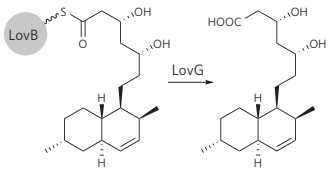
BIOSYNTHESIS

Set me free

Angew. Chem. Int. Ed. Engl., published online 7 May 2013; doi:10.1002/anie.201302406



Biosynthesis of the polyketide lovastatin requires the action of two highly reducing iterative type I polyketide synthases to construct nonaketide and diketide fragments that are coupled together in late stages of the pathway. However, the mechanism by which the nonaketide intermediate dihydromonacolin L acid is released from the LovB synthase is not known as there are no annotated thioesterases in the biosynthetic gene cluster that would be expected to fill this role. Xu *et al.* reinvestigated the *lov* gene cluster and identified *lovG*—annotated as a member of the esterase-lipase family of serine hydrolases—as a possible candidate. A *DlovG* mutant of *Aspergillus terreus* showed substantially attenuated production of lovastatin. *In vitro* assays and heterologous expression of LovG, LovB and an enoylreductase LovC directly demonstrated production and release of dihydromonacolin L acid in the presence of LovG, supporting the proposed activity. To determine whether LovG might have a proofreading function, akin to that described for the thioesterase domain in aflatoxin biosynthesis, the authors examined the products formed in the absence of LovC. LovB alone primarily released two incomplete products, but the addition of LovG yielded three additional products that were consistent with incorrectly formed structures. These results identify the missing thioesterase in the pathway and, as shown in preliminary experiments, will facilitate microbial production of this important natural product. *CG*

CELL BIOLOGY

Viruses get an ESCRT

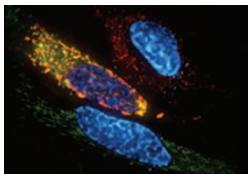
Dev. Cell, published online 28 May 2013; doi:10.1016/j.devcel.2013.04.003

Lysobisphosphatidic acid (LBPA) is a phospholipid abundant in the cargo-containing intraluminal vesicles (ILVs) that are contained within late endosomes and have the characteristic appearance of multivesicular bodies (MVBs). Viruses such as vesicular stomatitis virus (VSV) enter cells via endocytosis into endosomes, where fusion of the viral envelope with the ILV membrane releases the viral RNA into ILVs. To avoid degradative lysosomes, virus components enter the cytoplasm by back-fusion with the MVB membrane, but the details of this mechanism are unclear. Bissig *et al.* found that the interaction of ALIX with LBPA-containing membranes is enhanced by electrostatic and hydrophobic interactions and is calcium dependent, with Ca^{2+} and LBPA sharing the same binding domain as ALIX, but at distinct sites. The authors applied an algorithm to predict a membrane-interaction site within this domain of ALIX and could also predict the Ca^{2+}-binding site; both sites were verified with mutational analysis and competition studies. Biochemical studies, combined with Trp fluorescence, small-angle X-ray scattering and CD experiments, suggest that a conformational change in ALIX, controlled and stabilized by Ca^{2+}, leads to a conformational state of ALIX that may insert into the late endosome bilayer during LBPA engagement. The authors mapped domains in ALIX important for binding to Ca^{2+} and LBPA and monitored infection by VSV to conclude that LBPA recruits Ca^{2+}-bound ALIX onto late endosomes and that these interactions as well as the ALIX–ESCRT-III interaction are necessary for delivery of viral components to the cytosol during infection. *MB*

CELL DEATH

NonALKylating activity

Genes Dev. **27**, 1089–1100 (2013)



JENNIFER JORDAN & DRAGONY FU

ALKBH7 is a mammalian homolog to the bacterial AlkB dioxygenase that repairs alkylated DNA, but it lacks detectable DNA repair activity, so its biological function remains unknown. Fu *et al.* show that stable genetic knockdown of ALKBH7 confers resistance to cell death in response to DNA-damaging agents. Overexpression of ALKBH7 restored sensitivity and necrotic cell death to ALKBH7-depeleted cells. However, in distinct cell lines, ALKBH7 depletion did not confer protection against apoptosis, indicating that ALKBH7 depletion is specifically protective against necrotic death. In cells that would normally undergo necrosis, DNA-damaging agents induced a short-lived drop in ATP and NAD^+ when ALKBH7 was depleted, indicative of transient hyperactivation of PARP, an enzyme activated both in response to DNA damage and during necrosis. Supplementation with NAD^+ or application of PARP inhibitors blocked necrosis, whereas small-molecule inhibitors of the NAD^+ salvage pathway restored necrosis to ALKBH7-depleted cells. These experiments indicate that ALKBH7 disrupts cellular bioenergetics and may therefore affect mitochondrial function. Additional experiments indicated that blockade of mitochondrial respiration (but not glycolysis) disrupted ALKBH7-dependent necrosis. Although the precise activity of ALKBH7 in necrotic cell death remains to be determined, these studies indicate that selective activation of ALKBH7 might complement DNA-damaging chemotherapeutic agents to promote tumor cell death. *AD*

MICROBIOLOGY

I'm beginning to see the light

Nat. Struct. Mol. Biol., published online 2 June 2013; doi:10.1038/nsmb.2597

Rhodobacter sphaeroides is able to generate energy via aerobic respiration (when O_2 is abundant) or photosynthesis (when O_2 is scarce). The O_2- and light-sensing protein AppA and the transcriptional repressor PpsR regulate the expression of photosynthetic genes in this microorganism. Winkler *et al.* determined the X-ray crystal structures of AppA, PpsR and the AppA–$PpsR_2$ complex, performed hydrogen-deuterium exchange experiments in the presence and absence of light, and carried out functional studies to characterize the structural changes that govern the transition from aerobic respiration to photosynthetic energy production. Their data suggest that $PpsR_8$ and the AppA–$PpsR_2$ complex are able to bind the promoter region of specific genes. When O_2 is abundant, expression of AppA is low, and the predominant species *in vivo* are $PpsR_2$, $PpsR_4$ and a $PpsR_8$–DNA complex; this $PpsR_8$–DNA complex directly represses the transcription of photosynthetic genes. When the bacterium is in a dark, O_2-scarce environment, expression of AppA is increased; AppA can bind PpsR and DNA, forming an AppA–$PpsR_2$–DNA complex that prevents formation of the $PpsR_8$–DNA complex and allows transcription of photosynthetic genes to occur. A shift of environmental conditions from darkness back to light reduces the affinity of AppA–$PpsR_2$ for DNA, increasing the likelihood that the $PpsR_8$–DNA complex will reform, once again repressing the transcription of photosynthetic genes. *JMF*

© 2013 Nature America, Inc. All rights reserved.