Channelrhodopsin-2, a directly light-gated cation-selective membrane channel

Supporting Text

Supporting Methods

Expression of ChR2 in Oocytes or Mammalian Cells. A full-length and a C terminally truncated *chop2* variant were produced from a full-length cDNA template (GenBank accession no AF461397) by PCR, using a proofreading polymerase (pfu, Promega) with primers containing *Bam*HI and *Hin*dIII restriction sites. The products were inserted into pGEMHE and expressed in oocytes of *X. laevis* as described for BR (1, 2). After verification of the sequences the following constructs were tested in oocytes: *chop2*-737 (encoding all 737 aa of Chop2) and *chop2*-315 (encoding amino acids 1–315 of Chop2). Both constructs led to a light-gated cation conductance. Oocytes were injected with 20–30 ng of *in vitro*-transcribed cRNA (Ambion) and incubated in an *all-trans* retinal (1 μ M, from a 1 mM stock in ethanol) containing oocyte Ringer's solution for 2–5 days. Oocytes were examined by using two electrode voltage-clamp techniques (1, 2). *chop2*-315 was additionally subcloned into pBK-CMV and expressed in HEK293 or BHK cells by transient transfection. Mammalian cells were examined with the whole-cell patch-clamp technique (3).

Light Activation of ChR2. In two-electrode voltage-clamp experiments, ChR2 was activated with a cw HeCd laser of 442 nm wavelength (10 mW/mm^2), a mercury arc lamp and 450 ± 25-nm band filter (5 mW/mm^2), or a xenon arc lamp and 450 ± 25 nm band filter (3 mW/mm^2). The action spectrum for ChR2 or ChR2-315 was obtained with a xenon arc lamp and narrow bandwidth filters (± 10 nm).

In giant patch experiments (4–6), ChR2 was activated with a cw HeCd laser of 442 nm wavelength (>100 mW/mm^2) or a dye laser, which is pumped by a 10-ns, 308-nm laser pulse from an excimer laser (Lambda Physik, Acton, MA). In whole-cell (HEK293) patch experiments, ChR2 was activated with a cw HeCd laser of 442 nm wavelength (up to 100 mW/mm^2),

Opsin Quantification. The *chop1* cDNA fragment encoding amino acids 309–547 and the *chop2* cDNA fragment encoding amino acids 617–723 were cloned into the vector pMAL-c2X (New England Biolabs). The expressed maltose binding protein (MBP)-fusion proteins were affinity purified and used for antibody production in rabbits. Responses of algal fractions against the MBP tag were excluded by using a polyclonal anti MBP-antiserum (New England Biolab). *C. reinhardtii* cells (strain 806 and CW 2) were grown under different light conditions and harvested after 3 days. Cells were disrupted by sonication, and the total membrane fraction was collected by centrifugation for 40 min at 150,000 *g*. Membrane proteins were separated by PAGE, and Chop1 and

Chop2 were analyzed by protein blotting using the antisera at a dilution of 1: 2,000 and the second antibody at a dilution of 1:5,000.

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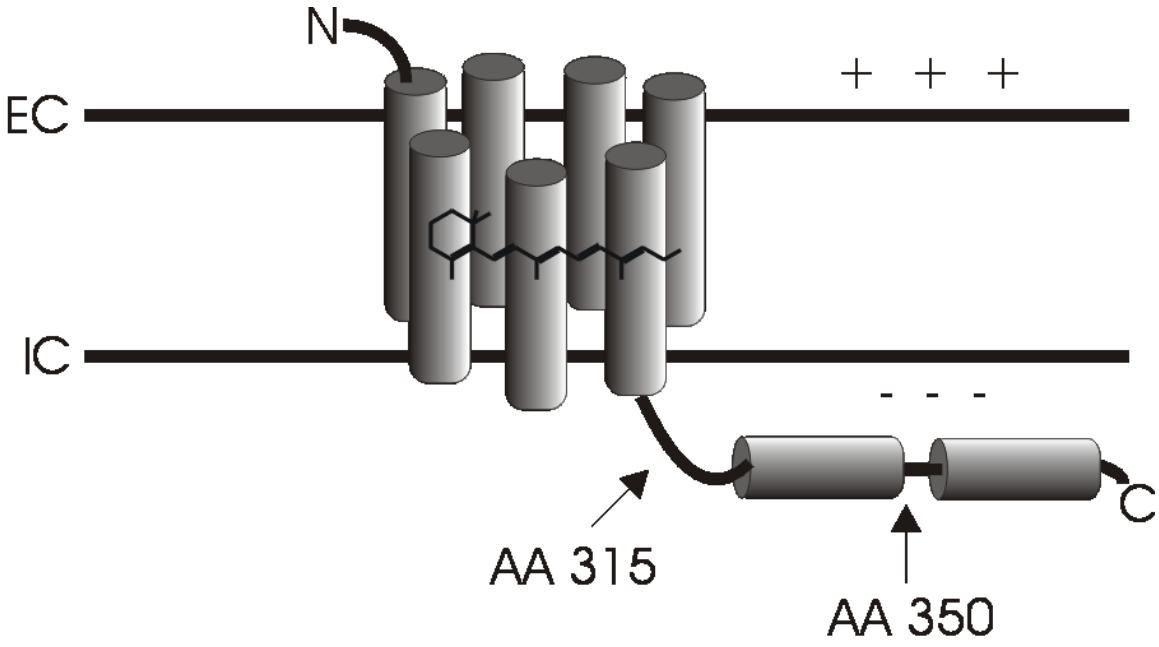
Fig.5 a

Fig. 5. (*a*) Comparison of the amino acid sequences of Chop1 (GenBank accession no. AF385748), Chop2 (GenBank accession no. AF461397), and bacterioopsin (Bop) from *Halobacterium salinarum* (GenBank accession no. P02945). Amino acids that are known from the BR structure to interact directly with retinal are indicated by *. Amino acids that are conserved in most microbial opsins are highlighted in green; those that are functionally homologous in microbial opsin sequences are in yellow, other identities are seen in blue. Amino acids that contribute to the H^+ conducting network in BR are shown in red. Residues that are part of the transmembrane H^+ network are red and bold. The key substitutions, D^{85}, D^{96}, and E^{204} in BR to E^{162}, H^{173} and S^{284} in Chop1, and E^{123}, H^{134}, S^{245} in Chop2 are seen as white letters on red background. Underlined regions indicate identified or hypothetical transmembrane regions. Amino acid sequences indicated in bold were expressed in *E. coli* and used for antibody preparation. (*b*) Cartoon of the ChR2-structure comprising a 7-TM segment, a rab-like domain (320–350), and a Gly-rich domain (350–737).


N
EC
IC
+ + +
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C
AA 315
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