Integrin triplets of marine sponges in the murine and human MHCI-CD8 interface and in the interface of human neural receptor heteromers and subunits

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Abstract
Based on our theory, main triplets of amino acid residues have been discovered in cell-adhesion receptors (integrins) of marine sponges, which participate as homologies in the interface between two major immune molecules, MHC class I (MHCI) and CD8αβ. They appear as homologies also in several human neural receptor heteromers and subunits. The obtained results probably mean that neural and immune receptors also utilize these structural integrin triplets to form heteromers and ion channels, which are required for a tuned and integrated intracellular and intercellular communication and a communication between cells and the extracellular matrix with an origin in sponges, the oldest multicellular animals.

Keywords: Neural receptor-receptor interactions, Receptor interface, Marine sponges, Triplet homologies

Introduction
Based on a mathematical approach, Tarakanov and Fuxe (2010, 2011) have deduced a set of triplet homologies (so called ‘triplet puzzle’) that may be responsible for protein-protein interactions, including receptor heteromers and human immunodeficiency virus (HIV) entry. For example, the triplet of amino acid residues ITL (Ile-Thr-Leu) appears in both receptors of any of six receptor heteromers: GABAB1-GABAB2 (GABAB receptor), GABAB1-mGluR1, GABAB1-CXCR4, CXCR4-CCR2, 5HT1B-5HT1D, and MHC class I MHCI-CD8. At the same time, this triplet ITL does not appear in both receptors of any of known non-heteromers (GABAB2-A2A, A2A-D1, A1-D2, NTSR1-D1, TSHR-D2, and CD4-D2; see Tarakanov and Fuxe 2010). According to recent biochemical studies (Borroto-Escuela et al. 2010, 2011, 2012a,b; Romero-Fernandez et al. 2011), such triplets exist in the interacting domains forming the receptor interface. Furthermore, a ‘guide-and-clasp’ manner of receptor-receptor interactions has been proposed where the ‘adhesive guides’ may be the triplet homologies (Tarakanov and Fuxe, 2010). According to recent bioinformatic studies (Tarakanov et al. 2012 a,b,c,d), several triplet homologies of such receptor heteromers in human brain may be the same as in cell-adhesion receptors of marine sponges, known to be highly conserved from the lowest metazoa to vertebrates (Gamulin et al. 1994; Muller 1997; Pancer et al. 1997; Buljan and Bateman 2009). Interactions between such triplets probably represent a general molecular mechanism for receptor-receptor interactions (Fuxe et al 2012) and may play an important role in human learning (Agnati et al. 2003) and some diseases (Tarakanov et al. 2009).

In the current paper, many of such triplets have been found in integrins of marine sponges together with human alpha and beta integrins. This means that such triplet homologies may play a role in alpha-beta heterodimeric complexes forming integrin receptors and interact with extracellular matrix proteins (Barcyk et al. 2010). Of especial interest is that the same integrin triplets exist also in the murine and human MHCI interface with CD8, in human neural receptors and in the interface of both protomers of several receptor heteromers. The presence of such triplet homologies in several receptor subunits building up the neuromuscular nicotinic cholinergic...
| Protein | Species | Type | Accession code |
|---------|---------|------|----------------|
| ITGA    | Sponge (Geodia cydonium) | Metazoan adhesion receptor subunit Integrin-α | CAA65943 |
| ITGB    | Sponge (Geodia cydonium) | Metazoan adhesion receptor subunit Integrin-β | CAA77071 |
| ITGB4   | Sponge (Marichromatium purpuratum) | Metazoan adhesion receptor subunit Integrin-β4 | ZP_08774040 |
| MHC1    | Mouse (Mus musculus) | H-2 class I histocompatibility antigen | NP_0010001892 |
| CD8a    | Mouse | T-cell surface glycoprotein chain CD8α | NP_001074579 |
| CD8b    | Mouse | T-cell surface glycoprotein chain CD8β | NP_00339886 |
| MHC1    | Human (Homo sapiens) | H-2 class I histocompatibility antigen | AAA595999 |
| CD8a    | Human | T-cell surface glycoprotein chain CD8α | NP_001139345 |
| CD8b    | Human | T-cell surface glycoprotein chain CD8β | NP_7573623 |
| CXCR4   | Human | Chemokine receptor | P61073 |
| TSHR    | Human | Thyroid stimulating hormone receptor | NP_000360 |
| FGFR1   | Human | Fibroblast growth factor receptor | NP_075598 |
| SHT1A   | Human | Serotonin receptor | AAHE69159 |
| Collagen | Human | Matrix protein | P02452 |
| ITGAIIB | Human | Integrin receptor subunit-αIib | P08514 |
| ITGAL   | Human | Integrin receptor subunit-αL | P20701 |
| ITGAM   | Human | Integrin receptor subunit-αM | NP_001139280 |
| ITGAV   | Human | Integrin receptor subunit-αV | EAX10934 |
| ITGAX   | Human | Integrin receptor subunit-αX | NP_000878 |
| ITGB2   | Human | Integrin receptor subunit-β2 | NP_000202 |
| ITGB3   | Human | Integrin receptor subunit-β3 | NP_000203 |
| ITGB4   | Human | Integrin receptor subunit-β4 | NP_000204 |
| ITGB5   | Human | Integrin receptor subunit-β5 | NP_000205 |
| ITGB6   | Human | Integrin receptor subunit-β6 | P18564 |
| ITGB8   | Human | Integrin receptor subunit-β8 | P26012 |
| ACHA    | Human | Acetylcholine receptor subunit-α | P02708 |
| ACHB    | Human | Acetylcholine receptor subunit-β | P11230 |
| ACHD    | Human | Acetylcholine receptor subunit-δ | Q07001 |
| ACHF    | Human | Acetylcholine receptor subunit-ε | Q04844 |
| mGluR1  | Human | Metabotropic glutamate receptor | NP_000829 |
| GABAB2  | Human | γ-aminobutyric acid receptor subunit-2 | O75899 |
| GABAB1  | Human (Homo sapiens) | γ-aminobutyric acid receptor subunit-1 | NP_001461 |
| GABAB1  | Mouse (Mus musculus) | * | NP_062312 |
| GABAB1  | Norway rat (Rattus norvegicus) | * | NP_112290 |
| GABAB1  | Western clawed frog (Xenopus (Silurana) tropicalis) | * | NP_001107291 |
| GABAB1  | Green puffer (Tetraodon nigroviridis) | * | uniprot/Q459D9 |
| GABAB1  | Zebrfish (Danio rerio) | * | NP_001070794 |
| GABAB1  | African malaria mosquito (Anopheles gambiae) | * | uniprot/Q7PM55 |
| GABAB1  | Drosophila pseudoobscura | * | XP_01357356 |
| GABAB1  | Human body louse (Pediculus humanus corporis) | * | XP_002430445 |
| GABAB1  | Caenorhabditis elegans | * | ACE63490 |
| Protein | Species | Type       | LLG | GLL | ITL | RPA | GDR | RDG | DGR |
|---------|---------|------------|-----|-----|-----|-----|-----|-----|-----|
| ITGA    | Sponge  | Integrin-α | -   | -   | +   | +   | +   | -   | -   |
| ITGB    | Sponge  | Integrin-β | +   | +   | -   | -   | -   | -   | -   |
| ITGB4   | Sponge  | Integrin-β | -   | -   | -   | -   | -   | +   | +   |
| MHC Class I | Mouse    | Immune receptor | + | - | + | + | - | - | + |
| CD8a    | Mouse   | Immune receptor | + | - | + | - | - | - | - |
| CD8b    | Mouse   | Immune receptor | - | - | - | - | - | - | - |
| MHC Class I | Human    | Immune receptor | + | - | + | + | - | + | + |
| CD8a    | Human   | Immune receptor | - | - | + | + | - | - | - |
| CD8b    | Human   | Immune receptor | - | - | + | - | - | - | - |
| CXCR4   | Human   | Endocrine receptor | - | - | - | - | + | - | - |
| TSHR    | Human   | Endocrine receptor | - | - | - | - | - | - | - |
| FGFR1   | Human   | Receptor tyrosine kinase | - | - | - | - | + | - | - |
| SHT1A   | Human   | Neural receptor | + | - | - | - | - | - | - |
| Collagen| Human   | Matrix protein | - | - | - | - | + | + | + |
| ITGALB  | Human   | Integrin-α | + | - | - | - | - | + | + |
| ITGAL   | Human   | Integrin-α | - | + | - | - | - | - | - |
| ITGAM   | Human   | Integrin-α | + | + | - | - | - | - | - |
| ITGAV   | Human   | Integrin-α | + | - | - | - | - | - | - |
| ITGAX   | Human   | Integrin-α | + | + | - | - | - | + | + |
| ITGB2   | Human   | Integrin-β | - | + | - | - | - | - | - |
| ITGB3   | Human   | Integrin-β | - | + | - | - | - | - | - |
| ITGB4   | Human   | Integrin-β | + | - | - | - | - | - | - |
| ITGB5   | Human   | Integrin-β | + | - | - | - | - | + | + |
| ITGB6   | Human   | Integrin-β | - | + | - | - | - | - | - |
| ITGB8   | Human   | Integrin-β | - | + | - | - | + | - | - |
| ACHA    | Human   | Neural receptor subunit | + | - | - | - | - | - | - |
| ACHB    | Human   | Neural receptor subunit | + | - | + | + | + | - | - |
| ACHD    | Human   | Neural receptor subunit | - | + | + | - | - | - | - |
| ACHE    | Human   | Neural receptor subunit | + | + | - | - | - | - | - |
| GABA    | Human   | Neural receptor | + | - | - | - | - | - | - |
| GABAB2  | Human   | Neural receptor | - | + | + | - | - | - | - |
| mGluR1  | Human   | Neural receptor | - | + | + | - | - | - | - |

(+ yes, - no).
| Receptor heteromer | Reference               | Function                           | LLG | GLL | ITL | RPA | DGR |
|-------------------|-------------------------|------------------------------------|-----|-----|-----|-----|-----|
| MHCI-CD8a         | Gao et al. (1997)       | Adaptive immune response           | -   | -   | #   | +   | -   |
| MHC1-CD8b         | Wang et al. (2009)      | Adaptive immune response           | -   | -   | #   |     |     |
| CD8a-CD8b         | Wang et al. (2009)      | Coreceptor of T cells              | -   | -   | +   | -   |     |
| ITGAVB-ITGB3      | Barczyk et al. (2010)   | RGD (Arg-Gly-Asp) receptor         |     |     |     |     | #   |
| ITGAVB-ITGB5      | Barczyk et al. (2010)   | RGD receptor                       | #   | -   |     | -   | -   |
| ITGAVB-ITGB6      | Barczyk et al. (2010)   | RGD receptor                       |     |     |   # |     | -   |
| ITGAVB-ITGB8      | Barczyk et al. (2010)   | RGD receptor                       |     |     |   # |     | -   |
| ITGAB-ITGB2       | Barczyk et al. (2010)   | Leukocyte receptor                 | -   | +   |     |     |     |
| ITGAB-ITGB2       | Barczyk et al. (2010)   | Leukocyte receptor                 | -   | +   |     |     |     |
| GABAB1-GABAB2     | Marshall et al. (2001)  | Activation of the potassium channels and regulation of receptor trafficking | -   | #   |     | -   | -   |
| GABAB1-mGluR1     | Hirono et al. (2001)    | Modulation of excitatory transmission | -   | #   |     | +   | -   |
| GABAB1-CXCR4      | Guyon and Nahon (2007)  | Modulation of neuroendocrine systems | -   | -   |   # |     | -   |
| ACHA-ACHB         | Changeux et al. 1984    | Part of the neuromuscular nicotinic receptor | +   | -   |     |     | -   |
| ACHA-ACHE         | Changeux et al. 1984    | Part of the neuromuscular nicotinic receptor | +   | -   |     |     | -   |
| ACHB-ACHD         | Changeux et al. 1984    | Part of the neuromuscular nicotinic receptor | -   | -   |     |     | #   |

(+ yes in both receptors, # may mediate their interaction, - no in any receptor).
receptors has also been demonstrated. At least one of the homologies may have a role in the intermolecular subunit interactions of this ion channel receptor.

**Methods**

Amino acid codes of receptors and other proteins have been obtained from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) and the Universal Protein Resource (http://www.uniprot.org). Table 1 summarizes data on proteins used. In abstract mathematical terms, any protein is just a word coded by a 20-letter alphabet where triplet is any 3-letter subword. Thus, triplet homology is any triplet which exists in both given words. Our theory of triplet puzzle supposes some basic set of triplets as a code that determines whether two receptors bind or not (Tarakanov and Prokaev 2007; http://youtu.be/1DevThU5fyM).

No experimental research has been performed on humans and/or animals.

**Results**

The triplets ITL (Ile-Thr-Leu), RPA (Arg-Pro-Ala), DGR (Asp-Gly-Arg), LLG (Leu-Leu-Gly), and GLL (Gly-Leu-Leu) of the integrin receptors of marine sponges appear as homologies in murine (underlined) and human MHCI-CD8 complex, human collagen (DGR triplet), and human receptor heteromers: TM1, TM2 and TM7 are the first, the second and the seventh transmembrane α-helices of ACHB, CXCR4, and GABAB (GABAB1-GABAB2 heteromer) receptors, respectively, and contain the ITL triplet. The RPA triplet is also found in the TSHR and FGFR1; the RPA but not the ITL triplet homologies are in a position to contribute to the physical interaction between the beta and delta subunits of the neuromuscular nicotinic receptor (ACHB-ACHD); light-shaded letters are positively charged amino acids (R, K, and H), whereas dark-shaded white letters are negatively charged amino acids (D and E); bold letters are main players of leucine-rich motifs (L, S, and C).
This triplet homology exists also in three GABAB1 receptor heteromers of human brain: GABAB1-GABAB2 forming the GABAB receptor (Marshall et al. 2001), GABAB1-mGluR1, and GABAB1-CXCR4 and may mediate the interaction in two of them (see Table 3 and Figure 1). In the first two heteromers also triplet GLL (Gly-Leu-Leu) may participate in the interaction (see Table 3 and Figure 2).

The triplet DGR (Asp-Gly-Arg) is in fact the inverse triplet of RGD (Arg-Gly-Asp) that provides the binding site for integrin RGD-binding receptors (see Table 3). Moreover, a small peptide ligand RGD (Arg-Gly-Asp) that mimics extracellular matrix protein binding to integrins also causes impairments in plasticity at glutamatergic synapses (Wiggins et al. 2011).

The evolution of the ITL triplet in the GABAB1 receptor subunit is displayed in Figure 3. In phylogeny, it appears to begin in fish (Tetraodon) and then continues to man, while it is missing in zebrafish (Danio rerio). Thus, the usefulness of the ITL triplet in recognition is rediscovered in the fish GABAB1 receptor.

Furthermore, the RPA triplet homology in the beta and delta interacting nicotinic subunits of the neuromuscular nicotinic receptor (see Changeux et al. 1984) is in a location...
(N-terminal parts of ACHB and ACHD) where it may participate in forming part of their interface (see Figure 1 and Table 3).

Discussion
The triplet ITL (Ile-Thr-Leu) found in integrins of marine sponges is presented as a homology in the interface between MHC Class I and CD8αβ heterodimer (coreceptor in T cells). It is postulated that this triplet homology can contribute to the formation of the MHCI-CD8 heteromeric complex which leads to a strong activation of the T cell by guiding the T-cell receptor into relevant self-MHC recognition (see Wang et al. 2009). Thus, it seems possible that the ITL triplet may have a critical role in the interaction between these two immune receptors which is necessary for appropriate T cell function. A mutation of the ITL triplet in these immune receptors will be of value to test this hypothesis. The indications have also been obtained that triplet homology ITL in the N-terminal of beta and delta nicotinic receptor subunits of the neuromuscular nicotinic receptor may help mediate their interaction in the subunit interface.

Conclusion
Integrin triplets of marine sponges found in the interface of human receptor heteromers and even in the interface between two major immune molecules MHCI-CD8 seem to confirm once more our theory. This triplet puzzle arose as a surprising merger of pure mathematics and most recent biochemical studies of receptor-receptor interactions. As a result, it appears that neural and immune receptor heteromers in humans may also utilize these structural elements originating in sponges, the oldest multicellular animals. Thus, the triplet puzzle may be an ancient and general mechanism for protein-protein recognition.

Competing interests
Both authors declare that they have no competing interests.

Authors’ contributions
AT carried out the mathematical studies and computations. KF carried out the biomedical interpretation of the results. All authors read and approved the final manuscript.

Acknowledgement
The authors have not received any support for this work.

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Received: 23 October 2012 Accepted: 11 March 2013 Published: 22 March 2013

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doi:10.1186/2193-1801-2-128

Cite this article as: Tarakanov and Fuxe: Integrin triplets of marine sponges in the murine and human MHCI-CD8 interface and in the interface of human neural receptor heteromers and subunits. SpringerPlus 2013 2:128.

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