Supporting Information

Oka et al. 10.1073/pnas.0809420106

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$Dr_G\alpha_{v1}$	MG	L <mark>Č</mark> LGSEVTT-EED	KKAKIHSSQIDRDLYEYÂKRÊLŴVKILLL <mark>G</mark> AÄE <mark>SGKST</mark> ÛV <mark>KQ</mark> MKIIHŜHGF	66
Ga_{v1}	MG	L <mark>C</mark> LGTEV-T- <mark>EE</mark> Q	KKARTHSAEIDRDLYECAKREMNVVKILLLGAAE <mark>SGK</mark> STLV <mark>KQ</mark> MKIIHSHGF	65
$Tr_G\alpha_{v1}$	MG	L <mark>C</mark> LGTEL-T-EE	KKARIRSAKIDRDLYEFAKMEMNVVKI <mark>LLLG</mark> AAE <mark>SGK</mark> STLV <mark>KQ</mark> MKIIHSNGF	64
Tn_Gα _{v1} *	MG	L <mark>C</mark> LGTEL-T- <mark>EE</mark>	KKARIRSAGIDRDLHELAKMEMNVVKILLL <mark>G</mark> AAE <mark>SGK</mark> STLV <mark>KQ</mark> MKIIHSHGF	64
01_Gα _{v1}	MG	L <mark>C</mark> LGIEL-P-ED	REARIRSAGIDRELLERAKRDMKEVRV <mark>LLLG</mark> AAE <mark>SGK</mark> STLV <mark>KQ</mark> MKIIHSGGF	64
Sa_Ga _{v1} *	MG	L <mark>C</mark> LGSES-NAEE	RQARLRSEKIDRALYEYAKQEL NVVKI LL<u>LG</u>AAE<mark>SGK</mark>STLV<mark>KQ</mark>MKIIHSHGF	65
$Cm_G\alpha_{v1}$ †	MG	L <mark>C</mark> LGSDA-SAEE	REARIRSEKIDRALYEFAKQEFNVVKILLLGREARIRSEKIDRALYEFAKQEFNVVKILLLG	44
$Bf_G\alpha_v$	MG	<mark>AC</mark> LSLDS <mark>EE</mark>	RKARIRSDEIDRQLQYMAKEDSSVIKI <mark>L</mark> L <mark>LG</mark> AGE <mark>SGK</mark> STLV <mark>KQ</mark> MKIIHSQGF	63
$Sp_G\alpha_v +$	MG	T <mark>C</mark> VSLDHDQ	KRARHRSEEIDRNLVAMARQEENIVKILL <mark>LG</mark> AGE <mark>SGK</mark> STLV <mark>KQ</mark> MKIIHSDGF	63
0I_Gα _{v2}	MG	MCPDI-S-EED	KRAKIQSSKIEQALNEHARAEINTVKI <mark>LLLG</mark> AAE <mark>SGK</mark> STVI <mark>KQ</mark> IKIIHSNGF	63
Ga_{v2}	MG	MCSHPKL-T-EEG	KKAKLRSSKIEQDLYEHARNEMNVVKI lmlg aae <mark>sgk</mark> stli <mark>kq</mark> ikiihsRgf	65
$Tr_G\alpha_{v2}$	MG	MCLDQDV-S-EES	KRAKLQSSKIEQDLCEHARTEMNVVKI <mark>LLLG</mark> PAE <mark>SGK</mark> STLI KQ IKIIHSHGF	65
$Tn_G\alpha_{v2}$	MG	T <mark>C</mark> LHQDV-T- <mark>EE</mark> S	KKAKLQSSKIEQDLCEHARAEMNVVKI <mark>LMLG</mark> PAE <mark>SGK</mark> STLI <mark>KQ</mark> IKIIHSHGF	65
$Lg_G\alpha_v$	MG	L <mark>C</mark> FSLDQ <mark>EE</mark>	LKARARSDVIDKNLQTWAKQDSNVIKILL <mark>LG</mark> AAE <mark>GGK</mark> STLV <mark>KQ</mark> MKIIHNDGF	63
Tc_Gα _v	MG	A <mark>C</mark> LSLEREE	GKARKRSEEIDRQLGELAKQQSNVIKI <mark>LLLG</mark> AGE <mark>SGK</mark> STLV <mark>KQ</mark> MKIIHADGF	63
$Csp_G\alpha_v$	MG	S <mark>C</mark> LSYDFED	RKAQARSVEIDKQLMDMAHEDRRVIKL <mark>L</mark> LL <mark>G</mark> AGE <mark>SGK</mark> STLV <mark>KQ</mark> MKIIYTSGF	63
$Gc_G\alpha_v *$	MG	G <mark>C</mark> VSATPEE	REAKTRSSVIDRQQRQDARQYENTIKI <mark>LLLG</mark> AGE <mark>SGK</mark> STVV <mark>KQ</mark> MKIIHGDGY	63
Ef_Gα _{v1} †			<mark>KI</mark> IHG <mark>DG</mark> Y	8
$Ef_G\alpha_{v2}^{\dagger}$			<mark>KI</mark> IHG <mark>DG</mark> Y	8
$Hs_G\alpha_{t1}$	MG	AGA <mark>S</mark> AEE	KHSRELEKKLKEDAEKDARTVKL <mark>LLLG</mark> AGE <mark>SGK</mark> STIV <mark>KQ</mark> MKIIHQDGY	57
$Hs_G\alpha_{i1}$	MG	CTL <mark>S</mark> AED	KAAVERSKMIDRNLREDGEKAAREVKL lllg age <mark>sgk</mark> stiv <mark>kq</mark> mkIIHeagy	61
$Hs_G\alpha_q$	MTLESI	IMA <mark>CC</mark> LSEEA	KEARRIN deier Qlrrdkrdarrelkl l l g tge <mark>sgk</mark> stfi kq mriihgsgy	67
$Hs_G\alpha_{12}$	MSGVVRTL	_SRCLLPAEAGGARERRAGS0	GARDAEREARRRSRDIDALLARERRAVRRLVKILLLGAGE <mark>SGK</mark> STFL <mark>KQ</mark> MRIIHGREF	85
$Hs_G\alpha_s$	MG	<mark>C</mark> lgnsktedqr	NEEKAQREANKKIEKQLQKDKQVYRATHRLLLL <mark>G</mark> AGE <mark>SGK</mark> STIV <mark>KQ</mark> MRILHVNGFNGEGGEEDPQAAR	SN 83

Fig. S1. Amino acid alignment of $G\alpha_y$ proteins: alignment of deduced amino acid sequences of 19 $G\alpha_y$ proteins from teleosts, sharks, a cephalochordate (lancelet), a sea urchin, a red flour beetle, an annelid (polychaete worm), a mollusk (limpet), and sponges together with human Gat1, Ga1, Ga2, Ga12, and Gas sequences as representatives for each class. Conservation was analyzed between all human, mouse, zebrafish, fruit fly, and sponge $G\alpha$ protein sequences. Residues conserved among all Ga proteins are in red boldface type. Residues in 100%, 80–99%, and 60–79% conservation within a class are in red, blue, and green, respectively. Atypical residues at conserved positions in sequences depicted here are shaded in gray. A comparison of the amino acids conserved in neoteleosts to the corresponding positions in shark and zebrafish sequences shows a 2-fold larger distance from Gav2 compared to Gav1, consistent with the topology of the shark and zebrafish genes as qnav1 orthologs in the phylogenetic tree using the NJ algorithm (Fig. 1B). Residues suggested for contact with regulators of G protein signaling/GTPase-activating proteins, effectors, and both (see refs. 1–13) are shaded in blue, green, and yellow, respectively. In the case of atypical residues at these contact sites the residues are not shaded, but boxed. Known acylation sites in the N-terminal region and Cys residues of Gi proteins, ADP-ribosylated by Pertussis toxin, are shaded in purple. G-boxes (G1-5) are indicated with bars above the alignment. Switch regions (SW1-3) are shaded with hatch marks. Solid and open circles indicate putative N-linked myristoylation and thio-palmitoylation sites for Gay proteins, respectively. Solid and open arrowheads are splicing junctions in a codon and between codons for teleost qnav genes, respectively. Gv-specific junctions are in red. Stars are Gv-specific motifs color coded for degree of conservation. Dr, zebrafish; Ol, medaka; Ga, stickleback; Tr, fugu; Tn, tetraodon; Sa, dogfish shark; Cm, elephant shark; Bf, lancelet; Sp, sea urchin; Lg, limpet; Tc, red flour beetle; Csp, polychaete worm annelid; Gc, sea water sponge; Ef, fresh water sponge; Hs, human. Asterisks and daggers indicate corrected and partial sequences, respectively. Two single-base ambiguities were resolved as follows: for Sa.Gav1, an ambiguous nucleotide (x) at the position 584 in the sequence DV496403 was replaced with cytosine (X333 to A333); for GC. G α_v , an ambiguous nucleotide (w) at the position 251 of the sequence Y14247 was replaced with thymine (X68 to L68). A potential pseudogene was rescued as follows: for Tn_Gav1, guanine between L180 and R181, adenine in G188, and guanine between E191 and T192 were removed. The sea urchin predictions GaoL and GaoL2 (14) exhibit partial overlap in the C-terminal region, with 99% nucleotide identity and no difference in amino acid sequence, but exhibit different exons in the N-terminal region of the sequence. We conclude that Gaol and Gaol2, which are assigned to the same contig, are different haplotypes of the same gene, a common assembly error in unfinished genomes constructed from two haplotypes of noninbred species. We therefore combined both predictions into a single prediction, $Sp_{-}G\alpha_{v}$. Furthermore, we found two lancelet sequences with 97% nucleotide identity, but encoding the identical protein, Bf_Gα_V. We concluded that these sequences constitute 2 alleles of a single gnav gene locus as well. TC_G α_{v_1} Ef_G α_{v_1} and G α_{v_2} , and GC_G α_v were renamed from G α_{i2} , G α_6 and G α_7 (15), and G α_o (16), respectively.

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$Dr_G\alpha_{v1}$	TKQELTSFKPAVLDNLLTSMKFVLHGMGVLRINLANPKNKVHAHSVLSC	GRĈFDED	QMLFPFIAHALCCLWADPGVRSSAARGYEYEL 154
Ga_{v1}	TKQELCSFKPAVLDNLLTSMKFVLHGMGVLRINLANTRNKVHAHSVLSC	GRCFDED	TVLFPFLGHALSCLLADQGVRAAAARGYEYEL 153
Tr_Gα _{v1}	TKQELITFKPAVLDNLLTSMKFVLHGMGVLRINLANSRNKVHAHSVLSC	GRCFDED	QVLLPFLSHAFSCLWSDEGVRSAVARGYEYEL 152
Tn_Gα _{v1} *	TRQELLTFKPAVLDNLLTSMKFVLHGMGALRINLTNSRNKVHAHAVLSC	GRCFDED	QVLLPVLGHAFTCLWSDQGVRSAVARGYEYQL 152
0I_Gα _{v1}	SGHELLSFKPAVLDNLLTSMKFVLHGMGLLHINLANSRNKVHARCVLAC	GRCFDEE	QVLRPFVGHALSCLWADQGVRAAAAQGCEYEL 152
Sa_Ga _{v1} *	TEEELTSFKPAVLDNLLSSMKFVLQGMGILRINLANPKNTIHAQTVLSC	GRCFDED	YALFPFMAHALRCLWADQRVKLAASRGYEFEL 153
$Cm_G\alpha_{v1}$ †	KPAVLDNLLSSMKFVLQGMGILRINLAIPRNTTHAQTVLSC	GRCFDED	ETLLPFVGHALRCLWADPAVRLAASRGYEYEL 124
$Bf_G\alpha_v$	SQEELNSFKPTLMDNLLSTMKFVLSGMGLLRINLSNPNNKIHAQTVLSS	RRGFGED	LIMFPFVTHALRCLWSDQGVRLAVARGYEYEL 151
$Sp_G\alpha_v +$	TDYELMSFKPAVLDNLLNSMKYVLNGMGLLRIPLANSKNK		103
$01_G\alpha_{v2}$	SKEELSSFKPAVLDNLLTSMKIVLRGMGKLRINLANQKNKVHACSILSC	SQCLGED	QELHPFIAHAFCALWADLGVKVAAARGYEFQL 151
Ga_{v2}	SETELLSFKAAVLDNLLTSMKFVLRGMGTLRINLANKNNKTWARSILSC	GQCLGDD	QELLPFVAHAICALWADQGVRAAAARGYEFEL 153
$Tr_G\alpha_{v2}$	SKQELTTFKPAVLDNLLTSMKFVLRGMGMLRINLANKKNKMHARSVLSC	SQCFGED	QELLPFVAHAFCALWSDHGFRAAAARGYEFEL 153
$Tn_G\alpha_{v2}$	SKQELISFKPAVLDNLLTSIKFVLQGMGMLRINLANRKNRTHARALLSC	DRCAGDD	QELLPFVAHAFCALCSDHGFRAAVARGHEFEL 153
$Lg_G\alpha_v$	SKHELRSFKTAVLDNLVSSMKFVLAGMGMLRVNLENPKNKLYAQKILSC	MCCYDKE	FHAMLPEIYEALKSLWKDRGIRLAVSRGYEFEL 152
Tc_Gav	THAELSSFRPTVLDNLLASMKYVLAGMGILRINLEQQRNKSHAQAVLMS	RSCFDMS	FTVLPNMAASLQALWSDRGVRLAVARGYEYEL 151
$Csp_G\alpha_v$	SENELMAFRPAVLDNLLFSMKFVLSGMGMLRINLERPYNRANAQIILSC	QRCYDDH	LIILPNVAVSLQSLWKDGGVRRAISRGYEFEL 151
$Gc_G\alpha_v *$	SQTELRSFKSVIYGNLAASMRVVLNAMEKLGIPYGNQASQEQARVILSL	SNSLSSY	ESFPPDVTSAFISLWRDAGVQECFSRAYEYQL 151
Ef_Gα _{v1} †	SKDELESFRPVIYGNLAASMRVVVSNMENLGIPFSDTTNREYANMILSL	STSIPNC	NSLPSEVAEAFRRLWNDQGVRACFSRAYEYQL 96
$Ef_G\alpha_{v2}$ †	SNEELDAFKHVVYKNLVASMAAIVRNMERLGISFSDPSNSVHADTLLAL	SSN-QDF	SSMPPKLAEAIKHLWSDQGVKACFKRAYEYQI 95
Hs_Ga _{t1}	SLEECLEFIAIIYGNTLQSILAIVRAMTTLNIQYGDSARQDDARKLMHM	ADTIEEG	TMPKEMSDIIQRLWKDSGIQACFERASEYQL 144
$Hs_G\alpha_{i1}$	SEEECKQYKAVVYSNTIQSIIAIIRAMGRLKIDFGDSARADDARQLFVL	<mark>A</mark> GAAEEG	FMTAELAGVIKRLWKDSGVQACFNRSREYQL 148
Hs_Ga _q	SDEDKRGFTKLVYQNIFTAMQAMIRAMDTLKIPYKYEHNKAHAQLVREVD	/EKVSAFENP	YVDAIKSLWNDPGIQECYDRRREYQL 153
Hs_Ga ₁₂	DQKALLEFRDTIFDNILKGSRVLVDARDKLGIPWQYSENEKHGMF	LMAFENKAGL	PVEPATFQLYVPALSALWRDSGIREAFSRRSEFQL 175
$Hs_G\alpha_s$	SDGEKATKVQDIKNNLKEAIETIVAAMSNLVPPVELANPENQFRVDYILSV	MNVPDFD	FPPEFYEHAKALWEDEGVRACYERSNEYQL 171

Fig. S1. continued

	*▼ ** * <u>G2 *</u> SW1 ▼ * <u>G3</u> SW2 * * * *SW3* ⊽
$Dr_G\alpha_{v1}$	NDSALYFFENMGRII-ADDYMPTET-DVLRVRLRTTGVIETQFKVKHLVFRMYDVGGQRTERRKWISCFEYVRSVLFVVSLSGYDMTLVEDPSMNRLQES 252
Ga_{v1}	NDSALYFFQNLTRIT-SPDYVPTET-DVLRVRLRTTGV1ETQFKVNRLIFRMYDVGGQRTERRKW1GCFEDVRAVLFVVALSGYDMTLVEDPSVNRLQES 251
$Tr_G\alpha_{v1}$	NDSALYFFENMIRIT-SPEYVPTEM-DVLRVRLRTTGVIETOFKVKHLVFRMYDVGGORTERKWIGCFEDVRAVLFVVSLSGYDMTLVEDPSMNRIQES 250
Tn_Gα _{v1} *	NDSALYFFENMSRISLSETTIPIRNPDVLRVRLRTTGVIETOFKVKHLVFRMYDVGGQRTERRKWIGCFEDVRAVLFVVALSGYDMTLVEEPSTNRIQES 252
$0I_G\alpha_{v1}$	NDSALYFFENLSRIT-SPDYVPTEA-DVLRVRLRTTGVIETOFKVNHLIFRMYDVGGQRTERRKWMGCFEDVRVVLFVVSLSGYDMTLLEDPHTNRLQES 250
Sa Ga _{v1} *	NDSAHYFFQNMNRIT-APEYKPTQM-DLLRVRLRTTGVIETQFKINNLIIRLYDVGGQRTERRKWIGCFEDVRAVLFVAALSGYDMTLLEEPSMNRLQES 251
$Cm^{-}G\alpha_{u1}$ +	NDSARMYDVGGORTERRKWIGCFEEVRAVLYVAALSGYDMTLLEELTVNRLOES 178
Bf Gα.	NDSALYLFENMDRIC-HEKFOPNSE-DVIRARVRTTGILETEFAISGIMFRMEDVGGORSERKWIOCFDDVKAILFVTALSGYDMTLLEDSNVNRLEES 249
$Sp G\alpha_{v} +$	YFENMERLT-SEKYKPDTQ-DVLRARVRTTGILETHFKIRGVIFRLYDVGGQRSERRKWIQCFDDVKALLFVAALSGYDMVLFEDPEVNRLQES 195
$0I_G\alpha_{y^2}$	NDSALYFFENISRII-APNYVPTET-DVLRVRVRTCGIIETOFQVNEMTFRLYDVGGORGERKWLNCFDSVHAVLFVVALSSFDLKLMEDPSVSRLQES 249
$Ga_{\alpha_{v2}}^{-}$	NDSALYFFENISRII-APKYVPTET-DVLRVRVRTCGI1ETOFQLNEMIFRLYDVGGQRSERRKWLRCFDCIQAVLFVVALSSYDMTQAEDPSGNRLQES 251
$Tr_G\alpha_{v_2}$	NDSALYFFENMNRII-APLYVPTET-DVLRVRVRTCGVVETOFQLNOMIFRLYDVGGQRSQRRKWLKCFEGIQAVWFVAALSSYDTTLMEASPVNRLQES 251
$Tn_G\alpha_{v_2}$	NDSALYFFENMTRII-APLYVPTEA-DVLRVRVRTCGIVETHFQHKQTIYRLYDVGGQRSGRKWLSCFEGVQAVWFVVALSSYDQMLAELPPMNRLKES 251
$Lg_G\alpha_v$	NDSAIYYFENMDRIC-SIKFOPSCT-DVLRARVRTTGVIETCFKIDGGVIRMFDVGGORSERRKWIQCFDDVRCILFVAALSCYDLTLFEDPSVNRLVES 250
Tc_Gav	NDSALYLFENMERIC-DPKYVPTPT-DVLRARVRTQG11ETHFRINDMIVSMYDVGGQRSQRRKW1YCFDDVRAVLFVVSLSGYDMTLLEDPSVNRLDES 249
Csp_Ga	NDSAIYYFENMHRLC-SEKFVPTVT-DVLRARVRTQGVIETCFKFRHCMFRMFDVGGQRSERRKWIHCFDNVHAIIFVAALSGYDMTLAEDPSINRLEES 249
Gc Ga *	NDSAPYYFQNMDRLL-REDYVPDEQ-DVLRSRVQTTG11ETSFRVKQLTYRVVDVGGQRSERRKW1QCFDDVRAVLFVCALSGYDMTLFEDGKTNRLEES 249
$Ef G\alpha_{u1} +$	NDSAPYYFENMDRLL-KPDYVPDEQ-DVLRSRVQTTG11ETSFRVEKLVYRVVDVGGQRSERRKW1QCFDDVKAVLFVVALNGYDMTLFEDGKTNRLEES 194
$Ef_G\alpha_{v2}^+$	EDSASYFLGDMDRLL-QSGYVPNEQ-DVLRSRVQTTG11ETSFRVKKLIYRIVDVGGQRAERSKWIQCFDDVKAVLFVVALNGYDMTLAEDGVTNRMREA 193
Hs_Ga _{t1}	NDSAGYYLSDLERLV-TPGYVPTEQ-DVLRSRVKTTG11ETQFSFKDLNFRMFDVGGQRSERKKW1HCFEGVTC11F1AALSAYDMVLVEDDEVNRMHES 242
Hs_Ga _{i1}	NDSAAYYLNDLDRIA-QPNYIPTQQ-DVLRTRVKTTGIVETHFTFKDLHFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSDYDLVLAEDEEMNRMHES 246
Hs_Gaa	SDSTKYYLNDLDRVA-DPAYLPTQQ-DVLRVRVPTTG11EYPFDLQSV1FRMVDVGGQRSERKWIHCFENVTS1MFLVALSEYDQVLVESDNENRMEES 251
Hs_Ga ₁₂	GESVKYFLDNLDRIG-QLNYFPSKQ-DILLARKATKGIVEHDFVIKKIPFKMVDVGGQRSQRQKWFQCFDGITSILFMVSSSEYDQVLMEDRRTNRLVES 273
Hs_Gas	IDCAQYFLDKIDVIK-QADYVPSDQ-DLLRCRVLTSGIFETKFQVDKVNFHMFDVGGQRDERRKWIQCFNDVTAIIFVVASSSYNMVIREDNQTNRLQEA 269

Fig. S1. continued

	★ V <u>G4</u> ★★ ★★	*	<u>G5</u>
$Dr_G\alpha_{v1}$	LKLFSSICNNIFFRGTSM-ILFMNKIDLFQEKILHSGRHLRHYLPQFR	GADCDVDAAARFIADMFVSLNASPSI	(L-IYHHFTTATD 336
Ga_{v1}	MKLFSSICNNIYFHSTSV-ILFMNKIDLFQDKILHSGRHLRLYLPQFK	<mark>G</mark> ADCDVDSSARFIAATFLSLNAAPSI	KL-VYHHFTTATD 335
Tr_Gα _{v1}	MNLFSAICNNVFFRSTSM-ILFMNKLDLFQDKVLHSGRHLRLYLPQFK	<mark>G</mark> ADCDVDSAAHFMASTFVSLNATPSI	(L-IYHHFTTATD 334
Tn_Gα _{v1} *	MNLFSSICNNVFFRSTSV-ILFMNKLDLFEEKILHSGRHLRLYLPQFK	<mark>Gadcdvdaaarfvasaf</mark> vslnttp{	KL-IYHHFTTATD 335
$01_G\alpha_{v1}$	MNLFSSICNSLFFCSTSM-VLFMNKIDLFQDKILHSGRHLRFYWPQFK	<mark>G</mark> ADRDVDSAARFITAR <mark>F</mark> LSLNKTPSI	RL-IYHHFTTATD 334
$Sa_G\alpha_{v1}*$	LRLFSSICNNIFFSSTSM-ILFLNKIDLFQEKILHSGRHLRLYFAQYR	<mark>Gadcdvdsaaryvanqf</mark> lslnrspA	(M-VYHHFTTATD 335
$Cm_G\alpha_{v1}$ †	LKLFSSICNNVFFRSTSMVILFLNKIDLFQEKILHSERHLRLYFSEYR	<mark>GadCDVD</mark> AGARFVANQFLSLNRNVA	KL-VYHHFTTATD 263
$Bf_G\alpha_v$	LRLFSSICNNLFFKDTSM-ILFMNKVDLFQEKILNSGRHLRYYFPSYT	<mark>g</mark> SDCDVDSAARy I QHM <mark>F</mark> QGCNKNPSI	(V-IYPHFTTATD 333
$Sp_G\alpha_v +$	LKL <mark>F</mark> ESICNNCFFRQTTM-I L FL NKVD LFQF K ILHTSRQLQHYFPDFQ	<mark>g</mark> pdydIdaaakyIqrr <mark>f</mark> qqcnrnpki	KE-VYPHFTTATD 279
$01_G\alpha_{v2}$	LQLFSSVCTNTLFQKISL-ILFLNKMDLFHKKILHSGRHLRFYLSSYK	<mark>G</mark> ADGDVDAAACHITDL <mark>F</mark> FACNN-FDF	RP-IYHHFTTATN 332
Ga_{v2}	LELFKSICTNTVFRSTSL-ILFMNKTDLFRDKILHSGRHLRFYLSSYK	<mark>G</mark> ADCDVDAAAHHIAAM <mark>F</mark> SLCNCSPAI	EP-VYHHFTTATD 335
Tr_Gα _{v2}	LELFASTCANGIFRRTSV-ILLMNKTDLFQEKIRHSGRHLRLYFSEYQ	<mark>G</mark> DDGDVDAAAHF I TAM <mark>F</mark> SSCSRGPDF	RP-VYHHYTTATD 335
$Tn_G\alpha_{v2}$	LDLFASVCTNSIFRGTSM-ILLLNKTDLFQEKIRHSGRHLRLYFSNYK	<mark>Gadgdvdaaahf</mark> itam <mark>f</mark> sscghF	RA-AFHHYTTATD 332
$Lg_G\alpha_v$	LKLFRGIANNRFFNNTAT-ILFLNKLDLFQDKIRHSGRHLRYYFPDFS	<mark>g</mark> pdydvdsaaryiqhlftmqcnnpsi	(V-IYPHFTTATD 334
Tc_Gα _v	LNLFGQIVNNPFFRDASF-VLFLNKFDLFREKILYSQRHLRLYFPDYK	<mark>g</mark> pdrdvdrgalfiqhk <mark>f</mark> vlknadsri	(V-LYPHFTTATD 333
$Csp_G\alpha_v$	LRIFKQICSIPFFRRAIL-ILFLNKMDLFTDKITRFNRHLKYYFHNFK	<mark>G</mark> PIHEAQPAAEFIRDM <mark>F</mark> ISHAIEAGI	KHHVYSHVTTATD 334
$Gc_G\alpha_v *$	LNLFQAICNNKFFVKTSM-ILFLNKADLFRDKITNSDRHLRLYFTQYT	<mark>g</mark> pdrdveaasrfigsefmernlnkqi	(I-IYPHLTTATD 333
Ef_Gα _{v1} †	LNLFQAICNNKFFVKTSM-ILFLNKVDLFENKVLTSDRQLRNYFSSYQ	<mark>gpdrnpeeakkfiqsqflernmskn</mark> i	(I-IYPHFTTATN 278
$Ef_G\alpha_{v2}^{\dagger}$	LVLFEDICNKPIFARTSM-ILFLNKVDAFETKILDTDRHLRTFFPEFS	<mark>g</mark> pdhdveaakeyiksq <mark>f</mark> lacnksrgi	Q-ITPHFTTATN 277
Hs_Gat1	LHLFNSICNHRYFATTSI-VLFLNKKDVFFEKIKKAHLSICFPDYD	GPN-TYEDAGNYIKVQFLELNMRRD'	KEIYSHMTCATD 324
$Hs_G\alpha_{i1}$	MKLFDSICNNKWFTDTSI-ILFLNKKDLFEEKIKKSPLTICYPEYA	<mark>GSN-</mark> TYEEAAAYIQCQFEDL <mark>NK</mark> RKD	IKEIYTHFTCATD 328
Hs_Ga _a	KALFRTIITYPWFQNSSV-ILFLNKKDLLEEKIMYSHLVDYFPEYD	<mark>G</mark> PQRDAQA <mark>AREFILKMFVDLNPD</mark> SDI	(I-IYSHFTCATD 333
Hs_Ga ₁₂	MNIFETIVNNKLFFNVSI-ILFLNKMDLLVEKVKTVSIKKHFPDFR	GDPHRLEDVQRYLVQCFDRKRRNRSH	KP-LFHHFTTAID 355
Hs_Gas	LNLFKSIWNNRWLRTISV-ILFLNKQDLLAEKVLAGKSKIEDYFPEFARYT	TPEDATPEPGEDPRVTRAKYF1RDEFLR1STASGDG	RHYCYPHFTCAVD 368

Fig. S1. continued

	* *
$Dr_G\alpha_{v1}$	TSNVQVVFQVVMDTIIKENLEÄVSLL 362
Ga_{v1}	TSNVEVVFEVVMDTIVKENLEAVSLL 361
$Tr_G\alpha_{v1}$	TSNIQVVFQVVMDTIIKENLEAVSLL 360
Tn_Gα _{v1} *	TSNIQVVFQVVMDTIIKENLEAVSLL 361
$0I_G\alpha_{v1}$	TSSIQVVFQVVMDTIIKENLQAMSLL 360
Sa_Ga _{v1} *	TSNVQIVFQGRHGHHYKRELEAVRLL 361
$Cm_G\alpha_{v1}$ +	TSNVQVVFQVVMDTIIKENLEAVSLL 289
$Bf_G\alpha_v$	TSNIQVVFQVVMDTIIRENLEAASLL 359
Sp_Gav +	TGNMEVVFQVVTNTIVKDNLEAAALM 305
$0I_G\alpha_{v^2}$	TANVQVVLHVVLNQIIEGNLAAFQPF 358
Ga_{V^2}	TTNVQVVFHMVIDQVMKGNLAAVQLL 361
$Tr_G\alpha_{v_2}$	TASVRLVFHMVVDQIVKDNLASVQLL 361
$Tn_G\alpha_{v_2}$	TAAVRVVLHMVVDQISKDNLASVQLL 358
$Lg_G\alpha_v$	TSNIQVVFQVVMDSVLRENIKAVSIL 360
Tc_Gav	TANVQVVFQAVMEMVISTNLGQVTLL 359
$Csp_G\alpha_v$	TNQVQSVFCQVVEGIVQANLSQAQLL 360
$Gc_G\alpha_v *$	TTNIKVVFGVVLDTIIRENLEAANLL 359
$Ef_G\alpha_{v1}$ †	TSNIRVVFQAVLDAIIRENLEAANLL 304
$Ef_G\alpha_{v2}^{\dagger}$	TSNVRVVIDAVIEAIIRENLESIGLIREDE 307
Hs_G α_{t1}	TQNVKFVFDAVTDIIIKENLKDCGLF 350
Hs_Glpha_{i1}	TKNVQFVFDAVTDVIIKNNLKDCGLF 354
$Hs_G\alpha_q$	TENIRFVFAAVKDTILQLNLKEYNLV 359
Hs_Ga ₁₂	TENVRFVFHAVKDTILQENLKDIMLQ 381
Hs_G α_s	TENIRRVFNDCRDIIQRMHLRQYELL 394

SANG SANG

Conservation

Red Bold: conserved among all G alpha classes
Red: 100% conserved within a class
Blue: 80-99% conserved within a class
Green: 60-79% conserved within a class
Shaded in grey: atypical residue at the conserved position
★★★: Gv specific conservation (color coded as above)

Modification

Shaded in purple (N-terminal): acylation sites (C-terminal): ADP-ribosylation site by Pertussis toxin

Contact sites

Shaded in blue: RGS/GAP contact sites Shaded in green: effector contact sites Shaded in yellow: both RGS/GAP and effector contact sites Boxed: atypical residue at contact sites (color coded as above)

Splice sites

 ∇ : junction between two codons

- ▼: junction within a codon
- **⊽**: Gv specific junction

Fig. S1. continued



Fig. S2. Single-site d_N/d_S analysis of teleost *gnav* genes: sequence plot of neutral (open boxes) and negatively selected (shaded boxes) sites (codons) in each *gnav* family. Dark and light shading corresponds to significance levels of P < 0.1 and P < 0.2, respectively. Amino acid numbers are shown to the right. G-boxes and switch regions are indicated. Asterisks indicate Gv-specific motifs.

DNAS



Fig. 53. Expression pattern of *gnav1* in zebrafish larvae: whole-mount in situ hybridization of *gnav1* probe (A–C and G–J) and negative control (sense probe, *D–F* and *K–N*) with 3 dpf zebrafish larvae. Embryos were grown in 0.0045% of phenylthiourea (Sigma) from 12 h postfertilization until used to prevent pigmentation. Probes were synthesized with T3 RNA polymerase (Roche) by following manufacturer's instructions. Templates for in vitro transcription were amplified using primers listed in Table S3. Probes were hybridized at 65 °C overnight. Specific probe hybridization was detected with anti-DIG antibody conjugated with alkaline phosphatase (1:5,000; Roche), using Nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate (Roche) as substrates. Stained larvae were observed and photographed with a Nikon SMZ-U binocular and an attached Nikon CoolPix 950 digital camera. Stained larvae were cryo-sectioned (8 μm), counterstained in 0.001% methyl green, mounted with VectaMount (Vector), and documented on a Zeiss Axioplan microscope and an attached AxioCamMRc5. (*A* and *D*) Lateral view of whole larvae. (*B* and *E*) lateral view around the developing inner ear. (*C* and *F*) Ventral view of head region. Dotted circle, mouth. (*G–N*) Cross-sections of stained larvae at the levels of developing inner ear (*G* and *K*), mouth (*H* and *L*), pectoral fin (*I* and *M*), and branchial arches (*J* and *N*). Dorsal is to the top. White and gray arrowheads indicate the cell clusters near the lip and midbrain–bindbrain boundary, respectively. White, yellow, and black arrowheads point to labeled cells within pectoral fin (pf), otic vesicle (ov), and branchial arches (ba), respectively. e, eye; hb, hindbrain; mo, mouth cavity; op, operculum; ph, pharynx; y, yolk. (Scale bars: 50 μm.)



Fig. 54. Expression pattern of *gnav* compared to *gna* genes of the other 4 classes. Whole-mount in situ hybridization of *gnav*, *gnas*, *gnat2*, *gnao1*, *gna11a*, and *gna13a* probes with 3 dpf zebrafish larvae was performed as described in Fig. S3. All images are lateral views, and anterior is to the left. Note that expression patterns are characteristically different and none from the other 4 classes is similar to that of *gnav1*. Colored frames enclose genes from the same class (red, Gv; blue, Gs; purple, Gi; green, Gq; yellow, G12). Primers used to clone *gna* genes are as follows: gnas-fw, 5'-agaactgaggaccagcgaaa-3'; gnas-rv, 5'-gctggacaggctaactggac-3'; gnat2-fw, 5'-ctggtgaagctgccacagta-3'; gnat2-rv, 5'-gcttcctcacaagcgccatt-3'; gna01-fw, 5'-ccagtccaacgctgtctttt-3'; gna01-rv, 5'-cgctccttgtcccgtactc-3'; gna11a-fw, 5'-cgatcaggttctggtggaat-3'; gna11a-rv, 5'-tgaaaggcgagttggagtct-3'; gna13a-fw, 5'-agaaactgcacatcccttgg-3'; and gna13a-rv, 5'-ttttggctgggcaagtagtc-3'.

DNAS

Table S1. List of teleost gnav genes

Gene	Protein	Ensembl	Chromosome	Start	End	Orientation
Dr_gnav1	Dr_Gα _{v1}	ENSDARG00000043006 ⁺	22	9,130,267	9,186,364	_
Ol_gnav1	$OI_G\alpha_{v1}$	ENSORLG0000012122	1	31,556,132	31,583,202	_
Ol_gnav2	$OI_G\alpha_{v2}$	ENSORLG0000007929	8	10,625,829	10,632,451	+
Ga_gnav1	$Ga_G\alpha_{v1}$	ENSGACG00000019015 ⁺	Group IX	15,176,864	15,193,316	-
Ga_gnav2	$Ga_G\alpha_{v2}$	ENSGACG00000011158 ⁺	Group XI	9,238,874	9,244,473	+
Tr_gnav1	$Tr_G\alpha_{v1}$	SINFRUG00000129994 [±]	Scaffold 189	438,137	448,743	_
Tr_gnav2	$Tr_G\alpha_{v2}$	SINFRUG00000135273 [‡]	Scaffold 115	694,830	698,835	+
Tn_gnav1*	$Tn_G\alpha_{v1}$	GSTENG00026255001 [±]	18	2,116,654	2,124,011	_
Tn_gnav2	$Tn_G\alpha_{v2}$	GSTENG00018570001*	3	10,269,269	10,272,711	+

The gene list of teleost gnav orthologs is shown. Gene identification numbers (IDs) and locations given here are derived from Ensembl release 48 (December 2007). Dr, zebrafish; Ol, medaka; Ga, stickleback; Tr, fugu; Tn, tetraodon. Note that a rare alternative start codon is used for Tn_Gα_{v2} and that a noncanonical splicing donor sequence (GC) instead of the canonical one (GT) is used for $Tr_{-}G\alpha_{v2}$ in intron 3 and for $Tn_{-}G\alpha_{v2}$ in introns 3 and 8. Other sequences used for phylogenetic analysis (Fig. 1) are as follows: XM_968117 (Tc_Gα_v, previously predicted as Gα_{i2}); AB006548 (Ef_Gα_{v1}, previously named Gα₆); AB006549 (Ef_Gα_{v2}, previously named Ga₇); Y14247 (Gc_Ga_v, previously reported as Ga₀); EE049147 and DV496403 (Sa_Ga_{v1}); AAVX01181130, AAVX01051239, AAVX01180564, AAVX01051240, AAVX01416005, AAVX01144285, and AAVX01077290 (Cm_Gα_{v1}); JGI protein ID 57428 (Bf_Gα_v); SPU_024792 and SPU_024793 (Sp_Gα_v, combined from previously named $G\alpha_{oL}$ and $G\alpha_{oL2}$); JGI protein IDs 185322 and 227716 (Lg_ $G\alpha_v$ and Csp_ $G\alpha_v$); XP-685500 (Dr_ $G\alpha_s$); AAS92627 (Dr_ $G\alpha_{olf1}$); ENS-DARG00000045415 (Dr_Gα_{0lf2}); ENSDARG00000044199 (Dr_Gα_{t1}); ENSDARG00000042529 (Dr_Gα_{t2}); ENSDARG00000016676 (Dr_Gα₀₁); ENSDARG00000036058 (Dr_Gα₀₂); ENSDARG00000021647 (Dr_Gα_{11a}); ENSDARG0000044760 (Dr_Gα_{11b}); ENSDARG00000017294 (Dr_Gα_{12a}); NP_001001818 (Dr_Gα_{12b}); ENS-(Dr_Gα_{11b}); ENSDARG0000025013 (Dr_Gα_{14a}); BC077106 (Dr_Gα_{14a}); BC077141 (Dr_Gα_{15a}); ENSDARG00000056654 (Dr_Gα_{15b}); ENSDARG00000031543 (Dr_Gα_{15c}); ENSDARG00000063231 (Dr_Ga_{15d}); ENSDARG00000025826 (Dr_Ga₁₂); NP_001012243 (Dr_Ga_{13a}); ENSDARG00000037924 (Dr_Ga_{13b}); NP_000507 (Hs_Ga_s); NP_002062 (Hs_Gα₀₁f); NP_000163 (Hs_Gα₁₁); NP_005263 (Hs_Gα₁₂); NP_001095856 (Hs_Gα₁₃); NP_066268 (Hs_Gα₀); NP_002060 (Hs_Gα₁₁); NP_002061 (Hs_Gα₁₂); NP_006487 (Hs_Gα₁₃); NP_002064 (Hs_Gα₂); NP_002063 (Hs_Gα_q); NP_002058 (Hs_Gα₁₁); NP_004288 (Hs_Gα₁₄); NP_002059 (Hs_Gα₁₅); NP_031379 (Hs_Gα₁₂); NP_006563 (Hs_Gα₁₃); NP_477502 (Dm_G-iα65A); NP_523684 (Dm_G-oα47A); NP_725191 (Dm_dgq); NP_477506 (Dm_G-sα60A); NP_524118 (Dm_Gfα); NP_001036421 (Dm_cta); CG30054 (Dm_CG30054); CG17760 (Dm_CG17760); CG40005 (Dm_CG40005); GCY14248 (Gc_Gα₀); GCY14249 (Gc_Gα₂); AB006543 (Ef_Gα₁); AB006544 (Ef_Gα₂); AB006545 (Ef_Gα₃); AB006546 (Ef_Gα₄); AB006547 (Ef_Gα₅); AB006550 (Ef_Gα₈); and AB006551 (Ef_Gα₉).

*A possible pseudogene (see Fig. S1).

[†]The predictions that lack a small part of the protein sequence (mostly N-terminal).

[‡]The predictions that lack \ge 1 exons.

Table S2. List of EST clones for teleost gnav genes

PNAS PNAS

Gene	EST accession	Length (bp)	Coverage of gnav CDS (%)	Nucleotide identity (%)
Dr_gnav1	CT736382.2	817	74	99
-	EE310328.1	848	73	99
	EB899666.1	650	58	99
	AL916853.1	539	18	98
	DT073850.1	853	14	100
	CD595967.1	650	19	90
	BI983956.1	569	13	100
	BG799669.1	444	10	98
	CK674763.1	447	4	100
Ol_gnav1	AM344465.1	815	68	99
-	AM304638.1	770	64	99
	AM303462.1	758	61	99
	AM317248.1	737	59	99
	AM336808.1	735	58	99
	AM326993.1	689	55	99
	AM353138.1	700	54	99
	AM315079.1	510	47	99
	AM367625.1	577	43	100
	AM370671.1	350	26	100
	AM322694.1	369	27	98
	AM383108.1	402	26	96
Ol_gnav2	AM149874.1	494	43	99
Ga_gnav1	DN665837.1	1323	81	93
	DN662698.1	1371	77	94
Tr_gnav2	CA846482.1	795	32	99

The list of EST clones for teleost gnav genes identified from the NCBI database is shown. Dr, zebrafish; OI, medaka; Ga, stickleback; Tr, fugu.

Table S3. List of primers used for RT-PCR, cloning for full-length *gnav1*, and amplification of in vitro transcription templates

Primer	ner Sequence	
Dr-gnav1-fw	5'-gtgtggccgttgttttgatga-3'	RT-PCR
Dr-gnav1-rv	5'-catgtcgtatccactcagag-3'	RT-PCR
Dr-b-actin-fw	5'-ccccattgagcacggtatt-3'	RT-PCR
Dr-b-actin-rv	5'-agcggttcccatctcctg-3'	RT-PCR
Dr-gnav1–5′UTR-fw	5'-ccaactggacctttagctcttc-3'	Full-length cloning
Dr-gnav1–3′UTR-rv	5'-ccagcatatgcttggctcatc-3'	Full-length cloning
gnav1-N-fw	5'-atgggtctgtgtttgggctc-3'	Template amplification
gnav1-N-rv	5'-catcaaaacaacggccacac-3'	Template amplification
gnav1-M-fw	5'-ggtgtgcgttcgtctgcggc-3'	Template amplification
gnav1-M-rv	5'-catgtcgtatccactcagag-3'	Template amplification
T3-promoter site	5'-tattaaccctcactaaagggaa-3'	Template amplification

Reverse (rv) or forward (fw) primers were attached with the T3 RNA polymerase promoter site at their 5' end for the anti-sense or sense probes, respectively.