STEROIDS

Building biosynthetic routes in yeast

In 1952, it involved around 40 steps of synthesis. Now, hydrocortisone production can be completed without any chemical synthesis at all by engineering the yeast *Saccharomyces cerevisiae* to biosynthesize the complex product from scratch, according to new research in the February issue of *Nature Biotechnology*.

Hydrocortisone is the major adrenal mammalian glucocorticoid and is an important intermediate in steroid drug synthesis that is used in large quantities in industry. Although the original chemical synthesis of the molecule was very cumbersome, hydrocortisone manufacture now requires just nine steps, including some bioconversions. In mammals, biosynthesis takes place in the adrenal cortex in two different compartments, the ER and the mitochondria. The pathway starts in the mitochondria with the cleavage of the cholesterol side chain to make pregnenolone, which then moves to the ER where it is oxidized and hydroxylated by three more enzymes to make 11-deoxycortisol, before moving back to the mitochondria for the final conversion to hydrocortisone.

Yeast neither makes cholesterol, nor takes up exogenous sterols from the medium during aerobic respiration. In order to produce molecules resembling cholesterol from a simple carbon source, such as glucose or ethanol, biosynthesis of its major sterol, ergosterol, was rerouted using the plant enzyme Δ7 reductase. The resulting products, campesterol and brassicasterol, were converted to pregnenolone by heterologous mitochondrial enzymes. Additional engineering mimicked the adrenal biosynthesis of pregnenolone to hydrocortisone (through intermediates progesterone, 17-hydroxyprogesterone and 11-deoxycortisol) and involved mainly membrane-bound enzymes: overall, three members of the P450 superfamily of monooxygenases, 3β-hydroxy steroid dehydrogenase/isomerase and three electron carriers were expressed. During the conversion, two major unwanted side reactions were identified: the esterification of pregnenolone and the ketoreduction of 17-hydroxyprogesterone. The endogenous yeast genes responsible for these changes were inactivated, bringing the total number of engineered genes in a single micro-organism to 13. The major sterol product of the engineered yeast was hydrocortisone (up to 70% of total steroids), with some by-product of 11-deoxycortisol and corticosterone.

This work represents the most complex engineered pathway described to date and the first example involving several coupled membrane enzymes in a eukaryotic cell. Such engineered microbes could be developed for low-cost industrial processes to substitute chemical approaches in the synthesis of corticoid drugs. In addition, they represent a powerful tool for deciphering steroid synthesis regulation and balance.

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References and links

ORIGINAL RESEARCH PAPER
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FURTHER READING
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WEB SITES
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History of cortisone and related compounds