ACCELERATED COMMUNICATION TRIADs: A new class of proteins with a novel cysteine-rich signature

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Abstract

Triad1 was recently identified as a nuclear RING finger protein, which is up-regulated during retinoic acid induced granulocytic differentiation of acute leukemia cells. Here we show that a cysteine-rich domain (C6HC), present in Triad1, is conserved in at least 24 proteins encoded by various eukaryotes. The C6HC consensus pattern C-x(4)-C-x(14–30)-C-x(1–4)-C-x(4)-C-x(2)-C-x(4)-H-x(4)-C defines this structure as the fourth family member of the zinc-binding RING, LIM, and LAP/PHD fingers. Strikingly, in 22 of 24 proteins the C6HC domain is flanked by two RING finger structures. We have termed the novel C6HC motif DRIL (double RING finger linked). The strong conservation of the larger tripartite TRIAD (two RING fingers and DRIL) structure indicates that the three subdomains are functionally linked and identifies a novel class of proteins.

Keywords: LAP/PHD domain; LIM domain; protein–DNA interaction; protein–protein interaction; RING finger

Since the initial discovery of the zinc finger as a DNA-binding protein structure (Miller et al., 1985), several other zinc-binding structures involved in protein–DNA or protein–protein interactions have been identified (Freemont, 1993; Schwabe & Klug, 1994; Aasland et al., 1995; Saha et al., 1995; Saurin et al., 1996; Dawid et al., 1998). The common denominator relating these motifs is the regular spacing of zinc-binding cysteine and histidine residues that form a three-dimensional structure that is stabilized by zinc, thereby providing an interface for interaction. Within this group of zincbinding motifs, there are three related structures that each bind two zinc atoms. These are the RING (really interesting new gene) (Freemont, 1993), the LIM (for the proteins in which it was first discovered: Lin11, Isl1, and Mec3) (Schwabe & Klug, 1994), and the LAP (leukemia-associated protein) (Saha et al., 1995) /PHD (plant homeo domain) fingers (Aasland et al., 1995). All these structures use eight cysteine/histidine residues for zinc-binding. In contrast to the classical zinc finger that interacts with both DNA and proteins (Mackay & Crossley, 1998), the LIM and RING fingers are thought to provide interfaces for protein interactions only (Freemont, 1993; Schmeichel & Beckerle, 1994; Schwabe &

Klug, 1994; Saurin et al., 1996; Dawid et al., 1998). LIM and RING finger proteins may have either cytoplasmic or nuclear functions and are implicated in various processes such as cell lineage determination, oncogenesis, and embryogenesis (Freemont, 1993; Schwabe & Klug, 1994; Saurin et al., 1996; Dawid et al., 1998). RING fingers are often found in conjunction with other cysteinerich domains (Saurin et al., 1996). In the RING finger B-box coiled coil (RBCC) proteins, the RING fingers are linked to a specific cysteine-rich zinc-binding motif (B-Box) and a coiled coil domain (Reddy et al., 1992). Interestingly, the three RBCC proteins PML, TIF1, and Rfp were all identified as oncogenic products that are disrupted by chromosomal translocations (Saurin et al., 1996). So far, LAP/PHD proteins appear to be confined to the nucleus. They have been shown to be involved in gene regulation through chromatin interaction or by functioning as bridging factors between transcription factors and the basal transcription machinery (Aasland et al., 1995; Giles et al., 1998). We have identified a novel conserved cysteine-rich motif (DRIL) that constitutes the fourth member of the family of the zinc-binding LAP/PHD, LIM, and RING finger domains.

Results

Identification of a novel cysteine-rich motif belonging to the family of LAP/PHD, LIM, and RING finger domains

We have recently identified Triad1 as a nuclear protein that is up-regulated during all-*trans* retinoic acid induced granulocytic

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Abbreviations: DRIL, double ring finger linked; LAP, leukemia-associated protein; LIM, Lin11, Is11, and Mec3; PHD, plant homeo domain; RBCC, RING finger B-box coiled coil; RING, really interesting new gene; TRIAD, two RING fingers and DRIL.



Fig. 1. Triad1 RING finger and C6HC domain conservation in the human (hTriad1), mouse (mTriad1), *D. melanogaster* (dTriad1), and *C. elegans* (eTriad1) orthologs. For clarity only the conserved cysteine/histidine residues related to the C6HC and RING finger domains are indicated. Numbers indicate Triad1 amino acid positions. Human, mouse, *D. melanogaster*, and *C. elegans* Triad1 GenBank accession numbers are respectively, AF099149, AJ130975, AJ01069, and U61944.

differentiation of acute promyelocytic leukemia cells (B.A. van der Reijden et al., in prep.). Triad1 contains two RING fingers, two C-terminal coiled coil domains and an acidic N-terminal region. In addition to the two RING fingers, it contains a cysteine-rich domain (C6HC) that is conserved in the murine, Drosophila melanogaster, and Caenorhabditis elegans orthologs (Fig. 1). To determine whether this domain is conserved in other proteins, we performed database searches using the human amino acid and pattern sequences and found 20 additional C6HC proteins. These proteins are encoded by various eukaryotes ranging from baker's yeast (Saccharomyces cerevisiae) to human. Alignment of the obtained sequences defined the C6HC consensus pattern as C-x(4)-C-x(14-30)-C-x(1-4)-C-x(4)-C-x(2)-C-x(4)-H-x(4)-C (Fig. 2). This pattern is reminiscent of the zinc-binding LAP/PHD, LIM, and RING finger consensus patterns. These motifs are characterized by the presence of eight zinc-coordinating cysteine and histidine residues that bind two zinc atoms (Fig. 2). The spacing between these zinc-coordinating residues is small (1–5 amino acids) with the exception of two loops that vary in length (4-48 amino acids). The C6HC motif differs from these patterns with respect to the histidine location and the absence of length-variation in the C-terminal loop (Fig. 2). The strong resemblance of the C6HC domain to the zinc-binding LAP/PHD, LIM, and RING fingers

C-x ₂ -	C-x ₍₁₇₋₁₉₎ -H-x ₂ - C-x	x ₂ - C-x ₂ -C-x ₍₁₆₋₂₀₎	- H- x ₂ -C	LIM pattern
C-x ₂ -	С-х ₍₉₋₃₉₎ - С-х ₍₁₋₃₎ - Н -х	x ₍₂₋₃₎ -C-x ₂ -C-x ₍₄₋₄₈₎ -	C-x ₂ - C	RING finger pattern
C-x _(1,2) -	· C-x ₍₉₋₂₁₎ - C-x ₍₂₋₄₎ -C-x	$x_{(4-5)}$ -H- x_2 -C- $x_{(12-46)}$	-C-x ₂ - C	LAP/PHD pattern
C-x4-	C-x ₍₁₄₋₃₀₎ -C-x ₍₁₋₄₎ -C->	x4- C-x2-C-x4-	H-x ₄ -C	DRIL pattern

Fig. 2. The novel C6HC pattern (DRIL) consists of seven regularly spaced cysteine and one histidine residues and closely resembles the LAP/PHD, LIM, and RING finger patterns. Zinc-binding ligands are C = cysteine, H = histidine (bold), whereas x can be any residue. In virtually all RING fingers the residues upstream of C5 and downstream of C6 are hydrophobic (Freemont, 1993). C8 in the LIM domain consensus can also be an aspartic acid or histidine residue (Dawid et al., 1998).

suggests that C6HC is a novel metal-binding motif possibly involved in protein–protein or protein–DNA interactions (Fig. 2).

C6HC motif conservation identifies a novel class of proteins

Remarkably, alignment of the 24 C6HC containing proteins revealed that the novel domain is flanked by two RING finger structures in 22 of the 24 proteins (Fig. 3). Given this association we propose the name DRIL (double RING finger linked) for the C6HC domain. The striking conservation of the two RING fingers and DRIL (TRIAD) motifs defines a novel conserved tripartite motif. The preservation of both the order and the distance of these three domains suggests that the spatial organization has functional significance. Downstream of the C-terminal RING finger an additional conserved cysteine and histidine residue is present (Fig. 3). Notably, many TRIAD RING fingers differ from the RING finger consensus pattern (Saurin et al., 1996). In the majority (18 of 22) of the N-terminal RING fingers, the spacing between C7-C8 is four instead of two amino acids, whereas in the C-terminal RING fingers the spacing between C5-C6 varies between 1-4 amino acids instead of two amino acids. In the latter finger the histidine residue is less conserved (14 of 22), whereas downstream of C6 a preference (12 of 22) for a basic as opposed to the consensus hydrophobic residues is found (Figs. 2, 3). In addition, several other conserved noncysteine/histidine residues are present, particularly in the C-terminal RING finger. While some of these residues are unique to the TRIAD structure, other residues can also be found in several RING fingers of non-TRIAD proteins indicating that they are not unique to the TRIAD structure but rather may identify a subclass of RING fingers in general.

To identify TRIAD domain conservation, an identity and similarity matrix was calculated (Fig. 4) (Thompson et al., 1994). Cross-species conservation of nonligand binding amino acids was found for at least four distinct groups of TRIAD domains. The very high similarity (61–100%) within these groups indicates that TRIAD domains are well preserved during evolution, and suggests that they have important biological functions.

1	5	5	9
т	J	J	2

Hu_THC22474	KLFLGS#GFCEKLG-SECMYF#EGRHVYGKAGLKDHFEIQ-IRDGQVQCLNGPEPKGPSVATPGQ#KELHEAE#FARHDRLLLQSS#DLM
Mo_TC36872	ĸlflqszqfceklg-sdcmyfzegkhvygkaglkdyfeiq-ikdgqvkclNgpepqgpsvatpqqykelyeadsfamydrlllqstyddk
Hu_164695	
Aa_U84248	iagegpæydnlpagdeffalængkeyageglenglmie-isesrtdisgpqgpesmhptdæqtlækafpaäitævedfmvrrvælad
Ce U80847/C17H11.6	KMKEQPEGAAKMPG-SAFPKLKGGOHRSGRAGLROWVELS-ITENRVEVPQPE-GSSYLHPNDEKMLEGDIPTEIERVEAFSLRRYEMTE
Hu AF099149/Triad1	PPHERA WOMOFVRK ENLLSEAGOHOFORSOWEOHCSVLVKDGVGVG-VSOMAODOPLRTPEDFEFPLEPN-EEEREKVRRYLFRDYEESH
Mo 3.1130975	PPHHON WORVER ENLLS A CONFERENCE OF CRACK AND A DOMESTIC SOMA OD A DEPLET PEDE FPL
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Ce_001944/T12612.1	
Ce_AF003137/C27A12.7	GDEEDECSMDELSGESENERAGAEGWOANLTNKIVSDAOSE-IEGMAPNGKILIEDEK¥LAYYKD-PTTIAAMKKMNVASYEEIN
Ce_AF003137/C27A12.8	GDAEGDECSLGELSGISGNERAGTOGWRAMLTNRIANNAOSE-IEGMAPNORLLIEDERGMFYTTD-PTGIATWRRLIVASYWETN
Ce_AF003137/C27A12.6	GDAEGDIGCSMDELSGISGNHRAGAEGWQAMLTNKIVSDAQSE-IEGMAPNGKLLIEDEKILSYISD-PTHVSKURKLMVASYVEIN
Dm_X98310	QCEEGEEGFSQLPPDSMAGEEGGHRFGMPGWHENLSTKIVAEGLGQTISGAAHGGDILVDDVTWANLWTD-ARWRWQQLITNSFWECN
At_AB018114	KTMKODYGMEDDLP-SNVMTRHEGGERFONDGWIGHFTVK-INEGESKRILGMAHEGKAICDEDVYRKLYSPEYADHUDRFLIESYYEDN
Ce U13644/F56D2.5	TLYDGOWGFESONG-OHCIKFOPOSEVFOKSGTFNYYISI-AKGFVSKPMSGLAEGGENEAQQGMWQEABGEEBFAWWEAHMLEKABREM
At AC004512/T8F5.21	WOLDGGE GETFLSDKLHAAAGHPFGDSGWEGMITTAINDGPGCLTLRGPDPSGRAAVGODMENLLAPDKDKORWTSYFVRSYFEDN
Sc P36113	NDFT@TECDKKDTETFATEGGHEYGINGYRHMIKDKLHEGNIITGMDGSLALKNEDTDKV#GHPSSSTLMDSSIKSFTOKH
Df 17.008982	EXET NOT , FLECOT EDTYTIS GUEYSKENLKNNLKTSLENDFEDDTTTKENTDLKENKTTKKNDWKNTCE EXDYFWLYTLLHTY KKS
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At_AL024486/T16H5.30	PIENOS ECEDROS EMMLS KOTHKFOSHOMKTOVEGKVKTSEVPIROPQVQGKHYLSAAECKSFEP~ #TT-EKSFEEANVCSKN
Motif	1 *2 * 3 4 5 6 & 7 8 * * * \$& *
Hu_THC22474	- ADVVXIIIR - PCGQLPXMQEPGCTMGIQSSQNFAQQTLQR-LTXIIGVSPQKVTAEKLMDLRNEYLQADEANKKLLD
Mo TC36872	- ADVY TOR- PCOOLPHMO
Hu 164695	-PDCRWGPA-PDGGYATTAYGCASCPK#TGEREGGOTEGGYHCK-OIWIPNOTGDMAROORAOTLR
Ap 1184248	
CA 1120247/017811 6	
HU_AFUFFIGF/IIIEUI	
MO_AJI309/5	
Dm_AJ010169	- PELREGIG-PNCQII TOOSSEISAKKAICKACHTGIGFKGG-MDEHAPTDGQ
Ce_U61944/T12E12.1	- PHLKEQVG-NEQPVIERSTEVKPKRTQMQQHTSIQVKQG-ADMHAPTSQE
Ce_AF003137/C27A12.7	-ALLK #CPG~VDCGRT #KVSHGEPRL #VCTCGSR FCFSCG-QD #HEPVNCR
Ce_AF003137/C27A12.8	-RLLK#GEG-IDGGKA&RVS
Ce_AF003137/C27A12.6	
Dm X98310	
At AB018114	-NMVKNOPSKPHCGSATRKIEDGHDVVEVGGSGGLOFCFSGL-SESHSPCSGL
Ce U13644/F56D2.5	-DDSMEQDN-ENCOMVAYLTDSORNLYEGSYONYSECNLOK-GTHIGVSRCKFRKEDEERIMKEWNEADEAGKEEMY
ht hc004512/#895 21	PETERS A DECENTION OF
AL_ACUVAJIA/10FJ.AI	
PT_AL008982	
Dg_Ca1215	- PASK WOR - PAGANAKIGD
Hu_AB014608	-SNLTWOTNPQCCDRIMCRQGLGCGTTCSKOGWASCFNCSFPEAHYPASCG
Hu_P50876	- PCRT號包2A-STCQAVCQLQDVGLQTPQP禁QQKAQRMECSTQK-AS號目PQQGQP
At_AL024486/T16H5.30	-NGKI CCY-PNGSFLEDPQECLSSGRASSSSTQSENSCCYEGPVGERFVGVDGG-VPMHASMSGE
Motif	#1+ 2 * * *3 4 ~5 6 #7 8
Hu THC22474	orygkryžokaleemeskewle – Knäksigigigigigi (goto do the state) – – Eightigitigi – – Moslsranpykiifindpg – – Spistra
Mo TC36872	ORYGKRYTOKALEEMESKDWLE KNSKSGPCGGTPHOKLD - GGNKWTGTG MOYJGWEG MGSLSRANPYRHFTDSE - SPGFNRL
Hu 164695	-VRTKHTSGLSYGOESGPDDIKY PRESAYHIKMNDGSGNEMTMAY
AA_0004240	
Hu_AFUJJI4J/Triadi	
MO_AJ130975	TIRKWETKCADDSETANJISAHERIDOSKONICIPHIKNGGONHBOOSKOKHDROWBOEGDWKTHGSEYEOSRYK
Dm_AJ010169	VIKKW發TKCADDSETANYISAH型MOGIKCHICHIMKNGGONH如QGFNGKHDDOGMG發GDWKTHGSEYYEGSRYK
Ce_U61944/T12E12.1	TIKQW#TKCADDSETANYISAH#KDCDQCHSCHPKAGCCNH#QCTRCRHHDCW#CFGDWKSHCSEYYEGSRYK
Ce_AF003137/C27A12.7	LLKLWäkkCNDDSETSNWINSNÄLCORKOMATIIIKNGGONQÄTCKNTGCKFQFOWÄCÄGPWTVBANAWYKCNKFD
Ce AF003137/C27A12.8	LLKLWäkkCNDDSETSNWINANTREGEKGMITIERDGGONHDTGKNTAGRFERGWEGEGOPWEPEGSSWYSENRFD
Ce AF003137/C27A12.6	HLKKWEKKCODDSETMNWINANEWDOPKOMIPHPKNGCONRMLGTNSGORYEFOWEGEEPWTKHGYQYAGNGYD
Dm X98310	WLKKWIKKCDDDSETSNWIAANINEGIRGSVTHIKDGCONHUVGKNONGKNEIZGWWGIGSWFP
AF AB018114	- WWKIWKKCEDESETUNWITUNWKIMBESKPIOKSDCANIMTES GOUNDANKEGOATCPD
Co W13644/#56D3 5	
At_AC004512/T8F5.21	TVSKWELKNSAESENNNWILANSKIPUSKNKKPITIKNUCOMHETUTPF-GKFERGWEGEGAWTEGGEKTGGFIACNRYE
Sc_P36113	ITTAWEKKARKESEILNWVLSHEMEGERGSVNMAMNGCOMHDVGSSGKYEDGWEGEGPWAPHGKNFFOCTWYK
Pf_AL008982	YIKKWYELENNDDHNMKWIHAY蘂冠MOBNONKPIEMSNSOOMN貘KOIOGYSIOOO蘂O蘂DNWKNIKGCFYKONKYL
~ * ~ ~ ~ ~ ~ ~	
Dd_C91572	-QWSEFRYLKETSNORFEEWAREN <u>EKPCE</u> KCKSK IDK DG <mark>GONHMTC</mark> KL C KHO <u>FCWRG</u> YDVYTKTHFSGGK G KQNS
Dd_C91572 Hu_AB014608	- Qwsefkwlket Snqrfeewar Energedrok Skidkdg Conhinterl Ckhorgwig Dvytkt Hfs Ggkokons Wyddggyydgmsveaqskelak Li <u>skropso</u> qaphdkne Cclemtgar - Cnhorgwrg Ekswkpn Ekd Yyngsamv
Dd_C91572 Hu_AB014608 Hu P50876	-QWSEFKULKETSNQRFEEWAREMFRYCERGKSKIEKDGGCNEMTCKLCKEQFCWEGEDVYTKTHFSGGRGQNS WyddgyydgmsveaqskulakLiskrCBSCQapterneGCLEMTCKLChkGFCWRGExsWKPNHKDYNGSAMV ETMFIFF9GETSAFYMEEDDAFIKCERCKYYBERDSGGLQMMGKNCHkAFGYVGLESEDDDFLLIHYDKGPGRNKL
Dd_C91572 Hu_AB014608 Hu_P50876 At AL024486/T16H5.30	-QWSEFKMLKETSNQRFEEWARENTRPCPRCKSKTEKDGGCNHMTCKLCKHQFCWLCDVYTKTHFSGGKCKQNS WVDGGYYDGKSVEAQSKHLAKLISKRCPSCQAPTEKNSCCLEMTCAKGNRGFCWRCDKSWKPNHKDYYNCSAMV ETMPITFHPGETSAAFKMEEDDAFIKRCPKCKVYIERDEGCAQMMCKNCKHAPCWYCLESDDDFLLIHYDKGPCNKL -EFOILPYDERYPDDITLHRLARYKWKRCQCCRIMTELAOCGNRUTCRGCHEFC%SGCAEYFEGOTCTCAFWD

Fig. 3. TRIAD conservation identifies a novel protein family. This TRIAD alignment contains five human (Hu), six C. elegans (Ce), three Arabidopsis thaliana (At), two murine (Mo), two D. melanogaster (Dm), one Aedes aegypti (Aa = mosquito), one S. cerevisiae (Sc), one Plasmodium falciparum (Pf), and one Dictyostelium discoideum (Dd) sequences. Names consist of GenBank, Swissprot, or TIGR database accession numbers. In cases where the GenBank database file contains more than one protein sequence, the accession number is followed by the name of the protein that contains the TRIAD domain. The TRIAD domains found in GenBank database files (accession numbers I64695, U84248, C91572, and AB014608) and TIGR database files (accession numbers THC22474 and TC36872) are derived from cDNA sequences. The TRIAD domains found in the sequences with GenBank accession numbers AB018114 and AL008982 are derived from genomic sequences (TRIAD coding positions are 44513-45157 and 6529-7158, respectively). Numbers (1-8) in the motif indicate conserved cysteine/histidine residues in the N-terminal RING (upper panel), DRIL (middle panel), and C-terminal RING (lower panel). Minimal variations regarding cysteine/histidine conservation and spacing are observed in P50876 (cysteine instead of histidine in the N-terminal RING), AB014608 (five instead of four amino acids between C1-C2 and C6-H in DRIL), AC004512/T8F5.21 (five instead of four amino acids between C1-C2 in DRIL), P36113 (serine instead of cysteine in DRIL), and AL008982 (leucine and serine instead of cysteine in the N-terminal RING). Symbols indicate the following conserved residues: * hydrophobic leucine, isoleucine, valine, or methionine; # aromatic hydrophobic phenylalanine, tryptophan, or tyrosine; \$ basic lysine or arginine; & tyrosine; ~ phenylalanine; + proline; @ isoleucine; % glutamic acid; ! glycine; ^ asparagine; = serine or threonine; 0 = cysteine or histidine. Two DRIL proteins that do not harbor a complete TRIAD domain (F56D2.2 and T19L18.6, accession numbers U13644 and AC004747, respectively) are not depicted.

Discussion

Here we describe a new class of proteins that is characterized by a novel tripartite domain designated TRIAD. TRIAD is a cysteinerich domain of approximately 200 amino acids that consists of a DRIL motif, which is flanked by two RING fingers (Fig. 3). The structure of the DRIL motif closely resembles those of the zincbinding LAP/PHD, LIM, and RING fingers and is therefore a putative metal-binding protein structure (Fig. 2). The conserved organization of the DRIL and RING fingers suggests that these

	Hu_THC22474	Mo_TC36872	Hu_164695	Aa_U842486	Ce_U80847/C17H11.6	Hu_AF099149/Triad1	Mo_AJ130975	Dm_AJ010169	Ce_U61944/T12E12.1	Ce_AF003137/C27A12.7	Ce_AF003137/C27A12.8	Ce_AF003137/C27A12.6	Dm_X98310	At_AB018114	Ce_U13644/F56D2.5	At_AC004512/T8F5.21	Sc_P36113	Pf_AL008982	Dd_C91572	Hu_AB014608	Hu_P50876	At_AL024486/T16H5.30	
Hu_THC22474 Mo_TC36872 Hu_I64695 Aa_U84248 Ce_U80847/C17H11.6 Hu_AF099149/Triad1 Mo_AJ130975 Dm_AJ010169 Ce_U61944/T12E12.1 Ce_AF003137/C27A12.7 Ce_AF003137/C27A12.8 Ce_AF003137/C27A12.6 Dm_Y98310	 92 24 23 26 23 22 25 22 23 23 23 23 25	96 23 22 26 22 21 25 21 25 21 22 23 23 24	33 32 61 54 22 22 24 20 24 24 24 25 27	32 31 67 50 22 24 21 23 23 24 26	36 35 64 61 22 22 22 22 22 24 25 24 25	34 34 31 33 98 58 53 36 39 39 39	34 34 32 31 33 100 59 54 37 40 40 39	36 36 33 34 34 66 66 56 38 41 42	33 34 32 33 65 65 66 37 38 38 34	36 35 34 32 35 49 51 50 80 79 51	33 33 31 35 50 50 52 51 88 77 57	36 34 34 50 50 51 49 88 85 53	35 33 33 33 51 52 48 63 66 63	35 36 33 30 32 41 41 41 51 51 50 49	47 47 26 29 30 33 32 33 30 29 31 31 32	31 32 32 32 47 47 46 44 46 47 48 49	27 26 31 28 32 43 43 43 42 44 46 44	31 30 31 28 30 38 38 38 39 42 40 41	40 39 38 36 33 38 36 41 40 40 39	32 31 31 31 42 42 38 37 36 35 37	29 28 31 33 34 34 31 30 30 31 30 28	26 27 28 27 30 30 31 34 31 33 31 32	% similarity
At_ABO18114 Ce_U13644/F56D2.5 At_AC004512/T8F5.21 Sc_P36113 Pf_AL008982 Dd_C91572 Hu_AB014608 Hu_P50876 At_AL024486/T16H5.30	27 35 24 21 23 28 22 21 18	28 34 23 20 22 27 22 19 18	24 19 23 22 21 27 23 24 18	21 20 23 21 20 27 24 24 19	23 24 23 23 20 27 23 23 19	34 24 36 30 29 25 31 25 21	35 23 36 30 29 26 31 25 21	34 22 35 31 32 30 30 25 20	32 21 30 28 30 25 28 21 23	36 22 35 33 31 29 28 21 20	39 22 36 34 30 28 28 28 24 23	37 22 37 34 32 28 28 22 21	37 23 38 34 30 31 28 24 21	 22 34 29 26 32 25 20 20	29 24 19 24 26 21 23 15	43 31 33 32 30 30 23 20	36 25 43 28 25 23 20 20	36 30 43 37 27 27 27 22 17	43 34 40 33 38 25 29 22	34 27 35 32 35 32 23 19	28 32 29 27 30 36 29 	28 23 31 29 29 28 28 28	

% identity

Fig. 4. Percentages of TRIAD similarity and identity. Names are explained in Figure 3. TRIAD orthologs/paralogs are in bold and boxed (identity \geq 50%). Related TRIAD family members (\geq 48% similarity) are in italics and boxed (dashed line). The similarity (\geq 40%) of lower eukaryotes (*S. cerevisiae*, *D. discoideum*, and *P. falciparum*) to several different conserved TRIAD proteins (dotted boxes) further illustrates the evolutionary preservation of TRIAD.

domains operate together as one functional unit that may mediate complex protein or DNA interactions. Both TRIAD RING fingers differ from the classical RING finger consensus pattern with respect to cysteine spacing and nonligand binding amino acid conservation (Fig. 3). These characteristic differences may result in structural variations that are essential to TRIAD functioning.

To date, the function of TRIAD containing proteins remains unknown. However, within the set of 22 TRIAD domains reported here, at least four groups with strong cross-species conservation are present, suggesting that they have an important function. More distant TRIAD homologies were found for parkin, a protein mutated in juvenile parkinsonism (Kitada et al., 1998), and RBCK, a novel protein kinase C interacting protein (Tokunaga et al., 1998) (data not shown). Neither protein completely matches the TRIAD consensus nor do they share certain cysteine/histidine or other conserved residues, formally disqualifying them as TRIAD proteins. Like TRIAD proteins, the exact functions of parkin and RBCK are unknown.

The TRIAD proteins described here vary in size from 292 to more than 1,754 amino acids (data not shown). In the smallest member (P50876), the TRIAD domain spans almost the entire length of the protein. If TRIAD represents an interface for multiple protein interactions, the principal role of the smaller TRIAD proteins may be to integrate the function of other proteins, whereas the larger ones may have additional roles.

Protein structure analysis predicts that two-thirds of the TRIAD proteins are nuclear (data not shown). Interestingly, all these nuclear TRIAD proteins also contain predicted coiled coil regions (Lupas, 1997), whereas all but two cytoplasmic proteins do not (data not shown). Apparently, the presence of coiled coil domains is essential to nuclear TRIAD protein functioning, perhaps providing additional surfaces for protein interaction. Since nuclear proteins may be involved in various processes that are pertinent to cell-cycle regulation, cellular differentiation, gene expression, and oncogenesis, it will be of interest to determine in which nuclear processes TRIAD proteins are implicated.

Materials and methods

Detection of C6HC/DRIL proteins

C6HC/DRIL proteins were identified by screening the nonredundant, the expressed sequence tag, and the Swissprot GenBank databases (version 2.0.6) at the National Center for Biotechnology Information (NCBI) (http://ncbi.nlm.nih.gov), and the tentative human and mouse consensus databases at The Institute for Genomic Research (TIGR) (http://www.tigr.org), with the human Triad1 DRIL protein sequence using the TBLASTN and BLASTP algorithms (Altschul et al., 1997). The pattern C-x(4)-C-x(4,25)-C-x(1,4)-C-x(2)-C-x(4,25)-H-x(4)-C was used to screen the ISREC prosite database by the pattern search algorithm (Hofmann et al., 1999).

Alignment of TRIAD domains and matrix calculations

Protein sequence alignments and identity/similarity matrix calculations were performed using the CLUSTAL W (version 1.74) algorithm (Thompson et al., 1994) at IBCP (http://www.ibcp.fr), using default parameters, except for the gap-penalty (8 instead of 10), and were adjusted by hand. TRIAD gene expression was confirmed by screening the expressed sequence tag database at NCBI with putative TRIAD protein sequences (that were derived from genomic DNA sequences) using the TBLASTN algorithm (Altschul et al., 1997). All proteins were shown to match to expressed sequences, except for AF003137/C27A12.7, AC024486/T16H5.30, and AB018114.

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