letters to nature

Expression in HEK293 cells and oocytes

HEK293 cells were plated on polylysine-coated coverglass or Lab-Tek II CC2 chamberslides (Nalgene-Nunc). Cells were transfected with Lipofectamine 2000 (Invitrogen) using 5–25 ng rat or human ANKTM1 plasmid per cm²; pcDNA3 vector was added to bring the total amount of plasmid DNA to 100 ng cm $^{-2}$. For muscarinic activation, 100 ng cm $^{-2}$ human mAChR plasmid was co-transfected with 10 ng cm $^{-2}$ ANKTM1 plasmid. Sixteen hours after transfection, cells were loaded with Fura-2AM (5 μM) for 30 min and imaged in Ringer's solution. For oocyte expression, constructs were linearized with Mlul and transcribed with T7 polymerase (Ambion). Oocytes were incubated in ND96 containing 5 μM ruthenium red as described*. Currents were recorded in ND96 (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl $_2$, 1 mM MgCl $_2$ and 5 mM HEPES pH 7.6) or calcium-free ND96 (containing $100\,\mu\text{M}$ BaCl $_2$) as indicated.

Preparation of natural extracts

For brown mustard, 3 g of ground brown mustard seeds were suspended in 10 ml ND96 and incubated for 2 h at room temperature. Extract was cleared by centrifugation at 3,000g for 20 min. The supernatant fluid was filtered through a 0.2 μm filter and diluted 20-fold in ND96. For wasabi, 0.5 g of 100% pure wasabi paste (Pacific Farms, Oregon) was suspended in 1 ml ND96, clarified by centrifugation at 18,000g, filtered and diluted 50-fold in ND96.

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erratum

Structure and conserved RNA binding of the PAZ domain

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Nature 426, 469-474 (2003).

In this Letter, the last two sentences of the second paragraph of the left column on page 470 should read: "Preliminary data using an immobilized 5' phosphorylated ssRNA with a 3'-biotin modification showed that this RNA failed to pull down purified Ago1 PAZ domain (data not shown). By NMR titration, this RNA had a reduced binding affinity ($K_{\rm d}$ greater than 20 μ M), suggesting that the 3' end may also play a role in PAZ domain interaction."

corrigendum

Eya protein phosphatase activity regulates Six1-Dach-Eya transcriptional effects in mammalian organogenesis

Xue Li, Kenneth A. Ohgi, Jie Zhang, Anna Krones, Kevin T. Bush, Christopher K. Glass, Sanjay K. Nigam, Aneel K. Aggarwal, Richard Maas, David W. Rose & Michael G. Rosenfeld

Nature 426, 247-254 (2003).

In this Article, K. A. Ohgi's surname was misspelled. It is presented correctly here and has been amended in the HTML version of the paper on *Nature*'s website (http://www.nature.com/nature/).