BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **235,** 162–165 (1997)
ARTICLE NO. RC976751

B30.2-like Domain Proteins: A Growing Family

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Received April 14, 1997

The B30.2 domain is a conserved domain of around 170 amino acids. It is found associated with different protein domains: immunoglobulin domain in the case of butyrophilin and Ring Finger domain in the case of Ret Finger Protein. B30.2 should therefore be considered a migratory domain. We here report new members of these families as well as new protein families having the B30.2 domain, and we tentatively propose a general function for this domain.

During the search for new coding sequences in the chromosomal region containing the human class I histocompatibility complex, we have identified an exon which encodes for a protein domain (170 amino acids) named B30.2. This domain belongs to a multigenic family. The B30.2 like domain is associated with 2 different types of N-terminal domain (Figure 1A) in few other proteins (Vernet et al, 1993). The first type, contains a BBox motif associated with the RING motif and has been found in a subset of the RING finger proteins. This subset included the Ret Finger Protein (RFP) (Takahashi et al, 1988)), the autoantigen Ro/SSa (52kDa) (Chan et al, 1991), the nuclear factor of the Xenopus (XNF7) (Reddy et al, 1991), the nuclear factor of pleurodele (pAw33) (Bellini et al, 1993), the Acid Finger Protein (Chu et al, 1995) and RING Finger B30 protein (Henry et al, 1997).

The second N-terminal domain associated with B30.2 like domain corresponds to two immunoglobulin like domains (IgV-IgC1) found in the extracytoplasmic part of the butyrophilin (Jack& Mather, 1990) and the BT2 protein (Henry et al, 1997).

We here report new members of these families as well as new protein families having the B30.2 domain, and tentatively proposed a general function for this domain.

RESULTS AND DISCUSSION

The sequence alignments between B30.2 like domains are shown in Figure 2. Sequence similarities are found for the whole domain. Moreover three highly conserved motifs are present. In order to identify new members of this family, searches in databases were performed using the B30.2 domain with tBLASTn (Altshul et al, 1990), FASTA (Pearson& Lipman, 1988) or BLOCK (Henikoff et al, 1995) algorithmic program softwares. Search was also performed using the three consensus motifs named LDP, WEVE and LDYE described in table 1A. The new members found by these ways are shown in figure 1B and their conserved motifs in tables 1B and 1C. Some of them were already known as RING finger proteins (i.e.: the Estrogen finger protein (Efp) (Inoue et al, 1993) and Staf50 protein (Tissot & Metchi, 1995)) but the authors had not pointed out the fact that they possess a B30.2 like domain. Other proteins were described as having no similarity with other proteins present in databases. This is the case for:

(1) the Stonustoxin a and b (Ghadessy et al 1996). The Stonustoxin a and b are the two subunits of a lethal protein isolated from the venom elaborated by the stonefish Synanceia horrida. The a and b subunits have a respective molecular masse of 71 and 79 kDa and display 50 % amino acid identities. We show here that they possess a B30.2 like domain at their C terminal end. The N terminal end of Stonustoxin a and b share no significant similarity with other proteins in databases.

(2) the KIAA0129 gene product (GB D50919), previously described has having no similarity with protein in the databases. We show here that the conceptual

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0006-291X/97 $25.00

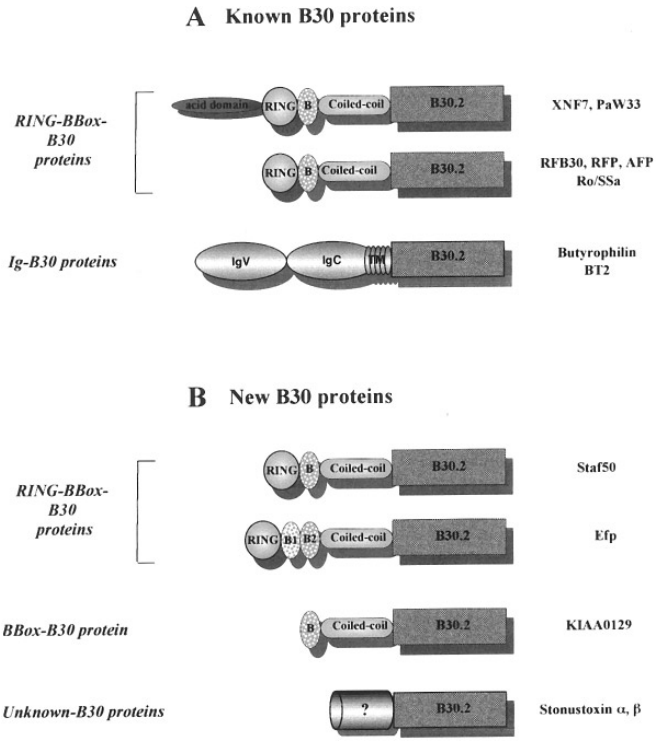

FIG. 1. Schematic representation of proteins which possess B30.2 like domain. The RING finger domain is shown in red, the BBox zinc fingers in red with white points, the coiled coil domain in yellow, the B30.2 domain in dark blue, the IgV domain in blue, the IgC domain in purple, and the unknown module is a grey cylinder.

protein is composed of a BBox associated with a B30.2 domain by a coiled coil region.

Moreover, several ESTs (Express Sequence Tags) were found (table 2). As ESTs correspond to partial cDNA sequences, there is no information available so far for the domain associated with the B30.2 like domain. Therefore the B30.2 like domains are found in at least twenty different conceptual human proteins.

There are convincing evidences that nature has assembled many large genes from sequence modules, defined as structural and/or functional units used repeatedly in different contexts (Dorit and Gilbert 1991). The B30.2 domain is an example of such a module. To the best of our knowledge the B30.2 domain is the only known domain found in transmembrane proteins (butyrophilin, BT2 protein), intracellular proteins (RFP, Ro/SSa, etc . . .) and secreted proteins (Stonustoxin a and b).

It would be therefore interesting to look for B30.2 domain ligands. These potential ligand(s) could belong to a same multigenic family, as described for other couples of ligand/receptor (Fryxell, 1996). Recently Ishii et al (Ishii et al 1995) showed that xanthine oxidase interacts with the B30.2 domain of the butyrophilin. It is mandatory to look if xanthine oxidase (or members of xanthine oxidase family) binds to other B30.2 like domain. In this context it is interesting to note that Stonustoxin inoculated to anaesthetized rats leads to a a release of nitric oxide or nitric oxyde yielding substances (Low et al, 1993). As xanthine oxidase is known

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afp          VSVTLDPQ-SASGYLQLSE-DWKCVTYTSLYKSAYLHPQQFWCEPGVLGSKGFTWGKVYWEVERE*GVLESCMVGVA---RDSVKRKGD--SLLRPE
hbt          VDVTLDPDT-AHPHLFLYE-DSKSVRLEDSRQKLPEKTERFDSWPCVLGRETFTSGWHYWEVEV--GDRTDWAIGVC---RENVMKKGFD--PMTPE
rfp          VDVTLDPDY-AYPSLILSD-NLRQVRYSYLQQDLPDNPERFNLFPCVLGSPCFIAGRHYWEVEV--GDKAKWTIGVC---EDSVCRKGGT--VSAPQ
ros          VHITLDPDT-ANPWLILSE-DRRQVRLGDTQQSIPGNEERFDSYPMVLGAQHFHSGKHYWEVDV--TGKEAWDLGVC---RDSVRRKGHL--FLSSK
xnf          TPMLLDPTS-AHPNLHLSD-GLTSVRYGENKLSLPDNPKRFSQCILVLGSQGFDSGRHYWEVEV--GDKTAWDVGMA---SESSNRKGKK--ILNPK
pAw33        APLTLDPNT-AHPNLVLSE-GLTSVKYTDTKQQLPDNPKRFSQCILVLGSQGFDSGRHYWEVEV--GNKTAWDVGMA---SESSNRKGKK--ILNPK
rfb30        AHISLDPQTSAHPKLLLSE-DHQRAQFSSKWQNSPDNPQRFDRATCVLAHTGITGGRHTWVVSIDLAHGASCTWGVV---SEDVQRKGE--RLLRPE
bt2          ADVTLDPDT-AHPELFQTE-DRRSVRAGPYRQRVPDNPERFDSQPCVLGRESFASGKHYWEVEV--ENVIEWTVGVC---RDSVERKGEL--VLIPQ

efp          IKVILDYNT-AHNKVALSECYTVA-SVAEMPQNYRPHPQRFTYCSQVLGLHCYKKGIHYWEVELQKNNFCG--VGIC---YGSMNRQGP--SERLGR
Staf50       VDVMLNPGS-ATSNVAISVDQRQVKTVRTCTFKNSNPCDFSAFG--VFGCQYFSSGKYYWEVDV--SGKIAWILGVHSKISSLNKRKSSGFAFDPS

αstonus      CDLTFDRNT-INNWISLSDNDTFAASEHGKRQNYPKHPERFVSFNQVLCNEGL-MGKHYWEVEW--NGYID--VGIA---YISIPRKEIDFASAFGY
βstonus      CELTLDPET-AHQVLTLSEGNKKAVSGNTKSP--TDHLEKFSHFQQVMCTKGL-SGRHYWELEW--SGYVG--AGVT---YKGIGRKTSTSDSSLGK
KIAA0129     RTPTLDPDT-MHARLRLSA-DRLTVRCGLLGSLGPVPVLRFDALWQVLARDCFATGRHYWEVDVQEAGAGWWVGAAYASLRRRGASAAARLGCNRQ-

Consensus    ..h.LDP.T-AHP.L.Las-...V.......Q..PD.PpRF.....VLG...F.SGRHYWEVEV.......W..Gh.....a...RKG.........
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afp          -DGVWALR-LSSS-GIWANTSP-EAELFPALRP-RRVGIALDYEGGTVTFTNA--ESQELIYTFT-ATFTRRLVPFLWLKWPGTR-
hbt          -NGFWAVE-L-YGNGYWALTPL-RTPLPLAGPP-RRVGIFLDYESGDISFYNMN-D-GSDIYTFSNVTFSGPLRPFFCLWSSGKKPL
rfp          NNGFWAVS-LWYGKEYWALTSP-MTALPLRTPL-QRVGIFLDYDAGEVSFYNVT-E-RCHTFTFSHATFCGPVRPYFSLSYSGGK--
ros          -SGFWTIW-LWNKQKYEAGTYP-QTPLHLQVPP-CQVGIFLDYEAGMVSFYNIT-DHGSLIYSFSECAFTGPLRPFF-SPGFNDGG-
xnf          -NGYWAIW-LRNGNAYKALESP-SKSLSLSSHP-RKIGVYVDYEGGQISFYNAD-D-MTIIYTF-NATFTEKLYPYLSPFLHDSG--
PAW33        -NGYWAIWLRNGN-AFKALESP-SKTLNLTSKP-SKIGVYLDYEGGQVSFYNAD-D-MSPIYTF-NGSFTEKLYPYLSPFLQDSG--
rfb30        -EGVWAVR-LAWG-FVSALGSF-PTRLTLKEQP-RQVRVSLDYEVGWVTFTNA--VTREPIYTFT-ASFTRKVIPFFGLWGRGSS--
bt2          -NGFWTLE-M-HKGQYRAVSSP-DRILPLKESL-WRVGVFLDYEAGDVSFYNMR-D-RSHITYCPRSAFSVPVRPFF-FRGCEDXPI

efp          NSASWCVEWFNTKISAWHNNVEK----TLPSTKATRVGVLLNCDHGFVIFFAVA-DKVHLMYKFR-VDFTEALYPAFWVFSAGAT--
Staf50       +NGYWVIG-LQNTCEYNAFEDSSS-SDPKVLTLFMAVLPVVLGFS#

αstonus      NTYSWVLS-YNPKIGYIERHKKREYNVRAPNPGFKRLGLFLDWRYGSISFYAVSSDEVHHLHTFK-TKFTEPVYPAFSIGPAGN--H
βstonus      NEKSWLFE-YSTKSGYQQIHNSKKTRVTVSSTGFKLLGVYLDWPAGTLSFYMVNKAWVTHLHTFH-TKFNEAVYPAFLIGDAQQKVN
KIAA0129     ---SWCLKRYD--LEYWAFHDGQR-SACGPATTSTGSASSWTTRPASSPSTT#

Consensus    -.GhWsf.-L.....o.A...P-...L.L.......hGahLDYa.G.f.FYN...a....ho.F....F...f.Poh.........
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FIG. 2. Sequence alignments and comparison of the conserved regions between the members of the B30 family. Over a span of 195 amino acids, each of the B30.2 like domains shows ± 40 % identity with one another. Lowercase letters identify the position of amino acids with functional or physical properties as follows: a, acidic (D, E); f, aliphatic (L, I, V); h, hydrophobic (L, I, V, M, Y); o, aromatic, (Y, F, W); p, polar (K, R, H, D, E, Q, N, T, S); s, small (A, G, S, T, V, N, D).(+) Indicates an insertion of 12 amino acids in the sequence of Staf50. (*) Indicates the position of the insertion of the glutamic acid-rich polypeptide of AFP (64 amino-acids).

TABLE 1

11Sequence Signatures" of the B30.2 Domains

Proteins	Database id	LDP motif	WEVE motif	LDYE motif
A				
(hum) RFP	gb: J03407	VDVTLDPDTAYPSLILSDN	FIAGRHYWEVEV	PLQRVGIFLDYDAGEVSFYN
(mice) RFP	pir: S37583	VDVTLDPDTAYPSLILSDN	FMAGRHYWEVEV	PLQRVGIFLDYDAGEVSFYN
(hum) Ro/SSa 52	Pir: A37240	VHITLDPDTANPWLILSED	FHSGKHYWEVDV	PPCQVGIFLDYEAGMVSFYN
(mice) Ro/SSa 52	gb: L27990	VDVTLDPDTAHPHLFLYED	FSSGKMYWEVDV	PPCQVGIFLDYEAGMVSFYN
(hum) AFP	gb: U09825	VSVTLDPQSASGYLQLSED	FTWGKVYWEVEV	RPRRVGIALDYEGGTVTFTN
(xenopus) XNF7	pir: A43906	TMPLLDPTSAHPNLHLSDG	FDSGRHYWEVEV	HPRKIGVYVDYEGGQISFYN
(pleurodele) pAw33	sp: Q02084	LDVTLDPNTAHPNLVLSEG	FDSGKHYWEVEV	PLAEYGVFLDYDAGDISFYN
(bov) butyrophilin	sp: P18892	VDVTLDPDTAHPHLFLYED	FTSGRHYWEVEV	PPRRVGVFLDYESGDIFFYN
(mice) butyrophilin	gb: S80642	VDVTLDPDTAHPHLFLYED	FTSGRHYWEVEV	PPRRVGVFLDYDAGDISFYN
(hum) butyrophilin	gi: U39576	VDVTLDPDTAHPHLFLYED	FTSGRHYWEVEV	PPRRVGIFLDYESGDISFYN
(hum) RFB30	Em: Y07829	AHISLDPQTSHPKLLLSED	ITGGRHTWVVSI	QPRQVRVSLDYEVGWVTFTN
(hum) BT2	in press	VDVVLDPDTAHPELFLTED	FASGKHYWEVEV	SLWRVGVFLDYEAGDVSFYN
B				
(hum) efp	pir: A49656	IKVILDYNTAHNKVALSEC	YKKGIHYWEVEL	KATRVGVLLNCDHGFVIFFA
(mice) efp	gb: D63902	FKVIFDYNTAHNKVSLSNK	YKNGIHYWEVEL	KATRVGVLLNCDHGFVIFFA
(hum) Staf 50	gb: X82200	VDVMLPNGSATSNVAISVD	FSSGKYYWEVDV	APCRIGVFLDYEAGIVSFFN*
C				
(hum) KIAA0129	gb: D50919	RTPTLDPDTMHARLRLSAD	FATGRHYWEVDV	PPRRLGVFLDYEAGVLAFYD*
(Synan. H.) stonustoxin α	gb: U36237	CDLTFDRNTINNWISLSDN	GLMGKHYWEVEW	GFKRLGLFLDWRYGSISFYA
(Synan. H.) stonustoxin β	gb: U32516	CELTLDPETAHQVLTLSEG	GLSGRHYWELEW	GFKLLGVYLDWPAGTLSFYM
Consensus		VDVT**LDP**D**TAHP**H**L**F**LSE**D	**F**TS**GRHYWEVEV**	PPRR**VG**VF**LDYE**S**G**DIF**FYN**

Note. This table was obtained by searching databases using the LDP, WEVE, and LDYE motifs. These proteins correspond to established members of the B30 family (for reference see the text). When these motifs were used in dbEST they matched at least 20 Sets different from the known members). For Staf50 and KIAA0129 product (*) the last motif LDYE can only be obtained by switching the open reading frame. This could be the result of overprinting events or a misreading in the published sequences.

to inhibit the nitric oxide synthase (Rengasamy and Johns, 1993), it is therefore possible that the binding of the B30.2 like domain of Stonustoxin with xanthine oxidase could prevent such an inhibition resulting in an increase of nitric oxide.

The three highly conserved motifs on the B30.2 like domains, described here, could be important for the binding to ligand(s) and/or for the domain folding.

TABLE 2

Other cDNA EST Clones Showing Similarities with the B30.2-like Domain; the B30.2-like Domain of the Butyrophilin Was Used as Template for These Comparisons

GenBank ID	Clones id	Identities with B30.2-like domain
F12347	c-38h03	55%
Z44417	c1zh10	42%
R31364	135543	38%
R61476	37677	46%
D79183	GEN 533E08	53%
H84023	249808	59%
H77331	233538	42%
R50145	153216	53%
R66237	141127	46%
H51728	194245	62%
R34643	37115	55%

ACKNOWLEDGMENTS

We thank H. Chap for helpful discussion and critical evaluation of the manuscript, and M. Record and R. Roubin for help in writing this paper. This work was supported by the French Ministère de la Recherche et de l'espace, the Centre National de la Recherche Scientifique, the European community, and the region Midi-Pyrénées.

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