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Structural plasticity of the coiled-coil interactions in SFPQ

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The proteins SFPQ (splicing Factor Proline/Glutamine rich) and NONO (Non-POU domain-containing octamer-binding protein), are mammalian members of the Drosophila Behaviour/Human Splicing (*DBHS*) protein family, which share 76% sequence identity in their conserved 320 amino acid DBHS domain [1, 2]. SFPQ and NONO are involved in all steps of post-transcriptional regulation [1] and are primarily located in *paraspeckles* in mammals: liquid phase-separated, ribonucleoprotein sub-nuclear bodies templated by NEAT1 long non-coding RNA [3]. A combination of structure and low-complexity regions provide polyvalent interaction interfaces which facilitate homo- and heterodimerisation, polymerisation, interaction with oligonucleotide, mRNA, long non-coding RNA, and liquid phase-separation. The strength and competition of these interaction modes defines their ability to dissociate from paraspeckles to fulfil functional roles throughout the nucleus or the cytoplasm. The imbalanced nucleocytoplasmic distribution of SFPQ has been shown to be an important factor in the neurodegenerative diseases ALS, FTLD and AD likely due to the disruption of the protein's essential nuclear functions [4,5]. We have defined and dissected the coiled-coil interactions which promote the polymerisation of DBHS proteins, via a crystal structure of an SFPQ/NONO heterodimer which reveals a flexible coiled-coil interaction interface which differs from previous studies [2]. We support this through extensive solution small-angle X-ray scattering experiments using a panel of SFPQ/NONO heterodimer variants which are capable of tetramerising to varying extents, ranging from null (quadruple coiled-coil mutant), through equilibrium (truncated proteins), to constitutive tetramerization (using an R542C mutation which mimics the pathological drosophila nonAdiss allele [6]). We demonstrate that coiled-coil interactions in addition to the previously-described canonical coiled-coil interface also play a role in determining the affinity for DBHS proteins which each other. The detail of these interactions and their relative strengths may be of significance to DBHS target recognition and protein oligomerisation *in vivo*, and ultimately disease pathology.

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