Minireview

STAM/EAST/Hbp adapter proteins – integrators of signalling pathways

Olli Lohi[a], Veli-Pekka Lehto[b,*]

[a] *Department of Pediatrics, University of Tampere, PL 2000, Tampere FIN-33521, Finland*
[b] *Department of Pathology, University of Oulu, PL 5000, Oulu FIN-90014, Finland*

Received 20 September 2001; revised 22 October 2001; accepted 23 October 2001

First published online 2 November 2001

Edited by Gunner van Heijne

Abstract STAM/EAST/Hbp family of proteins consists of eight members well conserved from yeast to mammals. The basic domain architecture is comprised of an N-terminal Vps27, Hrs and STAM homology domain, a ubiquitin-interacting motif and a central Src homology-3 domain. Vertebrate members also carry an immunoreceptor tyrosine-based activation motif. STAM/EAST/Hbp proteins become tyrosine-phosphorylated by a variety of cytokines and growth factors. STAM 1 and STAM 2A are involved in cytokine-mediated signalling for DNA synthesis and *c*-myc induction. EAST and STAM 2A/Hbp play a role in receptor-mediated endo- and exocytosis and probably also in the regulation of actin cytoskeleton. Knockout experiments implicate a role for STAM 1 in neural cell survival. A picture is emerging of STAM/EAST/Hbp proteins acting as integrators of thus far mechanistically disparate cellular signalling events.

Key words: Adapter; EAST; Endocytosis; Hbp; Receptor; STAM

1. Introduction

STAM (*s*ignal-*t*ransducing *a*dapter *m*olecule) and EAST (*E*GFR-*a*ssociated protein with *S*H3 and *T*AM domains) were originally discovered as tyrosine-phosphorylatable proteins involved in interleukin-2 (IL-2) and epidermal growth factor (EGF) receptor signalling, respectively [1,2]. Hbp (*H*rs-*b*inding *p*rotein) was identified based on its binding to Hrs, a hepatocyte growth factor-regulated tyrosine kinase substrate [3]. Thereafter, several homologs of the STAM/EAST/Hbp (referred henceforth as STAM) family have been discovered, and they now comprise a distinct and evolutionarily conserved protein family with members from yeast to mammals. Along with the growth of the family, the initial ideas of the functional roles of its members have diversified and become more accurate.

2. Domain organization

The schematic representation of the domain organization of STAMs is shown in Fig. 1. On the basis of the primary structure homology, the proteins can be grouped into three subgroups. Human STAM 1 is clearly distinct from the second subgroup with 53% identity with human STAM 2A (same as Hbp), 64% identity with human STAM 2B, and 60% identity with chicken EAST. Chicken EAST and mouse Hbp bear 78% and 86% identity, respectively, with human STAM 2A, thus putatively representing its homologs. STAM 2B is identical to STAM 2A, except for the lack of 183 amino acids in its C-terminus. The third subgroup consists of invertebrate orthologs of the family, bearing an identity of 20–40% to both vertebrate protein subgroups.

All STAMs have, in their N-terminus, a VHS (*V*ps27, *H*rs and *S*TAM homology) domain, an evolutionarily conserved domain of ~140 amino acids. It is also found in other proteins which are unrelated to STAMs and are mostly involved in vesicular trafficking [4]. Interestingly, in all proteins of its residence, the VHS domain occupies the N-terminus, suggesting the importance of this topology to its function [4]. The recently staged crystal structures of the VHS domains of Hrs and Tom1 proteins revealed an eight-α-helix superhelix. Based on the structural data, the VHS domain is predicted to be engaged in both inter- and intradomain interactions, and to function as a low-efficiency membrane-binding domain [5,6].

Recently, a novel ubiquitin-interacting motif (UIM) was identified in a wide variety of proteins, most of them involved in ubiquitination and ubiquitin metabolism [7]. Intriguingly, it is also present in STAMs (Fig. 1).

In their central portion, STAMs have an SH3 (*S*rc *h*omology 3) domain, a well-established protein–protein interaction domain [8]. In the C-terminal part of vertebrate STAMs (except for STAM 2B), there is an ITAM motif, an *i*mmunoreceptor *t*yrosine-based *a*ctivation *m*otif which in immunoreceptors serve as a docking site for SH2 domain-containing proteins [9]. ITAM overlaps with a region with a predicted propensity for coiled-coil arrangement [10].

3. Expression and localization

Northern and immunoblotting analyses showed that STAM 1, STAM 2A and EAST are widely expressed in a variety of tissues and cell lines, and that there is no change in STAM 1 expression during brain development in mouse [1,2,11,12]. In chicken embryos, EAST is highly expressed in neuronal cells, in the endothelium and smooth muscle throughout the tissues, and in the epithelial cell layer of the gizzard, intestine, lung and the skin (Lohi and Lehto, submitted). In *Drosophila*, STAM is prominent in both embryos and adults, with lower level of expression in first-, second-, and third-instar larvae

*Corresponding author. Fax: (358)-8-537 5953.
E-mail address: lehto@csc.fi (V.-P. Lehto).

PII: S0014-5793(01)03079-4

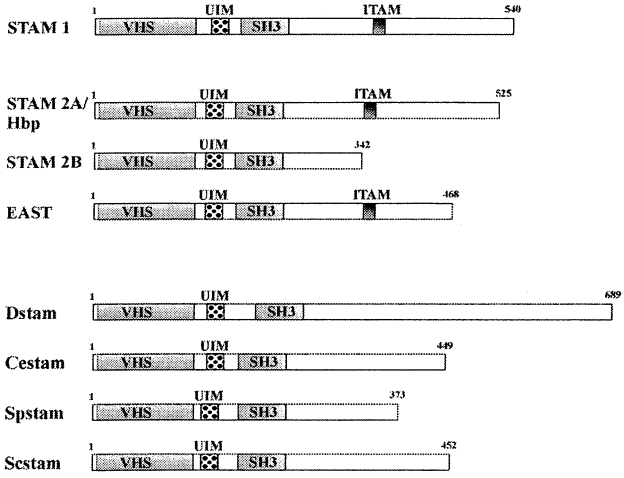

Fig. 1. Schematic representation of the domain structure of STAMs. The orthologs of STAMs are abbreviated as Dstam for the *Drosophila melanogaster*, Cestam for the *C. elegans*, Spstam for the *S. pombe*, and Scstam for the *S. cerevisiae* homologs.

and in pupae [13]. Thus, no distinct cell or tissue-specific expression pattern is to be seen.

Information on the subcellular distribution is available for STAM 1 and EAST. In primary hippocampal neurons of mouse brain, STAM 1 is associated with spot-like structures which are also positive for the synaptic markers GluR1, synapsin-I and SNAP-25 [12]. In subcellular fractionation studies, STAM 1 was enriched in the synaptosomal and especially in the synaptic vesicle fraction, suggesting a role in synaptic machinery [12].

In immunofluorescence microscopy studies, EAST displayed a staining pattern which is, at least partly, dependent on the fixation techniques. It was seen along the cell membranes, associated with the focal adhesion sites, along filamentous actin, and in vesicular structures which costained for clathrin [2,14]. All in all, it seems, based on the distinct localizations of STAM 1 and EAST, that either the members of the family differ also in their basic functional features or that they share functional features independent of their cellular localizations.

4. Role in signal transduction

A pivotal role for STAMs in signal transduction was originally and strongly suggested by the observation that they become readily tyrosine-phosphorylated upon stimulation with a variety of cytokines such that STAM 1 was phosphorylated upon stimulation with IL-2, IL-3, IL-4, IL-7, GM-CSF, EGF and PDGF; STAM 2A/Hbp with IL-2, IL-3, GM-CSF, EGF, PDGF and HGF; and EAST with EGF and PDGF [1–3,15]. Attesting to an association with specific pathways, no phosphorylation of EAST was seen upon treatment with lysophosphatidic acid, phorbol myristic acid or bradykinin [2]. The cytokine signalling pathways were further scrutinized and STAM 1 and STAM 2A were found to be associated with Jak2 and Jak3, downstream effectors of IL-2 signalling [11,16]. This binding is through the ITAM region of STAM 1/2A and is not dependent on ligand stimulation. Similarly, association between EAST and EGF-receptor was found and shown to be constitutive and only enhanced by EGF stimulation [2].

The molecular details of the tyrosine-phosphorylation of STAMs remain to be sorted out. There are, however, interesting differences in STAMs associated with distinct signalling pathways as to the site and number of the phosphorylatable tyrosine residues and to their regulation. For instance, phosphorylation by Jak1/2/3 of STAM 1/2A is dependent on the ITAM motif while phosphorylation of EAST/STAM 2A by EGF is not [2,15,16]. Our own studies also demonstrated that EAST is a direct substrate of both EGFR and Src kinases [2,17]. It still remains unresolved what the significance of the tyrosine-phosphorylation of STAMs is for downstream signalling and whether kinases other than Jaks, EGFR and Src are involved.

The original discovery of STAM 1 was based on an assumption that there is a STAT5-independent pathway from IL-2-receptor via Jak2 and Jak3 to *c*-myc induction and DNA synthesis, respectively [1,16]. A specific role of STAM 1 and STAM 2A in *c*-myc induction was later demonstrated in transfection studies which showed it to be dependent on the presence of the SH3 or ITAM domain [11,15,16]. SH3 deletion mutants of STAM 1 and STAM 2A acted in a dominant negative manner by suppressing cell growth signalling mediated by IL-2 and GM-CSF. Moreover, their coexpression induced an additive suppressive effect on DNA synthesis [11]. Since both STAM 1 and STAM 2A bind Jak2 and Jak3, this suggests that they contribute to the cytokine-mediated signal by comparable and compensatory mechanisms downstream of Jak2 and Jak3. Similar to SH3 domains, ITAM domains are known to serve mostly as docking sites of components of multimolecular assemblies. For instance, various SH2 domain-containing proteins, either adapter proteins or kinases, bind to ITAM(s) of immunoreceptors to further effect phosphorylation of the receptor itself or its downstream effectors [9]. Although not scrutinized in detail, ITAMs in STAM may serve a similar purpose in anchoring Jak kinases to the vicinity of their appropriate targets.

Based on the role of STAMs in cytokine signalling, it was to be expected that they are central players in, for example, lymphocyte development. Therefore, it came as a surprise that knockout of STAM 1 had little effect on the development in mouse of hematopoietic cells, including T-, B-, myeloid and erythroid cells, and on the proliferative responses of bone marrow cells and splenocytes in response to IL-2 and GM-CSF [12]. Obviously, this can be due to redundancy such that STAM 2A compensates for STAM 1. A curious observation was that the knockout animals showed an apoptotic loss of CA3 pyramidal neurons. This suggests that derangement of STAM 1-mediated signalling, while not compromising neural cell development, leads to a shortened cell survival and apoptosis [12]. Maybe, in these particular cells, in contrast to lymphocytes for example, STAM 1 is in a signalling pathway critical to survival and not balanced by compensatory pathways.

An interesting link between STAMs and signal transduction is provided by a recently discovered AMSH (*a*ssociated *m*olecule with the *SH3* domain of STAM) protein that was found via a far-western screen. It interacts with the SH3 domain of STAM 1 and STAM 2A [11,18]. AMSH lies in the signalling pathway from IL-2 and GM-CSF to *c*-myc induction and DNA synthesis, possibly as a component of a multimolecular complex comprised of cytokine receptors, Jaks, STAM and AMSH [18]. Quite recently, AMSH was found to be involved

in the bone morphogenetic protein (BMP) signalling [19]. By binding to and sequestering Smad6, an 'inhibitory Smad' and an antagonist of the BMP–Smad pathway, AMSH seems to be able to positively regulate BMP-induced responses.

The involvement of AMSH in various, otherwise disparate signalling pathways, suggests that, by way of binding to it, STAMs could be part of a network that sets parallel signalling pathways to balance and orchestrates their potencies and durations. Interestingly, Itoh et al. [19] reported that overexpression of AMSH in HS-72 cells resulted in an increased Fas-induced apoptosis. Could it be that the elevated level of apoptosis in CA3 cells in STAM 1 knockouts could be due to illicit levels of AMSH as a result of missing STAM 1?

Recently, a 72-kDa immunoanalog of STAM 1 was found in a search for tyrosine-phosphorylated proteins after PDGF treatment [20]. It was demonstrated to form a high-affinity complex with Eps15, Crk and Fyn [20], further pointing to a role of STAMs in signal transduction.

5. Role in endocytic trafficking

Several lines of evidence support a role for STAMs in endocytic trafficking. First, EAST is associated with EGF-receptor and Eps15, an EGFR substrate that binds to AP-2 and other endocytosis-associated proteins such as epsin and synaptojanin [2,21]. Second, STAM 1 and STAM 2A/Hbp are associated with Hrs, a VHS and FYVE domain-containing endocytosis-associated hepatocyte growth factor-regulated tyrosine kinase substrate (Fig. 2) [3,10,22]. Third, STAMs have a UIM motif which is found also in some proteins involved in endocytic receptor down-regulation. Fourth, the domain structure of STAMs (especially the presence of the VHS domain), and the subcellular localization studies (see above) are suggestive of STAMs participating in endosomal trafficking.

The role of Eps15 in endocytosis was established by experiments showing that perturbation of Eps15 function inhibits receptor-mediated endocytosis of EGF and transferrin [23]. EAST associates with Eps15 [2]. The significance of this interaction is, however, not known. It is independent of EGFR activation and is not mediated by the EAST's NPF-motif, a site responsible for Eps15 interactions with many other ligands [2,21]. On a more general note, there is an NPF-motif to be found in the N-termini of most STAMs. Thusfar, however, no binding partners have been found and their functional role remains to be studied.

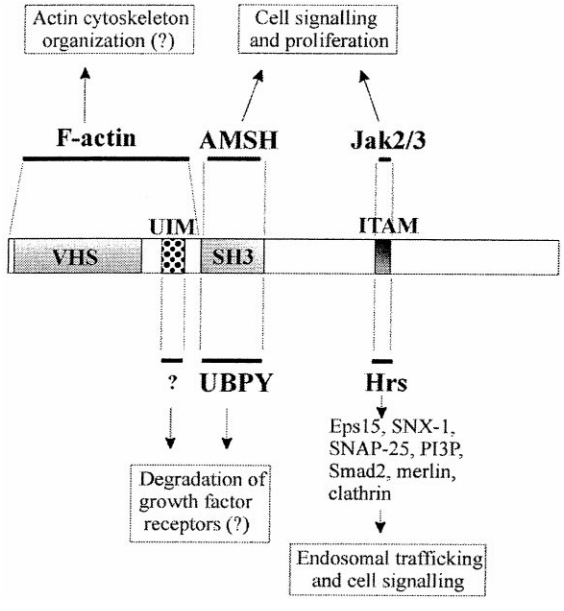

Fig. 2. STAMs and their binding partners.

The role of Hrs in endosomal trafficking is implicated by its presence in early endosomes and multivesicular bodies, and that it recruits clathrin to early endosomes [22,24]. Moreover, Hrs-2 (identical to Hrs) interacts with Eps15, and transiently overexpressed Hrs-2 or its Eps15-binding region inhibits receptor-mediated endocytosis [25]. Recently, Chin et al. [26] demonstrated a direct interaction between Hrs and sorting nexin 1 (SNX1), a protein that participates in lysosomal trafficking of EGFR. Overexpression of Hrs or its SNX1-binding domain, which overlaps with the site mediating binding to STAMs, inhibits ligand-induced degradation of EGFR. This together with the observation that a complex formed by SNX1 and Hrs excludes EGFR, suggest a role for STAMs in the regulation of the receptor degradation: displacement of SNX1 from a complex with Hrs by STAM could make SNX1 available for binding to EGFR and recruit it for degradation. Based on the experimental evidence by Chin et al [26], it can also be predicted that STAMs would act in a later, lysosome-targeting stage of endocytosis but not have a role in the early stages of endocytosis. On the other hand, association of EAST with Eps15, which operates in the formation of the coated pits, would suggest some function for STAMs also at this early stage of endocytosis.

More direct evidence for the role of STAMs in Hrs-mediated endocytotic events is provided by studies showing that Hrs and STAM 1/Hbp interact with each other through the coiled-coil sequence(s) present in both proteins [3,10]. Recently, in *Saccharomyces cerevisiae*, the Hrs homolog Vps27p and the STAM homolog were shown to interact [27]. In STAMs, the coiled-coil region (aa 350–377 in STAM 1) partially overlaps the ITAM domain (aa 370–387 in STAM 1), and it interacts with the second coiled-coil (CC2) region of Hrs [10,22]. Since both the FYVE and CC2 domains of Hrs are required for its early endosome localization [22], it is tempting to speculate that STAMs could, by competing for the endosome binding, interfere with the Hrs targeting.

Takata et al. [3] demonstrated that a transient overexpression of STAM 2A/Hbp mutants lacking either the SH3 or the Hrs-binding coiled-coil domains inhibited the intracellular degradation of PDGF and its receptor, corroborating the idea that STAM is a central player in Hrs-mediated endocytic events.

The role of STAMs in IL-2 signalling is generally associated only with the signalling cascade from the receptor to the downstream pathway. A recent observation that IL-2 receptors utilize a clathrin-independent endocytosis pathway that is coupled to detergent-resistant membrane domains makes it an intriguing possibility that STAMs would be involved also in this novel endocytic machinery [28].

The role of STAMs may not be restricted to endocytosis only but may be more widely applicable to vesicular trafficking. This is based on the studies of Murai et al. [29] with the mutant proteins lacking either the SH3 or the Hrs-binding coiled-coil domains. Their overexpression in mast cells inhibited the immunoglobulin E (IgE) receptor-triggered degranulation of secretory granules. These results suggest that STAMs

play a regulatory role in the IgE receptor-triggered exocytosis. In regard to its role also in endocytosis, it can be envisioned that STAMs could have an important regulatory function in coordinating, both spatially and temporally, the closely linked endo- and exocytic vesicle events.

The presence of the UIM motif, a consensus binding site for proteins carrying ubiquitin, in STAMs suggests its involvement in ubiquitin-mediated endocytosis. In this regard, it is interesting that EGFR and Eps15, both interacting with EAST, are ubiquitinated upon EGF binding [30]. That STAMs could be components of a larger complex of a ubiquitin machinery is suggested by a study of Kato et al. [31], showing that the deubiquitinating enzyme UBPY binds to the SH3 domain of STAM 2A/Hbp (Fig. 2). A specific sequence, Px(V/I)(D/N)RxxKP, which is distinct from the consensus SH3-binding PxxP motif, was identified as the SH3-binding motif in UBPY. A peculiar feature which further points to finely tuned specific interactions and possibly to a specific class of multiprotein complex-forming molecules, is that the proteins containing similar SH3-binding motifs are mostly members of multidomain linker proteins such as SLP-76 and Gab1 [31]. Intriguingly, this same SH3-binding motif is also present in AMSH, in which it mediates the interaction with the SH3 domain of STAM [31].

Ubiquitination of growth factor receptors regulate their intracellular degradation. SH3 deletion in STAM 2A/Hbp leads to an inhibition of receptor-mediated endo- and exocytosis. Thus, UBPY may play a positive regulatory role in receptor degradation by interaction with the SH3 domain of STAM 2A/Hbp.

6. Other functional features

It is well-established that the endocytotic machinery is closely linked with the dynamics and integrity of the actin cytoskeleton [32]. Of a special relevance to the present discussion is the observation that directly connects Pan1p, a putative yeast ortholog of Eps15, to actin polymerization [33]. Our own studies showed a direct interaction between the N-terminus of EAST, containing the VHS and UIM domains, and actin [14]. This is suggestive of STAMs functioning also as integrators between endocytosis and cytoskeletal events.

7. Concluding remarks

STAMs are closely involved, first, in the regulation of the cytokine-mediated signal transduction, and second, in the regulation of the receptor-mediated endocytosis. Accumulating evidence suggests that they probably act, in combination with positive and negative interactors, as regulators and integrators of parallel signalling pathways. STAMs may also have a role in regulating and driving later endocytotic events, especially those associated with the formation of early endosomes and their subsequent fate, in some exocytic events, and in a pathway regulating cell survival in neural cells.

References

[1] Takeshita, T. et al. (1996) Biochem. Biophys. Res. Commun. 225, 1035–1039.
[2] Lohi, O., Poussu, A., Meriläinen, J., Kellokumpu, S., Wasenius, V.-M. and Lehto, V.-P. (1998) J. Biol. Chem. 273, 14806–14816.
[3] Takata, H., Kato, M., Denda, K. and Kitamura, N. (2000) Genes Cells 5, 57–69.
[4] Lohi, O. and Lehto, V.-P. (1998) FEBS Lett. 440, 255–257.
[5] Mao, Y. et al. (2000) Cell 100, 447–456.
[6] Misra, S., Beach, B.M. and Hurley, J.H. (2000) Biochemistry 39, 11282–11290.
[7] Hofmann, K. and Falquet, L. (2001) Trends Biochem. Sci. 26, 347–349.
[8] Cohen, G.B., Ren, R. and Baltimore, D. (1995) Cell 80, 237–248.
[9] Isakov, N. (1997) J. Leukoc. Biol. 61, 6–16.
[10] Asao, H. et al. (1997) J. Biol. Chem. 272, 32785–32791.
[11] Endo, K. et al. (2000) FEBS Lett. 477, 55–61.
[12] Yamada, M. et al. (2001) Mol. Cell. Biol. 21, 3807–3819.
[13] Mesilaty-Gross, S., Reich, A., Motro, B. and Wides, R. (1999) Gene 231, 173–186.
[14] Lohi, O. and Lehto, V.-P. (1998) FEBS Lett. 436, 419–423.
[15] Pandey, A. et al. (2000) J. Biol. Chem 275, 38633–38639.
[16] Takeshita, T. et al. (1997) Immunity 6, 449–457.
[17] Lohi, O. and Lehto, V.-P. (1998) FEBS Lett. 432, 225–227.
[18] Tanaka, N. et al. (1999) J. Biol. Chem. 274, 19129–19135.
[19] Itoh, F., Asao, H., Sugamura, K., Heldin, C.H., ten Dijke, P. and Itoh, S. (2001) EMBO J. 20, 4132–4142.
[20] Hansen, K., Rönnstrand, L., Claesson-Welsh, L. and Heldin, C.H. (1997) FEBS Lett. 409, 195–200.
[21] Salcini, A.E., Chen, H., Iannolo, G., De Camilli, P. and Di Fiore, P.P. (1999) Int. J. Biochem. Cell Biol. 31, 805–809.
[22] Raiborg, C., Bache, K.G., Mehlum, A. and Stenmark, H. (2001) Biochem. Soc. Trans. 29, 472–475.
[23] Carbone, R., Fre, S., Iannolo, G., Belleudi, F., Mancini, P., Pelicci, P.G., Torrisi, M.R. and Di Fiore, P.P. (1997) Cancer Res. 57, 5498–5504.
[24] Raiborg, C., Bache, K.G., Mehlum, A., Stang, E. and Stenmark, H. (2001) EMBO J. 20, 5008–5015.
[25] Bean, A.J., Davanger, S., Chou, M.F., Gerhardt, B., Tsujimoto, S. and Chang, Y. (2000) J. Biol. Chem. 275, 15271–15278.
[26] Chin, L., Raynor, M.C., Wei, X., Chen, H. and Li, L. (2001) J. Biol. Chem. 276, 7069–7078.
[27] Uetz, P. et al. (2000) Nature 403, 623–627.
[28] Lamaze, C., Dujeancourt, A., Baba, T., Lo, C.G., Benmerah, A. and Dautry-Varsat, A. (2001) Mol. Cell 7, 661–671.
[29] Murai, S. and Kitamura, N. (2000) Biochem. Biophys. Res. Commun. 277, 752–756.
[30] Stang, E., Johannessen, L.E., Knardal, S.L. and Madshus, I.H. (2000) J. Biol. Chem 275, 13940–13947.
[31] Kato, M., Miyazawa, K. and Kitamura, N. (2000) J. Biol. Chem 275, 37481–37487.
[32] Qualmann, B., Kessels, M.M. and Kelly, R.B. (2000) J. Cell Biol. 150, F111–F116.
[33] Duncan, M.C., Cope, M.J., Goode, B.L., Wendland, B. and Drubin, D.G. (2001) Nat. Cell Biol. 3, 687–690.