Autophagy

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Autophagy gets a brake

DAP1, a novel mTOR substrate, is activated to suppress the autophagic process

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Autophagy, a highly regulated catabolic process, is controlled by the action of positive and negative regulators. While many of the positive mediators of autophagy have been identified, very little is known about negative regulators that might counterbalance the process. We recently identified death-associated protein 1 (DAP1) as a suppressor of autophagy and as a novel direct substrate of mammalian target of rapamycin (mTOR). We found that DAP1 is functionally silent in cells growing under rich nutrient supplies through mTOR-dependent inhibitory phosphorylation on two sites, which were mapped to Ser3 and Ser51. During amino acid starvation, mTOR activity is turned off resulting in a rapid reduction in the phosphorylation of DAP1. This caused the conversion of the protein into a suppressor of autophagy, thus providing a buffering mechanism that counterbalances the autophagic flux and prevents its overactivation under conditions of nutrient deprivation. Based on these studies we propose the "gas and brake" concept in which mTOR, the main sensor that regulates autophagy in response to amino acid deprivation, also controls the activity of a specific balancing brake to prevent the overactivation of autophagy.

Key words: DAP1, mTOR, autophagy, amino acid starvation, phosphorylation

Abbreviations: DAP1, death-associated protein 1; mTOR, mammalian target of rapamycin

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In recent years, many of the genes controlling and executing the autophagic process have been identified. Most of these genes act as positive mediators of the various steps of the process, including the ULK1 complex, which regulates the induction step, the Vps34-Beclin 1 complex that participates in the vesicle nucleation step and two ubiquitin-like pathways, the Atg12-Atg5 and the LC3-phosphatidylethanolamine (PE) conjugation steps, which play a central role in the vesicle elongation process. To date, only a few negative regulators of autophagy have been identified, including mTOR and the anti-apoptotic Bcl-2 family members. mTOR Ser/Thr kinase is a central suppressor of autophagy acting at the initiating regulatory steps of the process. Many signaling pathways act to inhibit mTOR activity, thus relieving its inhibitory effects on autophagy. The anti-apoptotic Bcl-2 and Bcl-X_L proteins, on the other hand, act at the nucleation step, by directly binding to Beclin 1's BH3 domain, thus reducing the activation of Vps34 and subsequent autophagy. This inhibition can be relieved through dissociation of the complex, following either JNK-1 mediated phosphorylation of Bcl-2 or DAP kinase-mediated phosphorylation of the BH3 domain of Beclin 1.

DAP1 is a small (~15 kDa), ubiquitously expressed protein, rich in prolines and lacking known functional motifs. DAP1 was isolated more than a decade ago in our laboratory using a functional approach to gene cloning aimed at identifying novel mediators of IFNγ-induced cell death in mammalian cell cultures. Until recently, very little was known about the cellular and molecular functions of DAP1, mainly due to the lack of homology to other known proteins and the lack of functional motifs that could indicate a possible cellular function and studies in mammalian systems were missing.

Recently, we discovered that DAP1 is another negative regulator of autophagy;

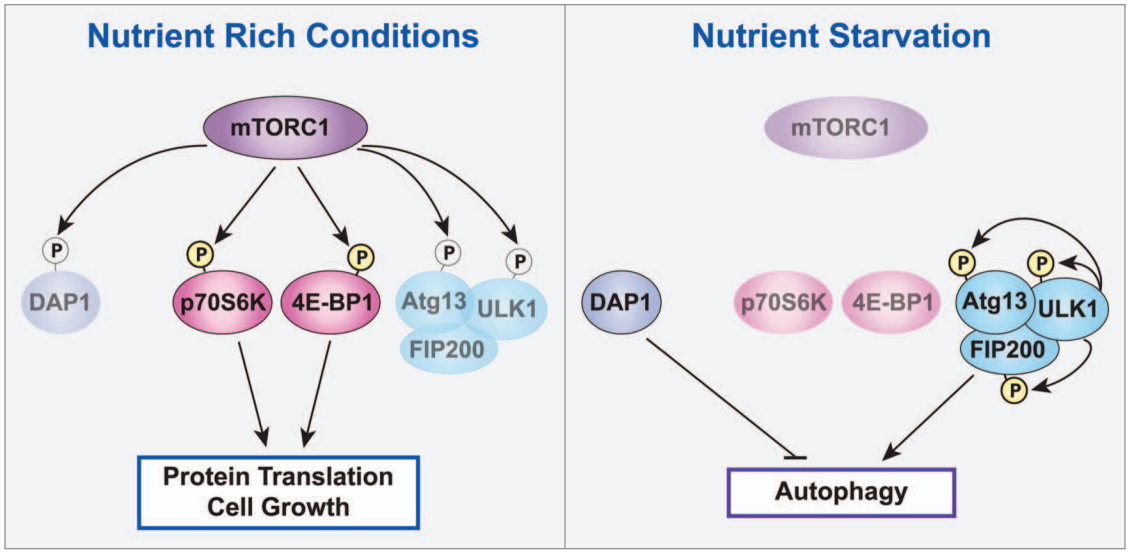

Figure 1. "Gas and brake" model. During nutrient-rich conditions, active mTORC1 phosphorylates and inactivates the components of the ULK1 complex, ULK1 and Atg13, thus preventing the induction of autophagy. DAP1 is also inactivated simultaneously by mTORC1-mediated phosphorylation on Ser3 and Ser51. In addition, mTORC1 phosphorylates and activates p70S6K and 4E-BP1, which mediate the protein translation and cell growth activities of mTOR. Upon nutrient starvation, mTORC1 activity is attenuated, leading to dephosphorylation and activation of ULK1. ULK1, in turn, undergoes autophosphorylation and phosphorylates Atg13 and FIP200 resulting in ULK1 complex activation and induction of autophagy. On the other hand, activation of DAP1 by dephosphorylation, results in suppression of autophagy, thus inserting a brake into the process of autophagy. Note that the inactive proteins/complexes are faded out.

yet, interestingly, its suppressive activity is selectively turned on during the autophagic process. Moreover, we found that DAP1 suppressive activity is tightly linked to the status of mTOR kinase activity. Under nutrient-rich culture conditions, DAP1 is phosphorylated by mTOR on two sites, Ser3 and Ser51, resulting in its inactivation. In response to nutrient deprivation, mTOR is inhibited and DAP1 undergoes rapid dephosphorylation. By knocking down the endogenous DAP1 and introducing either the phospho-mimetic or the nonphosphorylatible DAP1 mutants, we found that the dephosphorylation leads to activation of the autophagic suppressive function of DAP1, whereas the phophorylated form is inactive. These results led to a "gas and brake" model, in which at the same time that autophagy is induced, some brakes such as DAP1 are also activated to provide a buffering mechanism that counterbalances the autophagic flux and prevents its overactivation under nutrient-deprivation conditions (**Fig. 1**). Notably, balancing autophagy is extremely important, since deregulated or excessive autophagy has been implicated in the pathogenesis of diverse diseases, such as certain types of neuronal degeneration and cancer and also in cellular aging.

The current challenge is to identify the molecular basis of the suppressive functions of DAP1 on autophagy. We have recently shown that DAP1 knockdown enhances LC3 lipidation and autophagosome accumulation both during amino acid starvation and rapamycin treatment. In addition, preliminary data indicate that the knockdown of DAP1 has no effect on mTOR complex 1 (mTORC1) activity in cells, at least during the first hours of starvation. Accordingly, DAP1 may function between the mTORC1 and the LC3 conjugation systems. The potential targets may fall into one of the multiprotein complexes functioning downstream of mTOR such as the ULK1 complex, the Vps34-Beclin 1 complex and more. Future studies will be performed to identify the molecular mechanism by which DAP1 suppresses autophagy. The lack of known functional motifs in the DAP1 protein sequence suggests that this small proline-rich protein may function as an adaptor blocking autophagy by binding to critical protein partners that still await identification.

Although autophagy is primarily a protective process for the cell, it can also play a role in cell death. In response to prolonged starvation, autophagy can act either as a cell survival mechanism or be recruited as a cell death executer. In the future it would be interesting to examine whether the autophagy enhancement resulting from DAP1 knockdown contributes to increased cell death in our system or even may convert the survival properties of autophagy into death induction. This will fit the "gas and brake" model, in which autophagy, which is initially recruited as a cell survival mechanism, is converted into cell death machinery when a certain threshold is crossed due to the loss of the "brake" by the knockdown of DAP1.

To date, very little is known about the putative mechanisms that restrict the intensity of the autophagic flux to maintain the continuous benefits of this process under stress. Therefore, the ability of DAP1 to counterbalance and buffer the process in a manner that is tightly linked to the status of a central player in autophagy (i.e., mTOR) is an important discovery in this field and provides a target for future drug design.