AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756

Annu. Rev. Biochem. 2004. 73:925–51
doi: 10.1146/annurev.biochem.73.011303.073756
Copyright © 2004 by Annual Reviews. All rights reserved

RETURN OF THE GDI: The GoLoco Motif in Cell Division

Francis S. Willard, Randall J. Kimple, and David P. Siderovski

Department of Pharmacology, Lineberger Comprehensive Cancer Center, and UNC Neuroscience Center, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, 27599-7365; e-mail: fwillard@med.unc.edu, kimplera@med.unc.edu, dsiderov@med.unc.edu

Key Words asymmetric cell division, guanine nucleotide dissociation inhibitors, heterotrimeric G proteins, RGS proteins

■ **Abstract** The GoLoco motif is a 19-amino-acid sequence with guanine nucleotide dissociation inhibitor activity against G-alpha subunits of the adenylyl-cyclase-inhibitory subclass. The GoLoco motif is present as an independent element within multidomain signaling regulators, such as Loco, RGS12, RGS14, and Rap1GAP, as well as in tandem arrays in proteins, such as AGS3, G18, LGN, Pcp-2/L7, and Partner of Inscuteable (Pins/Rapsynoid). Here we discuss the biochemical mechanisms of GoLoco motif action on G-alpha subunits in light of the recent crystal structure of G-alpha-i1 bound to the RGS14 GoLoco motif. Currently, there is sparse evidence for GoLoco motif regulation of canonical G-protein–coupled receptor signaling. Rather, studies of asymmetric cell division in *Drosophila* and *Caenorhabditis elegans*, as well as mammalian mitosis, implicate GoLoco proteins, such as Pins, GPR-1/GPR-2, LGN, and RGS14, in mitotic spindle organization and force generation. We discuss potential mechanisms by which GoLoco/Gα complexes might modulate spindle dynamics.

CONTENTS

INTRODUCTION 926
STRUCTURE AND FUNCTION OF THE GoLoco MOTIF 927
Role of the Acidic-Glutamine-Arginine Triad 929
Role of Residues C-terminal to the Conserved Motif 934
Modulation of GDI Activity by Phosphorylation 935
GoLoco MOTIF PROTEINS IN GPCR PATHWAY MODULATION 936
GoLoco Peptides as Tools to Uncouple GPCRs 936
Loco and RGS12 936
Rap1GAP Isoforms 937
GoLoco MOTIF PROTEINS IN CELL DIVISION 937
Asymmetric Cell Division in *Drosophila* Neuroblasts 938

0066-4154/04/0707-0925$14.00

balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

Heterotrimeric G-Protein Involvement in *Drosophila* ACD 938
Mechanism of G-Protein Activation in *Drosophila* ACD 941
ACD in the *C. elegans* One-Cell Embryo 942
Heterotrimeric G-Protein Involvement in *C. elegans* ACD 943
A Cycle of GDI, GEF, and GAP Activities in *C. elegans* ACD? 944
Heterotrimeric G-Protein Involvement in Mammalian Cell Division 946
CONCLUDING REMARKS 948

INTRODUCTION

Signal transduction via heterotrimeric G-protein–coupled receptors (GPCRs) typically evokes a switch in the status of the G-protein alpha subunit (Gα), the guanine nucleotide-binding component of the G$\alpha\beta\gamma$ heterotrimer. Although normally tightly bound to its G$\beta\gamma$ partner when in the inactive, guanosine diphosphate (GDP)-bound state, Gα is converted to its active, guanosine triphosphate (GTP)-bound form via the guanine nucleotide exchange factor (GEF) activity of ligand-occupied GPCRs. GTP-bound Gα dissociates from G$\beta\gamma$, and thus both moieties become free to modulate the actions of a multitude of intracellular "effector" enzymes and ion channels (1, 2). Intrinsic guanosine triphosphate phosphohydrolase (GTPase) activity of Gα reverts the subunit back to the GDP-containing, G$\beta\gamma$-complexed form. The return of Gα to its ground state is dramatically hastened by "regulator of G-protein signaling" (RGS) proteins that serve as selective GTPase-accelerating proteins (GAPs) for various Gα subtypes (3, 4).

A common feature of RGS proteins is their possession of additional protein-protein interaction domains beyond their signature "RGS box" that exerts Gα-directed GAP activity (5, 6). In the original cloning of *loco*, the *Drosophila melanogaster* orthologue of *Rgs12*, Granderath, Klämbt, and colleagues identified a second, distinct interaction site for Gα subunits (region D) C-terminal to the RGS box of the encoded protein (7). Bioinformatic analyses of the region-D sequences from Loco and RGS12 led to our realization (8) that several other proteins, each previously identified as binding alpha subunits of the adenylyl-cyclase-inhibitory or Gi subclass (G$\alpha_{i1\text{-}i3}$, Gα_o, Gα_z), all harbored a highly conserved 19-amino acid polypeptide. Ponting (9) came to the same conclusion independently. We named this conserved polypeptide the GoLoco motif as an acronym for the G$\alpha_{i/o}$-Loco interaction (8). Several groups have since shown that binding of a Gα·GDP subunit to the GoLoco motif slows spontaneous nucleotide release (10–13). Hence, GoLoco motif-containing proteins are considered guanine nucleotide dissociation inhibitors (GDIs) for Gi-subclass alpha subunits.

Contemporaneously with the *in silico* discovery of the GoLoco motif, Cismowski, Lanier, and colleagues (14) identified three rat brain cDNAs in a yeast-based screen for receptor-independent activators of the G$\beta\gamma$-dependent pheromone signaling pathway. The third member of this disparate set of activator of G-protein signaling proteins (AGS3) was found to sequester GDP-bound Gα_{i2}

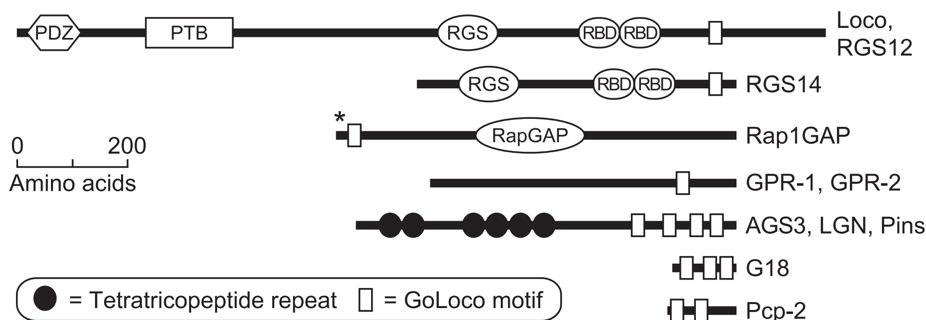


Figure 1 The $G\alpha_{i/o}$-Loco interaction or GoLoco motif is found singly, or in tandem arrays, in a number of different proteins. Domain abbreviations are PDZ, PSD-95/Discs large/ZO-1 homology domain; PTB, phosphotyrosine-binding domain; RGS, regulator of G-protein signaling box; RBD, Ras-binding domain; and RapGAP, Rap-specific GTPase-activating protein domain. Asterisk denotes N-terminal variation in GoLoco motif sequence between isoforms I and II of Rap1GAP.

in yeast, resulting in pheromone- and receptor-independent activation of $G\beta\gamma$-dependent responses (15). Lanier and colleagues (15) coined an alternate acronym, GPR for G-protein regulatory, to describe the highly conserved $G\alpha$·GDP-binding site found repeated four times within the C terminus of AGS3. However, the term GPR is already employed in the G-protein signaling field as one naming convention for orphan GPCRs (16). We will use the designation GoLoco for the rest of this review. [In the primary literature, the motif has never been called Loco homology domain contrary to use of that terminology in a recent review in this series (17)].

The GoLoco motif has now been identified in several distinct classes of proteins encoded in metazoan genomes (Figure 1), including: modulators of heterotrimeric and Ras family G-protein signaling (RGS12, RGS14, Rap1GAP), several variations on the tetratricopeptide repeat (TPR), multi-GoLoco architecture of AGS3 (LGN, Pins, GPR-1/-2), and two short polypeptides with multiple GoLoco motifs (G18, Pcp-2/L7). The GoLoco motif was first discovered in the context of plasma membrane-delimited GPCR signaling, and its ability to bind $G\alpha$·GDP to the exclusion of $G\beta\gamma$ is proving a useful tool in examining receptor/G-protein/effector coupling. However, a central role has recently emerged for GoLoco motif-containing proteins in otherwise unexpected arenas, the control of mitotic spindle force generation and the act of cell division (18).

STRUCTURE AND FUNCTION OF THE GoLoco MOTIF

An alignment of all currently known GoLoco motifs is presented in Figure 2. The entire motif was predicted to fold as an amphipathic α-helix (10, 15). However, in the structure of the RGS14 GoLoco motif (aa 496–531) bound to $G\alpha_{i1}$·GDP

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

Sequence name	Alignment	Residues	Accession
zAGS3.GL4	EDFFSLIQKVQSK.RMDEQR	80- 98	ens|ENSDARP00000026753
(f,h,m,r)AGS3.GL4	EDFFSLIQRVQAK.RMDEQR	579- 597	ens|SINFRUP00000127441
(m,r)LGN.GL4	EDFFSLILRSQAK.RMDEQR	622- 640	gb|AAL87447
(f1,z)LGN.GL4	EDFFSLIMRSQAK.RMDEQR	626- 644	ens|SINFRUP00000160450
hLGN.GL4	EDFFSLILRSQGK.RMDEQR	622- 640	gb|AAN01266
fLGN2.GL4	DDFFSLILRSQSN.RMEEQR	597- 615	ens|SINFRUP00000146023
aPins.GL4	EDFFSLIMRLQGG.RMEDQR	653- 671	gb|EEA14880
dPins.GL3	EDFFSLIMKVQSG.RMEDQR	613- 631	gb|AAF64499
(m,r)G18.GL2	EQLYSTILSHQCQ.RIEAQR	104- 122	gb|AAH21942
hG18.GL2	EQLYSTILSHQCQ.RMEAQR	105- 123	gb|AAF67476
aPins.GL3	DAFLDMLMRCQGS.RIEEQR	599- 617	gb|EEA14880
dPins.GL2	DDFLDMLMRCQGS.RLEEQR	552- 570	gb|AAF64499
zAGS3.GL3	DEFFNMLIKYQSS.RINDQR	51- 69	ens|ENSDARP00000026753
(m,r)AGS3.GL3	DEFFNMLIKYQSS.RIDDQR	595- 613	ref|NP_700459
hAGS3.GL3	DDFFNMLIKYQSS.RIDDQR	574- 592	gb|AAO17260
fAGS3.GL3	DDFFNMLIKCQSS.RIDDQR	545- 563	ens|SINFRUP00000127441
fLGN2.GL3	DVFFDMLVKCQGS.RLDDQR	563- 581	ens|SINFRUP00000146023
fLGN1.GL3	DDFFDMLVKCQGS.RLDDQR	592- 610	ens|SINFRUP00000160450
zLGN.GL3	DQFFDMLVKCQGS.RLEDQR	589- 607	ens|ENSDARP00000026291
(h,m,r)LGN.GL3	EDFFDILVKCQGS.RLDDQR	588- 606	gb|AAL87447
rRap1GAP2	AEFFEMLEKMQGI.KLEEQR	163- 181	ref|XP_220692
rPcp-2.GL1	EGFFNLLSHVQGD.RMEEQR	24- 42	ref|XP_221787
hPcp-2.GL1	EGFFNLLSHVQGD.RMEGQR	8- 26	gb|AAN52488
mPcp-2.GL1	EGFFNLLTHVQGD.RMEEQR	8- 26	gb|AAN52485
fLGN2.GL1	DGFFELLSRFQGN.RLEDQR	450- 468	ens|SINFRUP00000146023
(h,m)G18.GL3	QELLELLLRVQGGGRMEEQR	133- 152	gb|AAF67476
rG18.GL3	QELLELLLRVQGGGRMEDQR	131- 150	ref|XP_215346
aLoco	DELLEGLKRAQRS.RLEDQR	639- 657	gb|EAA08111
dLoco	DELLEGLKRAQLA.RLEDQR	1355-1373	gb|AAM50799
CbAGS3.GL3	DHLVEWLMRVQSQ.RLDEQR	521- 539	ens|ENSCBRP00000006892
CeAGS3.GL3	EHLVEWLMRVQGE.RLDEQR	522- 540	gb|AAL27247
(h,m,r)RGS14	EGLVELLNRVQSS.GAHDQR	498- 516	sp|O08773
dRap1GAP	QDLFELLERVQCS.RLDDQR	45- 63	gb|AAF52527
(h,m,r)RGS12	EEFFELISKAQSN.RADDQR	1188-1206	sp|O08874
fRGS12	EEFFELLSRAQSA.RANDQR	503- 521	ens|SINFRUP00000158915
(f1,h,z)LGN.GL1	EGFFDLLSRFQSN.RMDDQR	476- 494	ens|SINFRUP00000160450
mPins/mLGN.GL1	EGFFDLLRRFQSN.RMDDQR	484- 502	gb|AAL87447
rLGN.GL1	EGFFDLLRRLQSS.RMEDQR	481- 499	ens|ENSRNOP00000016617
(m,r)AGS3.GL1	ECFFDLLSKFQSS.RMDDQR	471- 489	gb|AAF08683
fAGS3.GL1	DCFFDLLSKFQSS.RMDDQR	445- 463	ens|SINFRUP00000127441
hAGS3.GL1	ECFFDLLTKFQSS.RMDDQR	473- 491	gb|AAO17260
(Cb,Ce)AGS3.GL1	EEFFDMLAKLQSK.RMNDQR	427- 445	gb|AAL27247
aPins.GL1	EDFFDLLTRSQSK.RMDDQR	484- 502	gb|EEA14880
dPins.GL1	DDFFEMLSRSQSK.RMDDQR	468- 486	gb|AAF64499
(Cb,Ce)AGS3.GL4	EDVTAIVMRMQAG.RLEDQR	560- 578	gb|AAL27247
CeRap1GAP	EDFLNMIERMQSN.RLDDQR	80- 98	gb|AAK71368
CeGPR-1/-2	VDMMDLIFSM.SS.RMDDQR	425- 442	ref|NP_498900;NP_499066
CbGPR	MDFMDLICKM.NS.RMDDQR	421- 438	ens|ENSCBRP00000007225
(h,m,r)G18.GL1	ELLLDLVAEAQSR.RLEEQR	62- 80	gb|AAH21942
zLGN.GL2	EPFLRLLANAQGR.RLDEQR	531- 549	ens|ENSDARP00000026291
(h,m,r)LGN.GL2	DEFLDLLASSQSR.RLDDQR	537- 555	gb|AAL87447
fLGN2.GL2	GHFLELLASSQAR.RLDDQR	506- 524	ens|SINFRUP00000146023
(h,m,r,z)AGS3.GL2	EEFFDLIASSQSR.RLDDQR	3- 21	ens|ENSDARP00000026753
fAGS3.GL2	EELFDLIASSQSR.RLDDQR	497- 515	ens|SINFRUP00000127441
CbAGS3.GL2	EVLIDLILNAQER.RMDDQR	474- 492	ens|ENSCBRP00000006892
CeAGS3.GL2	EVLIDLLLNAQGR.RMDDQR	475- 493	gb|AAL27247
mPcp-2.GL2	DNLMDMLVNTQGR.RMDDQR	27- 45	sp|P12660
rPcp-2.GL2	DNLMDMLANTQGR.RMDDQR	64- 82	ref|XP_221787
hPcp-2.GL2	DSLMDMLASTQGR.RMDDQR	27- 45	sp|Q8IVA1
(h,m,r)Rap1GAPII	TDLFEMIEKMQGS.RMDEQR	27- 45	ref|XP_233608
fRap1GAP	TELFEIIEKLQGS.RIDEQR	2- 20	ens|SINFRUP00000130548
aPins.GL2	NVLLEMIAHFQSE.RMDEQR	536- 554	gb|EEA14880
GoLoco motif consensus:	--ΦΦ-ΨΨ + Qπ RΨ--QR		

Alpha-helical — Triad

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

(19), only the first 13 residues (aa 496–508) adopted an α-helical configuration. This N-terminal α-helix is sandwiched within the Ras-like domain of the Gα subunit between the α3-helix and switch II (Figure 3), the latter being one of three flexible "switch" regions in Gα that adopt nucleotide-state-dependent conformations (20). Binding of the GoLoco peptide results in a significant displacement of switch II away from the α3-helix (19), thus deforming positions within GDP-bound Gα that normally serve as critical contact sites for Gβ in the G$\alpha\beta\gamma$ heterotrimer (21, 22). This observation supports early findings that formation of Gα·GDP-G$\beta\gamma$ and Gα·GDP-GoLoco complexes are mutually exclusive events (13, 15, 23).

The end of the GoLoco α-helix is anchored by burial into Gα of the nearly invariant glutamine found in the middle of the motif (Figure 2). C-terminal to this middle glutamine residue, the GoLoco peptide makes a more relaxed meander across the nucleotide-binding pocket and forms extensive contacts with the Gα all-helical domain (Figure 3*C*), contacts that are critical to Gα-binding selectivity (19). The spatial relationship between the N-terminal α-helix and the rest of the GoLoco motif is of critical importance to function because insertion of alanines between these two elements destroys all GDI activity (24).

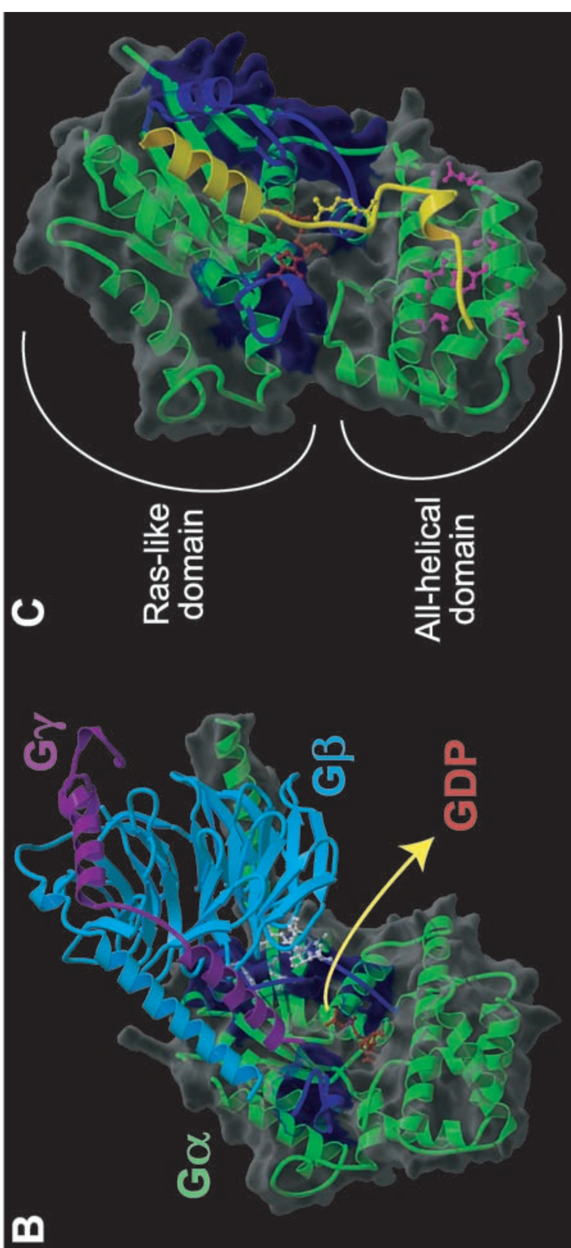
Role of the Acidic-Glutamine-Arginine Triad

Of all the positions within the GoLoco motif, the most highly conserved are the final three residues that comprise an acidic-glutamine-arginine triad (Figure 2). The structure of the RGS14 GoLoco-Gα_{i1}·GDP complex identified the final residue of this triad as an arginine "finger" (19) that is positioned by the

←

Figure 2 Comprehensive multiple sequence alignment of all known GoLoco motifs. Individual motifs from multi-GoLoco proteins are numbered from N to C terminus as GL#. The N-terminal α-helix and C-terminal acidic-glutamine-arginine triad, key features of the GoLoco motif fold as defined by the crystal structure of the RGS14 GoLoco/G α_{i1}·GDP complex (PDB accession 1KJY), are underlined below the GoLoco motif consensus. Species abbreviations are a, *Anopheles gambiae* (mosquito); Cb, *Caenorhabditis briggsae*; Ce, *Caenorhabditis elegans*; d, *Drosophila*; f, *Fugu rubripes*; h, human; m, mouse; r, rat; and z, *Danio rerio* (zebrafish). Sequence ranges and accession numbers from the Ensembl (ens), GenBank (gb), NCBI RefSeq (ref), and Swiss-Prot (sp) databases are denoted to the right of the sequence. Residues are colored according to side chain chemistry using Clustal-X defaults. Consensus symbols for amino acid character are hyphen (-), acidic; Φ, hydrophobic; Ψ, large aliphatic; (+), basic; and π, small side chain. Note that the GoLoco motif sequence denoted rRap1GAP2 is derived from a hitherto unpublished paralogue of Rap1GAP in the rat genome. The species abbreviation "f1" denotes sequence derived from the first of two LGN paralogues present in the *Fugu rubripes* genome (Figure 5). Based on sequence similarity and evolutionary relationships (Figure 5), mouse Pins should be called mouse LGN.

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

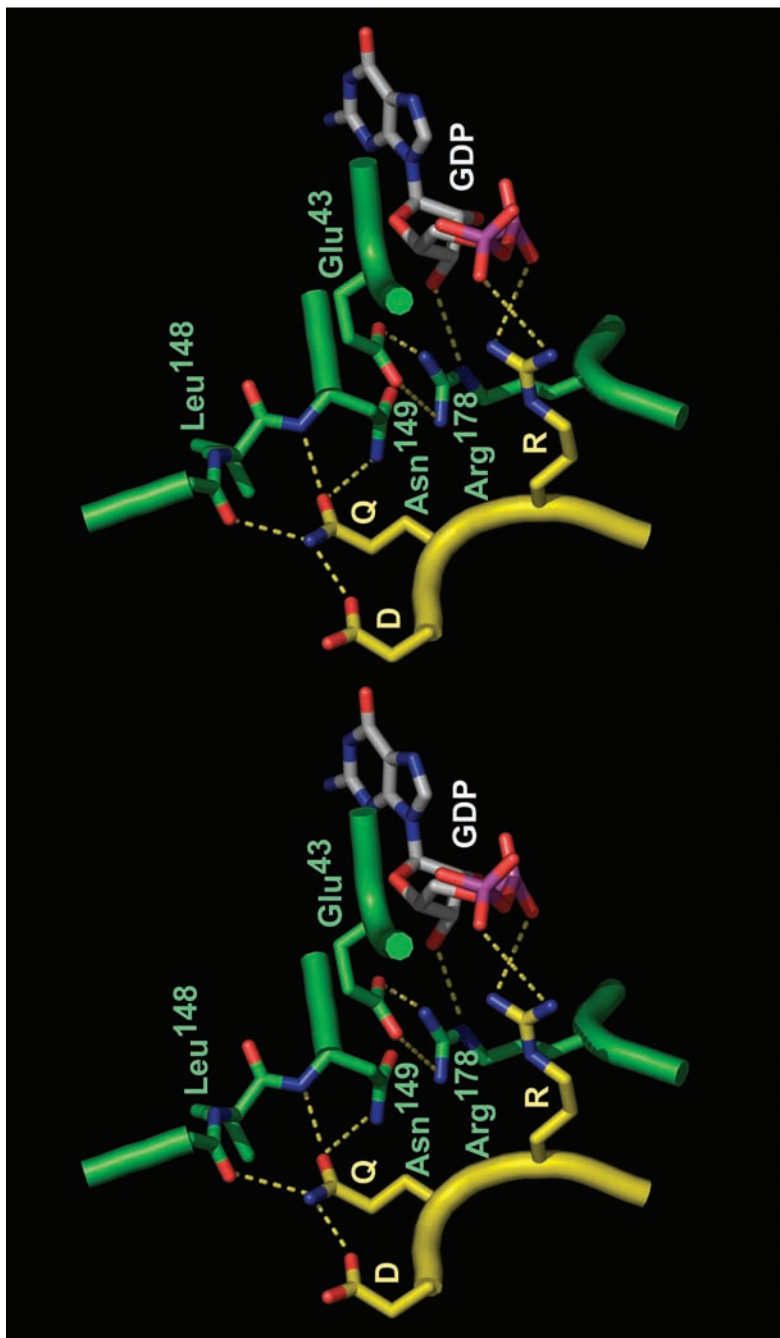


preceding two residues to reach into the nucleotide-binding pocket of Gα and contact the alpha- and beta-phosphate groups of GDP (Figure 4). This positioning of an arginine side chain in *trans* to contact the guanine nucleotide is reminiscent of the catalytic arginine fingers employed by GAPs for Ras- and Rho-family GTPases (25, 26). Arg-178 within switch I of Gα_{i1} works in *cis* like the arginine finger of a RasGAP or RhoGAP and stabilizes the developing negative charge on the gamma-phosphate leaving group during GTP hydrolysis (27). In the GDP-bound ground state of uncomplexed Gα, the Arg-178 guanidinium group contributes to GDP binding by forming hydrogen bonds with the alpha- and beta-phosphate oxygens (28). However, within GoLoco-bound Gα_{i1}, the Arg-178 side chain is displaced and instead contacts the 3′ hydroxyl of the GDP ribose sugar moiety (Figure 4), and forms a salt bridge interaction with the side chain of Glu-43. [This pairing of Arg-178 and Glu-43 is normally seen in G$\alpha\beta\gamma$ heterotrimers but not in the uncomplexed state of Gα·GDP (22).]

A principal aspect of GoLoco-mediated GDI activity therefore appears to be the creation of new contacts to GDP by remodeling Arg-178 and adding a second arginine in *trans* from the GoLoco motif triad. Replacing the triad arginine with a bulky phenylalanine eliminates all GDI activity of the fourth GoLoco motif of AGS3 (23) and of an AGS3-derived GoLoco consensus peptide (11); substitution with phenylalanine also completely eliminates the ability to bind Gi-class α subunits (15). Mutation of the triad arginine to less bulky alanine or leucine

←

Figure 3 Residues C-terminal to the core GoLoco motif contact the Gα all-helical domain and are important determinants of Gα binding specificity and GDI activity. (*A*) Alignment and sequence ranges of minimal functional GoLoco-motif polypeptides with their known Gα binding specificity indicated on the right. The core GoLoco motif is boxed in black. Alpha symbols (α) and asterisks (*) denote alpha-helical secondary structure and GoLoco contacts with Gα, respectively, as observed in the 2.7 Å crystal structure of the RGS14 GoLoco/Gα_{i1}·GDP complex (1KJY). Contacts to Gα_{i1}-specific residues in the all-helical domain are identified by connecting lines. The plus sign (+) after residue 80 in the human Rap1GAP isoform II sequence denotes the fact that the minimal functional G α_z-interacting domain of Rap1GAP isoform I (40) has only been minimized to the first 74 amino acids of that isoform (which starts at methionine 32 relative to the illustrated Rap1GAPII sequence). Other abbreviations follow from Figure 2. (*B*) Model of the G$\beta\gamma$ lever hypothesis, as proposed by Rondard et al. (32), to explain GPCR-mediated guanine nucleotide exchange activity. Gα_{i1} (*green* with translucent space-filling shell; switch regions in *blue*) makes side chain contacts (*white*) with residues in Gβ (*cyan*) of the G$\beta\gamma$ dimer (Gγ in *purple*). Outward movement of switch I and switch II by receptor-induced rotation of G$\beta\gamma$ is proposed to allow the egress of GDP (*brown*) from the nucleotide-binding pocket (32). (*C*) The RGS14 GoLoco-motif peptide (aa 496–531; *yellow*) binds across the Ras-like and all-helical domains of G α_{i1}, trapping GDP within. Note the relative position of the GoLoco triad arginine finger (ball-and-stick representation). Gα_{i1}-specific contacts to the GoLoco peptide, as denoted in part (*A*), are illustrated in pink.


GDP
Glu43
Leu148
Asn149
Arg178
Q
D
R
GDP
Glu43
Leu148
Asn149
Arg178
Q
D
R

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

causes, in contrast, a significant decrease in the GDI activity of the RGS14 GoLoco motif without a concomitant decrease in binding affinity for Gα (19). Since weak but measureable GDI activity still remains upon substituting the triad arginine with alanine or leucine (19, 24), the GoLoco arginine finger should be considered a principal, but not an absolute, determinant of guanine nucleotide dissociation inhibitor activity.

The invariant glutamine residue that just precedes the arginine finger in the triad (Figure 2) points away from the GDP binding pocket and makes extensive side chain and backbone interactions with Gln-147 and Asn-149 of Gα_{i1} (19), thus "kinking" the GoLoco peptide backbone and allowing full extension of the arginine side chain into the nucleotide-binding pocket (Figure 3*C* and 4). These interactions between triad glutamine and Gα_{i1} are critical for GDI activity. Replacing the triad glutamine with alanine eliminates the Gα-binding and GDI functions of the AGS3 consensus peptide (24), and replacing Asn-149 of Gα_{i1} with isoleucine leads to an insensitivity to GDI activity normally exerted by AGS3 and Pcp-2 (29). The latter mutation to Gα_{i1} was made in the context of a three-position exchange with Gα_s residues (Arg-144 to Asn, Asn-149 to Ile, and Ser-151 to Cys) and was originally interpreted as reflecting direct GoLoco motif interactions with the all-helical domain/switch III interface of Gα_{i1} (29). Reappraisal of this conclusion in light of the RGS14-Gα_{i1}·GDP crystal structure suggests that only the Asn-149 to isoleucine substitution is responsible for the observed GoLoco insensitivity. Arg-144 is far removed from the RGS14 GoLoco peptide-binding site, and the side chain of Ser-151 is involved in hydrogen bonds with the ribose sugar hydroxyl groups of the bound GDP (19).

Preceding the invariant glutamine of the GoLoco motif triad are two acidic residues (Figure 2). Only two GoLoco motifs lack an aspartic acid or glutamic acid at the position immediately adjacent to the invariant glutamine: the second motif of G18 (30) and the first motif of human Pcp-2 (31). Within the asymmetric unit of the RGS14/Gα_{i1} crystal (PDB accession number 1KJY), one of the two GoLoco/Gα dimers contains this acidic residue in a side chain hydrogen bond with the side chain of the following glutamine (Figure 4). This bond helps anchor the triad glutamine and supports the positioning of the arginine finger. This acidic residue of the GoLoco triad is also important for function; the second GoLoco motif of the triple-motif protein G18, which has an alanine residue at this position

←

Figure 4 Stereo view of the contacts made by the GoLoco motif acidic-glutamine-arginine triad to Gα and guanosine diphosphate (GDP), as determined by the crystal structure of the RGS14 GoLoco/Gα_{i1} ·GDP complex (PDB accession 1KJY). RGS14 triad residues aspartate-514 (D), glutamine-515 (Q), and arginine-516 (R) are drawn in yellow; Gα_{i1} side chains (Glu-43, Leu-148, Asn-149, and Arg-178) and the intervening backbone are in green; and GDP is rendered in the CPK (Corey-Pauling-Koltun) color scheme: carbon in white, nitrogen in blue, oxygen in red, and phosphorus in magenta. Dotted yellow lines represent hydrogen bonds.

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

(Ala-121) (Figure 2), neither exhibits GDI activity nor binds Gα subunits in vitro, and replacement with aspartate results in a robust gain of Gα_{i1}-binding and GDI activities (30). Similarly, increased activity is seen for the first GoLoco motif of human Pcp2 upon replacing the glycine residue at this triad position (Gly-24) (Figure 2) with glutamate (our unpublished observations), the amino acid normally present in this position in the rat and mouse orthologues.

Role of Residues C-terminal to the Conserved Motif

The minimal conserved GoLoco motif ends with the acidic-glutamine-arginine triad and thus is generally 19 residues long. Variants include the single, 18-residue GoLoco motifs present in *C. elegans* and *Caenorhabditis briggsae* GPR proteins, which lack the α-helix-ending central glutamine, and the 20-residue third GoLoco motif within G18, which possesses three glycine residues between the N-terminal α-helix and conserved triad regions (Figure 2). However, and importantly, this minimal conserved sequence is generally not sufficient to mediate interaction with Gα subunits. Residues C-terminal to the highly conserved motif (Figure 3*A*) are poorly conserved, yet required for robust GDI activity. (One study reports in vitro GDI activity exerted by an internal, 19-residue span of the AGS3 consensus peptide (24), GFFDLLAKSQSKRMD-DQRV. However, this activity was observed at an inordinately high molar excess of peptide (100 μM) versus Gα target (100 nM), and neither IC_{50} nor dissociation constant values for this interaction were reported.)

The structure of the RGS14 GoLoco/Gα_{i1}·GDP complex suggests a reason for the requirement of residues C-terminal to the acidic-Glu-Arg triad in GoLoco motif function—these residues make extensive contacts with the all-helical domain of Gα_{i1} (19), affording GoLoco motif proteins the ability to cross over the nucleotide-binding pocket and span both lobes of the Gα structure (Figure 3*C*). One proposed mechanism of GPCR-mediated nucleotide release involves agonist-bound receptor using G$\beta\gamma$ as its lever (32) to induce switch I and switch II of Gα to peel back and open the lip of the nucleotide-binding pocket found at the cleft between its Ras-like and all-helical domains (Figure 3*B*). Hence, beyond direct GDP contact by the arginine finger, other likely aspects of GoLoco-mediated GDI activity include blocking the route of nucleotide egress (29) and restricting any interdomain movement within Gα that might be necessary for nucleotide ejection (Figure 3*B* versus 3*C*).

Although the GoLoco regions of RGS12 and RGS14 preferentially interact with Gα_{i1}, Gα_{i2}, and Gα_{i3}, and not Gα_o (12, 19), other GoLoco-containing proteins can also interact with Gα_o (AGS3, LGN, Pcp-2, Rap1GAP isoform II) (13, 19, 23, 33–38), Gα_t (AGS3) (23, 39), and Gα_z (Rap1GAP isoform I) (40, 41). The RGS14 GoLoco-Gα_{i1}·GDP structure revealed that the primary determinants of Gα selectivity reside within the contacts made between the Gα all-helical domain and residues C-terminal to the 19-residue core conserved GoLoco motif (Figure 3*A* and 3*C*). Indeed, RGS14 can exert GDI activity on a chimeric Gα_o subunit containing the all-helical domain from Gα_{i1}, yet the same

RGS14 GoLoco peptide is unable to act as a GDI for a chimeric Gα_{i1} containing the all-helical domain from Gα_o (19). Moreover, exchanging the C-terminal residues (aa 496–531) (Figure 3*A*) of the RGS14 GoLoco peptide for those found C-terminal to the second GoLoco motif of Pcp-2 (aa 46–60) (Figure 3*A*) creates a chimeric GoLoco peptide with GDI activity on wild-type Gα_o nearly equal in potency to that of wild-type Pcp-2; conversely, the reciprocal chimera, containing the conserved second GoLoco motif of Pcp-2 with the C-terminal residues from RGS14, fails to interact with Gα_o (19).

Modulation of GDI Activity by Phosphorylation

Little is currently known about how GoLoco motif activity is regulated in vivo. Two recent reports have proposed that phosphorylation of GoLoco-motif proteins might be one mechanism by which GDI activity can be modulated. Hollinger and colleagues (42) found that RGS14 is phosphorylated in rat B35 neuroblastoma cells by cAMP-dependent protein kinase (PKA). In vitro phosphorylation of recombinant RGS14 protein by PKA occurs at two sites, Ser-258 and Thr-494; the latter site is just N-terminal to the start of the GoLoco motif. Mimicking PKA phosphorylation of Thr-494 by mutation to aspartate or glutamate increases nearly threefold the in vitro GDI activity of RGS14 toward Gα_{i1} (42). It is unfortunate that the structure of the RGS14 GoLoco motif peptide (aa 496–531) does not extend N-terminal to the Thr-494 residue (19); thus it remains conjecture whether phosphorylation at this site contributes directly to the interaction with Gα or results in structural changes within RGS14 that increase GoLoco motif accessibility. Increased cellular PKA activity is the principal outcome of G_s-coupled receptor stimulation (via adenylyl cyclase activation and the accumulation of cyclic AMP); hence, enhancement of Gα_i-directed GDI activity mediated by PKA phosphorylation could play a role in cellular cross-modulation of adenylyl cyclase-stimulatory (G_s) and adenylyl cyclase-inhibitory (G_i) GPCR signaling pathways, either by decoupling G_i-linked receptors and/or augmenting effector modulation by G$\beta\gamma$ subunits freed from G_i heterotrimers.

In a yeast two-hybrid screen for AGS3 interactors, Blumer and coworkers (43) identified LKB1/STK11, the mammalian homologue of serine/threonine kinases in *C. elegans* (PAR-4) and *Drosophila* (LKB1) required for establishing early embryonic anterior-posterior axis formation (44, 45). Immunoprecipitated LKB1 was found to phosphorylate a recombinant protein containing the four GoLoco-motif C terminal region of AGS3 (aa 463–650), a region containing 24 serine and threonine residues, only 9 of which are present within the conserved GoLoco motifs (AGS3.GL1–4) (Figure 2). It is currently unknown which specific serine/threonine residue(s) within AGS3 are phosphorylated by LKB1. Nevertheless, Blumer and colleagues (43) chose to phosphorylate the AGS3 consensus peptide (Figure 3*A*) at Ser-16 (C-terminal to the nearly invariant middle glutamine) and reported that phosphorylation at this site diminishes GDI activity in vitro. The physiological relevance of this finding is unknown in the absence of evidence that this serine is actually targeted for phosphorylation in vivo.

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

GoLoco MOTIF PROTEINS IN GPCR PATHWAY MODULATION

GoLoco Peptides as Tools to Uncouple GPCRs

The ability of the GoLoco motif to bind $G\alpha_{i1}$·GDP subunits and prevent concomitant $G\beta\gamma$ association has motivated investigations of whether GoLoco-motif proteins play a role in modulating cellular GPCR signaling pathways. It has been demonstrated, for example, that AGS3 can attenuate rhodopsin-catalyzed activation of transducin and high-affinity agonist binding to 5-HT_{1A} receptors (11, 38, 39). These studies were performed in cell-free systems that rely on reconstitution of GPCR signaling with purified or semipurified components; thus, it cannot be directly inferred that retinal phototransduction and/or serotonin signaling pathways are the direct targets of GoLoco protein modulation.

These studies do, however, highlight the utility of the GoLoco motif as a tool for selective decoupling of G_i-linked GPCRs. For example, intracellular microinjection of GoLoco peptides into AtT20 mouse pituitary corticotroph cells can selectively antagonize G_i-linked dopamine D2 receptor-mediated enhancement of G-protein gated inward rectifier potassium (GIRK/K_{ir}3.x) current without affecting somatostatin-induced (G_o-linked) GIRK current activation (46). In this system, the initial GIRK current response to quinpirole application remains unaffected by GoLoco peptide injection, yet subsequent applications of agonist elicit progressively reduced potassium currents. Mutation of the triad arginine within the GoLoco peptide to phenylalanine abrogates its ability to uncouple the D2 receptor. Hence, it appears that GoLoco-mediated decoupling of D2 receptors in this system depends on the GoLoco peptide having access to free $G\alpha$·GDP subunits, as afforded by agonist stimulation and the resultant cycle of receptor GEF activity, heterotrimer separation, and GTP hydrolysis.

These results in AtT20 cells argue against GoLoco motif peptides having any innate ability to displace the $G\beta\gamma$ subunit from a preformed receptor-coupled $G\alpha\beta\gamma$ heterotrimer in vivo. However, a recent, provocative report by Ghosh and coworkers (47) proposes just such an activity for the AGS3 consensus peptide. At a 20,000-fold molar excess of GoLoco peptide (1 μM) over heterotrimer (50 pM), Ghosh et al. observe an accelerated rate of G-protein subunit dissociation in vitro. It remains untested whether scrambled or loss-of-function point-mutant variants of the AGS3 consensus peptide also share this activity (47); moreover, it remains unknown whether such a high relative ratio of GoLoco motif to heterotrimer is ever achieved in a normal cellular context.

Loco and RGS12

Although the existence of the GoLoco motif was first inferred from the detection of a second $G\alpha_i$-binding site within the *Drosophila* protein Loco (7), the physiological role of the GoLoco motif within Loco has not yet been determined. Embryos lacking the *loco* gene display defects in glial cell differentiation and

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

consequently fail to hatch; rare adult escapers that do eclose show severe impairment of spontaneous locomotor activity (7). Loco was also found to be required for dorsal-ventral pattern formation in the *Drosophila* embryo (48). Yet despite this knowledge, no specific G-protein modulatory functions have been ascribed to Loco to help explain its particular role in these processes, nor has a GPCR been identified as having modulatory activity on Loco function.

In contrast, the avian Loco orthologue, RGS12, functions in the context of a GPCR-initiated signal transduction cascade: RGS12 controls the rate of desensitization from γ-aminobutyric acid (GABA)-mediated inhibition of the N-type calcium channel ($Ca_v2.2$) in chick dorsal root ganglion neurons (49). RGS12 is recruited, via its phosphotyrosine-binding (PTB) domain, to the α_{1B} pore-forming subunit of $Ca_v2.2$ in a tyrosine kinase-dependent manner (49). It is currently unknown, however, what specific role the C-terminal GoLoco motif within RGS12 plays in modulating $Ca_v2.2$ inhibition by the G_o-linked $GABA_B$ receptor.

Rap1GAP Isoforms

Currently, the clearest demonstration of a GoLoco/Gα interaction being involved in GPCR-mediated modulation of a cellular signaling pathway comes from studies of Rap1GAP, a negative regulator of the Ras-related GTPase, Rap1 (50). Meng and coworkers (40) previously found that Rap1GAP (isoform I) binds Gα_z in its activated, GTP-loaded form (a departure from the normal GoLoco motif requirement for a GDP-bound Gα subunit). Gα_z activation in PC12 cells, via agonist stimulation of α_{2A}-adrenergic receptors, was subsequently shown to recruit Rap1GAP to the plasma membrane (41). Gα_z-mediated recruitment of Rap1GAP attenuated Rap1-mediated ERK activation and neurite development (41), suggesting that G_z-linked GPCR signaling can antagonize the Rap1/B-Raf/ERK signal transduction cascade in PC12 cells via Rap1GAP translocation to the plasma membrane.

Similarly, Mochizuki and coworkers (37) have found that an N-terminally extended variant of Rap1GAP (isoform II) binds to activated Gα_{i1} and Gα_{i2} subunits. Activation of the Gi-linked M_2 muscarinic acetylcholine receptor was shown to recruit Rap1GAPII to the plasma membrane and lower cellular levels of GTP-loaded Rap1 (37). However, this reduction in activated Rap1 correlated with an increase in ERK activation. Differences between the findings of Meng et al. and Mochizuki et al. could be the result of differing Rap1GAP isoforms examined and/or differing operative Rap1-effector pathways in the cell lines used: PC12 rat pheochromocytomas (41) versus human embryonic kidney 293T fibroblasts (37).

GoLoco MOTIF PROTEINS IN CELL DIVISION

The seminal observation that first placed a GoLoco motif protein within an unconventional G-protein signaling paradigm was the finding that a complex comprising Gα and the multi-GoLoco protein, Pins, is a crucial component for

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

dictating asymmetric cell division in *Drosophila* neuroblasts (51). Asymmetric cell division (ACD) is used by many organisms during development to generate cellular diversity [reviewed by Knoblich (52)]. Conventional cell division produces two identical daughters, whereas in ACD, RNAs and proteins that determine cell fate are asymmetrically segregated into the two daughter cells. Consequently, daughter cells derived from ACD acquire different developmental potentials. The first step in ACD requires the establishment of an axis of polarity. The second step involves unequal distribution of cell fate determinants along this axis. The third and final step is the orientation of the mitotic spindle along this axis so that cell division segregates these cell fate determinants unequally to produce different daughter cells.

Asymmetric Cell Division in *Drosophila* Neuroblasts

Drosophila neuroblast mitosis is a commonly studied example of ACD (53, 54). Neuroblast cells delaminate from the ventral neuroectoderm and adopt an apical-basal axis of polarity. Subsequent asymmetric division produces a large apical neuroblast and a smaller ganglion mother cell (GMC). Apical neuroblasts can undergo further asymmetric divisions, whereas GMCs are committed to differentiating into neurons or glia. The apical determinants Bazooka (PAR-3), DmPAR-6, and atypical protein kinase C (DaPKC) form a protein complex that recruits the Inscuteable (Insc) protein to the apical cell cortex (55, 56), directs spindle orientation, and helps segregate the basal determinants Numb (a PTB domain-containing protein), Miranda (a coiled-coil protein), and Prospero (a transcription factor).

Insc is expressed during neuroblast delamination and localizes to the apical cell cortex; loss of Insc perturbs spindle orientation and randomizes axes of cell division (57). Deletional analysis has defined a central “asymmetry domain” within Insc as being sufficient to mediate all known functions of the full-length protein (58, 59). In the search for asymmetry domain-interacting proteins as potential Insc effectors, three groups independently discovered the Partner of Inscuteable (Pins/Rapsynoid) protein (51, 60, 61). Pins is the archetypal member of an evolutionarily conserved class of TPR and GoLoco motif-containing proteins involved in cell division processes (Figure 5). Pins is recruited by Insc to the apical cell cortex of delaminated neuroblasts; ablation of maternal and zygotic Pins results in defective spindle orientation, a failure to segregate determinants asymmetrically, and a limited asymmetry in the neuroblast division (51, 60, 61). These phenotypes are equivalent to those observed in *inscuteable*-deficient embryos (57), suggesting that Pins is the predominant effector for Insc function.

Heterotrimeric G-Protein Involvement in *Drosophila* ACD

Insc, Pins, and Gαi form an apical protein complex (Figure 6) essential for ACD in *Drosophila* neuroblasts (51, 62, 63). Pins is selective for GDP-bound Gαi, like

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

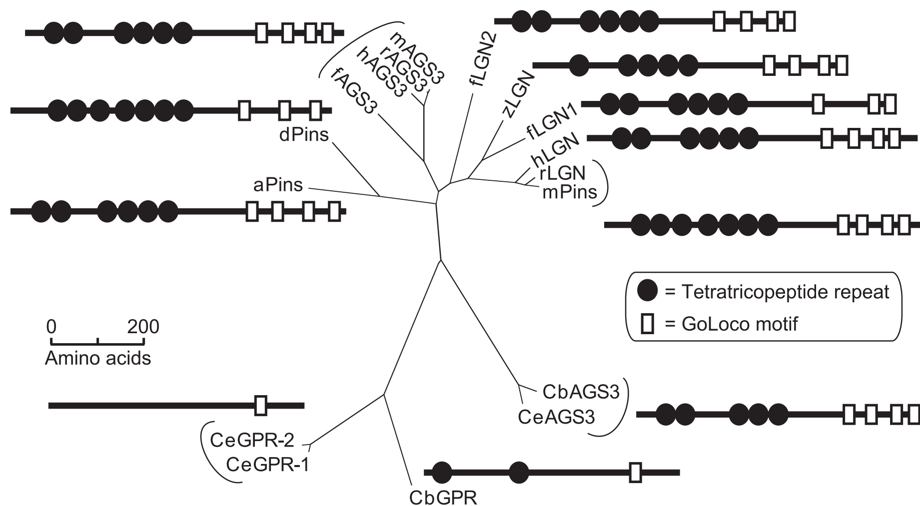


Figure 5 Phylogenic relationship and multidomain architectures of the Pins family of GoLoco proteins. Tetratricopeptide repeat (TPR) regions are illustrated based on detection by Pfam (http://pfam.wustl.edu/) or SMART (http://smart.embl-heidelberg.de/) hidden Markov models. The N termini of *C. elegans* GPR-1 and GPR-2 proteins are predicted to form stable all-alpha-helical folds comprised of TPRs via protein fold recognition algorithms, as implemented by the 3D-PSSM web-server (http://www.sbg.bio.ic.ac.uk/~3dpssm). Species abbreviations follow from Figure 2.

other GoLoco proteins (excluding Rap1GAP). Pins is also able to bind Gαo in vitro, but its partner in vivo appears to be Gαi (51, 62). Moreover, elements within the Insc/Pins/Gαi·GDP ternary complex appear mutually codependent for apical localization. Overexpression of wild-type Gαi inhibits polarization of asymmetry determinants and causes mitotic spindle misorientation (62). However, overexpression of constitutively active Gαi^{Q205L} has essentially no effect on neuroblast ACD. Thus, in the context of *Drosophila* neuroblast ACD, the active Gα species appears not to be GTP-bound Gαi but rather Gαi·GDP in complex with Pins. It is possible that G$\beta\gamma$ sequestration as caused by Gαi overexpression leads to the observed aberrations to neuroblast ACD. However, significant differences are seen with respect to relative size of daughter cells and Miranda mislocalization upon Gαi overexpression versus the loss of Gβ13F (the *Drosophila* orthologue of conventional mammalian Gβ subunits β1-β4) (62). These results suggest that the effects of wild-type Gαi overexpression on ACD are not due solely to Gβ13F subunit sequestration; however, this does not necessarily exclude a role for G$\beta\gamma$ signaling in ACD.

In contrast to the apical localization of Gαi, Gβ13F is uniformly expressed throughout the neuroblast cortex (62). Nevertheless, Gβ13F appears to be required for neuroblast ACD because neuroblasts in Gβ13F-deficient embryos

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

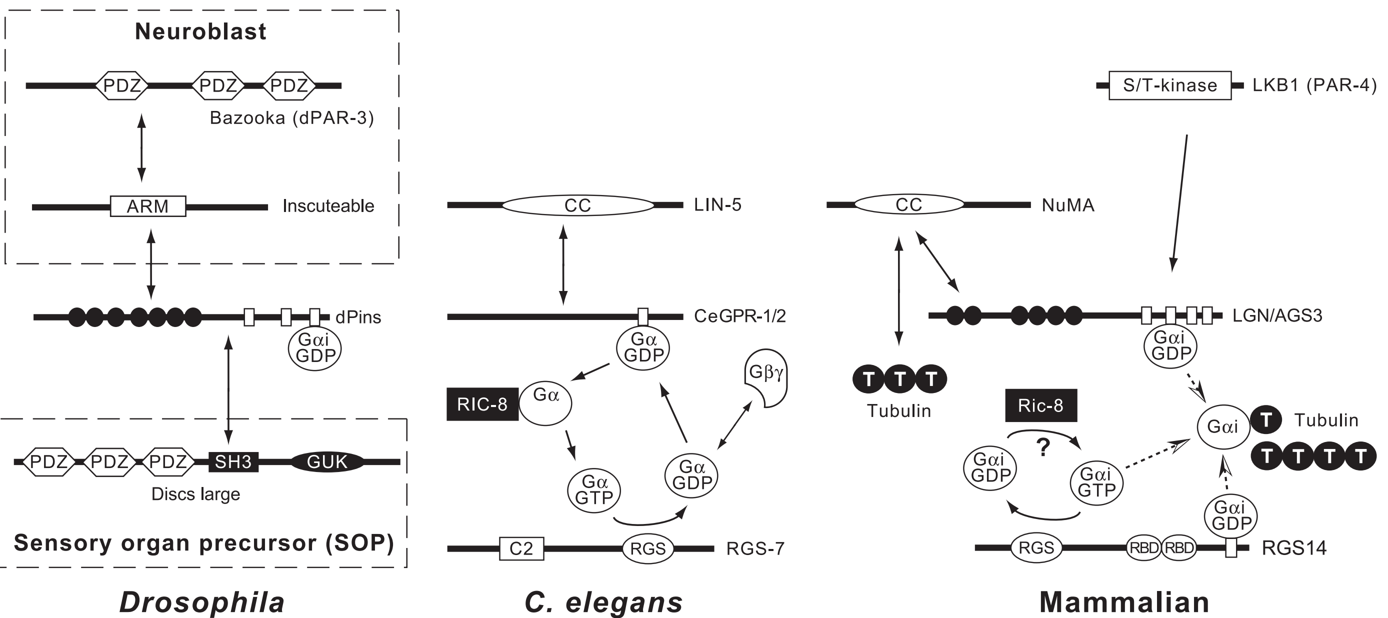


Figure 6 Comparable signal transduction complexes, centered around Pins family members, regulate *Drosophila* (*left*) and *C. elegans* (*middle*) asymmetric cell divisions and mammalian cell division (*right*). Interactions between signaling components are abstracted from genetic and direct biochemical evidence. Domain abbreviations are ARM, weakly predicted Armadillo repeats; C2, homology to conserved region 2 of protein kinase C; CC, coiled-coil region; GUK, membrane-associated guanylate kinase homology domain; PDZ, PSD-95/Discs large/ZO-1 homology domain; RBD, Ras-binding domain; RGS, regulator of G-protein signaling box; SH3, Src homology-3 domain; and S/T-kinase; serine/threonine kinase domain.

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

exhibit a high frequency of symmetric division (64). Fuse and colleagues (64) have found that ectopic coexpression of Gβ13F and Gγ1 reduces spindle size, in contrast to the large spindles seen in *Gβ13F*-null neuroblasts, suggesting that Gβγ signaling may act to suppress spindle formation. Confounding these analyses is the fact that one consequence of *Gβ13F* deletion is concomitant loss of Gαi expression (62). To overcome this, Yu and colleagues (65) recently generated and analyzed loss-of-function Gαi mutants, leading them to propose that Gβγ signaling is involved in ACD upstream of both Bazooka/DaPKC and Pins/Gαi complexes. Gβ13F appears to regulate the asymmetric localization and stability of both the Bazooka/DaPKC and Pins/Gαi pathway components, in a fashion not attributable to loss of Gαi expression.

In this light, recent genetic evidence from Cai et al. (66) suggests that *Drosophila* ACD may be defined by two, functionally overlapping, parallel pathways, one containing Bazooka/DaPKC and the other comprising Pins/Gαi. This is elegantly illustrated by the division of *Drosophila* sensory organ precursor (SOP) pI cells [a second model system for ACD; reviewed in (52)]. pI cells divide asymmetrically along an anterior-posterior axis to produce anterior pIIa and posterior pIIb daughter cells. The cell-surface serpentine receptor Frizzled acts to reorganize an initial apical-basal polarity into an anterior-posterior polarity during interphase. In sharp contrast to neuroblasts, Pins and Gαi localize in SOP pI cells to the anterior cortex whereas Bazooka/DaPKC localize to the posterior cortex (62, 67) and act antagonistically to Pins/Gαi (66). Division of SOP pI cells is asymmetric because the spindle is offset somewhat toward the anterior and resultant daughter cells have distinct fates as Numb is segregated solely to the pIIb cell (52).

The lack of Insc expression in pI cells can explain the phenotypic difference between SOP and neuroblast ACD. Ectopic expression of Insc in pI cells recruits the Bazooka/DaPKC complex to the anterior, and consequently, the anterior spindle becomes larger and cell division more asymmetric (66, 67). In the normal absence of Insc, the Pins/Gαi complex is recruited to the anterior by the SH3 domain of the Discs large (Dlg) protein, which can directly bind an unspecified target sequence within Pins (67) (Figure 6). Thus, weak spindle asymmetry in the SOP pI cell division, given opposing localizations of Pins/Gαi and Bazooka/DaPKC complexes, produces only a mild size difference in resultant pIIa and pIIb daughters, whereas in neuroblasts, strong asymmetry given cosegregating complexes produces a prominent size differential between GMC and neuroblast progeny.

Mechanism of G-Protein Activation in *Drosophila* ACD

In canonical G-protein signaling, the Gαβγ heterotrimer is activated by GPCR-mediated nucleotide exchange and the separation of Gβγ from Gα·GTP (1, 2). It is currently unclear whether this paradigm also holds true in ACD. In *Drosophila* neuroblasts, the Insc/Pins/Gαi·GDP complex is devoid of Gβ13F (62), consistent with the mutually exclusive nature of GoLoco- versus Gβγ-binding to GDP-

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

loaded Gα subunits. Schaefer et al. (62) report that preincubation of neuroblast lysates with recombinant Pins, or a peptide corresponding to its final GoLoco motif, can disrupt the Gαi/G$\beta\gamma$ interaction, as measured by the loss of Gβ from immunoprecipitated Gαi. Coupled with the recent in vitro work of Ghosh and colleagues (47) previously described, this finding leads to a provocative hypothesis that, in ACD, Pins acts to "activate" (i.e., separate) heterotrimeric G proteins without nucleotide exchange on the Gα subunit. An alternative hypothesis is that the addition of Pins or GoLoco peptide elicits an innate G$\beta\gamma$ releasing factor within the neuroblast lysate.

Current opinion suggests that G-protein action in ACD is independent of any GPCR-mediated GEF activity; recent biochemical and genetic evidence for a receptor-independent GEF for Gα subunits (i.e., Ric-8; see next section) supports the lack of a strict requirement for GPCR function if exchange activity is a requirement for ACD. However, GPCR signaling has not been formally disproven to be involved in ACD. Indeed, GPCR signaling can be peripherally linked with ACD, given that SOP polarization is directed by the actions of Frizzled receptors that bear more than a passing resemblance to canonical GPCRs (68). However, as previously stated, significant differences do exist between ACD in sensory organ precursors and in neuroblasts. In particular, expression of Gαi^{Q205L} in neuroblasts is without any effect on ACD, whereas in SOP cells, ACD is perturbed (62). This finding suggests that potential G-protein effectors of ACD may differ in SOP cells versus neuroblasts and that GTP-bound Gαi has a specific role to play in SOP division.

The functional interplay within the Pins/Gαi complex has yet to be clearly delineated. It may be that the sole function of Gαi (a lipidated and, thus, membrane-associated protein) is to recruit Pins to the plasma membrane. In this vein, it has recently been shown that the GoLoco region of Pins specifies cortical recruitment, whereas the first three TPRs mediate apical localization (63). An alternative speculation, however, is that specific effectors exist, but are hitherto unrecognized, for GoLoco-complexed Gαi subunits (18).

ACD in the *C. elegans* One-Cell Embryo

Studies in the nematode worm *C. elegans* have recently provided crucial insights into the mechanisms of G-protein signaling in ACD, as reviewed by Gönczy (69). The one-cell stage *C. elegans* embryo (P_0) divides asymmetrically to form a large anterior (AB) blastomere and a smaller posterior blastomere (P_1). Anterior-posterior polarity is thought to be initiated by the sperm aster shortly after fertilization. This paternally derived cue directs the polarized distribution of an evolutionarily conserved set of proteins that regulate cell polarity and ACD. This is exemplifed by the partitioning-defective (PAR) proteins, the analysis of which has illuminated the study of cell polarity in several metazoan organisms (70). In the *C. elegans* one-cell embryo, PAR-3, PAR-6, and the atypical PKC-3 are localized to the anterior cortex, whereas PAR-1 and PAR-2 are localized to the posterior cortex. The mitotic spindle at metaphase is symmetrically posi-

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

tioned along the anterior-posterior axis. During anaphase, the anterior spindle position is fixed, but the posterior spindle moves toward the posterior cortex. This movement is accompanied by vigorous rocking of the spindle as it elongates. Characteristically, the spindle flattens in telophase, and because the cleavage furrow forms to bisect the spindle, the daughter cells (AB and P_1) are destined to be of unequal size, with the resultant AB blastomere being larger (60% of initial cell volume) than the P_1 cell (40%). In concert with genetic studies, spindle-severing experiments demonstrate that polarity provided by PAR proteins results in an increased pulling force on the posterior spindle (71). These "extra-spindle" pulling forces act along astral microtubules and are exerted unequally on the spindle poles. This imbalance of extra-spindle pulling forces is under the control of polarity cues (72).

Heterotrimeric G-Protein Involvement in *C. elegans* ACD

The first evidence that heterotrimeric G-protein signaling pathways might function in the regulation of *C. elegans* cell division came when Zwaal and colleagues (73) observed that Gβ regulates centrosome migration in the *C. elegans* early embryo. Subsequently, Gotta & Ahringer (74) demonstrated that simultaneous RNA interference (RNAi)-mediated knockdown of two *C. elegans* Gα subunits, GOA-1 and GPA-16, causes a spindle positioning defect that results in a symmetric P_0 division and the production of equal sized AB and P_1 blastomeres. A genome-wide screen for cell division genes by Gönczy and colleagues (75) discovered two near-identical proteins (GPR-1 and GPR-2, for G-protein regulator; hereafter collectively called GPR-1/2), containing a single GoLoco motif that, when inactivated by RNAi, give a spindle positioning defect indistinguishible from that of *goa-1/gpa-16* (*RNAi*) embryos. This finding is reminiscent of the *Drosophila* ACD pathway with PAR-proteins translating polarity cues into spindle positioning via signal transduction through a GoLoco motif protein and heterotrimeric G proteins.

Subsequent to this, three groups have independently delineated the spatial and temporal signaling mechanisms of the GoLoco protein GPR-1/2 and its signal transduction via G proteins in the first cell division of *C. elegans* (76–78) (Figure 6). GPR-1/2 is located at the cortex and is enriched at the posterior pole. The asymmetric localization of the PAR proteins (PAR-1, -2, and -6) is not altered in *gpr-1/2* (*RNAi*) and *goa-1/gpa-16* (*RNAi*) embryos, indicating that the spindle displacement phenotype is not due to altered cell polarity. Genetic and spindle-severing studies (discussed below) have indicated that GPR-1/2 acts downstream of the PAR proteins to facilitate ACD.

It is worth contrasting *C. elegans* versus *Drosophila* ACD and their differential utilization of Gα/GoLoco signaling. In *Drosophila*, Gα signaling plays a role in establishing cell polarity and determinant segregation, whereas in *C. elegans*, Gα acts downstream of polarity cues to mediate spindle alignment. Recent studies indicate that the LIN-5 protein is another important component of the asymmetric spindle positioning pathway in *C. elegans*. LIN-5 was indepen-

dently isolated, both biochemically and genetically, as a GPR-1/2-interacting protein (77, 78) (Figure 6). Although GPR-1/2 is distantly related to *Drosophila* Pins (Figure 5), LIN-5 bears no sequence resemblance to *Drosophila* Inscuteable (Figure 6). *lin-5 (RNAi)* embryos have loss-of-function phenotypes similar to *gpr-1/2 (RNAi)* and *goa-1/gpa-16 (RNAi)* embryos, indicating they may all signal in the same pathway. LIN-5 also regulates the localization of GPR-1/2. Collectively, these findings suggest that LIN-5 may act as a scaffold or cofactor for GPR-1/2 and/or function in a pathway parallel to GPR-1/2 (77, 78). (Yet another component within this pathway, the DEP domain-containing protein LET-99, appears to function antagonistically to GPR-1/2 (79). Determining the epistatic relationships between these various signaling components is paramount.)

In vivo spindle severing with ultraviolet laser microbeams provided the definitive characterization of the role of GPR-1/2 and GOA-1/GPA-16 in force generation (76). Spindle severing experiments in wild-type embryos indicate that the peak velocity of the posterior spindle is 40% greater than the peak velocity of the anterior spindle; peak velocities are presumed to reflect the extent of pulling forces. In contrast, in *gpr-1/2* (*RNAi*) and *goa-1/gpa-16* (*RNAi*) embryos, peak spindle velocity is equivalent between the anterior and posterior spindles but dramatically reduced in magnitude. Therefore, the Gα-GPR1/2 protein complex is required to generate extra-spindle pulling forces.

What then is responsible for the force imbalance between the anterior and posterior poles? Elegant studies by Grill and coworkers (80) suggest that increased pulling forces on the posterior spindle pole result from an increased number of force generators at the cortex rather than an increase in the magnitude of the quantal force per se. Thus, asymmetric localization of GPR-1/2 provides a likely mechanism for the assembly of a force-generating protein complex, potentially including LIN-5, GPR-1/2, and Gα. Rather than GPR-1/2 asymmetry being unequivocal, there is instead a subtle bias (50% more posterior cortical GPR1/2 versus anterior) that correlates well with the differential spindle velocity (40% increase in posterior velocity versus anterior) (76) and the observation of 50% more active cortical force generators at the posterior (80). The force-generating complex must interact with the astral microtubules to generate pulling forces, presumably via microtubule depolymerization or motor activity (72). Direct modulation of microtubule dynamics by heterotrimeric G proteins is a potential mechanism for force generation that will be discussed in a subsequent section.

A Cycle of GDI, GEF, and GAP Activities in *C. elegans* ACD?

Although a role for GoLoco/Gα interations in both *Drosophila* and *C. elegans* ACD has been elucidated, the exact nature of the G-protein nucleotide exchange and hydrolysis cycle during ACD remains to be resolved. A combination of powerful genetic studies and in vitro biochemistry has described a new component of the G-protein cycle in the context of ACD and, potentially, for G-protein

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

signaling in general. Miller and colleagues (81, 82) isolated a novel gene *ric-8* (resistance to inhibitors of cholinesterase; also known as synembryn) as an upstream regulator of the *C. elegans* Gαq-like protein EGL-30. *ric-8* reduction-of-function mutants have high rates of embryonic lethality (29%), which could be augmented to almost 100% in embryos heterozyous for *goa-1* loss-of-function alleles, thus indicating that RIC-8 and GOA-1 signal in the same pathway during embryogenesis (83). Spindle rocking during the P_0 cleavage was diminished or nonexistent in *ric-8* mutant embryos, and a loss of spindle and blastomere asymmetry was also observed. Compellingly, *ric-8* mutant phenotypes are reminiscent of *gpr-1/2* (*RNAi*) and *goa-1/gpa-16* (*RNAi*) phenotypes.

A recent study has provided some insight into the possible role for RIC-8 in the G-protein cycle during ACD (Figure 6). Tall and coworkers (84) independently isolated rat Ric-8 in a yeast two-hybrid screen for Gα_s- and Gα_o-interacting proteins. Biochemical analyses of rat Ric-8A indicated it is a GEF for several mammalian Gα subunits. Ric-8, in contrast to GPCRs or Ras-superfamily GEFs, appears to have a unique mechanism of nucleotide exchange. Ric-8 preferentially interacts with GDP-bound Gα, in the absence of G$\beta\gamma$, and causes nucleotide release and the formation of a stable nucleotide-free complex (84). Binding of GTP to Gα then reduces its affinity for Ric-8 and the complex dissociates. Thus, it appears the G-protein cycle during ACD, certainly in *C. elegans* if not other organisms, utilizes Gα subunits, a Gα GDI (GPR-1/2), and a Gα GEF (RIC-8) (Figure 6).

The biochemical actions on isolated Gα subunits that are ascribed to GoLoco proteins and Ric-8 are opposing ones: GDI activity versus GEF activity, respectively. This then represents an enigma because these biochemically opposite activities, when individually removed by RNAi or mutation, result in the same phenotype of symmetric division. Thus, it appears from the genetics that GPR-1/2 and RIC-8 are each required to activate G-protein signaling in *C. elegans* ACD. To resolve this enigma, it has been proposed that RIC-8 may require interdiction by the GPR-1/2 GoLoco motif to function on GOA-1/GPA-16 (77, 78). Ric-8 is unable to act as a GEF when its substrate, Gα·GDP, is bound to G$\beta\gamma$ (84). The suggestion is, therefore, that G$\beta\gamma$ is somehow removed from Gα by the GPR-1/2 GoLoco motif. It is also possible that, subsequent to G$\beta\gamma$ displacement, the GoLoco motif of GPR-1 may present Gα·GDP to RIC-8 and, thus, act cooperatively with RIC-8 to facilitate nucleotide exchange (Figure 6).

It also appears that Gα·GTP deactivation is required for appropriate signaling during asymmetric P_0 division. Loss of RGS-7, a potential GAP for GOA-1 and GPA-16 (Figure 6), results in overly vigorous posterior spindle rocking, a more asymmetric cell division plane, and thus more exaggerated asymmetry between resultant AB and P_1 cells (M. Koelle and H. Hess; personal communication). This suggests that elevated Gα·GTP levels result in excess force generation and further implies that Gα·GTP is the active species for force generation. In this model, GPR-1/2 may be required for appropriate spatial localization and clus-

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

tering of the signaling complex. However, the notion of a GoLoco/Gα·GDP complex having specific effectors within the ACD signaling pathway cannot be readily dismissed.

Functional redundancy exists between GOA-1 and GPA16. To detect cell division defects, embryos must be lacking both GOA-1 *and* GPA-16 (78). Paradoxically, though, there appears to be differential interaction between the Gα subunits and their signaling regulators. Yeast two-hybrid studies indicate that GOA-1, but not GPA-16, interacts with GPR-1/2 (76), whereas both G proteins interact with RIC-8 (P. Gönczy, K. Colombo, and K. Afshar; personal communication). The Gα selectivity of RGS-7 has not been fully determined, but it does act as a GAP for GOA-1 (M. Koelle and H. Hess; personal communication). It is also important to note that, in addition to their role as negative regulators of G protein signaling by virtue of Gα GAP activity, RGS-box proteins can also act as effectors for GTP-bound Gα subunits (3); hence, an effector function for RGS-7 cannot be ruled out. Another intriguing possibility is that, with both GEF and GAP activities seemingly critical for proper ACD, the nucleotide cycling rate of GOA-1 and GPA-16 in this system may determine the signaling outcome for ACD (Figure 6), as is the case for the Rho-family GTPase Cdc42 (85). Studies from mammalian systems may be able to shed some light on possible mechanisms of force generation in ACD and also underscore the conserved usage of non-conventional heterotrimeric G-protein signaling paradigms in cell division.

Heterotrimeric G-Protein Involvement in Mammalian Cell Division

The functional interplay between components of plasma membrane-delimited GPCR signaling is well defined in mammalian systems (1, 2). In contrast, investigation into the role of heterotrimeric G-protein signaling in mammalian cell division has lagged behind studies in lower metazoans. A limited number of reports exist that preface a role for Gα signaling in mammalian mitosis [for example (86, 87)], but to a large extent, the field is open for investigation. Similarly, there is evidence that asymmetric cell division is important during mammalian neurogenesis [as reviewed by Cayouette & Raff (88)]; however, no role for heterotrimeric G-protein signaling has yet been elucidated in this realm.

The mammalian Pins orthologue LGN [named after the leucine-glycine-asparagine tripeptide present in its TPR regions (89)] has recently been described as a mitotic regulator, shedding light on possible effector systems for GoLoco proteins in cell division. Ectopic expression of LGN causes severe mitotic abnormalities in several mammalian cell lines (90). More compelling, however, is that RNAi-mediated knockdown of endogenous LGN levels disrupts microtubule organization and chromosome segregation during mitosis (90). Using a yeast two-hybrid screen, the nuclear mitotic apparatus (NuMA) protein was identified as an LGN-binding partner (90). The first two TPRs of LGN specify binding to NuMA in vitro and in vivo (Figure 6). This interaction provides a direct link between GoLoco/Gα signaling and the regulation of spindle dynamics

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

because NuMA has been shown to regulate spindle formation and organization at the level of the centrosome (91). Assays of aster formation in *Xenopus* mitotic extracts using recombinant NuMA fragments and anti-LGN antibodies indicate that LGN acts negatively on the intrinsic ability of NuMA to stabilize microtubules and form asters (90). Mechanistically, it appears that the LGN- and tubulin-binding sites on NuMA partially overlap, suggesting that LGN sterically inhibits NuMA-mediated microtubule stabilization (92) (Figure 6).

The only reported investigation into the cellular function of the mammalian LGN paralogue AGS3 has come from Pattingre and colleagues (93); this investigation found ectopic expression of AGS3 fragments can attenuate amino acid deprivation-induced autophagy. As previously described, an interaction between the mammalian PAR-4 homologue LKB-1 and AGS3 has recently been demonstrated by in vitro and cellular cotransfection studies (43), but the functional consequences of this interaction have yet to be determined. Little evidence currently exists that AGS3 regulates either GPCR or mitotic signaling in a bona fide physiological context, but given recent findings regarding LGN function, it appears likely that AGS3 may have an analogous function in cell division processes.

Seminal studies by Rasenick and colleagues (94–98) provide a compelling potential mechanism by which G-protein signaling may regulate spindle forces during mitosis. Mammalian Gα and G$\beta\gamma$ subunits have been shown to regulate microtubule dynamics both in vitro (95, 96) and in vivo (97, 98). Gα_i subunits can activate the GTPase activity of tubulin and consequently accelerate microtubule dynamics. Thus, the possibility exists that GoLoco/Gα complexes signal directly to tubulin to modulate spindle dynamics (Figure 6). Accordingly, in *C. elegans*, the cortical force-generating system could comprise GPR-1/2, Gα, and tubulin, with Gα acting to depolymerize astral microtubules at the cortex and with the asymmetric distribution of GPR-1/2 leading to unbalanced forces applied to the spindle poles. Indeed, Labbe and colleagues (99) have recently demonstrated that microtubules at the posterior cortex are less stable during spindle displacement in the *C. elegans* embryo. In contrast, microtubules are equally stable at the anterior and posterior cortex in *goa-1/gpa-16* (*RNAi*) embryos (99), thus reinforcing a role for heterotrimeric G proteins in the control of cortical microtubule dynamics.

In line with the findings in *C. elegans* and *Drosophila*, striking evidence from studies of RGS14 now implicate heterotrimeric G-protein signaling in mammalian embryogenesis. RGS14 contains an N-terminal RGS box, tandem Ras-binding domains, and a C-terminal GoLoco motif (12, 100) (Figure 6). RGS14 protein in the mouse embryo is detectable at 28 h postfertilization, concurrent with the loss of expression of RGS12 (a mammalian paralogue of RGS14) (Figure 1). RGS14 localizes to the first mitotic spindle. Homologous inactivation of *Rgs14* in the mouse results in early embryonic lethality (101). Mouse embryos lacking RGS14 undergo preimplantation lethality at the two-cell stage due to an apparent cell cleavage failure; the one-cell stage is characterized by absence of

a developed microtubular network and asymmetric distribution of the nucleus. Indications are that RGS14, via its GoLoco motif, controls microtubule dynamics at the mitotic spindle, and the loss of this function is sufficient to disrupt embryonic development (S.J.A. D'Souza and L. Martin-McCaffrey; personal communication).

CONCLUDING REMARKS

Originally identified as a putative Gα-binding sequence, trapped in a screen for GPCR modulators, and characterized biochemically as a guanine nucleotide dissociation inhibitor, the GoLoco motif was born into a presumed role as a modulator of plasma membrane-delimited G-protein–coupled receptor signal transduction. However, overwhelming recent evidence from *Drosophila*, *C. elegans*, and now mammalian systems suggest GoLoco motif proteins are crucial regulatory elements in animal developmental processes—serving a fundamental and evolutionarily conserved role in organism embryogenesis, apparently at the level of force generation and organization of the mitotic spindle. Future studies should therefore be targeted at the further exposition of the genetic, molecular, and cell-biological bases of GoLoco protein action in the machinery of cell division.

ACKNOWLEDGMENTS

We thank Dr. Pierre Gönczy (ISREC), and University of North Carolina colleagues Drs. Miller B. Jones, Christopher A. Johnston, and Christopher R. McCudden for critical reading of this manuscript. Our work is supported by NIH grants GM062338 and GM065533. F.S.W. is an American Heart Association Postdoctoral Fellow. R.J.K. gratefully acknowledges predoctoral fellowship support from the National Institute of Mental Health (F30 MH064319). D.P.S. is a Year 2000 Neuroscience Scholar of the EJLB Foundation (Montréal, Canada) and recipient of the Burroughs-Wellcome Fund New Investigator Award in the Basic Pharmacological Sciences.

The *Annual Review of Biochemistry* is online at http://biochem.annualreviews.org

LITERATURE CITED

1. Gilman AG. 1987. *Annu. Rev. Biochem.* 56:615–49
2. Hamm HE. 1998. *J. Biol. Chem.* 273: 669–72
3. Neubig RR, Siderovski DP. 2002. *Nat. Rev. Drug Discov.* 1:187–97
4. Kimple RJ, Jones MB, Shutes A, Yerxa BR, Siderovski DP, Willard FS. 2003. *Comb. Chem. High Throughput Screen.* 6:399–407
5. Siderovski DP, Strockbine B, Behe CI. 1999. *Crit. Rev. Biochem. Mol. Biol.* 34:215–51
6. Siderovski DP, Harden TK. 2003. In *Handbook of Cell Signaling*, ed. RA Bradshaw, EA Dennis, pp. 631–38. San Diego: Academic

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

7. Granderath S, Stollewerk A, Greig S, Goodman CS, O'Kane CJ, Klambt C. 1999. *Development* 126:1781–91
8. Siderovski DP, Diverse-Pierluissi M, De Vries L. 1999. *Trends Biochem. Sci.* 24: 340–41
9. Ponting CP. 1999. *J. Mol. Med.* 77: 695–98
10. De Vries L, Fischer T, Tronchere H, Brothers GM, Strockbine B, et al. 2000. *Proc. Natl. Acad. Sci. USA* 97:14364–69
11. Peterson YK, Bernard ML, Ma H, Hazard S 3rd, Graber SG, Lanier SM. 2000. *J. Biol. Chem.* 275:33193–96
12. Kimple RJ, De Vries L, Tronchere H, Behe CI, Morris RA, et al. 2001. *J. Biol. Chem.* 276:29275–81
13. Natochin M, Gasimov KG, Artemyev NO. 2001. *Biochemistry* 40:5322–28
14. Cismowski MJ, Takesono A, Ma CL, Lizano JS, Xie XB, et al. 1999. *Nat. Biotechnol.* 17:878–83
15. Takesono A, Cismowski MJ, Ribas C, Bernard M, Chung P, et al. 1999. *J. Biol. Chem.* 274:33202–5
16. Howard AD, McAllister G, Feighner SD, Liu Q, Nargund RP, et al. 2001. *Trends Pharmacol. Sci.* 22:132–40
17. Ross EM, Wilkie TM. 2000. *Annu. Rev. Biochem.* 69:795–827
18. Kimple RJ, Willard FS, Siderovski DP. 2002. *Mol. Interv.* 2:88–100
19. Kimple RJ, Kimple ME, Betts L, Sondek J, Siderovski DP. 2002. *Nature* 416: 878–81
20. Lambright DG, Noel JP, Hamm HE, Sigler PB. 1994. *Nature* 369:621–28
21. Lambright DG, Sondek J, Bohm A, Skiba NP, Hamm HE, Sigler PB. 1996. *Nature* 379:311–19
22. Wall MA, Posner BA, Sprang SR. 1998. *Structure* 6:1169–83
23. Bernard ML, Peterson YK, Chung P, Jourdan J, Lanier SM. 2001. *J. Biol. Chem.* 276:1585–93
24. Peterson YK, Hazard S 3rd, Graber SG, Lanier SM. 2002. *J. Biol. Chem.* 277: 6767–70
25. Scheffzek K, Ahmadian MR, Kabsch W, Wiesmuller L, Lautwein A, et al. 1997. *Science* 277:333–38
26. Rittinger K, Walker PA, Eccleston JF, Smerdon SJ, Gamblin SJ. 1997. *Nature* 389:758–62
27. Coleman DE, Berghuis AM, Lee E, Linder ME, Gilman AG, Sprang SR. 1994. *Science* 265:1405–12
28. Mixon MB, Lee E, Coleman DE, Berghuis AM, Gilman AG, Sprang SR. 1995. *Science* 270:954–60
29. Natochin M, Gasimov KG, Artemyev NO. 2002. *Biochemistry* 41:258–65
30. Kimple RJ, Willard FS, Hains MD, Jones MB, Nweke GN, Siderovski DP. 2003. *Biochem. J.* doi:10.1042/BJ20031686.
31. Zhang XL, Zhang HL, Oberdick J. 2002. *Mol. Brain Res.* 105:1–10
32. Rondard P, Iiri T, Srinivasan S, Meng E, Fujita T, Bourne HR. 2001. *Proc. Natl. Acad. Sci. USA* 98:6150–55
33. Redd KJ, Oberdick J, McCoy J, Denker BM, Luo Y. 2002. *J. Neurosci. Res.* 70: 631–37
34. Luo Y, Denker BM. 1999. *J. Biol. Chem.* 274:10685–88
35. Kaushik R, Yu FW, Chia W, Yang XH, Bahri S. 2003. *Mol. Biol. Cell* 14: 3144–55
36. Jordan JD, Carey KD, Stork PJ, Iyengar R. 1999. *J. Biol. Chem.* 274:21507–10
37. Mochizuki N, Ohba Y, Kiyokawa E, Kurata T, Murakami T, et al. 1999. *Nature* 400:891–94
38. Ma H, Peterson YK, Bernard ML, Lanier SM, Graber SG. 2003. *Biochemistry* 42: 8085–93
39. Natochin M, Lester B, Peterson YK, Bernard ML, Lanier SM, Artemyev NO. 2000. *J. Biol. Chem.* 275:40981–85
40. Meng J, Glick JL, Polakis P, Casey PJ. 1999. *J. Biol. Chem.* 274:36663–69
41. Meng J, Casey PJ. 2002. *J. Biol. Chem.* 277:43417–24
42. Hollinger S, Ramineni S, Hepler JR. 2003. *Biochemistry* 42:811–19
43. Blumer JB, Bernard ML, Peterson YK,

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

Nezu J, Chung P, et al. 2003. *J. Biol. Chem.* 278:23217–20

44. Watts JL, Morton DG, Bestman J, Kemphues KJ. 2000. *Development* 127: 1467–75
45. Martin SG, St Johnston D. 2003. *Nature* 421:379–84
46. Webb CK, Kimple RJ, Siderovski DP, Oxford GS. 2002. *Soc. Neurosci.* Abstr. 542:10 (Abstr.)
47. Ghosh M, Peterson YK, Lanier SM, Smrcka AV. 2003. *J. Biol. Chem.* 278: 34747–50
48. Pathirana S, Zhao D, Bownes M. 2001. *Mech. Dev.* 109:137–50
49. Schiff ML, Siderovski DP, Jordan JD, Brothers G, Snow B, et al. 2000. *Nature* 408:723–27
50. Rubinfeld B, Munemitsu S, Clark R, Conroy L, Watt K, et al. 1991. *Cell* 65: 1033–42
51. Schaefer M, Shevchenko A, Knoblich JA. 2000. *Curr. Biol.* 10:353–62
52. Knoblich JA. 2001. *Nat. Rev. Mol. Cell Biol.* 2:11–20
53. Knoblich JA. 2001. *Symp. Soc. Exp. Biol.* 53:75–89
54. Chia W, Yang XH. 2002. *Curr. Opin. Genet. Dev.* 12:459–64
55. Schober M, Schaefer M, Knoblich JA. 1999. *Nature* 402:548–51
56. Wodarz A, Ramrath A, Kuchinke U, Knust E. 1999. *Nature* 402:544–47
57. Kraut R, Chia W, Jan LY, Jan YN, Knoblich JA. 1996. *Nature* 383:50–55
58. Knoblich JA, Jan LY, Jan YN. 1999. *Curr. Biol.* 9:155–58
59. Tio M, Zavortink M, Yang XH, Chia W. 1999. *J. Cell Sci.* 112:1541–51
60. Yu FW, Morin X, Cai Y, Yang XH, Chia W. 2000. *Cell* 100:399–409
61. Parmentier ML, Woods D, Greig S, Phan PG, Radovic A, et al. 2000. *J. Neurosci.* 20:RC84 (1–5)
62. Schaefer M, Petronczki M, Dorner D, Forte M, Knoblich JA. 2001. *Cell* 107: 183–94
63. Yu FW, Ong CT, Chia W, Yang XH. 2002. *Mol. Cell. Biol.* 22:4230–40
64. Fuse N, Hisata K, Katzen AL, Matsuzaki F. 2003. *Curr. Biol.* 13:947–54
65. Yu FW, Cai Y, Kaushik R, Yang XH, Chia W. 2003. *J. Cell Biol.* 162:623–33
66. Cai Y, Yu FW, Lin SP, Chia W, Yang XL. 2003. *Cell* 112:51–62
67. Bellaiche Y, Radovic A, Woods DF, Hough CD, Parmentier ML, et al. 2001. *Cell* 106:355–66
68. Malbon CC, Wang H, Moon RT. 2001. *Biochem. Biophys. Res. Commun.* 287: 589–93
69. Gönczy P. 2003. *Med. Sci.* 19:735–42
70. Kemphues KJ, Priess JR, Morton DG, Cheng NS. 1988. *Cell* 52:311–20
71. Grill SW, Gönczy P, Stelzer EH, Hyman AA. 2001. *Nature* 409:630–33
72. Gönczy P. 2002. *Trends Cell. Biol.* 12: 332–39
73. Zwaal RR, Ahringer J, van Luenen HG, Rushforth A, Anderson P, Plasterk RH. 1996. *Cell* 86:619–29
74. Gotta M, Ahringer J. 2001. *Nat. Cell Biol.* 3:297–300
75. Gönczy P, Echeverri C, Oegema K, Coulson A, Jones SJ, et al. 2000. *Nature* 408:331–36
76. Colombo K, Grill SW, Kimple RJ, Willard FS, Siderovski DP, Gönczy P. 2003. *Science* 300:1957–61
77. Srinivasan DG, Fisk RM, Xu HH, van den Heuvel S. 2003. *Genes Dev.* 17: 1225–39
78. Gotta M, Dong Y, Peterson YK, Lanier SM, Ahringer J. 2003. *Curr. Biol.* 13: 1029–37
79. Tsou MF, Hayashi A, Rose LS. 2003. *Development.* 130:5717–30
80. Grill SW, Howard J, Schaffer E, Stelzer EH, Hyman AA. 2003. *Science* 301: 518–21
81. Miller KG, Alfonso A, Nguyen M, Crowell JA, Johnson CD, Rand JB. 1996. *Proc. Natl. Acad. Sci. USA* 93: 12593–98

AR REVIEWS IN ADVANCE 10.1146/annurev.biochem.73.011303.073756
balt1/z6w-arb/z6w-arb/z6w00104/z6w0089-04a fullers S=8 3/15/04 4:44 4/Color Figure(s): 2,3,4 Art: 73029

82. Miller KG, Emerson MD, McManus JR, Rand JB. 2000. *Neuron* 27:289–99
83. Miller KG, Rand JB. 2000. *Genetics* 156: 1649–60
84. Tall GG, Krumins AM, Gilman AG. 2003. *J. Biol. Chem.* 278:8356–62
85. Lin R, Bagrodia S, Cerione R, Manor D. 1997. *Curr. Biol.* 7:794–97
86. Willard FS, Crouch MF. 2000. *Immunol. Cell Biol.* 78:387–94
87. Crouch MF, Osborne GW, Willard FS. 2000. *Cell. Signal.* 12:153–63
88. Cayouette M, Raff M. 2002. *Nat. Neurosci.* 5:1265–69
89. Mochizuki N, Cho G, Wen B, Insel PA. 1996. *Gene* 181:39–43
90. Du QS, Stukenberg PT, Macara IG. 2001. *Nat. Cell Biol.* 3:1069–75
91. Gaglio T, Saredi A, Compton DA. 1995. *J. Cell Biol.* 131:693–708
92. Du QS, Taylor L, Compton DA, Macara IG. 2002. *Curr. Biol.* 12:1928–33
93. Pattingre S, De Vries L, Bauvy C, Chantret I, Cluzeaud F, et al. 2003. *J. Biol. Chem.* 278:20995–1002
94. Wang N, Yan K, Rasenick MM. 1990. *J. Biol. Chem.* 265:1239–42
95. Roychowdhury S, Rasenick MM. 1997. *J. Biol. Chem.* 272:31576–81
96. Roychowdhury S, Panda D, Wilson L, Rasenick MM. 1999. *J. Biol. Chem.* 274: 13485–90
97. Sarma T, Voyno-Yasenetskaya T, Hope TJ, Rasenick MM. 2003. *FASEB J.* 17: 848–59
98. Popova JS, Rasenick MM. 2003. *J. Biol. Chem.* 278:34299–308
99. Labbe JC, Maddox PS, Salmon ED, Goldstein B. 2003. *Curr. Biol.* 13:707–14
100. Snow BE, Antonio L, Suggs S, Gutstein HB, Siderovski DP. 1997. *Biochem. Biophys. Res. Commun.* 233:770–77
101. Martin-McCaffrey L, Natale DRC, Kimple RJ, Willard FS, Oliveira-dos-Santos AJ, et al. 2002. *Critical role of regulator of G-protein signaling-14 (RGS14) in early mouse embryogenesis.* Presented at Great Lakes G-protein– Coupled Recept. Retreat. 3rd Annu. Jt. Meet., Ann Arbor, MI