METABOLIC ENGINEERING

A run in nylon

Met. Eng. doi:10.1016/j.ymben.2014.05.007

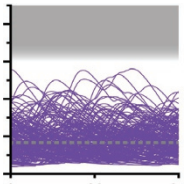
Bio-based production of polyamide (nylon) precursors stands to eliminate waste and inefficiencies from petrochemical-based processes. The material properties of polymers incorporating diaminopentane have made this building block a target of metabolic engineering processes, but existing strategies have demonstrated only limited yields or include undesired byproducts. Kind *et al.* now report a strategy beginning with a known strain, *Corynebacterium glutamicum* LYS-12, that overproduces lysine. The addition of lysine decarboxylase and deletion of an N-acetyltransferase led to the desired product in 37% yield, whereas deletion of the lysine exporter gene and overexpression of a permease to increase diaminopentane export further increased the molar yield to 41%. Testing of the new strain DAP-16 in a fed-batch process led to a titer of 88 g L^{-1} within 50 h, with trehalose as the only major byproduct. During this process, the authors noted the importance of supplying sufficient ammonium, presumably to support high flux en route to lysine through diaminopimelate dehydrogenase, which has a low affinity for ammonium. Testing of several extraction conditions identified *n*-butanol as a suitable solvent, with a two-step distillation leading to >99.6% pure product. Examination of the diaminopentane polymerized with sebacic acid showed favorable comparisons to other polyamides, including a lower density and higher transparency though slightly lower heat distortion temperature. *CG*

ANTIVIRALS

Turn up the noise

Science **344**, 1392–1396 (2014)

SCIENCE



The ability of HIV-1 to remain in a proviral latent state for extended periods, escaping antiviral drug action, necessitates lifelong therapy for infected individuals. Reactivation of latent virus to an actively replicating state in combination with traditional antivirals has been proposed as an eradication strategy. Small molecules exist that promote HIV reactivation, but these have all failed to fully reactivate latent virus. Dar *et al.* sought to capitalize on transcriptional noise—the stochastic nature of gene expression that can generate high variability in HIV gene products—to identify new compounds that can reactivate latent HIV. They proposed that compounds that could increase stochastic fluctuations in transcription from the HIV long terminal repeat (LTR) promoter would synergize with known transcriptional activators such as TNF that increase the mean expression level, to enhance HIV reactivation. The authors performed a small-molecule screen on a T-cell line containing GFP under the control of the LTR promoter with a library of 1,600 FDA-approved compounds and found 100 compounds that enhance GFP expression noise. Using this GFP system, the authors further showed that known chromatin modifiers that affect latency acted as noise enhancers, supporting their model that synergistic combinations of noise enhancers and activators can reactivate latent HIV. Among the new noise-enhancing compounds, >70% synergized with TNF to enhance reactivation. 'Noise screening' represents a new avenue to defining synergistic drug combinations in any system where fate choices are made in response to stochastic gene expression. *MB*

NEUROSCIENCE

Your channels on drugs

J. Neurosci. **34**, 8605–8611 (2014)

Voltage-gated calcium channels (VGCCs) are involved in regulating normal neurotransmission and have also been implicated in the mechanisms of plasticity and behavior that are associated with relapse to drug abuse. VGCCs are heteromeric complexes where the channel pore consists of a pore-forming subunit and ancillary subunits such as α2δ-1 that modulate channel kinetics as well as their trafficking and turnover. Repeated exposure to drugs of abuse such as ethanol and methamphetamine increase α2δ-1 expression in the cerebral cortex, and preliminary data suggest a similar upregulation with cocaine. Spencer *et al.* now verify α2δ-1 upregulation after cocaine self-administration and withdrawal (extinction) in the core subcompartment of the nucleus accumbens of rats, the reward center for motivationally relevant stimuli. They also found increased levels of TSP-1, an endogenous α2δ-1 ligand, suggesting that the increase in α2δ-1 is a functional one. The authors next studied an animal model of relapse, looking at drug-seeking by animals induced by drug-associated cues or drug injection. Using this model, they found that gabapentin, a pharmacological α2δ-1 antagonist that inhibits function of the channel, led to decreased cocaine-primed cocaine seeking. These results suggest that elevated α2δ-1 has a role in cocaine-reinstated drug seeking. *MB*

STEM CELLS

Clampdown

Mol. Cell. **54**, 1034–1041 (2014)

ELSEVIER

FGF signaling promotes the differentiation of mouse embryonic stem cells into primary endoderm through activation of downstream adaptor proteins such as Grb2 and Sos1, which stimulate the Ras-ERK pathway. Loss of Grb2 activity results in the maintenance of pluripotency. Grb2 is part of a protein network that interacts via its SH2 domain with many different tyrosine phosphorylated proteins during stem cell differentiation. However, determining which of its numerous interactions are sufficient for differentiation remained unclear. Yasui *et al.* applied the previously described affinity clamp approach to develop synthetic variants of the Grb2 SH2 domain that bound specifically to one single phosphotyrosine motif. They identified three SH2 domain variants, which they called pY-clamps, that respectively bound a phosphotyrosine motif of the tyrosine phosphatase Ptpn11 (Y580), the scaffold protein Shc1 (Y239/40) and the oncoprotein Bcr (Y177). Notably, the authors found that the chimeric Grb2 containing the Ptpn11 pY-clamp and not those with the other pY-clamps was sufficient to induce primary endoderm differentiation in a Grb2-deficient background. Further studies verified that upstream FGF signaling promoted the phosphorylation of Ptpn11 at Y580, which interacts with the Grb2 SH2 domain to activate ERK signaling. Future applications of this rescue-of-function approach with affinity clamps may reveal additional specific SH2-phosophotyrosine interactions that mediate unique biological processes. *GM*

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