## Channelrhodopsin-2, a directly lightgated 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  $\mu$  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 ( $10 \text{ mW/mm}^2$ ), a mercury arc lamp and 450 ± 25-nm band filter (5 mW/mm<sup>2</sup>), or a xenon arc lamp and 450 ± 25 nm band filter (3 mW/mm<sup>2</sup>). 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<sup>2</sup>) 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<sup>2</sup>),

**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.

1. Nagel, G., Möckel, B., Büldt, G. & Bamberg, E. (1995) FEBS Lett. 377, 263–266.

2. Nagel, G., Kelety, B., Möckel, B., Büldt, G. & Bamberg, E. (1998) *Biophys. J.* 74, 403–412.

3. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391**, 85–100.

4. Hilgemann, D. W. (1989) Pflügers Arch. 415, 247–249.

5. Hilgemann, D. W. & Lu, C. C. (1998) Methods Enzymol. 293, 267-280.

6. Weinreich, F., Riordan, J. R. & Nagel, G. (1999) J. Gen. Physiol. 114, 55-70.

Chop1.MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLEN 60 Chop2.....MDYGGALSAVGRELLFVTNPVVV 23 Bop.....MLELL \* \* Chop1.NGSVICIPNNGOCFCLAWLKSN-GTNAEKLAANILOWITFALSALCLMFYGYOTWKSTCG 119 Chop2.NGSVL-VP-EDOCYCAGWIESR-GTNGAOTASNVLOWLAAGFSILLLMFYAYOTWKSTCG 80 Bop. PTAVEGVSOAOITGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITTLVPAI 52 \* \* \* \*\* \*\* \* Chop1.WEEIYVATIEMIKFIIEYFHEFDEPAVIYSSNGNKTVWLRYA WLLTCPVILI LSNLTG 179 Chop2.WEEIYVCAIEMVKVILEFFFEFKNPSMLYLATGHRVQWLRYA WLLTCPVILI LSNLTG 140 BOD.AFTMYLSMLLGYGLTMVPFGGEO-----NPIYWARYADWLFTTPLLLLDLA-LLV 101 \* \* \* \* \* \* Chop1.LANDYNKRTM-GLLVSDIGTIVWGTTAALSKGY-VRVIFFLMGLCYGIYTFFNAA-KVYI 236 Chop2.LSNDYSRRTM-GLLVSDIGTIVWGATSAMATGY-VKVIFFCLGLCYGANTFFHAA-KAYI 197 Bop.-DAD-Q-GTILALVGADGIMIGTGLVGALTKVYSYRFVWWAISTAAMLYILYVLFFGFTS 158 \* \*\* \* \* # Chop1.EAYHTVPKGICRDLVRYLAWLYFCSWAMFPVLFLLGPEGFGHINQFN AIAHAILDLASK 296 Chop2.EGYHTVPKGRCRQVVTGMAWLFFVSWGMFPILFILGPEGFGVLSVYG TVGHTIIDLMSK 257 Bop.KAESMRPEV--ASTFKVLRNVTVVLWSAYPVVWLIGSEGAGIVPLNIETLLFMVLDVSAK 216 Chop1.NAWSMMGHFLRVKIHEHILLYGDIRKKQKVNVAGQEMEVETMVHEEDDETQKVP-TAKYA 356 Chop2.NCWGLLGHYLRVLIHEHILIHGDIRKTTKLNIGGTEIEVETLVEDEAEAGAVNKGTGKYA 317 Bop.VGFGLI--LLRSRAIFGEAEAPEPSAGDGA-AATSD..... Chop1.NRDSFIIMRDRLKEKGFETRASLDGDPNGDAEANAAAGGKPGMEMGKMTGMGMSMGAGMG 415 Chop2.SRESFLVMRDKMKEKGIDVRASLDNSKEVEOEOAARAAMMMMNGNGMGMGMGMMGMNGMNGMG 377 Chop1.MATIDS-----GRVILAVPDISMVDFFREQFARLPVPYELVPALGAENTL 460 Chop2.GMNGMAGGAKPGLELTPQLQPGRVILAVPDISMVDFFREQFAQLSVTYELVPALGADNTL 437 Chop1.QLVQQAQSLGGCDFVLMHPEFLRDRSPTGLLPRLKMGGQRAAAFGWAAIGPMRDLIEGSG 520 Chop2.ALVTQAQNLGGVDFVLIHPEFLRDRSSTSILSRLRGAGQRVAAFGWAQLGPMRDLIESAN 497 Chop1.VDGWLEGPSFGAGINQQALVALINRMQQAKKMGMMG-----GMGMGMGMGGGMG-MG 570 Chop2.LDGWLEGPSFGQGILPAHIVALVAKMQQMRKMQQMQQIGMMTGGMNGMGGGMGGGMNGMG 557 Chopl.MGMGMG-MAPSMNAGMTGGMGG---ASMGG----AVMGMGMGMOPMOOAMP--AMSPMM 618 Chop1.TQQPS-MMSQPSAMSAGGAMQAMGGVMPSPAP----GGRVGTNPLFGSAPSPLSSQ---- 669 Chop2.NGMSSGVVANVTPSAAGGMGGMMNGGMAAPQSPGMNGGRLGTNPLFNAAPSPLSSQLGAE 677 Chop1.-----PGISPGMATPPAATAAPAAGGSEAEMLQQLMSEINRLKNELGE 712 Chop2.AGMGSMGGMGGMSGMGGMGGMGGAGAATTQAAGGNAEAEMLQNLMNEINRLKRELGE 737 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<sup>+</sup> conducting network in BR are shown in red. Residues that are part of the transmembrane H<sup>+</sup> network are red and bold. The key substitutions, D<sup>85</sup>, D<sup>96</sup>, and E<sup>204</sup> in BR to E<sup>162</sup>, H<sup>173</sup> and S<sup>284</sup> in Chop1, and E<sup>123</sup>, H<sup>134</sup>, S<sup>245</sup> 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 Glyrich domain (350–737).

