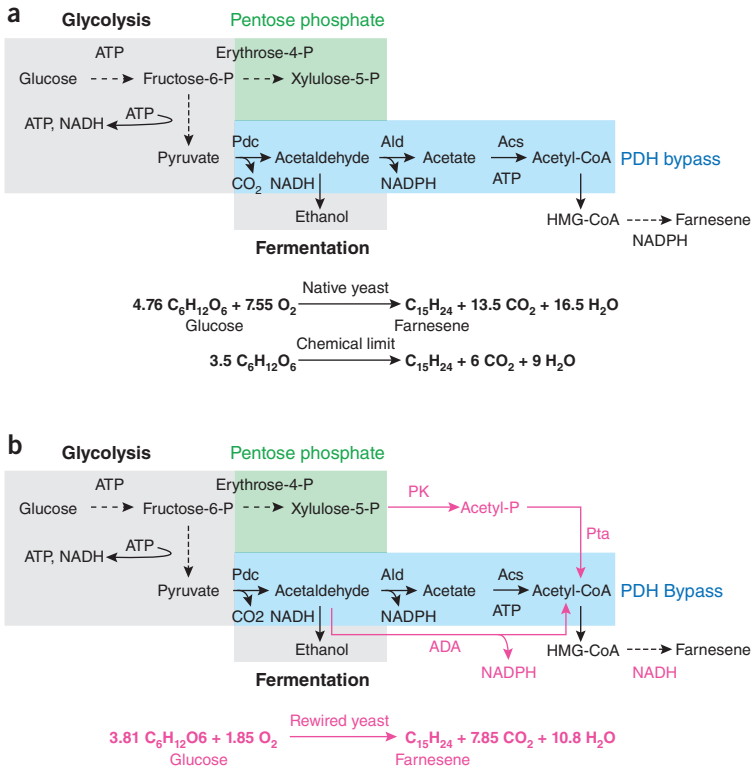

Figure 1 Carbon and oxygen use in yeast central metabolism. (**a**) Native yeast cannot efficiently produce the isoprenoid farnesene from acetyl-CoA owing to limitations in carbon and oxygen usage. An earlier-generation strain from Amyris uses an overexpressed PDHc bypass (shaded blue) to produce acetyl-CoA along with a NADPH-selective HMG-CoA reductase. (**b**) The simultaneous introduction of two new pathways in *S. cerevisiae* to introduce heterologous non-oxidative glycolysis and alternative pyruvate dehydrogenase (PDH) bypass pathways (pink) allows glucose flux to be balanced through these two pathways to achieve an optimal stoichiometry for farnesene production. Reaction stoichiometries are not balanced and the pentose phosphate pathway is simplified for clarity in this schematic. Dashed arrows indicate multiple steps that are not shown. P, phosphate; HMG-CoA, hydroxymethylglutaryl coenzyme A; Pdc, pyruvate decarboxylase, ACS, acetyl-CoA synthetase; PK, phosphoketolase, Pta, phosphotransacetylase; ADA, aldehyde dehydrogenase acylating.

pathway operates without loss of carbon dioxide, thereby increasing the carbon flux into farnesene but without producing the ATP and NADH needed to synthesize farnesene.

To balance the energetic deficiency caused by this shunt pathway, the authors added an acetaldehyde dehydrogenase (acylating), thereby avoiding the use of ATP in the native pyruvate dehydrogenase (PDH) bypass (**Fig. 1b**)[10]. As this enzyme generates NADH instead of NADPH, a compensatory cofactor switch was also made in the downstream isoprenoid pathway. Together, these two new connections redistribute glucose flux to balance each other out and reduce carbon loss and production of excess NAD(P)H, which reduces oxygen demand via aerobic respiration.

These changes were implemented to produce a next-generation isoprenoid strain after identifying candidates for these enzyme activities. Genes encoding phosphoketolase (PK), phosphotransacetylase (Pta) and aldehyde dehydrogenase acylating (ADA) were selected from various organisms and subjected to a genetics screen to choose the ortholog with the best *in vivo* performance in yeast. An NADH-selective hydroxymethylglutaryl-CoA reductase (HMGr) to replace the existing NADPH-selective HMGr in the mevalonate pathway was then found through *in vitro* biochemical characterization. These four new pathway components were incorporated to generate a new strain that was benchmarked against the previous generation containing only the overexpressed PDH bypass[8]. A direct comparison showed a 21% improvement in yield and a 77% improvement in productivity.

To confirm that these enhancements arose from an alteration in pathway stoichiometry, the authors compared the amounts of glucose and oxygen consumed with the amounts of biomass and farnesene produced. They concluded that the changes made to central metabolism enabled production of farnesene with 25% less glucose and 75% less oxygen.

The practical implications of this work are important for industrial fermentation as yields are key drivers of costs and profit margins. The success of the authors' approach also highlights the complexities in engineering central carbon pathways and the role of modeling in optimizing yield and productivity, as a single pathway may not provide the energetic balance required for commercially viable processes.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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