Letter to the editor

ISSN 1449-2288 www.biolsci.org 2006 2(4):194-196 ©2006 Ivyspring International Publisher. All rights reserved

# Does WAVE1 contain a GoLoco/GPR motif?

Francis S. Willard

Department of Pharmacology, CB# 7365, 1107 Mary Ellen Jones Building, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7365 USA

Correspondence to: Dr. Francis S. Willard, Tel: 919-843-9364 Fax: 919-966-5640 Email: fwillard@med.unc.edu

Received: 2006.07.05; Accepted: 2006.07.19; Published: 2006.07.25

Heterotrimeric G-protein alpha subunits (G $\alpha$ ) are molecular switches regulated by the binding and hydrolysis of guanosine 5-triphosphate [1]. Nonreceptor proteins that modulate the nucleotide binding and hydrolysis activities of  $G\alpha$  proteins have recently become of considerable interest [2, 3]. One class of Ga modulating proteins is defined by the presence of a GoLoco motif(s) in their primary sequence [4]. The GoLoco motif was discovered as a region of sequence homology within novel Gabinding proteins [5-7], and is also referred to as the Gprotein regulatory (GPR) motif [5]. The GoLoco motif is a peptide of 20-40 amino acids that binds  $G\alpha_{i/o}$ ·GDP and prevents the spontaneous release of GDP by  $G\alpha$ , thus acting as a guanine nucleotide dissociation inhibitor (GDI) (reviewed in [4]).

To date, GoLoco/GPR motifs are known to exist in four well-defined families of proteins (Fig. 1). (1) Single GoLoco motifs are present in the RGS and RBD domain containing proteins RGS12, RGS14, and Drosophila Loco (Fig. 1). (2) PCP-2/GPSM4 and GPSM3/G18 contain two or three GoLoco motifs respectively, but no other currently identifiable functional domains or sequence motifs (Fig. 1). (3) Single GoLoco motifs are present in the N-termini of RapGAP domain-containing proteins (Rap1GAP1a, -b and Rap1GAP2b, -c) (Fig. 1). (4) The GoLoco motif is found in multiple arrays in the tetratricopeptiderepeat (TPR) domain containing proteins PINS (Partner of Inscuteable), GPSM2/LGN, and GPSM1/AGS3 (Fig. 1).

Song *et al.*, recently described the presence of a GoLoco/GPR motif in the actin polymerization regulating protein WAVE1 [8]. A synthetic peptide corresponding to this putative WAVE1 GoLoco motif was found to have GDI activity for  $G\alpha_{i1,2,3}$  *in vitro*. Furthermore, in coimmunoprecipitation experiments full length WAVE1 was found to interact with  $G\alpha_{i3}$ ·GDP but not  $G\alpha_{i3}$ ·GTP $\gamma$ S in both COS-7 cell lysates and rat brain extracts [8].

Primary sequence analysis of WAVE1 (*a.k.a.* SCAR1) indicates that, in many ways, does conform to the GoLoco motif consensus sequence (Fig. 2A). WAVE1 contains a predicted alpha helical region that is terminated by a glutamine residue. Subsequently a

[R]-[K]-[aliphatic]-[DE]-[DE]-[Q]-[R] motif characteristic of many GoLoco motifs is present. However, upon further analysis of the primary sequence of WAVE1 it can be observed that the predicted GoLoco/GPR motif overlaps the WASP homology 2 (WH2) domain (Fig. 2B) [8]. The Cterminal domain architecture consisting of a WH2domain, central domain, and acidic domain (also referred to as the VCA region) is characteristic of proteins such as WAVE1-3, WASP, and N-WASP that stimulate Arp2/3-dependent actin nucleation [10]. The WH2 and central regions interact with actin monomers [11, 12] whereas Arp2/3 binds to the central and acidic domains of WAVE/WASP proteins [13] (binding sites are illustrated in Fig 2C). The structure and function of the WH2-central-acidic cassette in actin nucleating proteins is highly conserved throughout evolution [14].

As the WH2 domain of WAVE1 directly overlies the predicted GoLoco/GPR motif described by Song *et* al. [8] (Fig 2B, C), logic states that WAVE1 can either contain a WH2 domain or a GoLoco motif but not both. Available biochemical and structural evidence strongly suggests that WAVE1 contains a functional WH2 domain that binds actin monomers with low micromolar affinity, or with nanomolar affinity in the context of an extended WH2 domain containing the central region [12]. Given that WAVE1, 2 and 3 contain the only WH2 domains similar in sequence to GoLoco/GPR motifs, it must be considered highly unlikely that WAVE1 contains a *bona* fide GoLoco/GPR motif. A simple explanation is that WAVE1 contains a WH2 domain with fortuitous sequence similarity to GoLoco/GPR motifs. That is not to say that the WAVE1/G $\alpha_i$  interactions observed by Song et al. [8] should not be considered important. One could consider this intriguing dual usage of a WH2 domain as a possible case of convergent evolution. In terms of the known structural biology of WH2/actin and  $G\alpha/GoLoco$  complexes this is not out of the question. Both the WH2 domain and GoLoco motif are likely in a unstructured conformation when unbound to ligand [11, 15, 16]; thus it is possible that  $G\alpha_i$  and actin could compete for WH2 domain binding. It is worth noting, however, that despite high levels of sequence identity between the three human WAVE isoform WH2 domains (Fig. 1C) only WAVE1 but not

WAVE2 nor WAVE3 were found to have GDI activity on  $G\alpha_i$  subunits (the signature biochemical activity of a GoLoco/GPR motif). This therefore argues against a conserved role for WH2 domains in heterotrimeric Gprotein regulation, in general.

In summary, WH2 domains and GoLoco/GPR motifs are evolutionarily independent protein-binding sequences. Fortuitous sequence similarity between WH2 domains and GoLoco/GPR motifs should not be considered indicative of common ancestry and/or shared function.

# Abbreviations

Arp2/3: <u>Actin related protein 2/3</u>; GPSM: <u>G</u>protein <u>signaling modulator</u>; PCP-2: <u>Purkinje cell</u> protein-<u>2</u>; Rap1GAP: Rap1 <u>G</u>TPase <u>activating protein</u>; RBD: <u>Ras binding domain</u>; RGS: <u>regulator of G</u>protein <u>signaling</u>; SCAR: <u>suppressor of the</u> *Dictyostelium <u>cAMP</u> receptor*; WASP: <u>Wiskott-Aldrich</u> <u>syndrome protein</u>; WAVE: <u>WASP-family verprolin</u> homologous protein.

## **Conflict of interests**

Declared none.

### References

- 1. Gilman AG. G proteins: transducers of receptor-generated signals. Annu Rev Biochem 1987;56:615-649.
- Sato M, Blumer JB, Simon V, Lanier SM. Accessory proteins for G proteins: partners in signalling. Annu Rev Pharmacol Toxicol 2006;46:151-187.
- 3. Siderovski DP, Willard FS. The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. Int J Biol Sci 2005;1(2):51-66.
- Willard FS, Kimple RJ, Siderovski DP. Return of the GDI: the GoLoco motif in cell division. Annu Rev Biochem 2004;73:925-951.
- Takesono A, Cismowski MJ, Ribas C, Bernard M, Chung P, Hazard S, Duzic E, Lanier SM. Receptor-independent activators of heterotrimeric G-protein signaling pathways. J Biol Chem 1999;274(47):33202-33205.

- Siderovski DP, Diverse-Pierluissi M, De Vries L. The GoLoco motif: a Galphai/o binding motif and potential guaninenucleotide exchange factor. Trends Biochem Sci 1999;24(9):340-341.
- Ponting CP. Raf-like Ras/Rap-binding domains in RGS12- and still-life-like signalling proteins. J Mol Med 1999;77(10):695-698.
- Song KS, Peterson YK, Freidman A, Blumer JB, Sato M, Lanier SM. Identification and characterization of a G-protein regulatory motif in WAVE1. FEBS Lett 2006;580(8):1993-1998.
- 9. Schultz J, Milpetz F, Bork P, Ponting CP. SMART, a simple modular architecture research tool: identification of signaling domains. Proc Natl Acad Sci U S A 1998;95(11):5857-5864.
- 10. Bompard G, Caron E. Regulation of WASP/WAVE proteins: making a long story short. J Cell Biol 2004;166(7):957-962.
- Chereau D, Kerff F, Graceffa P, Grabarek Z, Langsetmo K, Dominguez R. Actin-bound structures of Wiskott-Aldrich syndrome protein (WASP)-homology domain 2 and the implications for filament assembly. Proc Natl Acad Sci U S A 2005;102(46):16644-16649.
- 12. Kelly AE, Kranitz H, Dotsch V, Mullins RD. Actin binding to the C domain of WASP/Scar proteins plays a critical role in the activation of the Arp2/3 complex. J Biol Chem; in press.
- Panchal SC, Kaiser DA, Torres E, Pollard TD, Rosen MK. A conserved amphipathic helix in WASP/Scar proteins is essential for activation of Arp2/3 complex. Nat Struct Biol 2003;10(8):591-598.
- Aspenstrom P. The verprolin family of proteins: regulators of cell morphogenesis and endocytosis. FEBS Lett 2005;579(24):5253-5259.
- Kimple RJ, Kimple ME, Betts L, Sondek J, Siderovski DP. Structural determinants for GoLoco-induced inhibition of nucleotide release by Galpha subunits. Nature 2002;416(6883):878-881.
- 16. Adhikari A, Sprang SR. Thermodynamic characterization of the binding of activator of G protein signaling 3 (AGS3) and peptides derived from AGS3 with G alpha i1. J Biol Chem 2003;278(51):51825-51832.
- 17. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215(3):403-410.
- Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res 2003;31(13):3497-3500.

### Figures

Figure 1. The predicted domain architecture of all human GoLoco motif containing proteins. Domain boundaries were determined using BLAST [17], SMART [9], and ClustalW [18].



Figure 2. (A) Structure-based multiple sequence alignment of quintessential GoLoco motifs and the putative GoLoco motif of human WAVE1. Alpha symbols ( $\alpha$ ) and asterisks (\*) denote alpha helical secondary structure and GoLoco motif contacts with  $G\alpha$  as determined in the X-ray crystallographic structure of the RGS14/Ga<sub>il</sub> GDP complex [15]. The alignment ClustalW constructed [18] and BOXSHADE3.21 was using v1.83 (http://www.ch.embnet.org/software/BOX\_form.html). Accession numbers are PCP-2 (AAH38715), GPSM2 (CAG33067), Rap1GAP1b (BAA83674), RGS14 (O08773), and WAVE1 (Q8R5H6). (B) Overall domain architecture of WAVE1. The position of the putative GoLoco/GPR motif in WAVE1 is indicated by arrows and amino acid annotations. Domain nomenclature: EVH1 (<u>Ena/VASP homology 1</u> domain; also known as the <u>WASP homology 1</u> (WH1) domain), <u>basic</u> domain (B), proline-rich domain (POLY PRO), WH2 (<u>WASP homology 2</u> domain), <u>central domain (C)</u>, <u>acidic domain (A)</u>. (C) Structure-based multiple sequence alignment of the VCA (verprolin homology (WH2)/central/acidic) region of WH2domain containing proteins, adapted from Chereau *et al.* [11], and Kelly *et al.* [12]. Alpha symbols ( $\alpha$ ) and red asterisks (\*) denote alpha helical secondary structure and actin monomer contacting residues as determined by NMR [12] and X-ray crystallography [11]. Blue circles (o) denote Arp2/3 interacting residues as determined by differential line broadening NMR [13]. Accession numbers are WAVE1 (Q8R5H6), WAVE2 (Q8BH43), WAVE3 (Q8VHI6), WASP (CAJ30264), N-WASP (BAA20128), WIP (NP 003378). The alignment was constructed as described in A.





С		WH2		CENTRAL		ACIDIC
		ααααααααααααα		0000000	000000000000000	00000
WAVE1	(498-559)	RSVILLEATRKGT-OLRKV	E	EAKHERIENDVAT	TILSRRI-AVEYSD	SEDDSEFDEV-DWLE
WAVE2	(436-497)	RSDLLSAIROGF - OLRRV	EEOREO	E-KRDVVGNDVAT	TILSRRI-AVEYSD	SEDDSSEFDED-DWSD
WAVE3	(440-501)	RSDLLAAIRMGI-OLKKV	OEOREO	EAKREPVGNDVAT	TILSRRI-AVEYSD	SDDDSEFDEN-DWSD
WASP	(431-502)	RGALLDOIROGI-QLNKT	PGAPESSALOPP	POSSECLVGALME	IVMOKRSRAIHSSD	EGEDQAGDEDED DEWDD
N-WASP	(434-505)	RDALLDQIRQGI-QLKSV.	ADGQESTPPTPA	PTSGIVGALME	VMQKRSKAIHSSD	EDEDEDDEEDFEDDDEWED
WIP	(43-60)	RNALLSDISKGK - KLKKT	VTNDRSAPILD-	<del></del> <del>.</del>	<del>.</del> <del>.</del> <del></del>	
MIM	(728 - 755)	GEDMLNAIRRGV-KLKKT	TTNDRSAPRFS-			
WIP	(97 - 125)	PPGLGGLFOACMPKLRST.	ANRDNDSGGSR-			