GENE REGULATION

On target

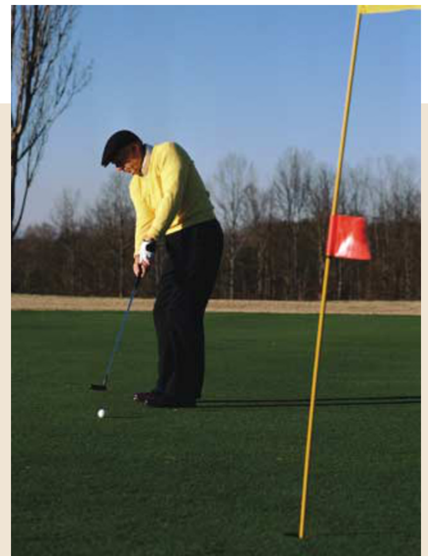
A recent paper in *Nature Cell Biology* describes the identification of a gene — *SMYD3* (*SET- and MYND-domain-containing-3*) — that encodes a histone methyltransferase, and that is overexpressed in colorectal and hepatocellular carcinoma cell lines and tissues.

In support of a role for SMYD3 in tumorigenesis, Yusuke Nakamura and colleagues found that SMYD3 overexpression induced cell growth *in vitro*, whereas knockdown of SMYD3 expression with small interfering RNAs suppressed the growth of several colorectal and hepatocellular carcinoma cell lines. In addition, microarray analysis highlighted the upregulation of several genes — including proto-oncogenes, cell-cycle regulatory genes and developmental genes — in cells that were overexpressing SMYD3.

Using yeast two-hybrid and immunoprecipitation analyses, the authors showed that SMYD3 binds to the heat-shock protein HSP90A and, indirectly, to RNA polymerase II. SMYD3 specifically methylates histone H3 at lysine 4 — this type of histone methylation is important for transcriptional activation. In addition, the catalytic activity of SMYD3 was induced in the presence of HSP90A, which indicates that it functions as a co-factor for histone-methyltransferase activity.

But how are these histone modifications targeted to a particular site? Nakamura and co-workers showed that SMYD3 binds directly to a specific DNA sequence. One of the target genes that is upregulated by SMYD3, the homeobox gene *Nkx2.8*, indeed contains such a SMYD3-binding sequence in its promoter region. And, chromatin-immunoprecipitation experiments showed that SMYD3 binds to this region *in vivo*.

So, SMYD3 seems to form a transcriptional complex with RNA polymerase II and targets its histone lysine-methylation activity to specific DNA sequences by binding to the



promoter region of target genes like "…a transcription factor containing histone methyltransferase activity". In addition, SMYD3 could be an ideal therapeutic target in the treatment of certain tumours.

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References and links

ORIGINAL RESEARCH PAPER Hamamoto, R. *et al.* SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells. *Nature Cell Biol.* 4 July 2004 (doi:10.1038/ncb1151)

CYTOSKELETON

Turn it on

They're either on or off. Rho GTPases regulate various cellular functions, and their ability to bind GTP or GDP, and hence their activity, is controlled by guanine nucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs), respectively. But even the most powerful GEF can't activate a Rho GTPase that is bound to a GDP-dissociation inhibitor (GDI). Gary Bokoch's group, though, have ascertained that the serine/threonine kinase p21-activated kinase-1 (PAK1), which is regulated by the Rho GTPases Rac and Cdc42, can. And they explain how.

After noticing that RhoGDI could be phosphorylated *in vitro*, the authors identified the kinase in question as PAK1, and found that PAK1 and RhoGDI strongly co-immunoprecipitated — but only when PAK1 was active. The carboxyl terminus (which contains the kinase domain), but not the amino terminus (which binds to Rac and Cdc42), of PAK1 mediated the interaction, so any indirect interaction through Rac or Cdc42 seems unlikely. The RhoGDI sequence contains two consensus PAK phosphorylation sites, and *in vitro* labelling experiments also identified two potential phosphorylation sites — at Ser101 and Ser174 — which turned out to be phosphorylated by PAK1 *in vivo*.

The burning issue is what phosphorylation by PAK1 does to RhoGDI that is complexed with Rho GTPases. Bokoch's group measured ^{35}S-labelled GTPγS incorporation by the GTPase–RhoGDI complexes with or without PAK1. Without PAK1, RhoGDI inhibits nucleotide exchange, so no [^{35}S]GTPγS was bound. However, adding PAK1 caused phosphorylation of the complex and increased [^{35}S]GTPγS incorporation by Rac1; this didn't occur using a non-phosphorylatable form of RhoGDI. In cells, expressing the catalytically active carboxyl terminus of PAK1 caused RhoGDI to dissociate from Rac1.

Bokoch's group thought that Cdc42 might activate PAK1, which would thereby induce Rac activation by RhoGDI dissociation. This proved correct — cells that expressed constitutively active Cdc42 and a particular non-inhibitory form of PAK1 had high levels of active Rac1; those with an inhibitory PAK1 mutant showed no increase. And the authors found that the activation of Rac1 that is induced by growth factors such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) required PAK-induced phosphorylation of Rac1 to dissociate Rac1 from RhoGDI. So they propose a model in which this PAK-induced dissociation of RhoGDI either initiates Rac signalling (probably through Cdc42-induced PAK activation) or feeds forward to enhance Rac activation.

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References and links

ORIGINAL RESEARCH PAPER DerMardirossian, C., Schnelzer, A. & Bokoch, G. M. Phosphorylation of RhoGDI by Pak1 mediates dissociation of Rac GTPase. *Mol. Cell* **15**, 117–127 (2004)