SIGNALLING

Friendly rivalry

Move over kinases — a new way of phosphorylating has been found. It comes courtesy of inositol pyrophosphates and is non-enzymatic, but its existence might be highly significant in terms of intracellular signalling. Solomon Snyder and his group report their evidence in *Science*.

Inositol pyrophosphates, such as diphosphoinositol pentakisphosphate (5PP-$InsP_5$, or $InsP_7$) and bis-diphosphoinositol tetrakisphosphate ($[PP]_2$-$InsP_4$, or $InsP_8$), are formed by inositol hexakisphosphate ($InsP_6$) kinases (InsP6Ks). The relatively high values for the free energy of hydrolysis of pyrophosphate bonds implied that inositol pyrophosphates might be able to donate phosphates.

To test this, the authors created an artificial $InsP_7$ — $5\beta[^{32}P]InsP_7$ — which was radioactively labelled at the β position of the pyrophosphate group. In the presence of $5\beta[^{32}P]InsP_7$, several proteins from mouse and fly tissues, and from yeast — but not bacteria — were phosphorylated. Such phosphorylation was enhanced by certain phosphatase inhibitors, consistent with the normal dephosphorylation of phosphorylated proteins by phosphatases; and the process required divalent cations, preferably Mg^{2+}.

So which proteins get phosphorylated by $5\beta[^{32}P]InsP_7$? NSR1, a nucleolar protein that is involved in ribosome assembly and export, and YGR130c, the function of which is unknown, were both phosphorylated by $5\beta[^{32}P]InsP_7$ in yeast. Both contain stretches of serines that are surrounded by acidic residues. Homology searching uncovered another nucleolar protein, SRP40, that has an acidic serine region, and this, too, was phosphorylated by $5\beta[^{32}P]InsP_7$.

Studies in kinase-mutant yeast cells ascertained that protein kinases weren't necessary for $5\beta[^{32}P]InsP_7$-mediated phosphorylation. Heating increased the rate of the phosphorylation, which also implied that the process was non-enzymatic (although an initial physiological temperature was needed to ensure the phosphorylation site was in the appropriate conformation). The authors suspected that phosphorylation occurred on serine residues, as a phosphothreonine antibody failed to label $5\beta[^{32}P]InsP_7$-phosphorylated proteins. They used the mammalian homologue of SRP40, Nopp140, for further analysis, and found by mutational analysis that multiple serines were phosphorylated. Acidic residues surrounding these serines, along with lysines, were also required.

Finally, Snyder and colleagues investigated the situation in intact cells. In yeast extracts that lacked InsP6K, NSR1 phosphorylation *in vivo* was reduced by ~60%, which indicates that endogenous $InsP_7$ physiologically phosphorylates NSR1 *in vivo*. The inositol pyrophosphate $InsP_7$ is therefore a physiological phosphate donor in eukaryotic cells, which not only increases the modes of inositide-mediated signalling, but also introduces some friendly rivalry with kinases when it comes to phosphorylation.

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ORIGINAL RESEARCH PAPER Saiardi, A. *et al.* Phosphorylation of proteins by inositol pyrophosphates. *Science* **306**, 2101–2105 (2004)

TECHNOLOGY WATCH

Fruitful fluorescence

Fluorescent proteins are invaluable tools in cell-biology research, with green fluorescent protein (GFP) being the most prominent. Red fluorescent proteins entered more common use, though, when monomeric (m)RFP1 was created from DsRed, a tetrameric *Discosoma* sp. red fluorescent protein. However, mRFP1 leaves room for improvement, as it has reduced brightness and photostability compared with DsRed. In *Nature Biotechnology*, Tsien and colleagues now describe how, starting with mRFP1, they have developed a range of new, improved fluorescent proteins.

The authors subjected mRFP1 to numerous rounds of directed protein evolution using manual and fluorescence-activated cell sorting (FACS)-based screening, and produced a range of monomeric yellow, orange and red fluorescent proteins — mHoneydew, mBanana, mOrange, mTangerine, mStrawberry and mCherry. As well as increasing the colour palette, these proteins show improvements in extinction coefficients, quantum yields and photostability, although no single protein is optimal by all criteria. For example, mOrange is the brightest, whereas mCherry offers the fastest maturation and highest photostability (it is ~10-fold more photostable than mRFP1). mCherry is also tolerant to N-terminal fusions (its N and C termini were replaced with those of GFP to prevent the fluorescence decrease that is seen on fusing proteins to mRFP1). This work has therefore filled a gap in the fluorescence rainbow, and the new proteins might well function as a starting point for yet more improvements.

REFERENCE Shaner, N. C. *et al.* Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. *Nature Biotechnol.* **22**, 1567–1572 (2004)

Talented tadpoles

The ability to detect and quantify small numbers of molecules is important to obtain a clear view of cellular processes and for early disease detection. Yet the techniques used to detect biological molecules other than nucleic acids have limited sensitivity and accuracy. So, the developments described by Brent and co-workers in *Nature Methods* are a welcome technological advance.

Although the idea of detecting target binding by bringing DNA sequences to the target and using DNA amplification has been around for some time, problems with antibody heterogeneity and the lack of control over the antibody–DNA attachment site have hampered progress. By adapting the chemistry of inteins — protein domains that are post-translationally excised from polypeptides by a self-catalytic protein-splicing process — Brent and colleagues were able to create chimeric 'tadpole' molecules that are composed of a protein head covalently coupled to a DNA tail. They made different tadpoles that could bind to specific targets, and measured the amount of bound target by quantifying DNA tails using T7-RNA-polymerase-driven transcription, real-time PCR and statistical evaluation. Using various proof-of-principle tests, they showed that this method has "…a dynamic range of detection of more than 11 orders of magnitude and distinguished numbers of molecules that differed by as little as 10%." These talented tadpoles therefore allow the sensitive detection and exact quantification of molecules other than nucleic acids.

REFERENCE Burbulis, I. *et al.* Using protein–DNA chimeras to detect and count small numbers of molecules. *Nature Meth.* **2**, 31–37 (2005)