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Comment on “A G Protein–Coupled Receptor Is a Plasma Membrane Receptor for the Plant Hormone Abscisic Acid”

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Abstract: Liu et al. (Reports, March 23, 2007, p. 1712) reported that the Arabidopsis thaliana gene GCR2 encodes a seven-transmembrane, G protein–coupled receptor for abscisic acid. We argue that GCR2 is not likely to be a transmembrane protein nor a G protein–coupled receptor. Instead, GCR2 is most likely a plant homolog of bacterial lanthionine synthetases.

G protein–coupled receptors (GPCRs) are commonly used by eukaryotic organisms for signal processing and homeostasis, but recognition of a bona fide plant GPCR has been elusive. Liu et al. (1) recently reported that the Arabidopsis thaliana gene GCR2 (TAIR gene name At1g52920) encodes a 401-amino acid GPCR for abscisic acid. Liu et al. predicted GCR2 as a seven-transmembrane protein (7TM), using the TMpred and DAS programs, but did not report score thresholds to evaluate the confidence of these predictions. TMpred and DAS are known to erroneously predict transmembrane helices within soluble proteins (55% and 83% false positive rates, respectively) (2). A newer version of DAS (the “DAS-TMfilter server”), containing a filter for false-positive predictions (http://mendel.impc.at/sai/DAS/DAS.html), does not predict transmembrane regions within GCR2. Two other algorithms, TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM) and SOSUI (http://bp.nuap.nagoya-u.ac.jp/sosui), also do not predict transmembrane helices in GCR2. Both TMHMM2.0 and SOSUI are robust transmembrane helix predictors with low false-positive rates (1% and 3%, respectively) (2). A diverse set of protein classification methods was recently used to identify potential Arabidopsis 7TM proteins, but GCR2 was not among them (3).

BLAST (4) analysis of GCR2 indicates significant sequence similarity to bacterial [expect (E) value, 2 × 10−7], plant (8 × 10−135), human (2 × 10−69), murine (3 × 10−69), and insect (3 × 10−53) lanthionine synthetase (LanC) enzymes. Prokaryotic LanC enzymes produce cyclized antimicrobial peptides (5). The function of the eukaryotic LanC proteins is unknown. Significant sequence similarities between GCR2 and various prokaryotic and eukaryotic LanC proteins (Figure 1) indicate that these proteins belong to an evolutionarily conserved protein family. Predicting the tertiary structure of GCR2, using the protein-fold recognition algorithm PHyre (http://www.sbg.bio.ic.ac.uk/p PHYRE),
GCR2 Homology Model
NisC [PDB ID: 2G0D]

Figure 2. GCR2 has a predicted tertiary structure consistent with a LanC protein. BLASTP search against the structural database (http://www.ncbi.nlm.nih.gov/Structure/3d馆/3d馆.cgi), using GCR2 as the query, identified nisin cyclase (NisC, PDB ID: 2G0D) as the only structural homolog producing a statistically significant alignment (E-value, 4 × 10^{-4}). Significant sequence similarity was noted between amino acids 216 and 282 of GCR2 and amino acids 209 and 386 of NisC. A homology model of GCR2 (amino acids 216 to 282) was then generated using Insight-II (http://www.accelrys.com/products/insight). Shown is a superposition of the GCR2 homology model (blue) and the corresponding region of NisC (green). The N and C termini are labeled accordingly. Alpha helices observed in the NisC structure are denoted H8 to H14. Arrows indicate two segments in which NisC contains extended inserts relative to GCR2 and are the only areas of the superposition that diverge between the molecules. The proposed catalytic residues are indicated in NisC (yellow sticks) and GCR2 (red sticks). The superposition and image were generated using PyMol (DeLano Scientific, Palo Alto, CA, USA).

Liu et al. reported solubilizing recombinant GCR2 from Escherichia coli using 0.1% Triton-X100 and purifying GCR2 to homogeneity. The apparent ease of this purification and the methods used are generally contrary to the known arduous biochemistry of GPCR purification, given 7TM helices (11), but are entirely consistent with purifying a soluble cytosolic protein from E. coli. In vitro protein–protein interaction was reported between GCR2 and the Arabidopsis Ga subunit GPA1 using surface plasmon resonance (SPR) (1). However, the presented SPR data are not representative of a bona fide interaction (12). Indeed, the data clearly demonstrate an absence of any GCR2/GPA1 interaction, as GPA1 binding to GCR2 is equivalent to that of the negative control BSA [figure S3 in (1)]. The presented sensorgrams are most likely bulk shift artifacts normally corrected by negative control subtraction (12). We were unable to determine how Liu et al. (1) measured their rate constants. However, simulated SPR sensorgrams based on their reported values (Figure 3) clearly demonstrate a discrepancy between the data of Liu et al. (1) and expected SPR results (12) based on their reported rate constants. The reported off-rate constant (3.9 × 10^{-5} s^{-1}) suggests that the GCR2/GPA1 complex has a binding half-life of 5 hours, thoroughly inconsistent with the raw data presented by Liu et al. (1), and also suggesting that a surface regeneration step would be necessary to obtain reliable dose-response data.

The classical in vitro assay for GPCR/Ga coupling is demonstration of agonist-promoted guanine nucleotide exchange factor activity, either by GTPγS binding or steady-state GTase activity (13). Reconstituting interactions between Ga subunits and their cognate GPCRs typically requires lipid-modified Ga and Gγy subunits and a model membrane (13). The binding of Ga and Gγy to GPCRs is synergistic, whereas isolated subunits have low affinity for receptor (14). These considerations were not addressed by Liu et al. (1). In summary, while it is possible that GCR2 is both an intracellular receptor for abscisic acid and a G protein modulator, we conclude that GCR2 is neither a transmembrane protein nor a G protein coupled receptor, but rather is an Arabidopsis homolog of bacterial lanthionine synthetases. We recommend that any putative plant GPCR be rigorously characterized as a bona fide G protein–coupled receptor using in vitro biochemical methods for demonstrating G protein coupling and activation that have been well-established for the analysis of mammalian GPCRs.
References and Notes

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