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The arrestin-like protein ArtA is essential for ubiquitylation and endocytosis of the UapA transporter in response to both broad-range and specific signals

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We investigated the role of all arrestin-like proteins of *Aspergillus nidulans* in respect to growth, morphology, resistance to drugs and specifically for the endocytosis and turnover of the well-studied uric acid-xanthine transporter UapA. All arrestin null mutants are viable and all, except one that is affected in conidiospore production (ArtG), show wild-type growth and morphology. A single arrestin, ArtA, is essential for UapA endocytosis and vacuolar turnover in response to several signals, such as the presence of ammonium, azole drugs or substrates. ArtA is shown to be required for $HulA^{Rsp5}$-dependent ubiquitination of UapA, occurring at a single C-terminal Lys residue (K572). We further show that the UapA cytoplasmic C-terminal region is sufficient for eliciting ArtA-dependent endocytosis of AzgA, a transporter that does not normally undergo internalization in response to ammonium. Mutational analysis further suggests that the region 564-571 in the UapA C-tail might interact with ArtA. Systematic deletions and mutational analysis of ArtA confirm the essentiality of both PY elements and show that the N-terminal region (2-123) is also necessary for its function. Immunoblot analysis and fluorescence microscopy of ArtA expressed from native, constitutive or strong-inducible promoters are suggestive of cytoplasmic localization, in all conditions tested. Finally, we show that ArtA is also essential for vacuolar turnover of transporters specific for purines (AzgA) or L-proline (PrnB), but not for an aspartate/glutamate transporter (AgtA). Given that these transporters are endocytosed in response to partially different stimuli, this observation opens the interesting issue of how ArtA is recruited for the turnover of specific transporters.