In the first year of the grant support, the Wade group, together with the Bork group, analyzed the yeast genome for motifs to identify potential WW domain-binding proteins. This was put in a structural context by analyzing structures of known WW binding motifs in the PDB. They have been put on a project web-server that was set up for sharing of data between the grant participants. A systematic comparison of all experimentally determined structures of WW domains has been performed. Studies to model yeast WW domain structures and investigate binding specificity and docking modes have been started.

Research in the Sudol laboratory has focused on the expression of all 11 yeast WW domains in bacteria and the analysis of complexes that are specifically pulled down by the WW domains from yeast lysates. Mass spectrometry has been used to identify these complexes.

The Fields laboratory has received 12 plasmids from Dr. Takashi Ito (Japan) encoding yeast WW domains as fusions to the Gal4 DNA-binding domain. Three proteins, Vid30, Ess1 and Ssm4, have been searched in a two-hybrid experiment against an array of nearly all yeast proteins as fusions to the Gal4 activation domain. Several positives have been identified for each WW protein, although no clear pattern has emerged that delineates a binding motif.

The Macias group has started with the structural determination of the U1 snRNP associated protein Prp40 (pre-mRNA processing) and the E3 ubiquitin-protein ligase Rsp5. We have determined the structure of the tandem WW domains found at the N-terminus of the Prp40 protein using heteronuclear multidimensional NMR spectroscopy. In addition to the structural work, we have also characterized by NMR and other biophysical techniques the interactions between the WW domains and two other splicing factors. At present we are solving the structure of the complex between Prp40 WW domains and the region of the branch binding protein that interacts with them. Rsp5 recognizes the carboxyl terminal domain (CTD) of the RNA polymerase II large subunit. In addition, the Sudol laboratory has found peptide sequences able to bind to the WW domains of Rsp5. We are therefore studying the interactions between WW domains of Rsp5 and seven peptides to understand the rules that govern the recognition of different peptide targets by one particular WW sequence. Together with the Wade group we are studying the stability of the WW fold by combining theoretical and mutational studies. So far we have successfully increased the midpoint temperature of a natural WW sequence to nearly the double of its initial value, by introducing only three mutations.

The WW domain page that has been maintained by the Bork laboratory at EMBL was updated last summer. Substantial additions, including structures of known WW domains and a link to SMART page of protein modules, were incorporated into the WW domain page on the worldwide web network. Dr. Sudol coordinated the update.

We have invited Dr. Ito to join our collaboration. An informal meeting was held in April 2001 at the University of Washington in the laboratory of Dr. Fields to coordinate the high-throughput yeast two hybrid screen with WW domains. Drs. Ito, Fields, Uetz and Sudol participated in that meeting.

RG0234/2000-M Publications

1. Chang, A., Cheang, S., Espanel, X., and Sudol, M (2000) Rsp5 WW Domains Interact Directly with the Carboxy-terminal Domain of RNA Polymerase II. *J. Biol. Chem.* **275**, 20562-20571.

2. Espanel, X., and Sudol, M. (2001) An Activated Form of the Yes Proto-oncogene Protein Kinase Regulates Interaction between Yes-Associated Protein (YAP) and p53BP-2. *J. Biol. Chem.* **276**, 14514-14523.

3. Strano, S., Munarriz, E., Rossi, M., Cristofanelli, B., Castagnolo, L., Shaul, Y., Sacchi, A., Oren, M., Sudol, M., Cesareni, G., and Blandino, G. (2001) Physical Interaction with Yes-Associated Protein (YAP) Enhances p73 Transcriptional Activity. *J. Biol. Chem.* **276**, 15164-15173.

4. Sudol, M., Sliwa, K., and Russo, T. (2001) Functions of WW domain in nucleus. *FEBS Lett.* **490**, 190-195.

5. Zhang, Y.Z.. Lindblom, T., Chang, A., Sudol, M., Sluder, A.E., and Golemis, E. (2000) Evidence that Dim1 associates with proteins involved in pre-mRNA splicing, and delineation of residues essential for Dim1 intercations with hnRNPF and Npw38/PQBP-1. *Gene* **257**, 33-43.

In preparation
1. Civera, C. Chang, A. Sudol, M., and Macias, M.J. (in preparation) Structural and binding studies on the second WW domain of Rsp5.

2. Wiesner, S., Stier, G. Sattler, M., and Macias, M.J. (in preparation) ^{1}H, ^{15}N and ^{13}C resonance assignments, secondary structure and binding specificity of the tandem WW domains from the splicing factor Prp40 from Saccharomyces cerevisiae.

3. Wiesner, S., Stier, G. Sattler, M., and Macias, M.J. (in preparation) High-resolution structure of the tandem WW domains of the splicing factor Prp40 from Saccharomyces cerevisiae.