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Site-directed mutagenesis for the RNA binding protein Bicc1 (Bicaudal C homolog 1) and its impacts in the protein overexpression

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Introduction: The Bicc1 (Bicaudal C homolog 1) is a RNA binding protein that is mutated in the Juvenile Congenital Polycystic Kidney Disease in the mouse model (jcpk) (Cogswell *et al.*, 2003), and is structurally characterized by presenting three K Homology domains (KH1, KH2 and KH3 domains) at the N-terminal end and a Sterile Alpha Motif (SAM domain) at the C-terminal end. The KH domains present features of RNA-binding capability while the SAM domain is involved in the direct protein-protein interactions (Bouvrette *et al.*, 2008). The KH domain (type I, found in eukaryotes) is characterized by the secondary structure β1-α1-α2-β2-β3-α3. Between the first and second alpha-helix there is a higher conserved motif, the GXXG (where "X" is a positively charged amino-acid residue and "G" the glycine) (Valverde, Edwards & Regan, 2008). According to Yoga *et al.* (2012), in the KH domain of the poly(C)binding protein 1, the oligonucleotides bind in the cleft formed across alpha-helix 1 and the two loops, and the GXXG motifs are essential for contact with the sugar-phosphate backbone. Indeed, mutations of GXXG motif to GAAG or AXXA in other proteins disrupts the interaction with the bound oligonucleotide (Walter *et al.*, 2002 and Siomi *et al.*, 1994) reinforcing the role of this loop in the interaction with the RNA target.

To explore structural and functional features of the Bicc1 domains, the GXXG motif of the KH domains was mutated by PCR mediated site-directed mutagenesis adapted from the procedure described by Wang & Malcolm (1999). The long-term aim of making various mutant constructs is focused on understanding if the binding properties of Bicc1 to mRNA targets is dependent on sequence and structural features. Initially, we evaluated if mutant proteins would allow *in vitro* expression of functional protein in HEK293 cells. For expressing constructs, we will follow up with protein production in E. coli and biochemical studies.

Results: Western-blot analysis pointed that mutations in the GXXG motifs of the KH domains 2 and 3 affected the protein overexpression in cells, whereas mutation in the KH domain 1 did not. *In silico* predictions indicate that the region mutated is intrinsically disordered only in the KH domain 1, being a structured loop in the KH domain 2 and 3, and the mutations introduced probably disrupt the normal protein folding.

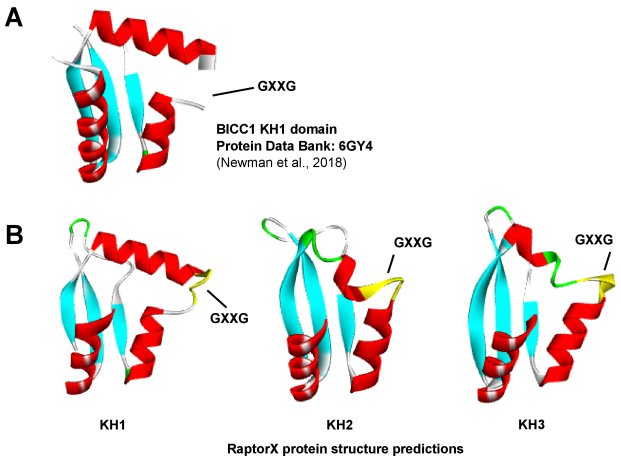

Figure 1 – Structural analysis of the three KH domains of Bicc1. In A – The crystallographic structure of the first KH domain of the human Bicc1 deposited by Newman *et al.* (2018) (DOI: 10.2210/pdb6GY4/pdb). GXXG missing coordinates in the X-ray structure probably by high degree of conformation freedom but still localized in a loop between α1 and α2. In B – Predicted 2ª and 3ª structures of the three KH domains by RaptorX protein structure predictor. The GXXG motifs (colored in yellow) were predicted as structured loops.

Conclusions: The traditional mutation of the GXXG motifs to study the role of each KH domain in the RNA binding function is not applicable to the Bicc1 protein and the mutations to GAAG in the KH domains 2 and 3 impair the expression of a full protein probably due to a disruption of the normal folding. We will continue to designed new mutants and to use the viable mutants to understand *in vivo* and *in vitro* properties of the RNA binding function of Bicc1.

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