

Vocke et al., <http://www.jgp.org/cgi/content/full/jgp.201311015/DC1>

RCBM:

The alignment shows the RCBM region of rat and zebrafish ANO proteins. The top sequence is rANO1, and the bottom sequence is zANO10-2. Red letters indicate RCBM sequences. Conservation levels are indicated by shading: black for 100%, dark gray for 80%, and light gray for 60%. The alignment highlights a highly conserved region across all sequences.

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rANO1    . . . NDRKLLYEEWASYGVFYK--YQPIDLVRKYF
zANO1-4   . . . NDRKLLYEEWASYSIFYK--YQPIGLIRKYF
rANO2    . . . NDRKLLYQEWARYGVFYK--EQPIDLIRKYF
zANO2-1   . . . NERQLLHDEWARYGAFYK--YQPIDLIRKYF
rANO3    . . . NNRHLLYERWARWGMWYK--HQPLDLIRRLYF
zANO3    . . . NHRHLLYERWARWGIWYK--YQPLDLIRRYF
rANO4    . . . NHRHLLYECWASWSVWYK--YQPLDLIVRRYF
rANO5    . . . NERYVLCKNWARFSYFYK--EQFEDLIRNYY
zANO5a   . . . SERYSLIYKNWARFSSFYK--EQPLNLIRKYY
zANO5b   . . . SERYHLLYRYWARFLCFYK--EQPLNLIKKYY
rANO6    . . . SERYLLYREWAHPRSIYK--KQPLDLIRKYY
zANO6    . . . NERYLLYSEWAHPKNFYK--MQPLDLIRKYF
rANO7    . . . TQRQVLFKHWARWGKWRK--YQPLDHVRRYF
zANO7    . . . NMROQILHHYWARWACWRK--YQPLDHIREYF
zANO7-2   . . . NKRVQLYHYWASWLKWCK--YQPLDHIREYF
rANO8    . . . RILNRLMKSWVQAVCE-N---QPLDDICDYF
zANO8-1   . . . RILGQLMKSWVQAVCE-R---QPLDDVCDYF
zANO8-2   . . . RILNQLMTSWVQAVCE-R---QPLDDVCDYF
rANO9    . . . KGEEDLSREWAQWRNVFO--PQPIDKIREYF
zANO9-1   . . . KEQKKLKKKWASWYSVLELFQOPVTDVKDYF
zANO9-2   . . . REQKEELKKSWARWSATEF--GQPITNVRNYL
rANO10   . . . EALKKLEDTWYTRFALKY--QPIDSIRGYF
zANO10   . . . EDLKRLSFSWYKKIKLSF---QPLDDIERSYF
zANO10-2  . . . ERLADLGKQWYSQKSLWQ--QPLDYIHNYF

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Segment c:

The alignment shows segment c of rat and zebrafish ANO proteins. The top sequence is rANO1, and the bottom sequence is zANO10-2. Red letters indicate segment c sequences. Conservation levels are indicated by shading: black for 100%, dark gray for 80%, and light gray for 60%. The alignment highlights a highly conserved region across all sequences.

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rANO1    . . . MEHWKRKQMRNYRWDLTGFEEEEAVKDHPRAEY
zANO1-4   . . . MEHWKRRQVRNYIWDLTGFEDTE--SHPRAEY
rANO2    . . . LENWKRLQMRIGYFWDLTGIEEEEERSQEHSRPEY
zANO2-1   . . . LEHWKRRRQISNYSWDLTGMEEEEE--EHPRPKY
rANO3    . . . LEFWKRRRSITTYTWDLIEWEEEE--ETLRPQE
zANO3    . . . LEFWKRRRAEITYDWDLIDWEEEE--EELRPQE
rANO4-2   . . . LEFWKQRQAREYEWDLVDFEEECQQQLQ--LRPEY
rANO5    . . . LEFWKQRQAREYEWDLVDFEEECQQQLQ--LRPEY
zANO5a   . . . LEFWKRRQAREYEWDLVDFEEECQQQLQ--LRPEY
zANO5b   . . . LEFWKRRQAREYEWDLVDFEEECQQQLQ--IRPEY
rANO6    . . . LEFWKRRQAEEYEWDTVELQQ--EQ---PRPEY
zANO6    . . . LEFWKRYQAKEYKWTDTVEFQQQEQ---PRPEY
rANO7    . . . LEYWKRKNATAYRWDCSDYEDIEPER---PRPQE
zANO7    . . . LEYWKRTSSLISHRWDCSEFEEEPER---PRPEF
zANO7-2   . . . LEYWKRKNATAHHWDCMDFHEDEPER---PRPEF
rANO9    . . . LEIWKRKRAREVQSWELYEWDEEE-----E
zANO9-1   . . . FEWKRHRSLYVSKWNVFDWCDDE-----E
zANO9-2   . . . LEFWKRHRSSFVCAWKVYDWCEEEE-----E
rANO8    . . . LEEWKRRGAEAYKWTLDSPGEAEE--PRPQE
zANO8-1   . . . LERWKPRRGAEAYKWTLNTPAESLEE--PRPQE
zANO8-2   . . . LERWKRREAEAYKWTLDTPAESLEE--PRPQE
rANO10-1  . . . LEVWKRGCANTYRWGTLVM-KRCFEE--PRPGF
zANO10-2  . . . MELWKRRSASISYHWGTFNL-AECFQE--PRPGF
zANO10   . . . LEVWKRCSATASWGTLGR-KKACFE--PRAGE

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Figure S1. Alignments of rat and zebrafish ANO proteins. The 10 rat homologues ANO 1–10 (prefix r) are compared with those orthologous zebrafish protein sequences (prefix z) that display the highest degree of conservation. Sequences were obtained from the Ensembl database. Indicated percentages of conservation are 100% (black), 80% (dark gray), and 60% (light gray). Red letters indicate RCBM sequences (top alignment) and segment c sequences (bottom alignment), each encoded by a separate exon.

TABLE S1

Composition of pipette solutions used for the electrophysiological experiments

Pipette solution	Composition
Measured free Ca ²⁺ : 62 μM	130 mM CsCl 9.98 mM CaCl ₂ 10 mM HEPES 10 mM HEDTA pH 7.0 using CsOH
Measured free Ca ²⁺ : 7.5 μM	133.474 mM CsCl 8.263 mM CaCl ₂ 10 mM HEPES 10 mM HEDTA pH 7.0 using CsOH
Measured free Ca ²⁺ : 2.6 μM	138.268 mM CsCl 5.866 mM CaCl ₂ 10 mM HEPES 10 mM HEDTA pH 7.0 using CsOH
Measured free Ca ²⁺ : 2.0 μM	143.582 mM CsCl 3.209 mM CaCl ₂ 10 mM HEPES 10 mM HEDTA pH 7.0 using CsOH
Measured free Ca ²⁺ : 0.7 μM	147.516 mM CsCl 1.242 mM CaCl ₂ 10 mM HEPES 10 mM HEDTA pH 7.0 using CsOH
Ca ²⁺ -free pipette solution	150 mM CsCl 10 mM HEPES 10 mM HEDTA pH 7.0 using CsOH
Bath solution	150 mM CsCl 10 mM HEPES 10 mM EGTA pH 7.4 using CsOH

Composition of pipette solutions used in whole-cell experiments. Chemicals used were of the highest purity available. Compositions were obtained from Reisert et al. (2003). The indicated free Ca²⁺ concentration of each solution was determined using a calcium electrode (KWIKCAL-2) that was calibrated with the CALBUF-1 series (World Precision Instruments). A single set of pipette solutions was used for the entire project.

TABLE S2
Primers used for cloning, RT-PCR analysis, and mutagenesis

Purpose	Orientation	Primer sequence (5'-3')
Cloning channels		
Full-length ANO1	Forward	AGAGCTAGCGGCCGCCGATGGGAGCGCGAGGCCACCATG
	Reverse	TGGATCCGGCTCAGCGGCCCATGGTA
Full-length ANO2	Forward	AAAGTCGATGCTAGCGACAATGGCAAGAGGAAGCCAG
	Reverse	TTCCCGGGCTCCTACGTTGGTGTGC
Testing for Segment c		
SegC+	Forward	AACGTTCCCAGAACACT
SegC-	Forward	GAAGAAGAACACTCCCG
SegCtest	Reverse	CAATGGAGAACGTCAGGGC
Remove/insert segment c		
ANO 1 remove c	Forward	GCTTCGAGGAAGAGGAGGATCATCCAGAGCAGAG
	Reverse	CTCTGCTCTGGATGATCCTCTCTTCCTCGAAGC
ANO2 insert c	Forward	GAAGAGGAAGAAGAACGTTCCCAGGAACACTCCGGCCTGAATAT
	Reverse	ATATTAGGCGGGAGTGTCTGGAAACGTTCTTCTCTTC
Point mutations		
ANO1 L323E	Forward	CAACGACAGGAAACTCGAGTATGAGGAATGGCAAGCTATGG
	Reverse	CCATAGCTTGCCTATTCTCATACTCGAGTTCTGCGTTG
ANO1 W327E	Forward	GACAGGAAACTCTGTATGAGGAAGAGGCAAGCTATGGAGTC
	Reverse	GACTCCATAGCTTGCTCTCCTCATACAGGAGTTCTGTC
ANO1 L323E/W327E	Forward	CAACGACAGGAAACTCGAGTATGAGGAAGAGGCAAGCTATGGAG
	Reverse	CTCCATAGCTTGCCTCTCCTCATACTCGAGTTCTGCGTTG
ANO1 V332E/Y336E	Forward	GGGCAAGCTATGGAGAATTCTACAAAGAGCAGCCATTGACCTGG
	Reverse	CCAGGTCAATGGGCTGCTTTGAGAATTCTCATAGCTTGC
ANO2 L309E/W313E	Forward	GAATGACAGAAAGTGGAGTATCAGGAAGAGGCACGCTATGGAG
	Reverse	CTCCATAGCGTGCCTCTCCTGATACTCCAACCTGTGATTC
ANO2 V318E/F322E	Forward	ATGGGCACGCTATGGAGAGTTTACAAGAACAAACCCATTGACCTCATC
	Reverse	GATGAGGTCAATGGGTTGTTCTTGAGAATTCTCATAGCGTGC
ANO2 F322E/L327E	Forward	CTATGGAGTGTACAAAGAGCAACCCATTGACGAGATCAGGAAGTATTTGGAGAG
	Reverse	CTCTCCAAAATACTCCTGATCTCGTCAATGGGTTGCTTTGAAACACTCCATAG
Chimaeras		
ANO1-NT Eco105I	Forward	CAGCCCCATTGACCTGGTACGTAATAACTTGGAGAAAAGGTTGGC
	Reverse	GCCAACCTTTCTCAAAGTATTACGTACCGTCAATGGCTG
ANO1-TMR3 EcoRI	Forward	GATTCCCAGCCTATTTCACGAATTCTGTCTCATCTTCATGATC
	Reverse	GATCATGAAGATGATGGAGACAGAATTCTGAAATAGGCTGGAAATC
ANO2-NT Eco105I	Forward	TTCAACCCATTGACCTCATCGTAAGTATTTGGAGAGAAAATTGGGC
	Reverse	GCCCAATTCTCCAAAATCTTACGTATGAGGTCAATGGTTGAA
ANO2-TMR3 EcoRI	Forward	GTTCAGGATACTTGATGATTCTGCTCCATCTGTTATG
	Reverse	CATAAACAGATGGAGACAGAATTCAAGTATCCTGGAAAC

List of the primer sequences used for the PCR protocols in this project. Forward and reverse primers are given for the various tasks. For generating the chimaera, we introduced restriction sites for the indicated endonucleases without altering the amino acid sequences.

REFERENCE

- Reisert, J., P.J. Bauer, K.W. Yau, and S. Frings. 2003. The Ca-activated Cl channel and its control in rat olfactory receptor neurons. *J. Gen. Physiol.* 122:349–363. <http://dx.doi.org/10.1085/jgp.200308888>