

A Cyclic Nucleotide-Gated Channel, HvCNGC2-3, is Activated by the Co-presence of Na⁺ and K⁺ and Permeates Na⁺ and K⁺ Non-selectively

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Supplementary Materials: Table S1: Accession number of nucleotide sequence and peptide sequence of barley CNGCs, Table S2: Gene name of Arabidopsis CNGCs and their Arabidopsis Genome Initiative (AGI) code, Table S3: Comparison of deduced ion selective motifs and S4 domains in CNG and HCN channels in human and group 2 CNGC in barley, Figure S1: Current/voltage relationship of HvCNGC2-3 expressing oocytes in the presence of 8Br-cGMP, Figure S2: Current/voltage relationship of water-injected oocytes, Figure S3: Effects of substitution of Na⁺ and K⁺ on the current of *HvCNGC2-3* cRNA-injected oocyte, Figure S4: Putative ion-selective pore-forming motifs of representative subgroup II CNGCs of glasses, Figure S5: Secondary structure prediction of the cyclic nucleotide binding domains.

Table S1. Accession number of nucleotide sequence and protein sequence of barley CNGCs

| Gene name | Accession number | |
|------------|---------------------|------------------|
| | Nucleotide sequence | Protein sequence |
| HvCNGC1-1 | AK366449.1 | BAJ97652.1 |
| HvCNGC2-1 | AK373237.1 | BAK04434.1 |
| HvCNGC2-2 | AK370933.1 | BAK02131.1 |
| HvCNGC2-3 | AK376248.1 | BAK07443.1 |
| HvCNGC3-1 | AK376248.1 | BAK07912.1 |
| HvCNGC3-2 | AK355803.1 | BAJ86607.1 |
| HvCNGC3-3 | AK371848.1 | BAK03046.1 |
| HvCNGC4a-1 | AK365205.1 | BAJ96408.1 |
| HvCNGC4b-1 | AK367927.1 | BAJ99130.1 |

Table S2. Gene name of Arabidopsis CNGCs and their Arabidopsis Genome Initiative (AGI) code

| Gene name | AGI code |
|-----------|-----------|
| AtCNGC1 | At5g53130 |
| AtCNGC2 | At5g15410 |
| AtCNGC3 | At2g46430 |
| AtCNGC4 | At5g54250 |
| AtCNGC5 | At5g57940 |
| AtCNGC6 | At2g23980 |
| AtCNGC7 | At1g15990 |
| AtCNGC8 | At1g19780 |
| AtCNGC9 | At4g30560 |
| AtCNGC10 | At1g01340 |
| AtCNGC11 | At2g46440 |
| AtCNGC12 | At2g46450 |
| AtCNGC13 | At4g01010 |
| AtCNGC14 | At2g24610 |
| AtCNGC15 | At2g28260 |
| AtCNGC16 | At3g48010 |
| AtCNGC17 | At4g30360 |
| AtCNGC18 | At5g14870 |
| AtCNGC19 | At3g17690 |
| AtCNGC20 | At3g17700 |

The data was acquired from The Arabidopsis Information Resource (<http://www.arabidopsis.org>).

Table S3. Comparison of deduced ion selective motifs and S4 domains in CNG and HCN channels in human and group II CNGC in barley

| Gene | Ion selective motif | S4 domain |
|--|---------------------|---------------------------|
| Human CNG channels (non-selective, voltage-independent) | | |
| CNGA1 | GGL | RLNRLLRFSRMFEFFQR |
| CNGA2 | GGL | RFNRLLHFARMFEFFDR |
| CNGA3 | GET | RFNRLLKFSRLFEFFDR |
| CNGA4 | GET | RLNRFLRAPRLF EAFDR |
| CNGB1 | GET | RLPRCLKYMAFF EFN SR |
| CNGB3 | GDT | RANRMLKYTSFFE FNHH |
| Human HCN channels (K ⁺ and Na ⁺ -permeable, voltage-dependent) | | |
| HCN1* | GYG | RALRIVRFTKILSLLRLLRLSRLIR |
| Barley group 2 CNGCs (K ⁺ and Na ⁺ -permeable, partially voltage-dependent) | | |
| HvCNGC2-1 | GQGL | KDRLLSIIIIAQYVPLVRIYPLS |
| HvCNGC2-2 | GQGF | KDALVWVVL CQYIPRLLRIFPVT |
| HvCNGC2-3 | AQGL | KTALFFIVLTQYLPRLVRFYPII |

Magenta and green indicate basic and acidic amino acid residues, respectively.

Accession numbers of human genes are as follow: CNGA1, NP_001136036.1; CNGA2, EAW99409.1; CNGA3, NP_001289.1; CNGA4, XP_016872706.1; CNGB1, XP_011521172.1; CNGB3, XP_011515440.1; and HCN1, NP_066550.2.

Accession numbers of barley genes are shown in Table S1. *Amino acid sequences of ion selective motif and S4 domain in HCN2 (NP_001185.3), HCN3 (NP_065948.1) and HCN4 (NP_005468.1) are identical to HCN1.

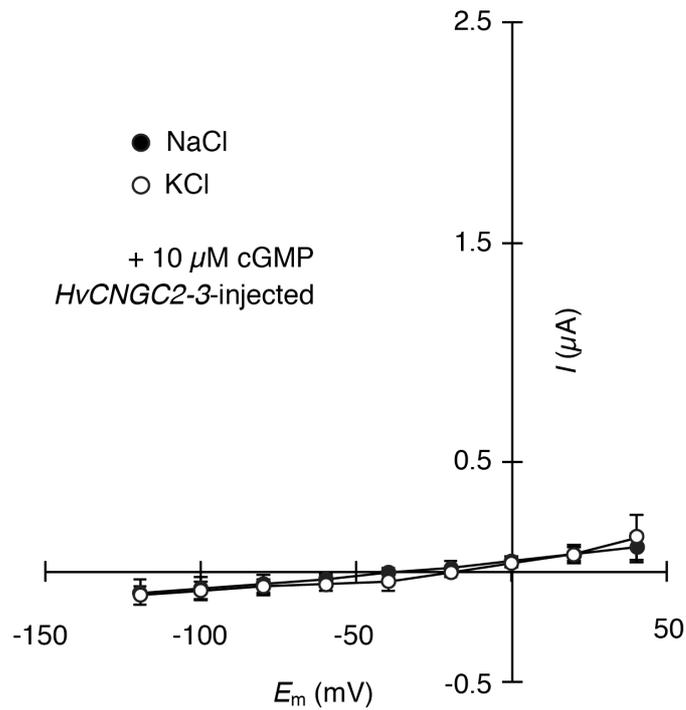


Figure S1. Current/voltage relationship of HvcNGC2-3 expressing oocytes in the presence of 8Br-cGMP. Currents were recorded by the two-electrode voltage-clamp. Bath solution contained 96 mM NaCl (closed symbols) or 96 mM KCl (open symbols). Oocytes were treated with 10 μ M 8Br-cGMP 30 min before measurements. Current values are means ($n=15$ [NaCl] and 10 [KCl]) \pm SD. I , membrane current. E_m , membrane potential.

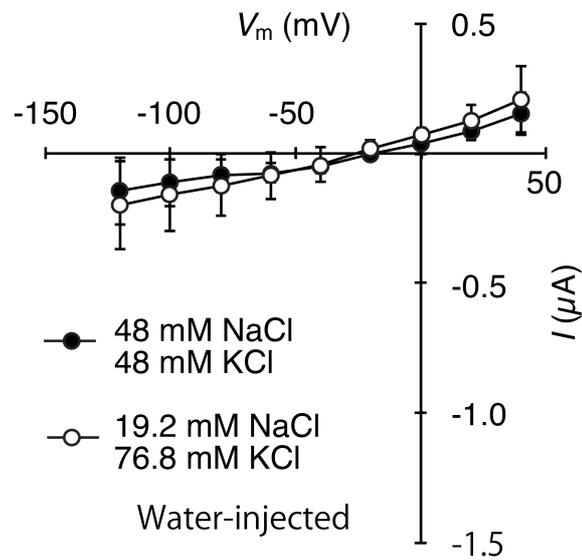


Figure S2. Current/voltage relationship of water-injected oocytes. Currents were recorded by the two-electrode voltage-clamp in bath solution containing 19.2 mM NaCl and 76.8 mM KCl (open symbols, $n = 10$), and 48 mM NaCl and 48 mM KCl (closed symbols, $n = 11$) in the presence of $10 \mu M$ 8Br-cAMP.

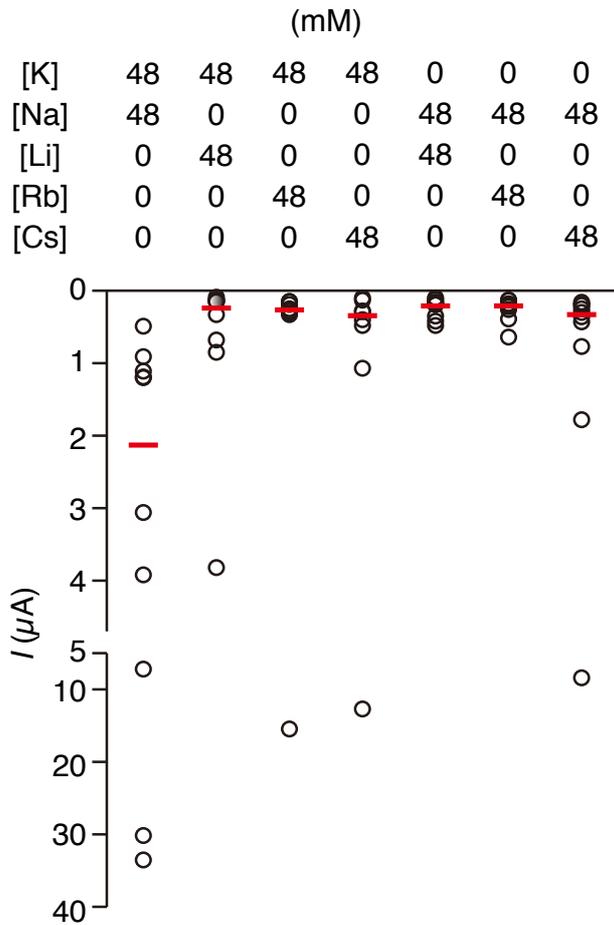


Figure S3. Effects of substitution of Na⁺ and K⁺ on the current of *HvCNGC2-3* cRNA-injected oocyte. Currents at -120 mV was plotted. Open circles indicate each measurement (n=10). Red lines indicate the median. Either of Na⁺ or K⁺ was substituted with Li⁺, Rb⁺ or Cs⁺. Mann-Whitney *u* test suggested that significant difference of the mean of current amplitude was observed in *HvCNGC2-3*-injected oocytes from water-injected control only in 48 mM K⁺ and 48 mM Na⁺ bathing solution ($\alpha = 0.05$).

Triticeae

Hordeum vulgare (Barley)

HvCNGC2-1
HvCNGC2-2
HvCNGC2-3

CLWWGLANLSTL**G**QGLK-TTIYTGESLF
CFWWGLQNLSTL**G**QGFV-TSTYPWEVLF
CLWWGLQNLST**G**AQGLE-TTHYKGEALF

Aegilops tauschii (Tausch's goatgrass)

XP_020165922.1
EMT29601.1
EMT29672.1

CLWWGLANLSTL**G**QGLK-TTIYTGESLF
CLWWGLQNLST**G**AQGLE-TTHYKGEALF
CLWWGLQNLSS**G**AQGLE-TTHYKGEALF

Triticum urartu (Red wild einkorn)

T1NH13_TRIUA

CLWWGLQNLST**G**AQGLE-TTHYKGEALF

Triticum aestivum (Common wheat)

TRIAE_CS42_2AL_TGACv1_097035_AA0322740.1
TRIAE_CS42_2BL_TGACv1_130157_AA0405260.1
TRIAE_CS42_2DL_TGACv1_160734_AA0553580.1
TRIAE_CS42_1AS_TGACv1_019890_AA0072520.1
TRIAE_CS42_1BS_TGACv1_049361_AA0150610.1
TRIAE_CS42_1DS_TGACv1_080801_AA0253910.1
TRIAE_CS42_5AL_TGACv1_375657_AA1225250.1
TRIAE_CS42_5BL_TGACv1_405743_AA1334310.1
TRIAE_CS42_5DL_TGACv1_437676_AA1467100.1

CFWWGLQNLSTL**G**QGFV-TSTYPWEVLF
CFWWGLQNLSTL**G**QGFV-TSTYPWEVLF
CFWWGLQNLSTL**G**QGFV-TSTYPWEVLF
CLWWGLQNLST**G**AQGLE-TTHYKGEALF
CLWWGLQNLST**G**AQGLE-TTHYKGEALF
CLWWGLQNLST**G**AQGLE-TTHYKGEALF
CLWWGLQNLSS**G**AQGLE-TTHYKGEALF
CLWWGLQNLSS**G**AQGLE-TTHYKGEALF
CLWWGLQNLSS**G**AQGLE-TTHYKGEALF

Brachypodieae

Brachypodium distachyon (Purple false brome)

Bradi5g23700.1
Bradi1g13740.1
Bradi1g78010.1

CFWWGLQNLSTL**G**QGFV-TSTYPGEVLF
CLWWGLANLSTL**G**QGLK-TTIYTGEALF
CLWWGLQNLSTV**G**QGLDQ-TTHYKGEALF

Andropogoneae

Zea mays (Maize)

GRMZM2G023037
AC197150.3

CFWWGLQNLSTL**G**QGLL-TSTYPGEVLF
CLWWGLQNLSTI**G**QGLE-TTHYKGEQLF

Sorgham bicolor (Sorgham)

Sb01g013500.1
Sb06g030420.1
Sb08g021830.1

CLWWGLANLSTL**G**QGLK-TSIYTGEALF
CFWWGLQNLSTL**G**QGLM-TSTYTGEVLF
CLWWGLQNLSTI**G**QGLE-TTHYKGEQLF

Oryzeae

Oryza sativa (Rice)

Os03g0646300
Os04g0643600
Os12g0468500

CLWWGLANLSTL**G**QGLQ-TSIYTGEALF
CFWWGLQNLSTL**G**QGLQ-TSIYPGEVLF
CLWWGLQNLSTV**G**QGLK-TTHYKGEALF

Figure S4. Putative ion-selective pore-forming motifs of representative subgroup II CNGCs of glasses. Twenty seven CNGCs showing >60% identity with HvCNGC2-3 was BLAST searched in two databases, EnsemblePlants (<http://plants.ensembl.org>) for *A. tauschii*, *T. urartu* and *T. aestivum* and PlantGDB (<http://www.plantgdb.org>) for *B. distachyon*, *Z. mays*, *S. bicolor* and *O. sativa*. Amino acid sequence of the putative ion-selective motif and their short flanking region were aligned manually. Glycine and alanine residues in the motif were highlighted blue and red, respectively. Gene names are as designated in each database.

