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TULA-family proteins: an odd couple

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Abstract Two members of the TULA family (TULA/STS-2/UBASH3A and TULA-2/STS-1/UBASH3B) recently emerged as novel regulators of several cellular functions. The degree of structural similarity between the TULA-family proteins is typical for proteins that belong to the same family. Furthermore, the experiments with knockout mice lacking these proteins may be interpreted as suggesting that functions of TULA-family proteins in T lymphocytes overlap. At the same time, TULA and TULA-2 exhibit clear functional dissimilarities, starting with the finding that a conserved phosphatase domain present in both proteins exhibits remarkable differences in enzymatic activity; TULA-2 is an active phosphatase capable of dephosphorylating multiple tyrosine-phosphorylated proteins, whereas the phosphatase activity of TULA is extremely low. In contrast, TULA, but not TULA-2, facilitates growth factor withdrawal-induced apoptosis in T cells. In spite of their apparent importance, the functional role of TULA-family proteins is not well understood. In particular, the role of functional dissimilarities between them remains unclear.

Keywords TULA · STS · UBASH3 · Ubiquitin · Phosphatase

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A new family is described

The genes/proteins of the family described here were discovered independently by several groups, and therefore multiple names are used to define them. First, the gene encoding one of the two proteins of this family was characterized in a study focused on the search for causative factors of autosomal recessive nonsyndromic deafness [1]. This study led to the isolation and characterization of a full-length transcript of the gene termed *UBASH3A* by its authors, because it encoded a product containing the ubiquitin-associated (UBA) and Src-homology 3 (SH3) domains (Fig. 1), which is unlikely to be involved in the development of the condition studied [1].

The product of this gene was characterized by us in the course of studies of c-Cbl, a multi-domain adaptor and an E3 ubiquitin ligase (reviewed in [2–4]). We determined that this protein interacts with c-Cbl through its SH3 domain [5]. We also demonstrated that this protein binds to ubiquitin via its UBA domain. Based on this finding and on the fact that this protein was isolated from T cells, we termed it T cell Ubiquitin LigAnd (TULA) [5].

An independent study by Carpino et al. [6] reported cloning this gene as a homologue of the gene they discovered previously as encoding the novel protein p70, which binds to a major autophosphorylation site of Jak2 kinase. The two proteins turned out to form a two-member family. These authors termed them Suppressor of T-cell receptor Signaling (STS)-1 and -2, since the lack of these proteins resulted in hyper-reactivity of T cells [7] (see below).

Finally, a study by Kowanetz et al. [8] indicated that UBASH3A/TULA/STS-2 was cloned by these authors as binding to the proline-rich sequences of c-Cbl in a yeast two-hybrid screen (Cbl-Interacting Protein 4; CLIP4).

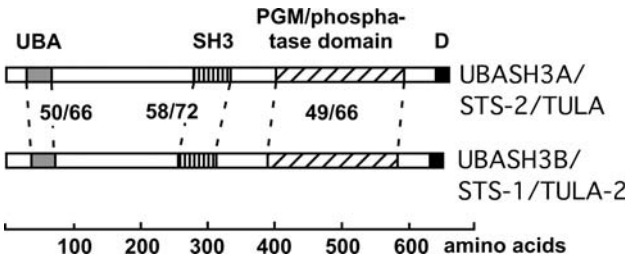

Fig. 1 Domain structure and homology between TULA and TULA-2. Major functional domains of TULA proteins are shown, including ubiquitin-associated domain (*UBA*), Src-homology domain 3 (*SH3*), PGM/phosphatase domain, and dimerization domain (*D*). The percent of sequence identity/percent identity + similarity (as defined in the Entrez Protein database) within major domains is shown. The overall homology between the long spice form of TULA and TULA-2 shown in this figure is 43% identity/59% identity + similarity

Thus, the proteins discussed in this article have multiple names, but the term TULA will be used for consistency with our previous publications. It should be noted, however, that the symbols currently used by Entrez Gene for the two family members are UBASH3A and UBASH3B.

Very similar, but considerably distinct

The family to which TULA and TULA-2 belong exhibits the unique domain architecture, which features an N-terminal UBA domain, a centrally located SH3 domain, and a domain located in the C-terminal half, which was originally termed HCD [1] and noted to display a homology to phosphoglycerate mutases (PGM) [6]. It was subsequently found out that the homology to PGM reflects the similarity of this domain to the superfamily of proteins with diverse activities and functions, termed the histidine phosphatase family due to the critical role of a histidine residue in the catalytic effects of its members [9]. This homology is consistent with substantial phosphatase activity detected for STS-1/TULA-2 [10].

The sequences of TULA-family proteins are ~45% identical (~60% identical plus similar). These values are in the range typical for many well-documented protein families, such as Src, Syk, and Cbl, and is fully consistent with the noteworthy similarity between the crystal structures of their C-terminal halves [11]. In particular, the structural similarity between these proteins is very strong in the substrate pocket, in which conserved catalytic residues of both proteins adopt identical configurations [11]. Likewise, similarities are clear for their regions outside the PGM/phosphatase domain. Thus, both TULA and TULA-2 bind to c-Cbl via SH3 and to ubiquitin via UBA [5, 8], and exert a UBA-dependent inhibitory effect on HIV-1 production [12].

In spite of these similarities, TULA and TULA-2 are quite different. First, they show distinct expression patterns. TULA-2 is expressed ubiquitously, whereas TULA has been detected only in lymphoid cells [5–7]. Second, it has been shown that TULA facilitates apoptosis induced in T cells by certain stimuli, such as growth factor withdrawal, whereas TULA-2 does not [13]. This pro-apoptotic effect of TULA appears to be linked to the interaction of TULA with AIF, a known apoptosis-inducing factor, to which TULA-2 does not bind [13]. Third, while TULA-2 has protein phosphatase activity comparable to that of known protein tyrosine phosphatases, the activity of TULA under identical conditions is not detectable either in vitro or in the cell culture [14, 15]. Enzyme kinetics analysis indicates that k_{cat} and k_{cat}/K_m of para-nitrophenyl phosphate (pNpp) hydrolysis for TULA is lower than that for TULA-2 by a factor of ~2,000- and ~9,000-fold, respectively [10, 11]. Although it has been shown that the pH-dependence of TULA's enzymatic activity is different from that of TULA-2, demonstrating the optimum at pH 5, k_{cat} for pNpp hydrolysis at pH 5 is still 200-fold lower for TULA than for TULA-2 [11]. The difference in k_{cat}/K_m becomes even higher at pH 5, reaching ~16,000-fold [11].

It appears reasonable to seek explanation of the difference between the catalytic activities of TULA-family proteins in the divergent nature of non-conserved non-catalytic residues in and around their active sites. Indeed, several residues fitting this description are different in TULA and TULA-2. These residues in TULA were mutated, singly and in pairs, to make its active site more TULA-2-like; these changes increased k_{cat}/K_m of TULA for pNpp hydrolysis both at pH 5 and 7.2 by ~10- to 40-fold [11]. The achieved increase is high, but significantly lower than the reported difference between k_{cat}/K_m of pNpp hydrolysis for wild-type (WT) TULA and TULA-2. It is therefore possible that non-conserved non-catalytic residues located away from the active site play a role in the differential activity of these proteins.

Finally, the possibility exists that TULA, while being poorly active with phosphoproteins and pNpp as substrates, acts on other phosphorylated molecules. Thus, the particular similarity of TULA-family proteins with ecdysone phosphate phosphatase (EPP) [16] argued that TULA might act on phosphorylated steroids. However, while both TULA-2 and EPP demonstrate ability to dephosphorylate phosphorylated steroids, pNpp, and phosphotyrosine-containing peptides/proteins [16, 17], TULA shows no activity towards either phosphorylated steroids or multiple other small phosphorylated molecules [11].

Reported effects: overlapping or not?

In spite of the obvious dissimilarities, both genes of the family should be deleted to cause phenotypic differences

between double knockout (dKO) and WT mice, while neither single KO (sKO) appears to be different from WT [7]. Consistent with the finding that the family members are co-expressed only in lymphoid cells [5–7] (notably, in mice, TULA is expressed primarily in T cells [7]), phenotypic changes have been reported only in dKO T cells, which become hyper-reactive to stimulation through T cell antigen receptors [7]. This finding may have alternative interpretations. Thus, functions of TULA and TULA-2 in controlling T cell reactivity may be highly overlapping, so either protein may compensate for the lack of its counterpart. On the other hand, TULA and TULA-2 may suppress T cell responses via non-overlapping routes, both of which have to be blocked to cause detectable differences. While the essential role of phosphatase activity in the T cell signaling-suppressing effect of TULA-2 has been shown [10], it remains unclear whether this activity is required for the effect of TULA. Based on the dramatic differences in activity and pH-dependence between TULA and TULA-2, one may reason that TULA either targets a highly specific, as yet unidentified, subset of phosphoproteins or is not a protein tyrosine phosphatase at all [11]. Considering the pro-apoptotic potential of TULA, which is unique for this family member [13], one may also speculate that the T cell reactivity-suppressing effect of TULA is related to its ability to maintain the appropriate level of T cell apoptosis, a process which leads to the removal of activated effector T cells during the shutdown phase of an immune response (reviewed in [18, 19]). Notably, the consumption of growth factors by T regulatory cells appears to cause growth factor deprivation-induced apoptosis of responder T cells in vitro and in vivo [20], while the pro-apoptotic effect of TULA is at least partially specific for factor withdrawal-induced apoptosis [13].

Differential sequence conservation: a clue?

Some observations related to the TULA-family gene structure in various species seem to provide an indirect support to the idea that TULA may exert phosphatase-independent biological effects. Genes encoding TULA and TULA-2 homologues exist in mammals, birds, amphibians, and fish, but the level of homology between TULA-2 orthologues is substantially higher than that between TULA orthologues. In addition, two genes of this family found in fish show more similarity to each other than do those of the avian and mammalian families, but both fish genes are more similar to TULA-2 than to TULA (see [21] for a more detailed discussion). Furthermore, the homology within PGM domain for TULA-2 from two different species is as high as that within UBA and SH3 and the overall homology between TULA-2 from these species. In contrast, the PGM homology for TULA proteins from different species is typically lower than that within other domains or the overall homology (Table 1). This observation suggests that, while the PGM/phosphatase domain is critical for the phosphatase-dependent conserved function of TULA-2, the function of TULA, unclear whether or not it is conserved in various species, is not as dependent on the phosphatase activity as the function of TULA-2. This notion does not exclude the possibility that the PGM/phosphatase domain is critical for some phosphatase-independent functions, which do not require high conservation of the sequences essential for phosphatase activity.

Table 1 Differential interspecies sequence conservation for various domains of the TULA-family members

Species	TULA				TULA-2			
	UBA	SH3	PGM	Total	UBA	SH3	PGM	Total
Mouse	83	91	76	83	100	98	96	97
Chicken	71	77	62	66	89	95	89	89

Percent identity between human protein sequences and their orthologues from mouse and chicken are shown for the entire protein sequence (*Total*) and major functional domains defined according to Entrez Protein. Mouse and chicken are presented as the examples, respectively, of a non-human mammal and a non-mammalian vertebrate that demonstrates intrafamily diversity comparable to that in mammals. (See details in the text and in [21])

Conclusion and future studies

Although the UBASH3/STS/TULA family was defined recently, considerable knowledge of structure, expression, interactions, and functions of its proteins has been accumulated. Interestingly, several disparate functions of TULA proteins have already been reported, some of which are mediated by phosphatase activity and some appear to be phosphatase-independent. In spite of the considerable structural similarity between TULA proteins and the possibility that their biological effects are overlapping, several profound differences between TULA and TULA-2 on the molecular level have been observed. Namely, TULA has extremely low phosphatase activity, but is capable of promoting caspase-independent apoptosis, whereas TULA-2 is an active phosphatase, demonstrating no pro-apoptotic activity.

Among the issues that have to be addressed to better understand the role of TULA proteins in cellular regulation and the molecular basis of their role, the following questions may be emphasized. (1) What are the physiological roles of TULA proteins? Why both of them should be deleted to exert a significant effect on T lymphocytes? To what extent their cellular effects are overlapping or, in

contrast, unique? (2) What is the structural/molecular basis of remarkable functional dissimilarities between TULA and TULA-2? (3) Is the phosphatase activity of TULA proteins regulated in the cell? To what extent regulation of TULA and TULA-2 differs functionally and mechanistically? (4) What are the best experimental systems to study biological significance of the effects of TULA proteins? Do systems exist that can demonstrate the TULA-independent role of TULA-2? (5) Do UBA and SH3 play any role in biologically relevant effects of TULA proteins? Are these domains important for phosphatase activity or do they exert phosphatase-independent regulatory effects?

Clearly, more structure–function studies of the molecular disparities between TULA and TULA-2 underlying dramatic differences in their activities, along with the identification of their biologically relevant substrates are needed for better understanding of the biological role of this family. Also, it is possible that further studies of TULA-2 sKO mice may reveal defects in cell types expressing only TULA-2 that were not profound enough to be detected upon the initial characterization of dKO and sKO mice. Such defects, if detected, may be instrumental in characterizing biological functions of TULA-2 in a relatively simple experimental system, in which TULA does not play a role.

To sum up, initial studies of TULA-family proteins suggested that these proteins may play an important regulatory role and pointed out their functional elements likely underlying this role. However, these studies also demonstrated apparent functional dissimilarities between these proteins that seem to be in contrast to a considerable degree of structural similarity between them. It remains to be determined what relevant physiological and pathogenic processes are dependent on TULA proteins and to what extent their involvement in these processes is specific.

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