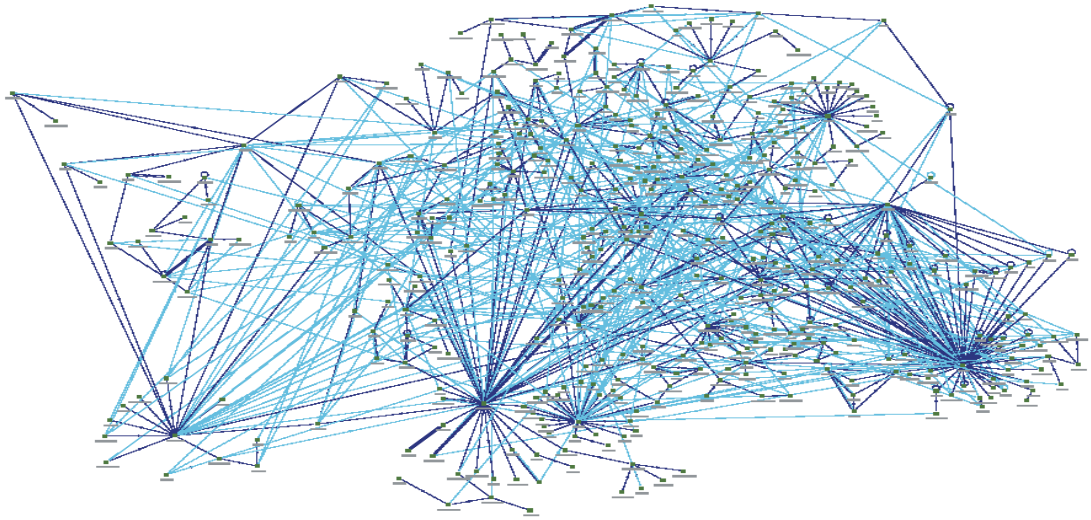
## Budding Yeast Protein Interactome

K. Ota, K. Kito, H. Kubota, Y. Yamaguchi, R. Matsuo, T. Chiba, K. Sakuraba, & T. Ito

We established a system for comprehensive two-hybrid analysis to examine all possible binary interactions between the 6,000 open reading frames encoded in the budding yeast genome. Consequently, 4,549 two-hybrid interactions, including 841 with higher reliability, were identified and made available from our web-site (http://genome.c.kanazawa-u.ac.jp/Y2H/). This is the very first protein interactome data, which have been providing numerous leads for novel findings in yeast cell biology and have also evoked new bioinformatics on protein interaction networks shown below.



We next intended to assign biological roles to the catalogued interactions. Toward this goal, we are taking two strategies, namely “interaction profiling” and “interaction targeting”. For interaction profiling, we pursue a combinatorial use of quantitative mass spectrometric analysis with isotopic labeling and tandem affinity-tag purification of protein complexes. As a variant of interaction profiling, we are also trying to profile polyubiquitinated proteins by means of a unique parallel affinity-tag purification approach. For interaction targeting, we are developing methods for “two-hybrid footprinting” and “guaranteed reverse two-hybrid screening” to map interaction domains and isolate interaction-defective alleles, respectively. These techniques have been successfully applied to the analysis of protein interactions involved in stress-responsive translational control as well as cell polarity establishment.