





Atg3, as the E2-like protein, together with E1-like Atg7 and E3-like Atg5-Atg12 complex covalently conjugate phosphatidylethanolamine (PE) to Atg8 (6). Atg8 lipidation reaction can be reconstituted in vitro with recombinant Atg7 and Atg3 (7). To exclude the possibility that K-R mutation may affect autophagy by directly disturbing Atg3 enzymatic activity rather than reducing Atg3 acetylation, we assayed the enzymatic activity of recombinant variants of Atg3 in an in vitro Atg8 lipidation reaction. *atg3*^{K19R}, *atg3*^{K48R}, and *atg3*^{K19R-K48R} catalyzed Atg8 lipidation, whereas *atg3*^{K183R} and *atg3*^{K19R-K48R-K183R} mediate little Atg8 lipidation (Fig. 3F). Thus, K183R mutation disrupts the enzymatic activity of Atg3, but K19R and K48R do not. Because both *atg3*^{K19R-K48R} and *atg3*^{K19R-K48R-K183R} block autophagy, we conclude that acetylation on K19 and K48 are important for regulation of autophagy, whereas K183 is important for Atg3 enzymatic activity.

Atg8 conjugation to PE requires the interaction of Atg3 with Atg8 (8). In *esa1-1*, the interaction between endogenous Atg3 and exogenous tagged Atg8 was reduced, suggesting that acetylation promotes Atg3-Atg8 interaction (Fig. 3G). The interaction between Atg8 and Atg3^{K19R-K48R} was also reduced (Fig. 3H). Thus, Esa1-mediated Atg3 acetylation appears to influence autophagy through controlling Atg3-Atg8 interaction. However, acetylation of other proteins by Esa1 may also affect autophagy.

Histone deacetylases (HDACs) participate in regulation of protein acetylation. Genetic analysis identified Rpd3 as a negative regulator of autophagy (fig. S7). Acetylation of Atg3 was increased in *rpd3*Δ cells (Fig. 4A), and autophagy was accelerated (Fig. 4B and fig. S8). Time-lapse imaging revealed that the duration of Atg8 puncta was reduced in *rpd3*Δ cells (Fig. 4C and fig. S9). Kinetic studies revealed that Atg3 acetylation was transiently induced by starvation (fig. S10). In contrast, Atg3 acetylation was more sustained in *rpd3*Δ cells (fig. S10), suggesting that attenuation of Atg3 acetylation is mediated by Rpd3. Starvation-induced autophagy was transient in wild-type cells (Fig. 4D). However, Atg8 puncta formed rapidly but more slowly attenuated in *rpd3*Δ cells (Fig. 4D), indicating that deacetylation of Atg3 by Rpd3 may contribute to the attenuation of formation of autophagosome during starvation. As a consequence of constitutive autophagosome formation, Atg8-GFP cleavage is increased in *rpd3*Δ cells (Fig. 4E and fig. S11). Thus, metabolic cues appear to regulate the duration and magnitude of autophagy through temporal control of Atg3 acetylation.

Abundance of Esa1, Epl1, and Rpd3 and the enzymatic activity of Esa1 were not changed during autophagy (fig. S12). We constructed yeast strains in which hemagglutinin (HA)-Atg3 was expressed with Esa1-Myc, Epl1-Myc, and Rpd3-Myc, respectively, in physiological amount. We found weak interaction between Atg3 with Esa1 and Epl1 in yeast before starvation. Starvation transiently increased the interaction between Atg3 and Esa1 or Epl1, which peaked 1 hour after star-

vation and subsided thereafter (Fig. 4F). We also detected interaction between Atg3 and Rpd3 under nutrient-rich conditions, and starvation enhanced this interaction (Fig. 4F). We proposed that the transient acetylation of Atg3 may result from the dynamic interaction between Atg3 and acetylases. In yeast, most of Atg proteins are located on pre-autophagosomal structures (PASs) under growth or starvation conditions (9). PASs are thought to be the site for autophagosome formation and can be labeled with vacuolar hydrolases aminopeptidase I (Ape1) (10). Most Esa1, Epl1, and Rpd3 was located on the nucleus with or without nitrogen starvation (11, 12); however, we found that in starved cells a small fraction of Esa1, Epl1, and Rpd3 was located in the cytosol in the form of puncta (Fig. 4G and fig. S13). These punctas appeared to colocalize with Ape1, indicating these punctas were PASs (Fig. 4G and fig. S13). Starvation-induced transient recruitment of Esa1-GFP and Epl1-GFP to the PAS peaked 1 hour after starvation and subsided to prestarvation amount after 7 hours of starvation (Fig. 4G and fig. S13). In contrast, Rpd3-GFP was recruited to PAS on a delayed manner (Fig. 4G and fig. S13).

Acetylation is emerging as an important metabolic regulatory mechanism (13, 14) and is itself tightly regulated in response to metabolism changing (15). The TOR pathway provides one signaling pathway to couple autophagy with metabolism status. Acetylation may provide another mechanism (fig. S14).

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2. Y. Ohsumi, *Tanpakushitsu Kakusan Koso* 1 (suppl.),