

vesicles for reconstitution assays has been described<sup>10,20–22</sup>. Briefly, the internal pH of the vesicles was set at 6.0 by dialysis. The final uptake solution contained 1 mM  $^{22}\text{Na}^+$ , 250 mM mannitol, 30 mM potassium gluconate,  $1\ \mu\text{g ml}^{-1}$  valinomycin and 50 mM Tris/MES at either pH 6.0 or 8.0. Sodium uptake was determined by application of the reaction mix to 1 ml Dowex 50X8 (Tris), 100-mesh columns and rapid elution with vacuum suction with 1 ml 300 mM mannitol (pH 8.0) at 0°C. Under these voltage-clamped conditions, the proton-stimulated component of sodium uptake was taken as a measure of  $\text{Na}^+/\text{H}^+$  exchange rate.

**Quantification of  $\text{Na}^+/\text{H}^+$  exchanger activity by cytosolic pH measurement.** The intracellular pH ( $\text{pH}_i$ ) of small groups of cells was determined by microphotometry of the fluorescence emission of the pH-sensitive dye, 2',7'-bis-(2-carboxyethyl)-5( and 6) carboxyfluorescein, using dual wavelength excitation<sup>15,16</sup>. Cells grown to 70–80% confluence on glass coverslips were concurrently loaded with 25 mM  $\text{NH}_4\text{Cl}$  and  $2\ \text{mg ml}^{-1}$  of the acetoxymethyl ester precursor of 2',7'-bis-(2-carboxyethyl)-5( and 6) carboxyfluorescein in PBS for 10 min at 37°C. Where indicated,  $10\ \mu\text{M}$  forskolin or  $50\ \mu\text{M}$  isoprenaline was also added. Acid loading was accomplished by washing cells with a  $\text{Na}^+$ -free medium containing (in mM): 117 N-methyl-D-glucammonium chloride, 1.66  $\text{MgSO}_4$ , 1.36  $\text{CaCl}_2$ , 5.36 KCl, 25 HEPES, 5.55 glucose, pH 7.5,  $290 \pm 10\ \text{mosM}$ . Sodium-dependent pH<sub>i</sub> recovery was then initiated by perfusing the cells with a  $\text{Na}^+$ -rich solution composed of (in mM): 117 NaCl, 1.66  $\text{MgSO}_4$ , 1.36  $\text{CaCl}_2$ , 5.36 KCl, 25 HEPES, 5.55 glucose, pH 7.5. To measure fluorescence, the coverslip was placed into a holding chamber attached to the stage of a Nikon Diaphot TMD inverted microscope equipped with a Nikon Fluor 40x/1.3 N.A. oil-immersion objective. Clusters of 6–12 cells were selected for analysis with an adjustable diaphragm. Excitation light provided by a Xenon lamp was alternately selected using  $495 \pm 10\ \text{nm}$  and  $445 \pm 10\ \text{nm}$  filters at a rate of 50 Hz and then reflected onto the cells by a 510-nm dichroic mirror. Emitted light was directed to the photometer through a  $530 \pm 30\ \text{nm}$  band-pass filter. Photometric data were acquired at 10 Hz using a 12-bit A/D board (Labmaster, National Instruments) interfaced to a Dell 486 computer and analysed with Felix software (Photon Technologies). Calibration of fluorescence intensity with pH<sub>i</sub> was done in the presence of 5 mM nigericin in high- $\text{K}^+$  medium (140 mM KCl, 20 mM HEPES, 1 mM  $\text{MgCl}_2$ , 5 mM glucose) as described<sup>15,16</sup>.

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## corrections

## Treatment of experimental encephalomyelitis with a peptide analogue of myelin basic protein

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In this Letter, “96P” indicates a proline at residue 96, and not phenylalanine as published. □

## A new pattern for helix–turn–helix recognition revealed by the PU.1 ETS-domain–DNA complex

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In the legend to Fig. 3, lines 11 and 12 should read: “Arg 232 (NH1) makes two hydrogen bonds with G9(06) and G9(N7).” In panel b, a dashed line indicating a hydrogen bond to base A10 should be deleted. The coordinates for the complex remain unchanged as originally submitted to the Brookhaven Protein Data Bank under accession number 1 pue. □