

Gene Section

Review

USP15 (ubiquitin specific peptidase 15)

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Identity

Other names: KIAA0529; UNPH4; Unph-2

HGNC (Hugo): USP15

Location: 12q14.1

DNA/RNA

Note

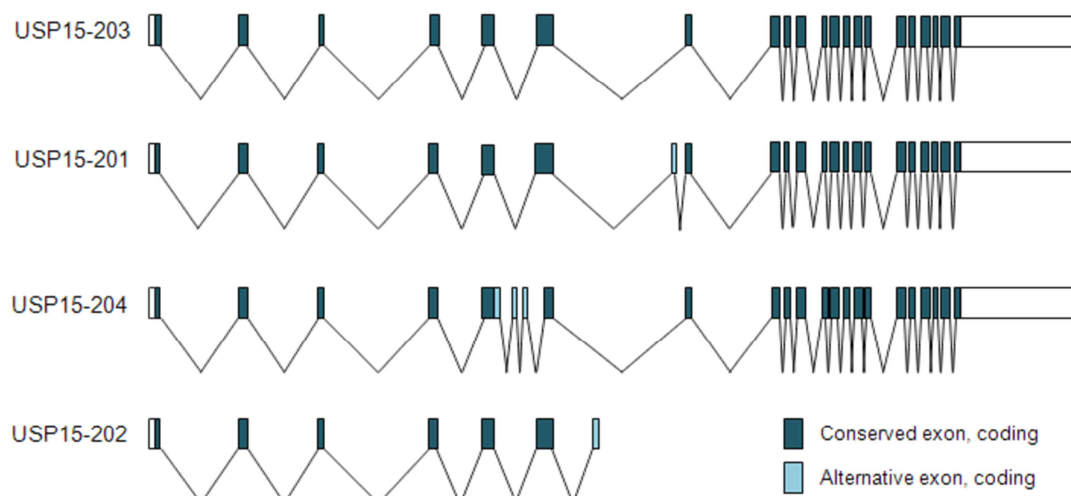
USP15 is a member of the ubiquitin-specific protease (USP) family; these cysteine proteases comprise the largest sub-group of deubiquitinase enzymes (DUBs). USP15 cleaves the isopeptide bonds of polyubiquitin chains, and can cleave linear ubiquitin fusion proteins (Baker et al., 1999).

Description

The USP15 gene spans 145 kb of genomic DNA.

Transcription

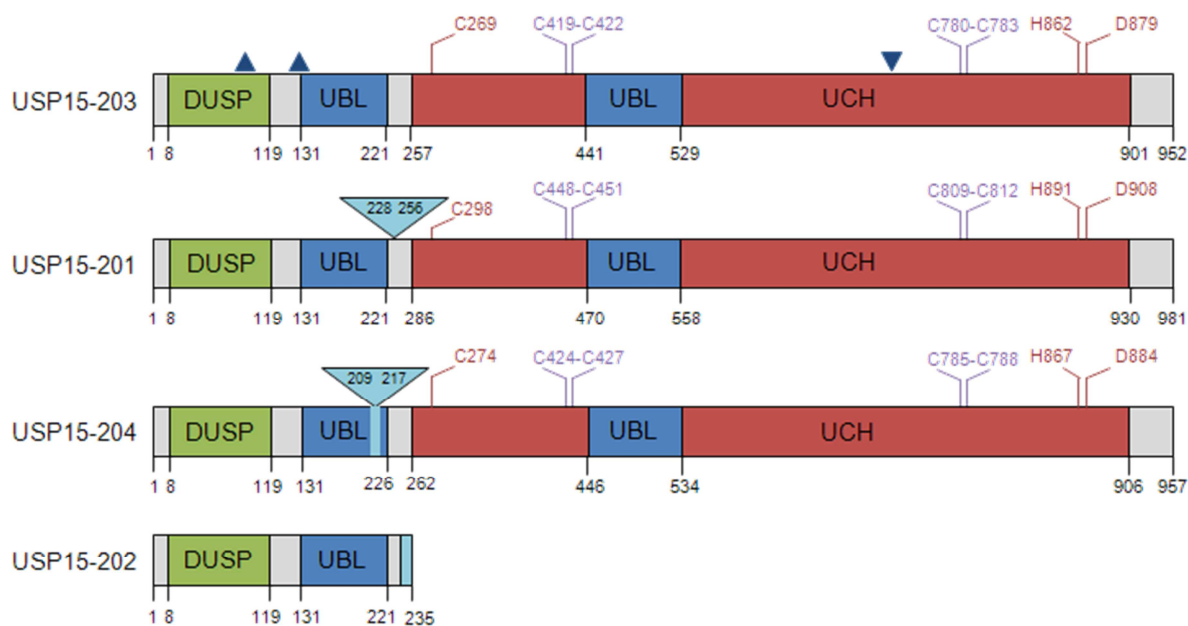
Four transcripts of the human USP15 gene are described by Ensembl and are summarized in the accompanying diagram and table. According to Entrezgene, USP15 encodes a single reference sequence mRNA of 4611bp (NM_006313.1) composed of 21 exons, which corresponds to USP15-203. However, three other USP15 splice variants utilise several alternative-splicing sites between exon 5 and exon 7 of this reference sequence. USP15-201, a 4698 bp mRNA comprised of 22 exons, is expressed at similar levels to the reference sequence (Angelats et al., 2003). Expression of the remaining variants, USP15-204 and the truncated USP15-202, is less well studied.



Schematic illustrating four human USP15 transcripts. The USP15 reference sequence mRNA (USP15-203) and three alternative splice variants are illustrated. The approximate position and size of exons within the USP15 gene, according to Ensembl, is shown for each splice variant.

Summary table of USP15 transcripts.

Name	Ensembl	Entrez	Aceview	Size (bp)	Exons
USP15-203	ENST00000353364	NM_006313.1	Variant b	4611	21
USP15-201	ENST00000280377		Variant a	4698	22
USP15-204	ENST00000393654			4626	23
USP15-202	ENST00000312635		Variant e	717	7



Schematic illustrating four human USP15 isoforms. The domain structure is shown for the reference sequence protein (USP15-203) and three alternative isoforms according to Ensembl. DUSP, domain present in ubiquitin-specific proteases; UBL, ubiquitin-like fold; UCH, ubiquitin carboxyl-terminal hydrolase. The cysteine motifs that form the zinc-binding site are shown in purple and the amino acids comprising the catalytic triad are shown in red. The approximate location of nuclear export sequences (triangles) and a putative nuclear localisation signal (inverted triangle) are shown above isoform USP15-203. Differences in amino acid sequence between isoforms are shown in light blue. The UCH is absent in isoform USP15-202, but USP15-201, USP15-203 and USP15-204 are predicted to be catalytically active.

Summary table of USP15 protein isoforms.

Name	Ensembl	Entrez	Size (aa)	MW (kDa)
USP15-203	ENSP00000258123	NP_006304.1	952	109
USP15-201	ENSP00000280377		981	112
USP15-204	ENSP00000377264		957	109
USP15-202	ENSP00000309240		235	40

Protein

Description

All USP15 isoforms encompass a single N-terminal DUSP (domain in USPs) characterised by a novel tripod-like fold with a conserved hydrophobic surface patch that is predicted to participate in protein-protein interaction (de Jong et al., 2006). The ubiquitin carboxyl-terminal hydrolase (UCH) catalytic core of the USPs is typically around 350 amino acids, but consists of six conserved boxes, interspersed by insertion sites for additional sequence that can confer diversity (Ye et al., 2009). The major insertion in USP15 is between boxes 3/4, and embeds an ubiquitin-like fold (UBL) domain within the catalytic domain. UBLs are commonly found in the USPs and in certain other DUB families (Zhu et al., 2007; Komander et al., 2009a). They exhibit low sequence conservation, but have high structural similarity with ubiquitin and have been proposed to play important roles in regulating DUB catalytic function or interactions (Zhu et al., 2007; Ye et al., 2009). The intercalation of a UBL between boxes 3 and 4 of the catalytic domain increases the spacing between two sets of zinc-coordinating cysteine motifs, which form a functional zinc finger that is required for activity (Hetfeld et al., 2005). In the case of USP15, a second UBL is located directly adjacent to the DUSP (Zhu et al., 2007; Ye et al., 2009).

The four USP15 splice variants encode four distinct protein isoforms, which are illustrated in the diagram and summarised in the accompanying table. As a consequence of alternative splicing, isoform USP15-201 has a 29 amino acid insert within the unstructured region between the first UBL domain and the start of the UCH domain, whereas USP15-204 has a substitution of 3 amino acids for 8 residues within the first UBL. Otherwise the three isoforms that retain the catalytic domain are identical. They also retain predicted nuclear export signals (NES) (Soboleva et al., 2005) and, by homology with rat, a functional nuclear localisation signal (NLS) (Park et al., 2000).

Expression

USP15 messenger RNA (mRNA) expression is prevalent throughout the tissues of the body, although its levels vary. Human USP15 is least abundant in brain, lung and kidney, consistent with observations for mouse *Usp15* and the rat ortholog *UBP109* (Park et al., 2000; Angelats et al., 2003). In each species, USP15 is most abundant in testes, and is variously enriched in spleen, heart, skeletal muscle or peripheral blood leukocytes.

Localisation

As USP15 harbours both putative NES and NLS, its sub-cellular distribution may in part depend on the cellular context. Rat *UBP109* localises to both the cytoplasm and the nuclear compartment, with the latter

dependent on a C-terminal NLS (Park et al., 2000) that is conserved across species. Using an *Usp15*/USP15-specific polyclonal antibody, Soboleva et al. demonstrated that in HeLa (human cervical cancer cells) endogenous USP15 localised to the cytoplasm and nucleolus, but was largely excluded from the nucleoplasm; whilst in NIH3T3 (mouse fibroblast cells), *Usp15* localised in the cytoplasm and was enriched proximal to the plasma membrane (Soboleva et al., 2005). Interestingly, GFP-tagged USP15 (isoform USP15-203) adopts a largely cytoplasmic distribution in human cancer cell lines (Urbé, unpublished observation).

Function

Human USP15 was cloned and characterized in 1999 (Baker et al., 1999) and belongs to the largest ubiquitin specific protease (USP) group of deubiquitinating enzymes (DUBs). Protein ubiquitination occurs at lysine residues through the concerted action of E1 activating, E2 conjugating and E3 ligase enzymes. Ubiquitin contains seven lysine residues (K6, K11, K27, K29, K33, K48 and K63), which allow poly-ubiquitin chains to assemble through alternative isopeptide bond linkages. In addition, linear ubiquitin chains may be assembled through the amino-terminus and substrate proteins may also be mono-ubiquitinated. Consequently, in addition to the classical K48-poly-ubiquitin tag that targets substrates for proteasome-mediated degradation, ubiquitination has multiple cellular functions including regulation of protein localisation and activity (Pickart and Eddins, 2004). The general role of the DUBs, in addition to processing inactive ubiquitin precursors and keeping the 26S proteasome free of inhibitory ubiquitin chains, is to reverse the ubiquitination of substrate proteins (Amerik and Hochstrasser, 2004). There are approximately 80 active human DUBs that are divided into five families (Komander et al., 2009a). These DUBs are steadily being assigned to specific substrates (Ventii and Wilkinson, 2008), which is increasingly revealing associations with signalling pathways in cancer (Sacco et al., 2010).

USP15 has activity against both mono-ubiquitinated and poly-ubiquitinated substrates; the zinc-binding domain is necessary for USP15 to process poly-ubiquitin chains, but is not required for USP15 to remove ubiquitin from linear ubiquitin-GFP fusion proteins (Hetfeld et al., 2005). Although USP15 is relatively promiscuous in showing little specificity between K48- and K63-linked poly-ubiquitin chains, or between K63 and K11 di-ubiquitin linkages, it has limited activity against K11-linked poly-ubiquitin chains or linear ubiquitin (Komander et al., 2009b; Bremm et al., 2010).

A recent endeavour to map protein partners of the DUBs by mass spectroscopy reported that, in common with USP4 and USP39, USP15 interacts with several proteins involved in mRNA processing and so may

play a role in ubiquitin-dependent control of splicing or mRNA decay (Sowa et al., 2009). In addition, various cancer-signalling pathways have been associated with USP15. For example, USP15 was one of twelve DUBs identified from an siRNA screen that impact on the hepatocyte growth factor (HGF)-dependent cell scattering response in non-small cell lung cancer and pancreatic cancer cells (Buus et al., 2009). A number of specific USP15 substrates have also been described, including the human papilloma virus (HPV) E6 oncoprotein (Vos et al., 2009), the RING-box protein Rbx1 (Hetfeld et al., 2005), the adenomatous polyposis coli (APC) tumour suppressor (Huang et al., 2009), and the NF- κ B inhibitor I κ Ba (Schweitzer et al., 2007).

The latter three examples are all connected with the COP9-signalosome (CSN), a conserved multi-protein complex that regulates the cullin-RING ligase (CRL) superfamily of ubiquitin E3 ligases (Wei et al., 2008). CRLs have a core complex comprised of a cullin scaffold and the RING-box protein Rbx1 that recruit alternative adapter and substrate recognition proteins to form diverse E3 complexes with different substrate specificities. The primary function of the CSN is to remove the ubiquitin-like modifier Nedd8 from the cullin component. This both terminates E3 activity and is required for the reassembly of new CRLs (Wei et al., 2008). The CSN plays a role in many cancer-associated pathways including the cell cycle and DNA damage repair, and both CSN and CRL components may be dysregulated in tumours (Richardson and Zundel, 2005). Ubp12p, an *S. pombe* ortholog of human USP15, was shown through a systematic mass spectrometry screen to bind the CSN (Zhou et al., 2003). This targets Ubp12p to nuclear cullins, where it is proposed to protect against auto-ubiquitination and degradation of CRL components, in particular the substrate-specific adaptors. (Zhou et al., 2003; Wee et al., 2005).

Human USP15 also co-purifies with the CSN complex and was reported to stabilise the CRL core component Rbx1 (Hetfeld et al., 2005), thereby acting as a positive regulator of these E3 ligase complexes. In contrast, other studies suggest USP15 may directly oppose CRL E3 ligase activity by deubiquitinating specific substrates. For example, the CSN is involved in ubiquitin-dependent turnover of the I κ Ba inhibitor that retains NF- κ B in the cytosol (Schweitzer et al., 2007). Phosphorylation of I κ Ba triggers CRL-mediated poly-ubiquitination of I κ Ba and subsequent proteasomal degradation, allowing NF- κ B to enter the nucleus and

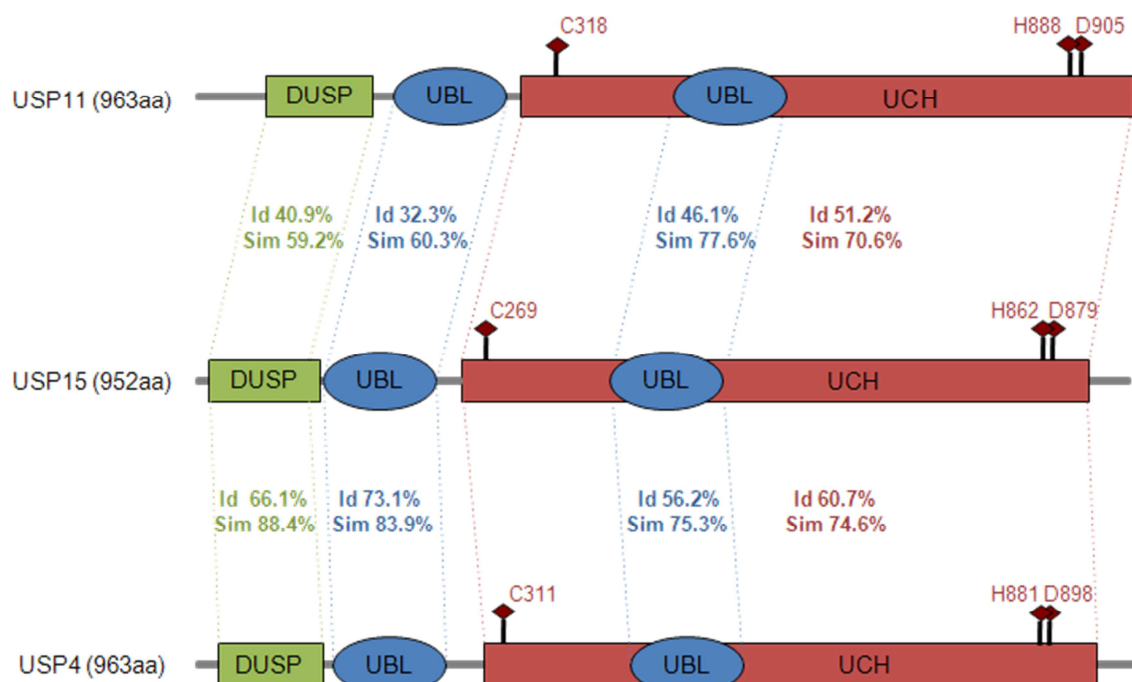
activate transcription (Karin and Ben-Neriah, 2000). In response to TNF α , I κ Ba has been reported to interact with the CSN leading to its deubiquitination and stabilisation by CSN-associated USP15 (Schweitzer et al., 2007).

The adenomatous polyposis coli (APC) tumour suppressor and the beta-catenin oncogene are frequently mutated in cancers, particularly of the intestine, leading to constitutive wingless and Int-1 (Wnt) signalling (Clevers, 2006). The CSN is proposed to control the balance of beta-catenin and APC through formation of a regulatory super-complex. Deneddylation by the CSN promotes assembly of the beta-catenin destruction complex, whilst CSN-associated USP15 stabilises APC (Huang et al., 2009). The APC also plays a role in mitotic fidelity through interaction with the plus end-binding protein EB1 that controls microtubule growth and dynamics. In contrast to APC, EB1 is destabilised by USP15, suggesting that this is not a direct substrate, but rather that USP15 stabilises a CRL that accelerates ubiquitination and degradation of EB1 (Peth et al., 2007). It is interesting to speculate that such links with microtubule regulation may underpin recent reports that USP15 levels can influence the taxol sensitivity of cancer cells (Xu et al., 2009; Xie et al., 2010).

VCP/p97 is a large AAA+-type ATPase that acts as a chaperone in many cellular processes. Its basic function is to segregate ubiquitinated proteins from macromolecular complexes, and VCP plays an important role in recognizing and handling misfolded proteins, which are then either handed over for degradation or recycled. The CSN directly interacts with VCP and USP15 can process VCP-bound poly-ubiquitinated substrates, which accumulate following USP15 depletion (Cayli et al., 2009). VCP is implicated in human neurodegenerative disorders where it co-localises with poly-glutamine aggregates and is proposed to act as both an aggregate-formase and an unfoldase (Kakizuka, 2008). Another established VCP-associated cofactor, the DUB Ataxin-3, is subject to polyglutamine repeat expansion, which causes Machado-Joseph disease (Madsen et al., 2009). Although the mechanism is as yet unclear, USP15 was recently associated with this same disorder (Menziez et al., 2010).

Homology

USP15 belongs to the peptidase C19 family. The closest paralogs based on sequence homology are USP4 and USP11.



USP15 and the paralogs USP4 and USP11. The highly similar domain structure is illustrated for USP15 (NP_006304.1), USP4 (NP_003354.2) and USP11 (NP_004642.2). The degree of identity (Id) or similarity (Sim) was derived using ClustalW (EMBL-EBI); note that the indicated UCH homology includes the internal UBL domain. Overall, USP15 shares 56.9% identity (71.2% similarity) with USP4, and 42.4% identity (60.2% similarity) with USP11.

Mutations

Somatic

No mutations have yet been reported for USP15 according to the COSMIC database, which includes data from four studies of 595 renal carcinoma, glioblastoma, breast and colon cancers.

Implicated in

Cervical cancer

Note

USP15 plays an oncogenic role in cervical cancer. Specific HPV strains are associated with cervical carcinoma and two HPV oncoproteins, E6 and E7, are expressed in these cancers. E6 hijacks a cellular E3 ubiquitin ligase and forms a complex with p53, whilst E7 binds the retinoblastoma (Rb) protein; in each case the viral oncoproteins facilitate degradation of the cellular tumour suppressor. It was recently found that USP15 interacts with the oncogenic HPV16 E6 protein (Vos et al., 2009). siRNA mediated depletion of USP15 led to a decrease in E6 protein, whilst overexpression of wild-type but not catalytically inactive USP15 promoted the stabilisation of E6. Interesting, another group has shown that E7 is regulated in a similar fashion by USP11 (Lin et al., 2008). Intriguingly, USP4 also has functional Rb binding motifs (Blanchette et al., 2001; DeSalle et al., 2001) that are conserved in USP11 and USP15 (Baker et al., 1999).

Ovarian cancer

Note

USP15 was identified from a genome-wide siRNA screen for Paclitaxel-resistance in the cervical cancer cell line HeLa and, in ovarian cancer samples, Paclitaxel-resistant cases (n=3) showed lower expression of USP15 mRNA than drug-sensitive cases (n=6) (Xu et al., 2009). Moreover, USP15 appeared to stabilise caspase-3, suggesting that reduced levels of USP15 may promote cell survival rather than apoptosis in response to drug treatment.

Gastro-intestinal cancers

Note

USP15 was also amongst four genes, identified by expression profiling of Docetaxel-sensitive versus resistant cells, which correlated with drug-sensitivity in a panel of gastric cell lines. However, no statistical correlation was established between elevated USP15 transcript levels and Docetaxel-sensitivity in 25 gastric cancer tissues (Xie et al., 2010).

Germline mutations in APC lead to inherited colon cancer and sporadic tumours are associated with beta-catenin stabilisation. Huang et al. show a role for USP15 in stabilizing APC levels through the action of the CSN (Huang et al., 2009).

Machado-Joseph disease

Note

USP15 was identified from microarray analysis of a mouse model of spinocerebellar ataxia type 3. In this

study, USP15 transcript and protein levels were decreased in both the ataxin-3 model, and in a second huntingtin transgenic model of a polyglutamine disorder; although overexpression of USP15 promoted the accumulation of protein aggregates, this was independent of its activity on poly-ubiquitin chains (Menzies et al., 2010).

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