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GENE EXPRESSION

Hunting out hypoxia

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A targeted approach to dosing a drug has long been an attractive prospect, with the potential to improve efficacy at a desired target while avoiding side-effects that might result from the drug's actions elsewhere. Now, writing in *ACS Chemical Biology*, Stuart Conway and co-workers describe small molecule drugs that induce gene expression in hypoxic (low oxygen) environments.

"I've been interested in targeting drugs for my entire independent career," says Conway. "We made several light-activated or 'caged' compounds, but it was when I started collaborating with Ester Hammond that I first became interested in drugs that would be activated under hypoxic conditions."

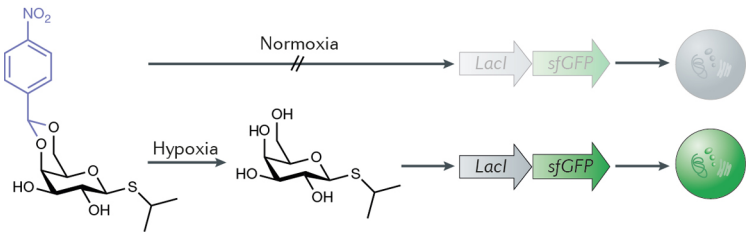
Although light activation can work well in cell studies, it is challenging to achieve in clinical settings as the short wavelengths required to activate organic molecules have low tissue penetration. "Solid tumours are the most likely to undergo metastasis, and also have limited blood supply, making them difficult to treat," explains Conway. "But the poor blood supply also results in a hypoxic environment and we wanted to use this difference in chemical environment to activate a prodrug."

Nitroreductase enzymes can catalyse a one-electron reduction of nitroaryl groups in prodrugs to form a radical anion. In oxygen rich conditions this radical is rapidly reoxidized, but in hypoxic conditions further reduction to an aminoaryl group occurs, ultimately leading to cleavage. This concept has been applied previously to prodrugs but Conway and co-workers are the first to apply the concept to turn on and off gene-expression. "We realized that this was potentially very powerful as it would mean we could place the production of any protein under the control of hypoxia," he says.

To begin, the team needed to show that hypoxia-induced cleavage of a nitroaryl group was possible in bacteria — it was known to work in mammalian cells but bacterial cells are known to be more reducing. They were quickly able to show that a nitrobenzyl group had the correct reactivity profile using a nitrobenzyl-protected fluorescent dye (resofurin). They then went on to prepare several prodrug versions of the well-known gene expression inducer isopropyl 1-thio-β-D-galactopyranoside (IPTG).

IPTG can induce gene expression by competitively binding to the protein LacI, which otherwise binds to DNA and inhibits transcription. Five prodrug versions of IPTG were prepared — four in which a single hydroxyl group was protected with the 4-nitrobenzyl group and a fifth in which two hydroxyls are tied up in a 4-nitrobenzylidene acetal.

These prodrugs were then tested to examine the expression of green fluorescent protein (GFP) in a plasmid containing an IPTG inducible promoter. If successful, no green fluorescence would be observed at normal oxygen conditions (21%), but in hypoxic conditions the IPTG would be released, beginning a series of reactions that ends in production of green fluorescence.



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The prodrugs were then tested in both normoxic and hypoxic conditions. The 6-hydroxyl protected benzyl ether induced green fluorescence under both conditions, indicating that this protection was insufficient to prevent the IPTG binding to LacI. The other three benzyl ether derivatives showed no production of GFP under normoxia, but no increase was observed under hypoxia either, which was attributed to low levels of IPTG being released. The acetal, meanwhile, showed no fluorescence with 1–21% O_2, partial induction at 0.5% O_2 and high levels of expression at 0.1% O_2.

Currently, the experiments have only been performed using bacterial cells. "Our next step is to try to transfer this technology into mammalian cells," says Conway. "We're then very excited to begin investigating the role of expressing genes that are down-regulated in hypoxic conditions, or genes that are not expressed in cancer cells."

Conway is keen to highlight that the work was all carried out by a DPhil student, Sarah Collins, who recently graduated from his research group. "This project, and much of our other work, is done in collaboration with Ester Hammond," he explains. "It is only through this collaboration, and our complementary skills, that we have been able to tackle problems that involve synthetic chemistry, chemical biology, molecular biology and cell biology."

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ORIGINAL ARTICLE Collins, S. L., et al. Hypoxia-activated small molecule-induced gene expression. *ACS Chem. Biol.* https://doi.org/10.1021/acschembio.8b00858 (2018)