Supplemental Material

Conserved Gene Microsynteny Unveils Functional Interaction Between Protein Disulfide Isomerase and Rho Guanine-Dissociation Inhibitor Families

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Supplemental Figure Legends

Suppl. Fig. S1 – The maximum likelihood phylogeny of the PDI protein family.

The tree was reconstructed using the LG model of protein sequence evolution, estimating the amino acid equilibrium frequencies from the data. The color code indicates that at least three PDI sub-families that can be traced back to the last eukaryotic common ancestor (LECA), indicated by the filled circles. Open circles denote the position of the last common ancestor of the animals. Black branches represent PDI orthologs that cannot be associated with any of the main PDI lineages. Branch labels denote percent bootstrap support.

Suppl. Fig. S2 – The maximum likelihood phylogeny of the RhoGDI protein family.

The tree was reconstructed using the LG model of protein sequence evolution. The color code indicates the RhoGDI subfamilies that can be traced back to the last common ancestor of the vertebrates (open circles). The filled circle represents the last common ancestor of the animals. Note that the RhoGDIβ lineage (represented in green) has been lost in ray finned fish (actinopterygii) and the shark *C. milii*. Branches in black represent RhoGDI lineages outside the vertebrates. Branch labels denote percent bootstrap support.

Suppl. Fig. S3: Alignment of genomic regions adjacent to RhoGDI genes in selected species in vertebrates.

Gene names and positioning are based upon the Genomicus database (see Methods). All species are aligned against *Homo sapiens*. The map is centralized in RhoGDI genes, focusing on species (particularly ray-finned fish) in which the PDI/RhoGDI arrangement is altered (vs. *Homo sapiens*) in some aspects (gene duplications, displacement of microsyntenic arrangement or absence of the clusters). Data are shown for RhoGDI α (ARHGDIA) (A and B), RhoGDI β (ARHGDIB) (in C) and RhoGDI γ (ARHGDIG) (in D). Genes are aligned in columms and kept in the order in which they appear in chromosomes (Chr) and scaffolds (Scf), without consideration for distance, while the transcriptional sense is represented by the pentagon tip. All orthologs are drawn with the same color and the lettering on the top refers to the *Homo sapiens* genes. In addition to the RhoGDIs, additional neighboring genes are included for reference.

Suppl. Fig. S4: Identity and similarity (between brackets) of amino acid sequence from human PDIs (A) and RhoGDIs (B) adressed in this study. Proteins are identified by UNIPROT ID.

Suppl. Fig. S5: Effects of PDIA1 or RhoGDIα silencing on the expression of the corresponding pair.

PDIA1 or RhoGDI α were silenced via siRNA transfection and their reduced level (48 h after transfection) was unassociated with alterations in their respective partner.

Suppl. Fig. S6: Physical interaction between PDIA1 and RhoGDI α by coimmunoprecipitation.

RhoGDIα were immunoprecipitated with anti-RhoGDI clone A-20 sc360, rabbit IgG. Epitope: N-terminus – against peptide derived of the 25 aminoacids from N-terminus. Western blots: Anti-PDI clone RL90-MA3019, Thermo; anti-RhoGDI clone A-20 sc360, rabbit IgG; anti-actin-B A5441, Sigma Aldrich, mouse IgG.

Suppl. Fig. S7: Physical interaction between PDIA1 and RhoGDIα in endothelial cells.

Confocal microscopic images depicting PDIA1 and RhoGDIα co-localization. In upper and lower panels, antibodies were targeted to the N- and C-terminus of RhoGDIα, respectively. Magnification 40x.

Suppl. Fig. S8: Uncropped western blottings.

A) Western blotting against PDI and RhoGDIα in rabbit VSMC transducing Tet-on system.
Representative of 3 samples from three independent experiments (From fig. 6A); B)
Western blotting against PDI and RhoGDIα in VSMC from PDI transgenic mice.
Representative of 9 samples from three independent experiments (From fig. 6C); C)
Western blotting against PDI and RhoGDIα in HUVEC transducing CRISPR activation
system dCas9-VP64 to PDI promoter region (From fig. 7A); D) Western blotting against
PDI and RhoGDIα in HUVEC transducing system dCas9-VP64 to
RhoGDIα promoter region (From fig. 7B);

Suppl. Fig. S9: Uncropped western blottings

A – **E)** Effects of PDIA1 overexpression upon RhoGDI α levels in a transgenic mouse model. Tissue from transgenic PDIA1-overexpressing mice: brain (A), kidney (B), liver (C), aortae(D)and heart (E). Data representative of n \geq 3, 2 (heart) or 1 (brain).,

Suppl. Fig. S10: Uncropped western blottings.

A) RhoGDIα were immunoprecipitated with anti-RhoGDI, ab5385, ABCAM, rabbit IgG. Epitope: N-terminus – against peptide derived of the 25 aminoacids from N-terminus. Western blots: Anti-PDI clone RL90-MA3019, Thermo; anti-RhoGDI ab5385, ABCAM, rabbit IgG; anti-actin-B A5441, Sigma Aldrich, mouse IgG (From Fig. 8A); **B)** RhoGDIα was immunoprecipitated with anti-RhoGDI clone B1- sc-13120, Santa Cruz, mouse IgG. Epitope: C-terminus - aminoacids 166-198.Western blots: Anti-PDI clone RL90-MA3019, Thermo; anti-RhoGDI clone A-20 sc360, rabbit IgG; anti-actin-B A5441, Sigma Aldrich, mouse IgG (From Fig. 8B); **C)** Western blotting against PDI and GST-tag (RhoGDIα) pulldown assay in HUVEC homogenate. Representative of 3 independent experiments (From fig. 8C); **D)** RhoGDIα was immunoprecipitated with anti-RhoGDI clone A-20 sc360, rabbit IgG. Epitope: N-terminus – against peptide derived from the N-terminal 25 aminoacids. Western blots: Anti-PDI clone RL90-MA3019, Thermo; anti-RhoGDI clone A-20 sc360, rabbit IgG; anti-actin-B A5441, Sigma Aldrich, mouse IgG (From Suppl fig. S6);

Protein	Organism	Protein name	Gene ID	mRNA and protein IDs	Genomic sequence	Location	Location Sense reading		Protein Identity/similarity vs human sequences ⁽¹⁾		
									RhoGDIα	RhoGDIβ	RhoGDlγ
RhoGDI	Trichoplax adhaerens	Putative uncharacterized protein	TRIADDRAFT_53598	XM_002109678.1 XP_002109714.1 ⁽²⁾	NW_002060944.1	180,991185,870 ⁽³⁾	reverse	6,180	53% / 67%	51% / 69%	47% / 65%
	Trichoplax adhaerens	Putative uncharacterized protein	TRIADDRAFT_20518	XM_002109679.1 XP_002109715.1	NW_002060944.1	189,570193,770	reverse	14,759	52% / 68%	51% / 69%	46% / 63%
	Nematostella vectensis	Predicted Protein	NEMVEDRAFT_v1g170702	XM_001628841.1 XP_001628891.1	NW_0018342601	150,219157,089	sense	7,246	47% / 64%	50% / 67%	40% / 62%
	Strongylocentrotus purpuratus	Sp-Rhogdi/Rho GDP dissociation inhibitor	SPU_025771	SPU_025771.1		Scaffold569: 461576467249	reverse	4,909	52% / 67%	49% / 67%	46% / 65%
	Patiria miniata	Sp-Rhogdi_1/Rho GDP dissociation inhibitor	PMI_007749	PMI_007749.1		Scaffold6534: 6586490774	sense	47,978	34% / 44%	34% / 43%	30% / 38%
	Patiria miniata	Sp-Rhogdi_2/Rho GDP dissociation inhibitor	PMI_007751.1	PMI_007751.1		Scaffold6534: 99795101554	sense	37,198	49% / 72%	55% / 71%	55% / 70%
									PDIA1	PDIA8	PDIA2
IQ	Trichoplax adhaerens	Protein Disulfide Isomerase	TRIADDRAFT_52862	XM_00210977.1 XP_002109713.1	NW_002060944.1	169,664174,811	reverse		49% / 69%	24% / 47%	37% / 59%
	Nematostella vectensis	Protein Disulfide Isomerase	NEMVEDRAFT_v1g118540	XM_001628843.1 XP_001628893.1	NW_001834260.1	142,973147,036	sense		58% / 76%	31%/ 51%	42% / 61%
	Strongylocentrotus purpuratus	Sp-Calseq/Protein Disulfide Isomerase ⁽⁴⁾	SPU_025772	SPU_05772.1		Scaffold569: 443358456667	reverse		46% / 58%	22%/ 41%	35% / 52%
	Patiria miniata	Pm-Calseq/Protein Disulfide Isomerase ⁽⁵⁾	PMI_007752	PMI_007752.1		Scaffold6534: 138752167074	sense		38% / 53%	29%/ 49%	35% / 58%

Supplementary Table S1: PDI/RhoGDI synteny in other organisms.

⁽¹⁾Uniprot IDs, GDIa, GDIB and GDIg respectively, P52562, P52566 and Q99819; and for PDIA1, PDIA8 and PDIA2 are P07237, Q96DN0 and Q13087, respectively.

⁽²⁾XP_002109714.1 vs XP_002109715.1 = 65% and 77% of identity and similarity, respecitevelly

⁽³⁾Intergenic distance between TRIADDRAFT_53598 and TRIADDRAFT_20517 = 3,700 pb.

(4)Sp-Calseq vs human calsequestrins - Casq1 (UNIPROT ID P31415) and Casq2 (UNIPROT ID O14958): identity/similarity are respectivelly, 21%/37% and 19%/33%

(5)Pm-Calseq vs human calsequestrins - Casq1 (UNIPROT ID P31415) and Casq2 (UNIPROT ID 014958): identity/similarity are respectivelly, 21%/39% and 21%/39%

T.Adhaerens and N. vectensis source of data: NCBI gene and protein database, UNIPROT.

S. Purpuratus and P. miniata source of data: EchnoBase

Organism	Name	ID	Source		
Homo sapiens	ARHGDIG, PDIP polycistronic mRNA for Rho GDP dissociation inhibitor gamma, pancreatic protein disulfide isomerase, complete cds	AB127078.1	GeneBank		
Mus musculus	Rho GDP dissociation inhibitor (GDI) gamma (strand-) contains the whole PDIA2 transcript	ENSMUST00000176961.7	Ensembl		
Macaca mulatta	Axin1 or PPP1R49 (strand -) contains exons of PDIA2	ENSMMUT0000000876.3	Ensembl		
Callithrix jacchus	PDIA2 contains Axin1 in the intronic region	ENSCJAT00000057730.1	Ensembl		
Canis familiaris	ARHGDIB contains exons of ERP27/PDIA8	ENSCAFT00000020458.3	Ensembl		
Meleagris gallopavo	PDIA2 (strand -) and part of an exon exhibit sequences of the gene RGS11, regulator of G-protein signaling (strand +)	ENSMGAG0000008956	Ensembl		

Supplementary Table S2: Polycistronic gene transcripts in different organisms







	PCYT2	NPB	ANAPC11	ALYR	F)-/	ARHGOIA	Р4НВ	PPP1R27	MCRIP1	GCGR	SLC25A10	RP13-1032/ 1.10	MRPL12	HGS	ARL16		DC137	C
-	NPB	ALYREF	ENSXETG00 000033135	ARHGD		5XETG00 00030259	Р4НВ	PPP1R27	FAM195B	GCGR	SLC25A10	HGS	slc16a3	FASN	DUS1L	- GP	9S1)-	B
}-	PCYT2	NPB	ALYREF	ENSXET0 0000331	35 A	RHGDIA	ENSXETG00 000030289	Р4НВ	PPP1R27	FAM195B	GCGR	SLC25A10	HGS	slc16a3	FASN		51L	(
3	ANAPC11	PPP (R278.)	P4HB	ENSTNIG 000141	36		FTR99	ENSTNIG00 000014133	PITPNC1	HELZ	CACNG5A	ENSTNJG000 00014129	ENSTN1G00 000014128	АРОН	NOL11	}- ВР	PTF	EN
}	NPTX1L	ENSTNIG00 000011596	PPPTR27A	ALYR	EF)		CDR2L	RECQL5	ENSTNIG00 000011591	XYLT2	RAB37	RAB37	SLC9A3R1	ATP2A1	RABEP2		DT9	N
в	ANAPC11	PRP18278	P4HB	ENSTRUE 0000030	500 051	ARHGDIA	FTR99	ENSTRUG00 000001894	ENSTRUG00 000001840									
4	ENSTRUG00 000008955	PEPIR27A	FAM195B	ALYR	EF)	ARHGDIA	ENSTRUG00 000008528	RECQL5	XYLT2	SLC9A3R1	ATP2A1	NUDT9	МАРКЗ	GDPD3B	ATP6V00	SPS	вза	м
3	CWC25	zgc:77358	SCRN2	ENSPFO 000017	G00 094		PPP1R12C	QPRT	zmp:0000 001082	ANAPC11	PLA2G12B	PPF(R278)	P4HB	ENSPF0G00 000016892	RPTOR	NP	TX1	{E
}	ENSPF0G00 000005947	PPP1R27A	FAM195B	ALYRE	F -		CDR2L	ARHGDIA	CDR2L	RECQL5	si:ch211- 120g10.1	si:ch73-3 64h19.1	XYLT2	RAB37	SLC9A3R	1) ATP	2A1	
	FAM195B	ALYREF	ARHGDIA		2L		CDR2L	RECQL5	si:ch211- 120g10.1	si:ch73-3 64h19.1	XYLT2	RAB37	SLC9A3R1	ATP2A1	RABEP2	2 sitch 1k	211-1 18.4	{
3	CWC25	zgc:77358	SCRN2	ENSXMA 000018	G00 086	ARHGDIA	PPP1R12C	QPRT	zmp:0000 001082	ANAPC11	PLA2G12B	PPP1R2/B	P4HB	ENSXMAG00 000017986	RPTOR	NP'	TX1	
}-	ENSXMAG00 000012237	PPPHR27A	FAM195B	ALYR	EF)	ARHGDIA	CDR2L	RECQL5	si:ch211- 120g10.1	XYLT2	RAB37	SLC9A3R1	ATP2A1	RABEP2) MA	РКЗ	G
}	FAM161A	XPO1	USP34	ENSORL 000001	600 620	ARHGDIA	ENSORLG00 000001632	ENSORLG00 000001635	zmp:000 0001082	ENSORLG00 000001655	PLA2G12B	ENSORLG00 000001668	ENSORLG00 000001670	ANAPC11	PRP1827	5 - (P4	HB	
-	PECAM1	PPPIRZIA	FAM1958	ALYR			CDR2L	RECQL5	ENSORLG00 000012256	si:ch73-36 4h19.1	XYLT2	RAB37	SLC9A3R1	ATP2A1	ENSORLGOD 000012024		RLG00 12015	< <p>[]</p>
-	PECAM1	\$\$\$\$1627A	FAM195B	ALYR	EF	ARHGDIA	CDR2L	RECQL5	si:ch211-1 10e21.3	si:ch211-1 10e21.4	CR352258.1	zgc:113030	si:ch211-1 10e21.4	CR352258.2	i:ch211-1 20g10.1	si:cf 64h	h73-3 19.1)
						ai Ca												
-	PCYT2	NPB	ANAPC11	ALYR	EF)	ARHGDIA	Р4НВ	PPP1R27	MCRIP1	GCGR	SLC25A10	RP13-1032	MRPL12	HGS	ARL	16 - (CCDC137	_
-	ENSMICG00 000033235	ZRANB2	PTGER3	UBE2		ENSMRCGQ 0000035290	СТН	ANKRD13C	SRSF11	LRRC7	-							
	PCYT2	NPB	ENSM1CG00 000026558	ALYR	F)-	INSMICGOO 000002895	P4HB	MCRIP1	GCGR	ENSMICG00 000008678	ENSMICG000 00028678	HGS	ARL16		37 - OXL	D1)-(TSPAN10	ŀ





Ipp RhoGDIα (anti-RhoGDIa clone A-20 – against N-terminus)







B 1th wblot RhoGDIa

2nd re-blot myc-tag

3th re-blot PDI

4th re-blot GAPDH









C PDI + Actin-β

RhoGDIa











с

RhoGDI-GST

Merge PDIA1+GAPDH PDS+RP -GDE-GS1 100kida 75Kda PDIA1 RhoGDI GST dimeric forms RhoGDI-GST homodimeric forms heterodimenic PDIAT RhoGDI-GST monomeric forms GAPDH 176.0 258.68 Free-GST

