Disabled-2 Structure and Function

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Contributor: Zoe K. Price , Noor A. Lokman , Masato Yoshihara , Hiroaki Kajiyama , Martin K. Oehler , Carmela Ricciardelli

Disabled-2 (*DAB2*), a key adaptor protein in clathrin mediated endocytosis, is implicated in the regulation of key signalling pathways involved in homeostasis, cell positioning and epithelial to mesenchymal transition (EMT).

DAB2 cancer metastasis

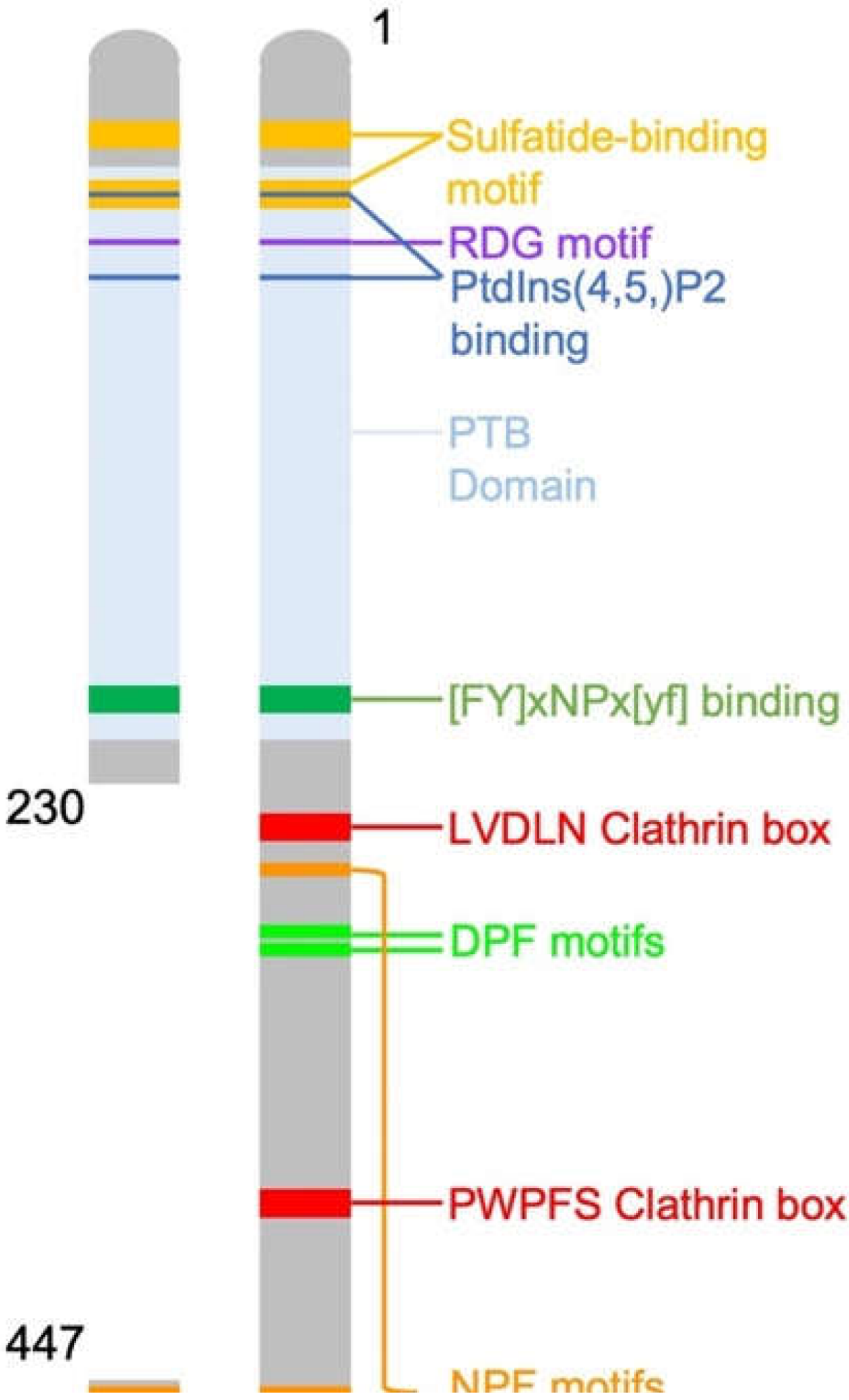
1. Introduction

Disabled-2 (DAB2) is a widely recognised tumour suppressor. It was initially discovered in 1994 when Mok et al. identified an 800bp cDNA fragment which was expressed in normal ovarian surface epithelial cell lines but not in ovarian cancer cell lines. They referred to it as differentially expressed in ovarian carcinoma 2 (DOC-2) [1]. The following year, Xu et al. identified a 96 kDa phosphoprotein in mouse macrophage cell line, BAC1.2F5 with an amino terminal end which shared homology to the Drosophila disabled gene [2][3]. The Drosophila disabled protein is important in embryogenesis and neural positioning [4]. *DAB2* is one of two human orthologs of the Drosophila disabled gene, Disabled-1 is expressed almost exclusively in neural cells whereas *DAB2* is expressed in a wide range of epithelial cells including those of the ovary, lung and breast [5][6][7]. Loss of *DAB2* expression has been reported in a range of malignancies including ovarian, lung and breast cancer [5][6][7]. The loss of DAB2 is associated with activation of key signalling pathways including wingless/integrated (Wnt), mitogen activated protein kinase (MAPK) and transforming growth factor beta (TGFβ) which is associated with enhanced cell proliferation, chemotherapy resistance and tumour progression, supporting its role as a tumour suppressor.

2. *DAB2* Structure and Function

The human *DAB2* gene, located on chromosome 5p13 consists of 15 exons, encoding a 770 amino acid protein [8]. The mouse *DAB2* gene has 83% homology with the human gene, it also consists of 15 exons and encodes a 766 amino acid protein [9]. There are two isoforms of DAB2, including full length p96 (also known as p82) and spliced p67 (also known as p59) that is missing the central exon. DAB2 contains binding domains and motifs which allow it to recognise and recruit proteins to clathrin coated pits for endocytosis (**Figure 1**). Two key binding domains of DAB2 are a phosphotyrosine binding (PTB) domain at the N-terminus and a proline rich domain (PRD) at the carboxy terminal end of the protein which contains a myosin interacting region (MIR). The main function of DAB2 is as a clathrin associated sorting protein (CLASP) in clathrin mediated endocytosis. DAB2 interacts with clathrin via multiple binding sites including a type I LVDLN and type II PWPYP sequence [10]. DAB2 can interact with both pre-assembled clathrin cages and also soluble clathrin trimers, indicating a possible role in clathrin cage assembly [10].

The DAB2 PTB binds to phosphoinositide(4,5)P2 (PtdIns(4,5)P2) containing liposomes, further suggesting it is involved in clathrin cage assembly and vesicle budding [10].


1
Sulfatide-binding motif
RDG motif
PtdIns(4,5,)P2 binding
PTB Domain
[FY]xNPx[yf] binding
230
LVDLN Clathrin box
DPF motifs
PWPFS Clathrin box
447
NPF motifs

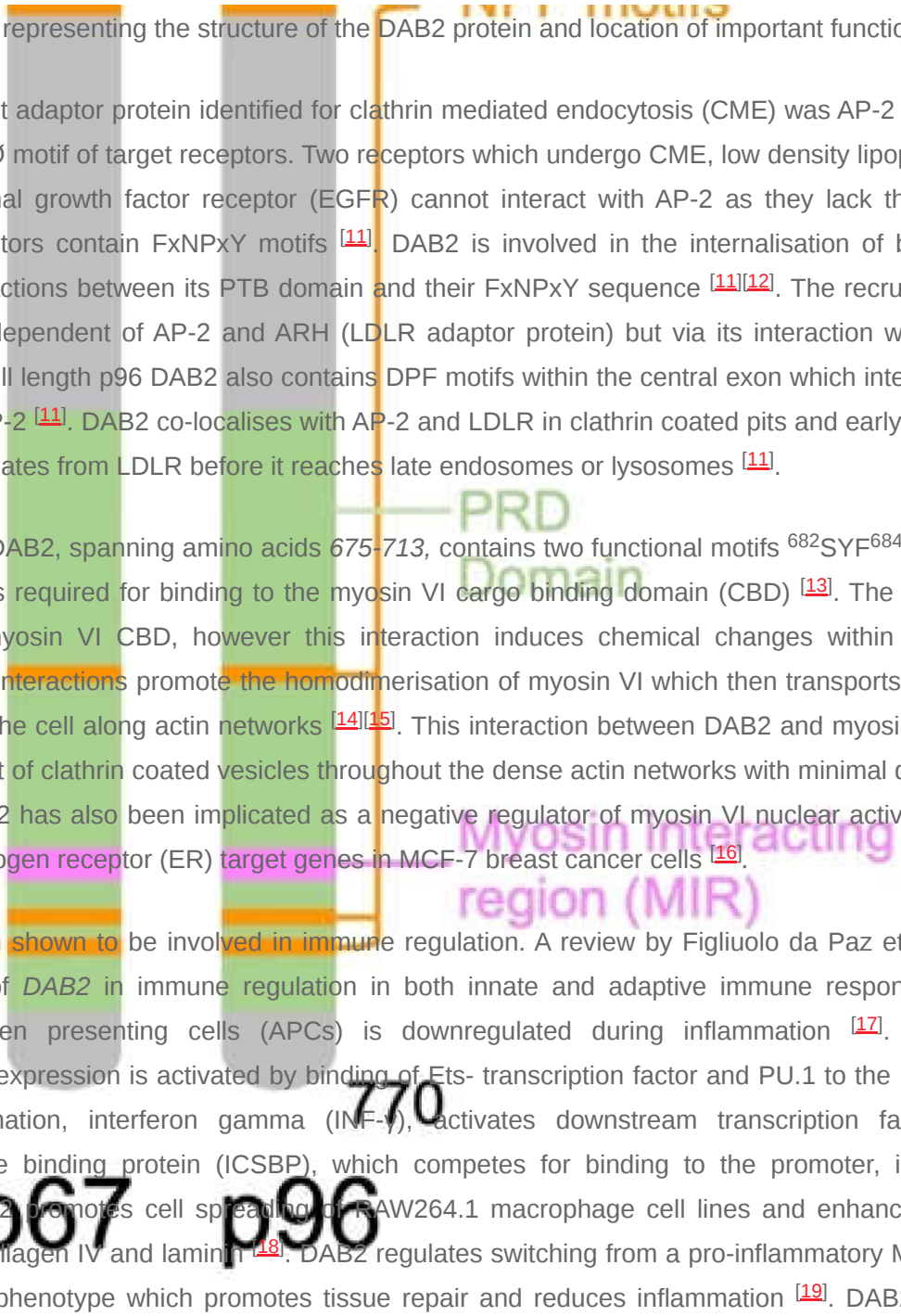


Figure 1. Diagram representing the structure of the DAB2 protein and location of important functional domains.

The principle and first adaptor protein identified for clathrin mediated endocytosis (CME) was AP-2 tetramer, which recognises the YXXØ motif of target receptors. Two receptors which undergo CME, low density lipoprotein receptor (LDLR) and epidermal growth factor receptor (EGFR) cannot interact with AP-2 as they lack the YXXØ motif, however, both receptors contain FxNPxY motifs [11]. DAB2 is involved in the internalisation of both LDLR and EGFR through interactions between its PTB domain and their FxNPxY sequence [11][12]. The recruitment of LDLR via DAB*2* occurs independent of AP-2 and ARH (LDLR adaptor protein) but via its interaction with clathrin and PtdIns(4,5)P2 [12]. Full length p96 DAB2 also contains DPF motifs within the central exon which interact with the α-adaptin subunit of AP-2 [11]. DAB2 co-localises with AP-2 and LDLR in clathrin coated pits and early endosomes. In the cell DAB2 dissociates from LDLR before it reaches late endosomes or lysosomes [11].

The MIR domain of DAB2, spanning amino acids *675-713,* contains two functional motifs ^{682}SYF^{684} and ^{699}DFD^{701} [13]. The SYF motif is required for binding to the myosin VI cargo binding domain (CBD) [13]. The DFD motif also interacts with the myosin VI CBD, however this interaction induces chemical changes within the myosin VI structure [13]. These interactions promote the homodimerisation of myosin VI which then transports clathrin coated vesicles throughout the cell along actin networks [14][15]. This interaction between DAB2 and myosin VI is dynamic allowing the transport of clathrin coated vesicles throughout the dense actin networks with minimal disruption to the actin fibres [14]. DAB2 has also been implicated as a negative regulator of myosin VI nuclear activity, such as the transcription of oestrogen receptor (ER) target genes in MCF-7 breast cancer cells [16].

DAB2 has also been shown to be involved in immune regulation. A review by Figliuolo da Paz et al. extensively explores the roles of *DAB2* in immune regulation in both innate and adaptive immune responses [17]. DAB2 expression in antigen presenting cells (APCs) is downregulated during inflammation [17]. Under normal homeostasis, DAB2 expression is activated by binding of Ets- transcription factor and PU.1 to the DAB2 promoter [18]. During inflammation, interferon gamma (INF-γ), activates downstream transcription factor, interferon consensus sequence binding protein (ICSBP), which competes for binding to the promoter, inhibiting DAB2 expression [18]. DAB2 promotes cell spreading of RAW264.1 macrophage cell lines and enhances adhesion to ECM components collagen IV and laminin [18]. DAB2 regulates switching from a pro-inflammatory M1 macrophage phenotype to a M2 phenotype which promotes tissue repair and reduces inflammation [19]. DAB2 interacts with tumour necrosis factor receptor associated factor 6 (TRAF6) via 2 domains at aa226 and aa689, preventing activation of Nuclear factor kappa B (NF-κB) and subsequent expression of pro-inflammatory genes in M1 macrophages [19]. Loss of DAB2 was required for pro-inflammatory responses to Toll-like receptor (TLR) ligands lipoteichoic acid (LTA) and lipopolysaccharide (LPS) [19][20]. DAB2 is highly expressed on $CD11b^{+}CD103^{-}$ dendritic cells (DC) which are involved in Th17 and Th1 responses in the gut [21]. Loss of DAB2 enhanced colitis in mouse models suggesting a role of DAB2 in immune tolerance [21]. TLR4 ligand LPS downregulates DAB2 expression and shifts the DCs to a mature, activated DC [21]. Furthermore, DAB2 silencing in bone marrow derived DC activates PI3K and NF-κB, enhancing the expression of pro-inflammatory cytokines IL-6 and IL-12 [22].

Overall, there is limited research on the role of DAB2 in T cells. DAB2 is expressed exclusively in $FOX3P^{+}CD4^{+}CD8^{-}$ T cells, with FOX3P promoting DAB2 expression by binding to its promoter [23]. DAB2 knock out (KO) in T cells has no effect on the overall number of T_{reg} cells *in vivo*, however, adoptive transfer of the DAB2 KO T_{regs} had diminished efficacy against colitis *in vivo* [23]. This suggests DAB2 is not crucial for maintenance of T_{reg} populations but is involved in their function.

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