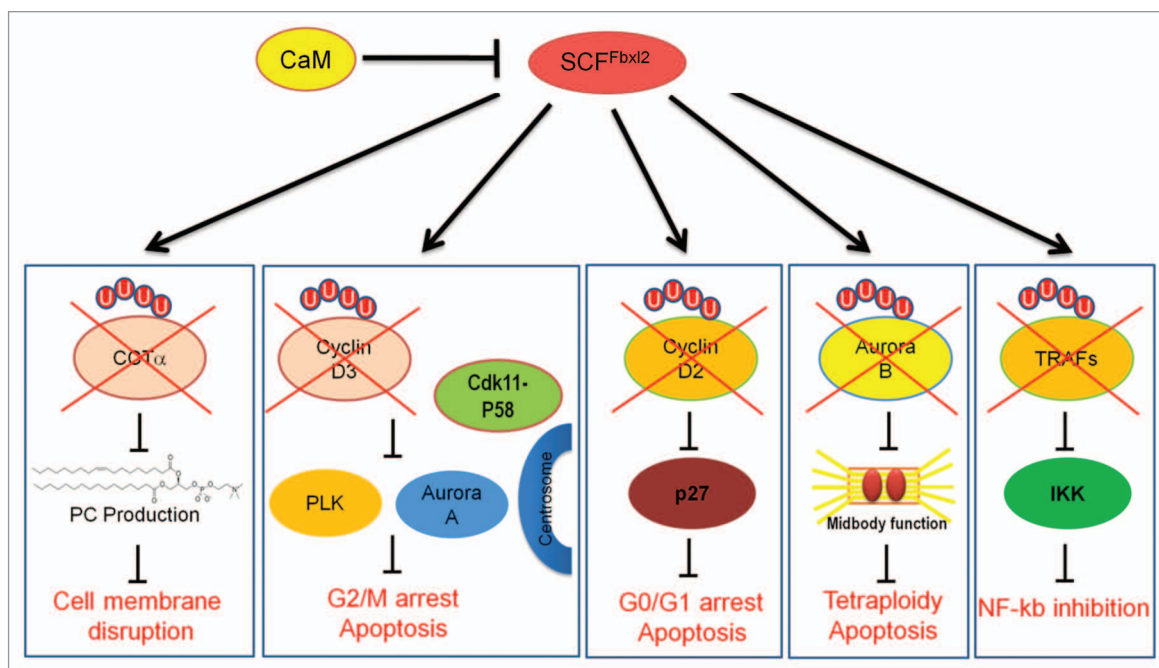
More recent studies reveal that the SCF<sup>Fbxl2</sup> complex might also play an important role in regulating the NF $\kappa$ B pathway and cytokine responses. Fbxl2 acts as a crucial, pan-reactive inhibitor of tumor necrosis factor receptor-associated factors (TRAF) function by mediating their polyubiquitination.<sup>10</sup> By eliminating TRAFs, Fbxl2 inhibits NF $\kappa$ B activity and secretion of a broad spectrum of Th<sub>1</sub> panel cytokines. Interestingly, TRAF proteins contain a conserved 1–10 CaM-binding motif in which a tryptophan residue is essential for Fbxl2 targeting.<sup>10</sup> In conclusion, studies in our laboratory have

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**Figure 1.** SCF<sup>Fbx12</sup> complex targets many substrates for ubiquitination and it is antagonized by CaM.

identified multiple substrates of Fbx12, all of which are CaM-binding proteins and are essential for either cell proliferative activity or innate immunity. The ability of Fbx12 to recognize a CaM binding signature appears to represent a unique molecular mechanism of F box protein substrate targeting that underlies its ability to exhibit tumorocidal activity and possibly play a role in immune suppression. Indeed, Fbx12 expression is significantly reduced in peripheral blood cells from leukemic subjects, where its substrates cyclin D3, cyclin D2, Aurora B are highly expressed.<sup>6</sup> Additional investigations are needed to assess the expression of Fbx12 in subjects with inflammatory illness, such as sepsis and pneumonia (Fig. 1).

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