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Structural basis of interaction between DhHal3p and fungal specific Ser/Thr phosphatase protein DhPpz1p having intrinsically disordered N-terminus from osmotolerant yeast *Debaryomyces hansenii*

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Fungal specific Ser/Thr phosphatase 1, DhPpz1p, is negatively regulated by DhHal3p to confer halotolerance in *Debaryomyces hansenii*. Here we report structurally important pockets and points of interaction between these two proteins. The *in silico* interaction identified domains and sites of molecular interaction which were corroborated *in vitro* by yeast two hybrid interaction. Remarkably, a hot spot region residing in the carboxyl end of DhPpz1p was identified to bind DhHal3p. Similarly, from DhHal3p, a region comprising 463-559 residues was identified as vital for binding interaction with DhPpz1p and also with secondary interaction points scattered over N-terminus of DhPpz1p. Loss of binding between proteins was observed for position D443A and D446A from DhPpz1p and R338A, H344A, R348A and R349A from DhHal3p confirming the importance of these docking sites. Our results also revealed the dynamic dual role of short Ser/Arg/Asn motifs from N-terminus of DhPpz1p in selective interaction with DhGlc8p and negative regulator DhHal3p. The DhPpz1$_{N\Delta 59\text{-}75}$ and DhPpz1$_{N\Delta 162\text{-}183}$ were clearly refractory to DhHal3p binding and exhibited stronger binding with DhGlc8p. Antagonistically, DhPpz1$_{N\Delta 27\text{-}36}$, DhPpz1NΔ81-104 and DhPpz1$_{N\Delta 106\text{-}120}$ showed stronger binding with DhHal3p and no binding with DhGlc8p. Therefore, this study is the first documentation to reveal the regulatory role of N-terminus from DhPpz1p in recruiting regulators via degenerate yet distinct regulatory motifs from its N-terminus. Our work has further opened the room for frontier research in the field of antifungal development against unique N-terminus from each Ppz1p enzyme from diverse fungal species.

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