The Sudol group has refined conditions for mass spectrometry analysis of proteins that are pulled down by 11 GST-WW domains derived from yeast proteins. This work is now in progress at the Mass Spectrometry Facility at Mount Sinai. In terms of functional studies, we have analyzed in detail two yeast WW domain-containing proteins: Ess1 and Rsp5, and showed that they have opposing effects on RNA polymerase II function in yeast. In our previous reports, we have shown that these WW domain-containing proteins have capacity to interact with the yeast Pol II carboxy-terminal region through their WW domains.

The Fields laboratory has performed two-hybrid screens with nine of the twelve WW domains identified in yeast and generated by Dr. Takashi Ito's laboratory. No biologically relevant interactions appear to have been identified with these nine domains. The remaining three domains are self-activators from the Rsp5 protein and thus cannot be used in the two-hybrid system. We are collecting ~40 genes that are predicted by the Wade laboratory to be potential interacting partners with WW domains and are creating a mini-array of these as hybrid proteins to screen against the yeast WW proteins.

The Bork group has mainly focused attention on a detailed search of putative WW domain interacting proteins in yeast. We applied two different kinds of pattern search analyses trying to extract from the entire yeast proteome sequences bearing one of the known WW domain binding motifs. The complete list of results is reported on the WW domain project web page, which we designed in collaboration with R. Wade's group.

1 First group (PPXY motif): due to the shortness and low complexity of this motif's composition, more than 300 proteins were retrieved. To reduce that number to the most probable, we scanned the database using more detailed WW binding motifs reported in literature. The most interesting results were a DNA topoisomerase I, in which the motif is located in an exposed surface, and a ubiquitin-conjugating enzyme for which a structural model suggests the exposition to the solvent of the putative WW binding region.

2 Second group (PPLP motif): we identified 12 proteins bearing a perfect match to the motif, most of them not functionally annotated. They represent good candidates for further experimental follow up.

3 Third type (PXXGMXPP motif): only two yeast proteins have been retrieved, one of unknown function and another annotated as a pre-mRNA polyadenylation factor interacting with poly(A)polymerase.

The Fields and Bork laboratories, together with other collaborators, compared various large scale approaches to the identification of protein-protein interactions to each other and to a reference set of interactions reported in the literature.

Mutations proposed by R. Wade in the relatively unstable YAP WW domain that would increase its stability have been made by the Macias group, and thermal stability measured by circular dichroism. The experiments are in accord with the predictions. These results show the importance of cooperative aromatic interactions for the stability of the structure, and how increased stability may adversely affect cognate peptide binding.

The Wade group has identified proteins in the yeast proteome containing known WW domain binding motifs by sequence analysis, supplemented by structural modelling where possible, and carried out in collaboration with F. Ciccarelli in P. Bork's group. A list of these proteins was deposited on the project web page. We compiled all available experimental data on the interactions of the proteins in yeast containing WW domains with other yeast proteins, and deposited them on the project web page. We analyzed the relation between sequence motif conservation in WW domains and functional subfamily. In particular, an interesting conserved potential phosphorylation site was identified. 3D models of 11 putative WW domains were constructed and used to investigate peptide binding properties. We investigated the physical basis of stability of the WW domain by molecular modelling and simulation in conjunction with experimental work by M. Macias, and developed and tested methodology for protein-protein docking. We have done validation test docking simulations for a WW domain-peptide complex as well as for other protein-protein complexes.

Collaborative Aspects: There have been frequent meetings for discussion between the Macias, Bork and Wade groups at EMBL. These groups also met together with Marius Sudol when he visited Heidelberg in May 2002. Contact throughout the collaboration has been maintained via e-mail and a project website.

Publications:

(from more than one collaborating laboratory)

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