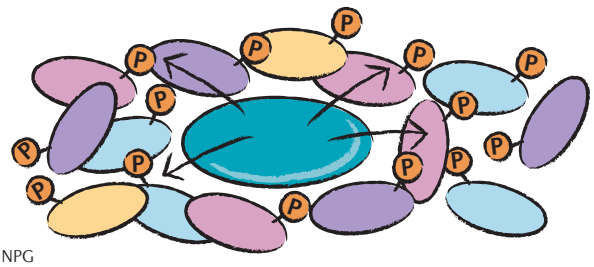
CELL SIGNALLING

One kinase targets many secreted proteins

Many extracellular proteins are phosphorylated, but research into these proteins has been hampered by the 'secret identity' of the kinases that phosphorylate them. Tagliabracci *et al.* now show that the secretory pathway kinase FAM20C phosphorylates the majority of secreted phosphoproteins.

Members of the family of FAM20C-related kinases phosphorylate secreted proteins and proteoglycans, but the extent and mechanism of their action is unclear. The authors characterized FAM20C and revealed that the secreted portion of this peptide is truncated, that *N*-linked glycosylation is required for its correct folding and secretion and that it undergoes autophosphorylation. Previous work suggested that FAM20C phosphorylates Ser in the motif S-X-E/pS (where X is any amino acid and p indicates phosphorylation), and the screening of a 198-peptide array showed that FAM20C strongly prefers Glu at the +2 position of this motif.

> "the secretory pathway kinase FAM20C phosphorylates the majority of secreted phosphoproteins"



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Other FAM20C-related kinases, and those related to the vertebrate lonesome kinase family of secreted kinases, could not phosphorylate S-X-E/pS, suggesting that the specificity of FAM20C is unique among secretory pathway kinases.

The authors next sought FAM20C substrates, first showing that FAM20C must phosphorylate proteins in the lumen of the secretory pathway. Mass spectrometry analysis of serum-free conditioned medium from wild-type and *FAM20C*-knockout HepG2 liver cells showed that more than 50 proteins secreted by the conventional secretory pathway were phosphorylated in wild-type samples; the phosphorylation of ~90% of these proteins was reduced by more than twofold in conditioned medium from *FAM20C*-knockout cells. Thus, most of the phosphoproteins secreted by HepG2 cells are likely to be phosphorylated by FAM20C. Different cell lines secrete different proteins. Indeed, across HepG2 cells, MDA-MB-231 breast epithelial cells and U2OS osteoblast-like cells, the phosphorylation of ~100 secreted proteins was markedly reduced when FAM20C was depleted. The authors propose that 80% of secreted phosphorylated proteins are FAM20C substrates.

Using several *in vitro* and *in vivo* kinase assays, the authors then confirmed the ability of FAM20C (but not a kinase-inactive mutant) to phosphorylate several FAM20C candidate substrates. They also assessed the phosphorylated motifs in FAM20C substrates from HepG2 cells and found that, although most corresponded to S-X-E/pS, FAM20C could also phosphorylate Ser residues outside this motif. Thus, the remit of FAM20C cannot be assessed by screening for this canonical motif alone.

What, then, is the biological significance of FAM20C-mediated phosphorylation? Analysis of gene ontology terms showed that genes involved in wound healing, lipid homeostasis, endopeptidase inhibitor activity, cell adhesion and cell migration are enriched in the list of FAM20C targets. To experimentally confirm some of these results, the authors generated *FAM20C*-knockout MDA-MB-231 cells and found that they were more adherent, and less migratory and invasive, *in vitro* than their wild-type counterparts. Culturing *FAM20C*-knockout cells in media from wild-type cells rescued their migratory defects, suggesting that FAM20C substrates secreted from MDA-MB-231 cells could control MDA-MB-231 cell migration.

The identification of FAM20C as the main kinase for secreted phosphoproteins, and the finding that it phosphorylates extracellular proteins involved in adhesion, cell migration and invasiveness, will potentially have therapeutic implications.

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ORIGINAL RESEARCH PAPER Tagliabracci, V. S. *et al.* A single kinase generates the majority of the secreted phosphoproteome. *Cell* **161**, 1619–1632 (2015)