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Dynamical Analysis of Yeast Protein Interaction Network During the Sake Brewing Process[§]

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Proteins interact with each other for performing essential functions of an organism. They change partners to get involved in various processes at different times or locations. Studying variations of protein interactions within a specific process would help better understand the dynamic features of the protein interactions and their functions. We studied the protein interaction network of *Saccharomyces cerevisiae* (yeast) during the brewing of Japanese sake. In this process, yeast cells are exposed to several stresses. Analysis of protein interaction networks of yeast during this process helps to understand how protein interactions of yeast change during the sake brewing process. We used gene expression profiles of yeast cells for this purpose. Results of our experiments revealed some characteristics and behaviors of yeast hubs and non-hubs and their dynamical changes during the brewing process. We found that just a small portion of the proteins (12.8 to 21.6%) is responsible for the functional changes of the proteins in the sake brewing process. The changes in the number of edges and hubs of the yeast protein interaction networks increase in the first stages of the process and it then decreases at the final stages.

***Keywords*:** protein interaction network, dynamical analysis, sake brewing, hubs, *Saccharomyces cerevisiae*

Proteins play essential roles in performing various biological functions of a cell. The most important to know are the basic cellular processes that they perform within each cell. Since the first complete DNA sequence of the yeast *Saccharomyces cerevisiae* was released in digital form (Goffeau *et al*., 1996), yeast has been widely used to study basic cellular processes. Studying Protein Interaction Networks (PINs) on a genome-wide scale has also been possible through advances in high-throughput experimental research. These experiments have generated large amounts of interaction data for several species including *S. cerevisiae* (Uetz *et al*., 2000; Ito *et al*., 2001; Ho *et al*., 2002; Gavin *et al*., 2006; Krogan *et al*., 2006), *Escherichia coli* (Butland *et al*., 2005), *Drosophila melanogaster* (Giot *et al*., 2003), *Caenorhabditid elegans* (Li *et al*., 2004), and *Homo sapiens* (Rual *et al*., 2005; Stelzl *et al*., 2005). The corresponding PINs are accessible through databases such as IntAct (Hermjakob *et al*., 2004), DIP (Salwinski *et al*., 2004) and BioGrid (Breitkreutz *et al*., 2003).

Many researchers have reported their results on the topological properties of PINs in yeast (Schwikowski *et al*., 2000; Hazbun and Fields, 2001; Jeong *et al*., 2001; Maslov and Sneppen, 2002). They mainly concentrated on characteristics of connections. They found that protein interaction networks follow the small world property. Cho and Wolkenhauer (2005) have studied system biology with the aim of unraveling the dynamic behavior of biochemical networks. Li and Jia (2006) studied the evolution of PINs in the yeast nucleus. They introduced a dynamic model for evolution of yeast PINs. Vázqueza *et al*. (2003) introduced a graph-generating model for representing the evolution of PINs. For modeling the evolution, they used the gene duplication and divergence hypothesis.

In a previous study, we proposed a method to identify non-hubs, intermediately connected, party hubs and date hubs of yeast PINs (Mirzarezaee *et al*., 2010). In this paper we take a step in studying dynamical changes of yeast PINs in the sake brewing process. During this process, rice starch is saccharified by enzymes produced by koji (*Aspergillus oryzae*), and the resultant glucose is fermented to ethanol by sake yeast (*Saccharomyces cerevisiae*). In the sake brewing process, yeast cells are simultaneously exposed to various stresses including hypoxia, low temperature, low pH, high osmotic pressure, and an increasing concentration of ethanol. Analysis of yeast protein interactions during the sake brewing process would be helpful for understanding how proteins adapt to these multiple stresses. It would also be useful to determine which processes are accompanied by stresses and thus should be manipulated to improve the fermentation performance of yeast cells in the sake mash (Wu *et al*., 2006).

In the present study, we used gene expression profiles obtained from DNA microarray analysis (Wu *et al*., 2006) to study the dynamical changes of the yeast PIN during the sake brewing process.

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Materials and Methods

Yeast protein interaction network

The protein interaction network of yeast was downloaded from the BioGrid website on Sep. 25, 2010. The downloaded file contains all curated interaction data processed prior to this date and reflects the most recent data to date separated by organism. In the downloaded file 243,055 interactions between 6,035 yeast proteins were reported.

Gene expression levels for yeast proteins in different stages of the sake brewing process

Gene expression profiles for yeast proteins at nine different stages of brewing were extracted and studied by Wu *et al*. (2006). They provided raw and normalized gene expression profiles of 6,349 yeast genes in the supplementary materials of their paper. We used that data to study the changes of yeast protein interactions during the sake brewing process.

Wu and his colleagues sampled the sake proteins during nine different stages of the brewing process. The brewing process, as they explained, consists of growth and the main fermentation phases. They used DNA microarray analysis to analyze the global gene expression profile of yeast cells during the sake brewing process. Yeast cells were cultured in YPD medium and sampling was carried out nine times during the brewing process (S2, N1, T1, T2, T3, T5, T8, T11, and T14). Total RNA was extracted from 3 to 5 g of yeast cells by the hot phenol method and the GeneChip yeast genome S98 array was used as the microarray in that study (Wu *et al*., 2006).

Mapping sake brewing and BioGrid protein names

The BioGrid database contains 6,035 proteins, while gene expression of yeast proteins is provided for 6,349 proteins. There were 314 proteins that are involved in the sake brewing process but whose names did not exist in the BioGrid database. There were 355 proteins that we could not match with names in either of the databases. As an example, the gene expressions of *YPR195C* and *YPR197C* that were provided in the supplementary materials of Wu *et al*. (2006) are dubious Open Reading Frames (ORFs) that are unlikely to encode proteins or functional proteins. That may be the reason why they do not exist in the BioGrid protein interaction data sets. In total, 669 protein names from this supplementary data could not be mapped to any of the BioGrid yeast proteins. Also 580 proteins from the BioGrid database did not have corresponding gene expression profiles in the data downloaded from Wu *et al*. (2006). We have omitted these proteins from our study. All the other proteins are fully mapped to their BioGrid correspondence. We did our experiments in both the original BioGrid PIN and the PIN where these 580 nodes were omitted.

Pruning the yeast protein interaction network in different stages of the sake brewing process

PINs of yeast were pruned based on available gene expression data during different stages of the process according to the following procedure: the variants of gene expressions for proteins in these nine stages were considered and if the gene expression of a protein was below a predefined threshold during any of the nine stages, we assumed that the corresponding protein was not expressed in that stage, thus all of its interactions with other proteins of the network (based on the BioGrid DB) were omitted in that stage.

Cut-off methods for identifying active and inactive genes during different stages of brewing

We calculated the cut-off values from gene expression data using seven different methods. We used *average*, *average – variance*, *average + 0.5variance*, *average + variance*, *average + 1.5variance*, *median*, and a *correlation-based method* for identifying a cut-off value for presence or absence of a gene. This means that if gene expression of a protein was above the calculated threshold, that protein was active in that stage, otherwise it was inactive and all of its interactions with the rest of the network were omitted in that stage.

Average cut-off method

In finding the cut-off values using the *average* method, we calculated average gene expression profiles of each gene during the nine stages of the brewing process. We used these calculated averages as cut-off values. For each stage of the brewing process, we compared the expression profile of each gene with its average within these nine stages. If the gene expression value in that stage was above the average of its values over the whole process, we considered the gene active, otherwise we considered it inactive.

Average-variance cut-off method

In finding the cut-off values using the average-variance method, we calculated average and variance of gene expression profiles for each gene during the nine stages of the brewing process. For each stage of the brewing process, we compared the expression profile of each gene with its *average minus variance* of its gene expressions over these nine stages. If the gene expression of the protein in that stage was above that threshold, we considered the gene active, otherwise we considered it inactive.

Cut-off values for *average + 0.5variance*, *average + variance*, *average + 1.5variance* were calculated in a similar manner.

Median cut-off method

In finding the cut-off values using the median method, we calculated the median of gene expression profiles for each gene during the nine stages of the brewing process. We used these calculated values as cut-off values. For each stage of the brewing process, we compared the expression profile of each gene with its median within these nine stages. If the gene expression value in that stage was above the median of its values in the whole process, the gene was active, otherwise it was inactive.

A correlation based cut-off method

This method consists of the following steps:

1. The sum of all nine expressions for each gene was calculated.
2. Correlation of each gene expression with sum was calculated.
3. Nine calculated correlations were divided into the sum of correlations and called w_is.
4. The cut-off value was computed according to the following formula:

$$m_j = \sum w_i \times gene - \exp_i \quad (1),$$

where *j=1:gene numbers* and *gene-exp*$_i$ stands for the expression of a gene in stage i of the brewing process. m contains j thresholds for each of the genes of the network. In each stage of the brewing process, we compared the expression profile of each gene with this threshold. If the gene expression value during that stage was above this value, the gene was active in that stage otherwise it was inactive.

Graph-related parameters

We calculated twelve graph-related parameters that Platzer *et al*. (2007) introduced in their paper. We used these parameters to calculate their values during different stages of the sake brewing process. We calculated the parameters of closeness, graph diameter, index of aggregation, entropy of distribution of edges, connectivity, number of edges divided by the number of vertices, entropy, graph centrality, sum of the Wiener number, modified vertex distance number, and Eigen values as defined by Platzer *et al*. (2007). More information on these parameters and their formulas can be found in Platzer *et al*. (2007).

Dynamical changes of interactions and hubs during different stages of brewing

The number of hubs and interactions of the constructed PINs during the nine different stages of sake brewing were calculated to see how proteins changed their interaction patterns during this process.

The number of interactions is the sum of all the interactions inside the whole protein interaction network. We assumed that if protein A has interaction with protein B, protein B also interacts with protein A. Therefore, the number of actual interactions calculated based on the sum of all network edges was divided by two.

Hub proteins are the proteins with the most interactions within the protein interaction networks. Usually proteins with more than eight interactions are called hubs (Komurov and White, 2007). We used this definition to separate hubs and non-hubs of the yeast for these nine stages. To identify low connectivity and high connectivity, hubs with eight to ten interactions and hubs with greater than ten interactions were also separated. Proteins with eight or fewer interactions were assumed to be non-hubs.

Behavior of hubs and non-hubs in the sake brewing process

To study how proteins and, especially hubs, change their partners during the sake brewing process, we classified proteins into five behavioral classes: Hubs, NHubs, Hubs-NHubs, NHubs-Hubs, and Hubs-NHubs-Hubs / NHubs-Hubs-NHubs as shown in Fig. 1. This categorization was done as follows.

Interactions of yeast proteins in the nine different stages of the brewing process were counted separately for each protein. Then hubs and non-hubs of each stage were identified. This way, a sequence of the nine stages for each of the yeast proteins was constructed. We classified the proteins into five classes based on these sequences, as shown in Fig. 1. In this figure, H stands for Hub and NH for Non-Hub respectively. Percentages of the proteins belong to each class were then specified.

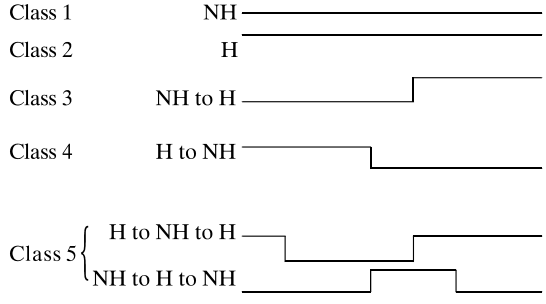

Fig. 1. Classification of yeast proteins to five classes based on their changes during different stages of sake brewing.

In this classification, if there was only one stage among the nine stages in which a transition from hub to non-hub or from non-hub to hub happened, this transition was ignored and it was assumed to be the result of unavoidable noise in PIN construction for different stages.

Identifying static and dynamic hubs of the yeast protein interaction network in the sake brewing process

We calculated variance of gene expressions for each protein in the nine different stages of the brewing process. If gene expression values for a protein varied significantly, it was assumed that this protein was dynamic, otherwise it was static (Komurov and White, 2007). This way, we labeled yeast proteins as static or dynamic and especially static and dynamic hubs of this process were identified.

We used three different cut-off methods for separating static and dynamic proteins of the brewing process. All of the methods used the gene expressions to establish a good cut-off value.

We calculated the cut-off value for separating static and dynamic proteins using three different correlation-based methods, histogram of variance of the gene expressions and histogram of variance divided by the average of gene expression values.

We also studied the variance of gene expressions among neighbors of static and dynamic nodes of a PIN.

Correlation based cut-off method

The procedure for calculating this threshold was the same as the one explained for cut-off methods for identifying active and inactive genes. The only difference was that each gene was assumed to be static if the variance of its gene expressions during these nine stages of the brewing process was below the calculated threshold. Otherwise, it was assumed that the gene was dynamic in the whole brewing process.

Cut-off method based on histogram of the data

In this method, the histogram of the gene-expression variances and gene-expression variances divided by their averages was plotted for all the genes. We cut the two histograms at their 95% value where the plotted figure was almost smooth. This cut-off points at a value where proteins with gene expressions greater than that were assumed to be dynamic and others to be static proteins of the brewing process. In these two methods, one cut-off value for all of the genes was computed.

Results

Dynamic analysis of PINs of organisms during different processes can give good insights into understanding how proteins collaborate in the specific process. We studied the dynamic changes of the yeast PIN within the sake brewing process. We downloaded the yeast PIN from the BioGrid website. We used the expressions of yeast genes during the nine stages of sake brewing (Wu *et al*., 2006) to study the dynamical changes of its PIN. We used the gene expressions to prune the original PIN. This way, nine different PINs, representing PIN structure in corresponding stages of the sake brewing process, were constructed. For this purpose, we used different methods of cut-off calculation to identify the active and inactive genes as described in 'Materials and Methods'. Active genes were the genes whose expressions were above a specified threshold.

Table 1. Number of proteins in each stage based on different cut-off methods

Sampling time/Cut-off method	S2	N1	T1	T2	T3	T5	T8	T11	T14
Avg. – Var.	4089	4745	6064	6312	5905	5605	5286	2855	1993
Median	2412	3208	5552	6145	5439	4011	3208	1221	551
Avg.	1753	1931	5021	5886	4629	2827	2237	833	367
Correlation based	1686	1826	4960	5878	4575	2596	2098	784	339
Avg. + 0.5Var.	1281	1051	4160	5503	3421	1723	1698	625	266
Avg. + Var.	980	607	2845	5018	2211	1016	1310	469	179
Avg. + 1.5Var.	682	354	1447	4026	1293	544	1021	328	118

To analyze the best cut-off values for identifying active and inactive PIN genes, seven different methods for identifying cut-off values for the gene expressions, including *average*, *median*, *average – variance*, *average + 0.5variance*, *average + variance*, *average + 1.5variance*, and a *correlation based method*, were applied. The number of active genes (proteins) for each stage was calculated and the results are shown in Table 1. More details on these results are provided as supplementary materials I. It can be inferred from Table 1 that the *average + 0.5variance*, *average + variance*, and *average + 1.5variance* are not good cut-off candidates because they omit many proteins of the network at each stage.

Analysis of protein interaction networks as graphs

Nine PINs obtained from four of the introduced cut-off methods, including average-variance, median, average and a *correlation*-based method were constructed and the trends of changes within their graph-related parameters were studied using twelve graph-related parameters: closeness, graph diameter, index of aggregation, entropy of edge distribution, connectivity, number of edges divided by the number of vertices, entropy, graph centrality, sum of the wiener number, modified vertex distance number, and Eigen values as introduced by Platzer *et al*. (2007).

As mentioned, there were 580 proteins from the BioGrid data set whose gene expressions were not studied by Wu *et al.* (2006). We constructed two sets of networks, one with these 580 proteins and one without them. We calculated the graph-related parameters for these two sets of networks. The results are shown in Figs. 2 and 3.

As shown in Figs. 2 and 3, the trends of changes within the two sets of networks were the same. The parameters' values for the introduced correlation-based method had the lowest values among the four methods, except for the closeness cen-

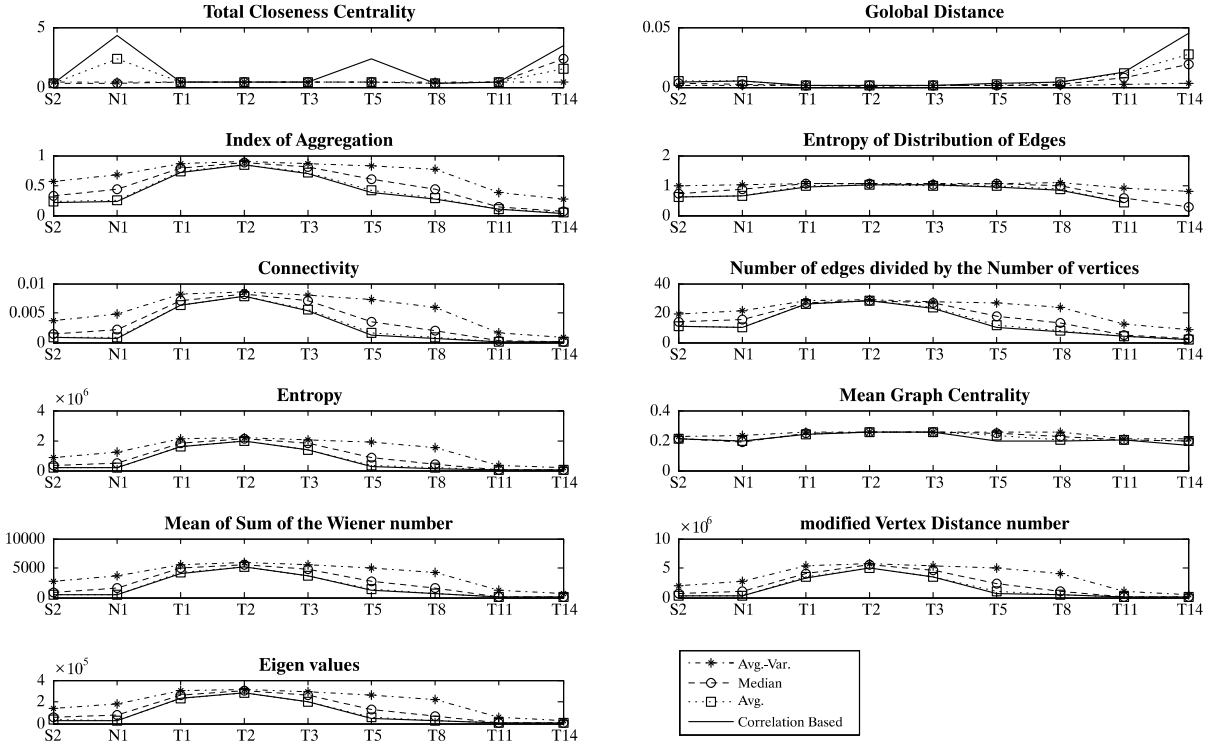

Fig. 2. Graph parameters for nine different stages of sake brewing when 580 genes are omitted.

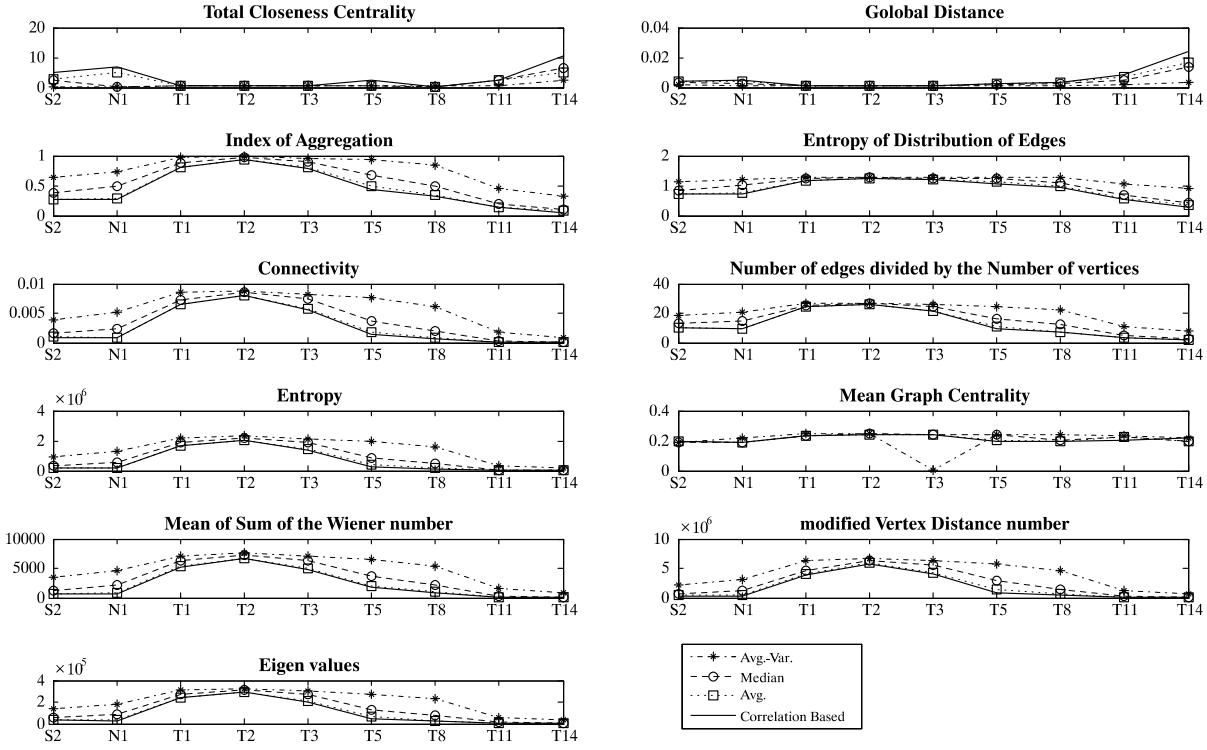

Fig. 3. Graph parameters for nine different stages of sake brewing when all the proteins are included.

trality parameter, and thus the corresponding dynamic networks constructed using the proposed correlation-based cut-off method showed very smooth variations. Correlation and average methods had very similar results, which showed the closeness of these methods. The important point in calculating the graph-related parameters based on the four proposed methods is that the trend of changes for these network parameters was almost the same in the proposed cut-off methods and the only difference was in their values, which was because of the number of available genes or proteins in the PINs using a specific cut-off method.

The closeness centrality parameter values showed different characteristics with different cut-off methods. For the *average-variance* method, this parameter showed a peak value at T1 and its value decreased within the remaining stages of the process. In the median method, the value of this parameter was almost the same in all the stages except for the last, T14. For the average cut-off method this parameter had two peak values, one in N1 and one in T14. In the proposed correlation method, this parameter had three peak values, one each in N1, T5, and T14. Its value in the other stages of this process was almost the same. As these variations cannot be fully understood from Fig. 2, the closeness parameter values for the networks with the omitted 580 genes are shown in Table 2. Results in Figs. 2 and 3 show that the graph diameter of the network within this process is increasing. The mean of the graph centrality parameter was also a special case. Its value increased within the sake brewing process to a point in the middle stages of the process. It remained almost the same for some stages and after that its value decreased in the final stages of the brewing process. This parameter was the only one that showed different variations when the 580 genes with unknown gene expressions existed in the network. In this case, the value of mean graph centrality decreased dramatically in T3 when the *average-variance* was used as the cut-off method.

The other eight studied parameters of the network showed the same trend in the whole process, which means that their values increased to a stage in the middle of the brewing and after that their values decreased.

Analysis of edges and hubs in different stages of sake brewing

We studied the changes of the protein interaction network during the sake brewing process based on the total number of edges, hubs and non-hubs and their variations within the stages of this process.

Table 2. Total closeness centrality parameter values for the networks with 580 omitted genes in different stages of sake brewing and for different cut-off methods

Cut-off method/stages	*Avg. – Var.*	*Median*	*Avg.*	*Corr. Based*
S1	0.373	0.3682	0.3599	0.3582
N1	0.377	0.3643	2.3437	4.3422
T1	0.4154	0.3900	0.3907	0.3916
T2	0.4150	0.4151	0.4143	0.4143
T3	0.4151	0.4150	0.4138	0.4136
T5	0.4137	0.3992	0.3865	2.3395
T8	0.4099	0.3694	0.3551	0.3534
T11	0.3986	0.3853	0.3867	0.3865
T14	0.3894	2.3574	1.5270	3.4449

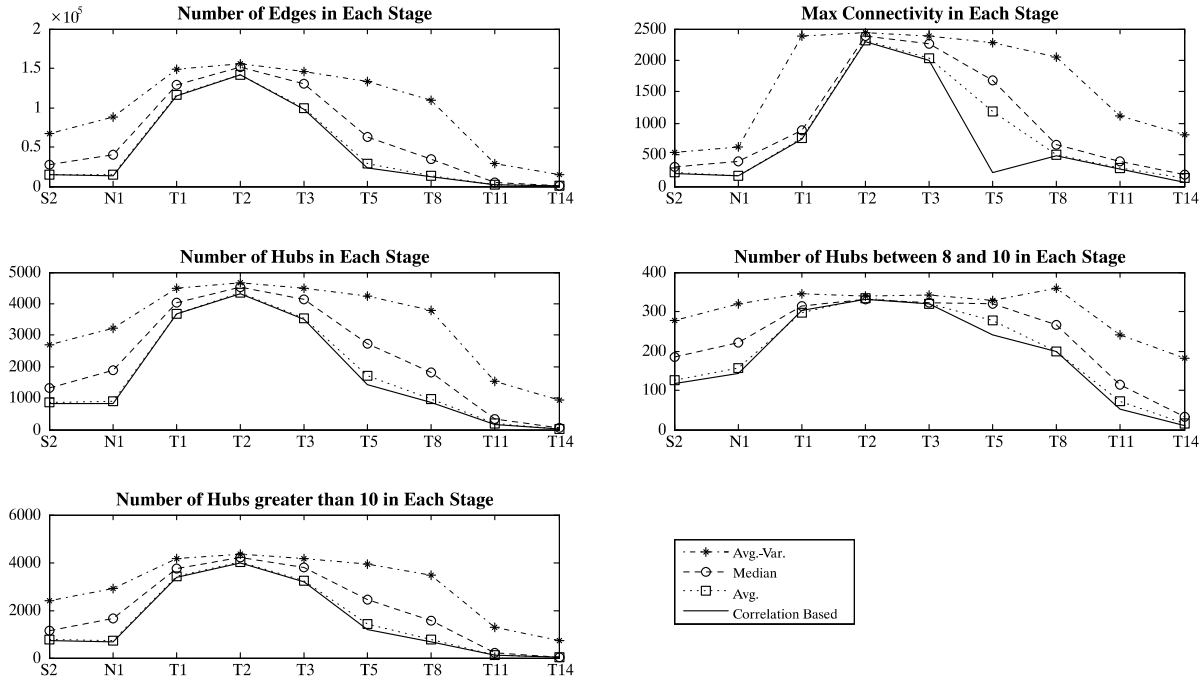

Fig. 4. Number of edges and hubs for nine different stages of the sake brewing process when 580 genes are omitted.

As the results in Figs. 4 and 5 show, the number of edges increased from the first stage of the brewing process to the middle of process and then it decreased.

The same trend was observed for the number of network hubs, *i.e*. proteins with more than eight connections. This means that in the first stages of the process, more protein interactions and hubs were observed. The number of available interactions and hubs increased to a maximum value in one stage of the process. After that, they showed a reduction to almost the same or lower values as the beginning of this process. This means that some of the protein interactions and hubs of the network became more active up to the middle

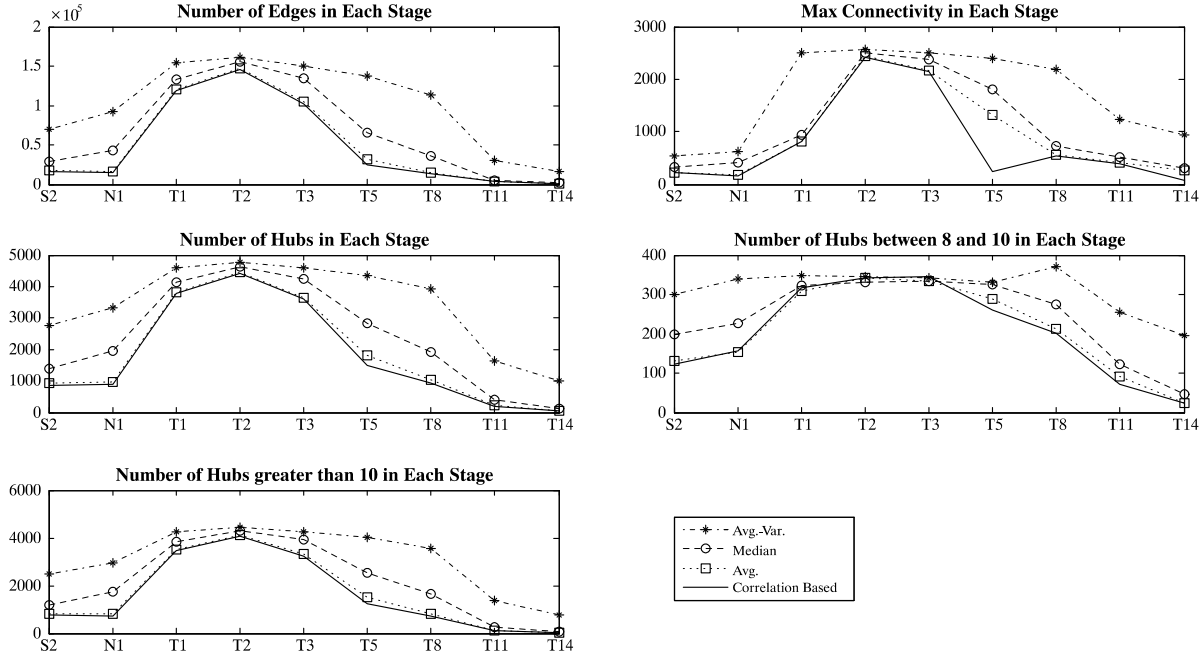

Fig. 5. Number of edges and hubs for nine different stages of the sake brewing process with all the proteins included.

stages of the process and after that some of the others or the same interactions and hubs were not needed any more, and they again became inactive at the final stages.

This result is reasonable because the yeast proteins are processed to produce a new material and the interactions among those proteins should have some changes so that they can perform the new functions for which they are now responsible.

Classifying yeast proteins based on their dynamic behaviors

To analyze how the number of hub interactions changed from one stage of the brewing to another stage, we classified the yeast proteins into the following five different classes:

- Hubs (H)
- non-hubs (NH)
- hubs to non-hubs (H-NH)
- non-hubs to hubs (NH-H)
- hubs to non-hubs to hubs or non-hubs to hubs to non-hubs (H-NH-H, NH-H-NH).

The result of this classification is shown in Table 3. More details on these results and protein behavioral classifications are provided as supplementary materials II. As the results in Table 3 show 20.7-to 24.2% of the proteins were hubs, i.e. they had more than eight interactions, and they also keep their number of interactions within that process.

None of the studied proteins that had below eight interactions kept their number of interactions during the sake brewing process. Hub or non-hub classes identify the proteins that were hubs or non-hubs and they remained hubs or non-hubs during the whole process. These classes encompass the proteins for which the number of interactions do not change significantly during the sake brewing process, which means that they might have routine functions, without any special changes during this process.

The third class of proteins contained the ones that had greater than eight interactions, but in one of the brewing stages, their number of interactions decreased to a number below eight and its number of interactions remained under that threshold after that stage until the end of the sake brewing process. This class contained at most 1.6% of all the yeast proteins.

The fourth class of proteins contained the ones for which the number of interactions was below eight, but in one of the stages of the process it increased to a number above eight and its number of interactions remained above that threshold after that stage until the end of the sake brewing process. This class contained 12.6 to 20.5% of all the yeast proteins, based on the used cut-off method. Classes three and four of the proteins showed the fraction of proteins whose interactions changed significantly during this process and they might be the proteins that have the main responsibility for changing the cell functions during the sake brewing process.

The fifth class contained the proteins whose number of interactions increased from eight but after one or more stages, their number of interactions decreased to below eight, and also the proteins that had above eight interactions but whose number of interactions dropped below eight and then, after one or more stages, their number of interactions increased to a number above eight. The fifth class of the proteins may contain the fraction of proteins whose partners change during this process and then they go back to their original states. This class contained between 54.5 to 62.6% of all the yeast proteins based on the used cut-off method.

From the results, it can be inferred that the proteins that most took part in the brewing process are the ones whose functions changed significantly during this process. In other words, the proteins that lost their previous interactions or gained more interactions during the process are the ones with more variety in their functionality and they might have more responsibilities for transforming the yeast cell to the final product. This group contained the proteins of class three and four which are just between 12.8 to 21.6% of all the yeast proteins. This means a small fraction of the yeast proteins were responsible for most of the changes during the sake brewing process, which is an interesting result.

It should have been mentioned that in the above classification, one slight change from hub to non-hub or non-hub to hub was ignored as noise in the available data. This way, almost all of the yeast proteins were classified in one of the five classes and only about 2% of the proteins did not belong to any of the above-mentioned groups, based on the used cut-off methods.

Static and dynamic proteins in the sake brewing process

We studied the variations of gene expressions during the nine stages of brewing to identify static and dynamic yeast proteins of this process (Komurov and White, 2007).

We used two different methods of correlation and a histogram for separating them as explained in 'Materials and Methods'. Depending on the cut-off method, different numbers of static and dynamic hubs were identified. We have also classified the proteins into hubs and non-hubs based on the number of their interactions in the BioGrid dataset. A complete list of the yeast protein labels in the sake brewing process

Table 3. Percentages of proteins belonging to each of five behavioral classes

Protein classes/Cut-off method	H%	NH%	H-NH%	NH-H%	H-NH-H, NH-H-NH%	Others%
Networks without 580 genes omitted						
Median	20.73	0.40	1.06	20.55	54.90	2.37
Average	22.07	0.35	0.60	13.87	61.87	1.24
Corr. Based	22.37	0.31	0.45	13.19	62.63	1.04
Networks with 580 genes omitted						
Median	22.49	0.00	0.83	19.83	54.50	2.34
Average	23.88	0.00	0.40	13.29	61.26	1.16
Corr. Based	24.21	0.00	0.23	12.58	61.99	0.99

are provided as supplementary materials III.

Ekman *et al*. (2006) classified proteins as non-hubs, intermediately connected, party hubs and date hubs based on their number of interactions and the co-expression profiles from five different conditions of stress response (Gasch *et al*., 2000), cell cycle (Spellman *et al*., 1998), pheromone treatment (Roberts *et al*., 2000), sporulation (Chu *et al*., 1998) and unfolded protein responses (Travers *et al*., 2000). They calculated the average PCC between each hub and its interaction partners for these five conditions and the combinations of them. They defined party (static) hubs as proteins that show high average PCC with their interacting partners in contrast to date (dynamic) hubs.

We compared the static and dynamic hubs obtained from our experiments on the sake brewing process with that of Ekman *et al*. (2006). Some of the proteins have similar labels in both studies; some of the others have been differently labeled. For example, *YEL009C* is classified as a date or dynamic hub in five conditions studied by Ekman *et al*. (2006) but this protein shows a static behavior in the sake brewing process. It may be concluded that the proteins show different reactions in response to different conditions. Therefore, the static and dynamic proteins of each process can be determined specifically for that studied process. What Ekman *et al*. (2006) reported were the average reactions of those proteins in five special conditions.

The relation of static and dynamic proteins with their neighbors was also studied. This study showed that the variance of gene expressions of a protein in most cases was smaller than the mean of variance of their neighboring partners in the sake brewing process. From the 6,035 studied proteins/genes, 1,906 proteins had gene expression variances greater than the mean of their neighboring proteins' variance. Neighboring proteins are the proteins that have interactions with the studied protein. The proteins with variance zero are the ones for which we do not have gene expression data in the studied sake brewing process.

Discussion and Conclusions

In this paper, we studied the proteins of *S. cerevisiae* during the sake brewing process to analyze how their functions may change during this process. The methods that are used here are quite general, and are applicable to other organisms or processes as well.

We studied the changes in the number of hubs and edges of the yeast PIN during the sake brewing process. We also calculated some of the graph- related features of the PINs to see how they were modified during this process. We studied two types of hubs called static and dynamic hubs based on the variance of gene expressions of the yeast proteins in these nine stages. The trend of changes in neighbors of static and dynamic hubs were also studied.

It was found that just a small fraction of the proteins (12.8 to 21.6%) were mainly responsible for the functional changes that happen during the sake brewing process and the number of edges and hubs of the yeast PINs increased in the first stages of the process and then decreased during the final stages.

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