Development of a genetic tool to map protein-protein interactions associated with neurological disorders

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The PDIA3 protein is an enzyme belonging to the family of protein disulfide isomerases (PDIs), oxidoreductases that catalyze the formation of disulfide bonds during protein folding in the endoplasmic reticulum (ER), a process known as redox folding. The PDI family plays a fundamental role in ER proteostasis, so alterations in their activity can lead to an accumulation of misfolded proteins and aggregates, giving rise to the condition of ER stress. PDIA3 interacts with two lectin-type chaperones, calnexin (Canx/CNX) and calreticulin (Calr/CRT), which are canonical interactors, to catalyze the redox folding of glycosylated substrates. In addition, a pathogenic mutant has been found in which cysteine 57 is replaced by a tyrosine, PDIA3^{C57Y}, which is related to intellectual disability.

There is evidence that alterations in PDIA3 activity are related to various pathological conditions involving the nervous system, such as the causal relationship between the loss of its enzymatic activity due to genetic mutation and the development of severe intellectual disability, its function in the differentiation of motor neurons and maintenance of the neuromuscular junction in amyotrophic lateral sclerosis (ALS), its neuroprotective effect by stimulating axonal regeneration in conditions of peripheral nerve damage and even in conditions such as Alzheimer's disease.

It is therefore important to try to understand how these proteins interact with each other and with other proteins in order to generate all the observed responses - this set of interactions is called interatome. Protein-protein interactions, known as PPIs, deal with physical contact *in vivo*, the way in which two or more proteins exchange information with each other through molecular coupling, this contact being directed by specific, non-generic interactions, typically related to the function that these proteins perform.

There are numerous methods used to study PPIs, including the set of techniques known as proximity-dependent biotinylation, PDB, which consists of targeting a mutant biotin-ligase enzyme that releases biotin in its reactive form, biotin-AMP, which reacts non-specifically with basic residues of nearby proteins (within a radius of up to 20 nm). Thus, by fusing this enzyme to a protein of interest (called bait) and treating the medium with biotin, a range of target proteins (preys) can be located that are possible candidates for bait interactors.

The PDB approach has a great advantage over other classical techniques for studying PPIs, not only because the proteins are biotinylated within their cellular compartments, avoiding artifacts originated in protein extracts, but also because it makes it possible to map transient interactions typical of those that occur between enzymes and their substrates.

In view of the above, we hope to build molecular networks of PDIA3 protein interactions that coordinate cognitive processes. It was possible not only to validate the PDIA3-BioID2 and PDIA3^{C57Y}-BioID2 chimera proteins by Western Blot against PDIA3 and V5, but also to validate their biochemical activity by verifying canonical interactions with CNX and CRT and the formation of molecular aggregates. In addition, we were able to establish conditions for the quantitative capture of biotinylated proteins.

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