CELL SIGNALLING

Responding to intracellular pH

pH-induced changes in ... Gpa1 decrease the downstream activation of mitogen-activated protein kinases at lower pH

Changes in intracellular pH in response to extracellular signals such as ischaemia and nutrient deprivation indicate that protons might function as second messengers to regulate cell signalling. However, with the exception of a few well-known examples such as haemoglobin, it has been difficult to identify the pH sensors that translate changes in intracellular proton abundance to changes in biological function. This study reports that G protein-coupled receptor (GPCR) signalling decreases in response to reduced pH through the binding of protons to Gα subunits.

To predict pH-sensing proteins, Dohlman and colleagues developed an algorithm ([pHinder](#)) that identifies proton-binding motifs sequestered within a protein core in the form of buried ionizable side-chains. Using pHinder they found that 10% of 11,890 non-redundant protein structures contain large proton-binding networks in their cores. In addition to several

known pH sensors, the algorithm predicted that 96% of the 129 Gα subunit structures analysed contain a large core proton-binding network at the interface between their RAS-like and helical domains, which is in close proximity to the catalytic residues in the RAS-like domain.

In support of a pH-sensing role for Gα subunits, the thermostability of GTP-bound yeast G protein α1 (Gpa1) and mammalian Gα_i was sensitive to changes in pH within a physiological pH range, becoming less stable as the pH dropped below 7. Using an intrinsic Trp fluorescence assay, the authors showed that both GDP- and GTP-bound Gpa1 and Gα_i exhibit changes in conformation as a function of pH that are similar in magnitude to those that accompany GPCR-mediated activation of Gα subunits. As the pH decreases, the conformational change of Gα_i exposes two protected Cys residues at the interface of its RAS-like and helical domains. This indicates that

a proton-driven conformational change in Gα_i displaces the helical domain from the RAS-like domain, as was confirmed by nuclear magnetic resonance (NMR) spectroscopy.

To assess the functional relevance of this conformational change, the authors used a yeast system in which the intracellular pH can be manipulated genetically, metabolically or biochemically and can be quantified using a genetically encoded pH sensor. Gpa1 was rapidly phosphorylated as the intracellular pH was decreased *in vivo*, with the same dynamics as the conformational change that was observed *in vitro*, and was dephosphorylated as the cellular pH increased back to baseline. The activity of the kinases responsible for Gpa1 phosphorylation was not affected by the pH, so the authors conclude that the proton-dependent conformational change of Gpa1 controls access to the kinase. Finally, they showed that these pH-induced changes in the conformation and phosphorylation status of Gpa1 decrease the downstream activation of mitogen-activated protein kinases at lower pH.

As the core proton-binding network of Gpa1 is widely conserved among Gα subunits, the authors speculate that “pH sensing is a general feature of GPCR-mediated signalling in a variety of physiological contexts.”

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ORIGINAL RESEARCH PAPER Isom, D. G. *et al.* Protons as second messenger regulators of G protein signaling. *Mol. Cell* **51**, 531–538 (2013)