A Balancing Act for Taxol Precursor Pathways in *E. coli*

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Science **330**, 44 (2010);
DOI: 10.1126/science.1195014

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

several functions of STIM proteins in activating store-operated Ca^{2+} channels (*11*).

In their studies, Wang *et al*. and Park *et al*. examined the role that STIM proteins might also play in regulating voltage-gated Ca^{2+} channels. Specifically, they examined one medically important member of this diverse family, known as the $Ca_V1.2$ subunit (α_{1C}). $Ca_V1.2$ forms the so-called L-type Ca^{2+} channels found in a wide array of cells, including those in the cortex, hippocampus, cerebellum, neuroendocrine system, heart, and arterial smooth muscle. Mutations in the $Ca_V1.2$ gene *CACNA1C* cause Timothy and Brugada syndromes, which are associated with life-threatening cardiac arrhythmias. A number of treatments, including the drugs verapamil, diltiazem, and various dihydropyridines, specifically target $Ca_V1.2$ channels.

The two groups found that the sequence of events involving STIM1 begins in the same way for both store-dependent and voltage-regulated channels, but the end result is very different. After Ca^{2+} store depletion in the ER, a structure known as the EF hand of STIM1 unbinds Ca^{2+}, and STIM1 oligomers translocate to form clusters immediately adjacent to the plasma membrane at ER-PM junctions. If voltage-gated Ca^{2+} channel proteins are expressed, they, like Orai subunit proteins, accumulate adjacent to STIM1. There, STIM1 and $Ca_V1.2$ interact via specific domains that protrude into the cytosol: a CRAC-activating domain (CAD) in STIM1 and the C terminus of $Ca_V1.2$. CAD opens Orai1 channels, but—as the two new papers show—it inhibits $Ca_V1.2$ channel activity in two ways: acutely (immediately) by physical interaction, and more slowly by causing internalization, a process that inhibits the channel and results in subsequent degradation of the channel protein. The two reports differ with respect to the relative importance of the acute and internalization phases (Wang *et al*. find that the acute phase is strong, Park *et al*. less so), but the outcome is the same: STIM1 regulates Orai1 and $Ca_V1.2$ reciprocally. In inexcitable lymphocytes, where STIM1 is relatively abundant, voltage-gated Ca^{2+} channels are inhibited and Orai1 is activated. In neurons and presumably other excitable cells, STIM1 is not so abundant and voltage-gated Ca^{2+} channel activity predominates.

As the activator for one Ca^{2+} channel (Orai1) and an inhibitor for another ($Ca_V1.2$), STIM1 assumes a new functional importance. In excitable cells, membrane depolarization (a change in electrical charge) activates Ca^{2+} influx through voltage-gated Ca^{2+} channels, but in lymphocytes depolarization actually inhibits Ca^{2+} influx through Orai channels, because the electrical driving force for Ca^{2+} to enter is reduced. This potentially resolves a long-standing controversy regarding the type of Ca^{2+} channel in lymphocytes (*12*). Even if subunits of voltage-gated Ca^{2+} channels are expressed, they are not functional because of inhibition by STIM1. More important, STIM1's reciprocal functional interaction guarantees that only one type of Ca^{2+} channel or the other will be active.

Many questions remain concerning the generality of the findings. Does STIM1 block other voltage-gated Ca^{2+} channel family members? In native tissue, $Ca_V1.2$ subunits interact with numerous other cellular proteins that help with subcellular localization and modulation. Do they physically or functionally protect the channels from being inhibited by STIM1? When STIM1 is knocked out in mice or in rare patients with mutations of STIM1, what are the consequences for voltage-dependent Ca^{2+} channel function in vivo? Answers to these questions should further improve our understanding of how Ca^{2+} signaling is regulated.

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10.1126/science.1196348

CHEMISTRY

A Balancing Act for Taxol Precursor Pathways in *E. coli*

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A fermentation process that balances the activities of several bacterial and plant enzymes makes a precursor to an anticancer drug in substantial quantities.

Taxol (paclitaxel) is a widely used cancer drug that was first isolated from the bark of the Pacific yew tree, *Taxus brevifolia*. In 1991, during early stages of its clinical use, 130 kg of Taxol were extracted from 1000 tons of bark, which required cutting down more than 500,000 mature Pacific yew trees (*1*). Fortunately, Taxol is presently made by less destructive methods, either through chemical conversion of a related molecule derived from needles of the more prevalent European yew, *T. baccata* (*2*), or from cultured plant cells (*3*). Nonetheless, both these plant-based processes are difficult, and this valuable drug remains expensive. On page 70 of this issue, Ajikumar *et al*. (*4*) have made progress toward making Taxol in *Escherichia coli*, biotechnology's workhorse bacterium, in substantial quantities by balancing several enzymatic pathways to make its complex multicyclic core (see the figure).

Taxol belongs to a large family of natural products called terpenes, which also includes artemisinin (a malaria drug), carvone (a flavoring agent), and pinene (the main constituent of turpentine). The carbon skeletons of all terpene molecules (taxadiene, in the case of Taxol) are made from two closely related five-carbon precursors, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are made in virtually all cells from substrates such as glucose. Each terpene skeleton then undergoes a series of further modifications by a set of enzymes that execute a sequence of reactions that leads to the complex natural product. The conversion of IPP and DMAPP into Taxol is a more complex process that so far has been seen only in cells from the Pacific yew bark.

Nature has two chemical processes for converting glucose into IPP and DMAPP. The "mevalonate pathway" operates in animal cells, as well as in microbes such as yeast. It was the target of a related recent effort aimed at reducing the cost of artemisinin manufacture (*5*). An unrelated process, the "nonmevalonate" pathway, synthesizes IPP and DMAPP in bacteria and plant cells.

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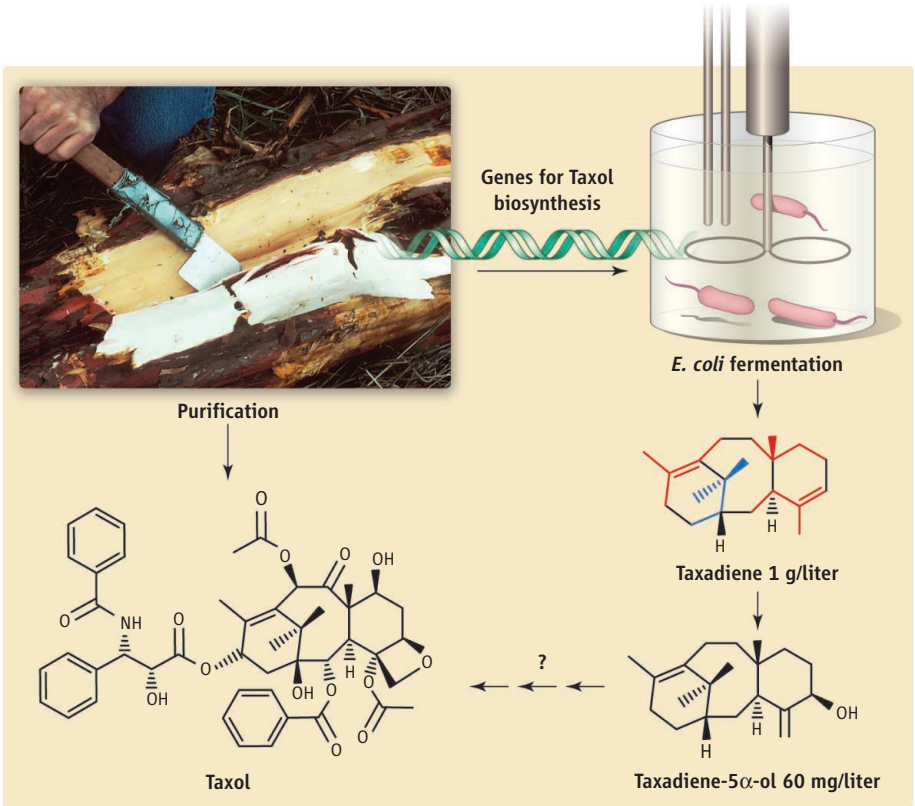

Rebuilding a biosynthetic pathway. The anticancer drug Taxol was originally obtained from the bark of the precious Pacific yew tree. Ajikumar *et al*. now report that a key intermediate in its chemical synthesis, taxadiene, can be abundantly obtained from *E. coli* by fermentation. The three terpene groups in the carbon-bond backbone from isopentenyl pyrophosphate are highlighted in red, and those from dimethylallyl pyrophosphate are highlighted in blue. The hydroxylation of this molecule to form taxadiene-5α-ol was also achieved, even though such reactions are usually difficult in fermentation processes. Further work will be needed to complete a chemical synthesis or an enzymatic pathway in *E. coli* that leads to Taxol.

Having chosen *E. coli* as their host for engineered Taxol biosynthesis, Ajikumar *et al*. targeted its native nonmevalonate pathway. They also genetically transplanted the first three enzymes in the Taxol pathway from the Pacific yew into *E. coli*. They developed a remarkably efficient fermentation process for taxadiene that also afforded respectable quantities of the next molecule in the Taxol pathway, taxadiene-5α-ol.

Specifically, *E. coli* synthesizes taxadiene with a volumetric productivity of 8 mg/liter per hour, and in lab fermentors, taxadiene accumulated to a final concentration of 1 g/liter. Previous attempts to make taxadiene in *E. coli* only yielded 1.3 mg/liter, with a correspondingly lower volumetric productivity (*6*). Although taxadiene-5α-ol only accumulated to a final concentration of 60 mg/liter, this is no ordinary accomplishment, in that chemical or genetic hydroxylation of inert hydrocarbons such as taxadiene is notoriously difficult. Several additional enzyme-catalyzed reactions are required to convert the taxadiene-5α-ol core into Taxol, although at least some of these transformations may be chemically feasible.

Multiple factors contributed to this engineering success. First, a dramatic improvement in the rate of IPP and DMAPP synthesis was attained through careful optimization of the absolute and relative levels of four key enzymes in *E. coli*. Second, the intracellular activities of two plant enzymes (GGPP synthase and taxadiene synthase) were enhanced substantially through codon optimization and deletion of an N-terminal plastid transit peptide. Third, the upstream segments to IPP/DMAPP and downstream segments to taxadiene were balanced by systematically varying promoter strength, plasmid copy number, and genotype. This endeavor appears to have set a new productivity standard in the field. Whereas most investigators typically choose optimal conditions by making and screening a relatively small number (<10) of alternative multigenic configurations, the authors balanced carbon flux through the two segments with far greater precision by evaluating more than 30 such constructs.

For the final step, that of making taxadiene-5α-ol in *E. coli*, the authors needed to coax a cytochrome P450 oxygenase from the Pacific yew to work in a different cellular environment. In plants, these metalloenzymes partner with dedicated, membrane-bound cytochrome P450 reductases. Because such reductases cannot be expressed as readily in *E. coli*, Ajikumar *et al*. used a previously developed strategy (*7*) to engineer a chimeric enzyme harboring both oxygenase and reductase activities without a membrane anchor.

The authors also took advantage of an unexpected observation—the concentrations of an unrelated metabolite, indole, correlated inversely with taxadiene productivity. Follow-up experiments revealed that this inverse correlation primarily affected IPP and DMAPP productivity, and the authors exploited this empirical relationship as they optimized taxadiene biosynthesis. Their observation raises several intriguing and still unanswered questions. What is the mechanistic link between IPP/DMAPP and biosynthesis of tryptophan, the likely source of indole? Is this regulatory strategy unique to the present problem, or does it represent a more general mechanism for controlling carbon flow through a cell's metabolic networks? Finally, what are its implications for producing other useful chemicals through fermentation and other biotechnology routes? *E. coli* still has more secrets to reveal when it comes to designing the perfect chemical factory.

The same approach of Ajikumar *et al*. could be applied to a number of important terpene natural products derived from plants. Synthetic chemists can convert pinene into Taxol (*8*, *9*) after many reaction steps, so there is no need to restrict possible targets to products that have been isolated from natural sources. Complex "green" chemicals could be made more affordably if feedstocks like taxadiene and taxadiene-5α-ol become commonly available.

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10. This work was supported by NIH grant R01 CA 077248 to C.K.

10.1126/science.1195014