**TÜBİTAK 110M692**

**Project Title :** Investigation of the effect of caffeine and rapamycin on lifespan and life quality using a system based approach


**Abstract**

Rapamycin is an immunosuppressive and anti-proliferative antibiotic that targets and inhibits the TOR kinase. It has been suggested that rapamycin mimics a signal generated by the amino acid starvation but that the signal is not likely to be indicating the absence of amino acids themselves. Rapamycin (Rapamune) has been approved as an immonusuppressant in 1999 by the United States Food and Drug Administration (FDA) and in 2000 by the European Commission (EC). Although the United States National Cancer Institute (NCI) has identified rapamycin to be effective against mass tumors possessing an anti-tumor activity, the progress in its use in cancer therapy has been slow due to the problems that have been encountered in the preparation of the composition and the stability of the drug. Although rapamycin is an immunosuppressant, which is frequently used in organ transplant patients, it is also preferred in anti-tumor therapies owing to its anti-angiogenetic properties and the relation between these two contradictory properties has not yet been elucidated. In addition to rapamycin, a number of other pharmacological agents have been shown to affect TOR, particularly in mammalian cells, such as caffeine. Caffeine has been shown to be involved in various cellular processes related to cellular growth, DNA metabolism, and cell cycle progression.

The target of rapamycin (TOR) kinase is an important regulator of growth in eukaryotic cells. The TOR signaling pathway, conserved from yeast to human, is involved in nutrient and growth factor signaling. Signaling through TOR kinase pathways regulates the nuclear localization of several transcription factors in response to the carbon and nitrogen sources in the nutritional environment. It is known that TOR-signaling pathway has roles in both glucose and nitrogen metabolisms, and this pathway acts both on transcriptional and posttranscriptional processes. The set of genes regulated by TOR affect cellular growth associated processes such as translation and nutrient utilization.

Sucrose non-fermenting 1 (Snf1p) protein kinase is a key component in the cellular response to fluctuations in the levels and the quality of the carbon source in the surrounding media. Snf1p belongs to a group of remarkably conserved Serine/Threonine kinase family that exists in all eukaryotes ranging from yeast, worm, fruit fly, plant and mammals. Snf1p (yeast) and AMPK (human), which play a central part in the regulation of energy metabolism and trancriptional mechanisms, interact with several regulatory pathways. It is known that the rapamycin-sensitive TOR negatively regulates SNF1 and the activation of Snf1p or the inhibition of Tor1p extends the life span of yeast and human. It has been conclusively shown in mammals that AMPK inhibits the mammalian TOR complex-1 but the hierarchy of the regulation between Snf1 and Tor1 in yeast remains elusive. PKA regulates cellular growth through transcriptional regulation. In addition to this PKA and Snf1p share the regulation of many processes such as the carboxylic acid metabolism, β-oxidation of fatty acids, stress response and filamentous growth but under different conditions since they are activated by excess glucose or by its depletion respectively. On the contrary, PKA and Tor1p are both active in response to the nutritional stimuli (glucose and nitrogen) thereby functioning in parallel. The apparent complexity and the high connectivity of these interacting signaling pathways and regulatory networks makes systems biology a very well suited approach for such studies.

The aim of this proposed project is the elucidation of processes including the nutrient metabolism, growth, cellular aging, lifespan in yeast as well as the regulation of the response to chemotherapeutic agents, in which the TOR pathway and the Snf1p are significant constituents. In the first step of this project, the concentrations of the rapamycin and the caffeine providing similar cellular growth response in yeast, which is a model system for higher organisms, will be determined in controlled and/or relaxed environmental conditions. In the next step, the effect of the pre-determined drug doses on the metabolic fluxes using genome scale metabolic models will be comparatively investigated and the transcriptome data generated using microarray analysis will be used to elucidate the similarities and the differences between the presence and absence of the drugs in the wild type metabolism. In the second step, in order to elucidate the relationship between the TOR pathway and the SNF1 gene, it is planned to run fully controlled batch fermentations in carbon/nitrogen limited and non-limiting media containing caffeine and rapamycin using the wild type strain and the deletion mutant of the SNF1 gene. As a result, the analysis of the transcriptome data and the integration of this data with metabolic information and flux predictions will lead to the elucidation of relationships between TOR signaling pathway and Snf1 and also the molecular mechanisms regarding lifespan, aging, cellular growth and nutrient sensing. This information will provide a basis for the construction of a model for higher organisms, specifically humans. The conducted studies will constitute a partial requirement of a PhD thesis and a basis for two MS theses.

**Keywords:** Life Span, Growth, Caffeine, Rapamycin, Whole Genome Stochiometric Models, Flux Balance Analysis, TOR pathway, Saccharomyces cerevisiae, Microarray Analysis, SNF1