Name of the Candidate:	**Md. Jahoor Alam**
Name of the Supervisor:	**Dr. R. K. Brojen Singh**
Centre:	**Centre for Interdisciplinary Research in Basic Sciences**
Title of the Thesis:	**Molecular Communication: Stochasticity and Synchronization in the Dynamics of p53 Pathway in Stress Cells.**

Abstract

p53 is found to be an important and key tumour suppresser protein which is either lack or frequent mutated in most of the human cancer cell. In normal cell, it induces due to the several types of cellular stress such as Dna Damage, Hypoxia, rNTP depletion, Spindle damage, Oncogenic activation, stress generated due to several virus inclusion etc. In the present work we have studied how the p53 stability is the affected, due to several stress inducer molecules, at molecular level. A system biological approach is used to understand the impact of the cellular stress on p53.

The integration of calcium and p53-MDM2 oscillators' model is studied using deterministic as well as stochastic approaches to investigate the impact of calcium wave on single cell dynamics and on inter-oscillators interaction. The high dose of calcium in the system activates nitric oxide synthase to synthesize nitric oxide which then down regulates MDM2 and influences drastically p53-MDM2 network regulation, lifting the system from normal to stressed state. The increase in calcium level switches the system to different states as identified by different behaviours of p53 temporal dynamics i.e. oscillation death to sustain oscillation state via a mixed state of damped and oscillation death states. Further increase of calcium dose in the system switches the system from sustained to oscillation death state again, while an excess of calcium shifts the cell to apoptotic state. Another important property of calcium ion is its ability to behave as synchronizing agent among the interacting systems. The time evolution of p53 dynamics of two diffusively coupled systems at stress condition via Ca^{2+} shows synchronization between the two systems. The noise contained in the system interestingly helps the system to maintain its stabilized state (normal condition). However, noise has tendency to destruct the synchronization effect which means that it tries to restrict the system from external signals to maintain its normal condition. However, at stress condition, synchronization rate is found to be faster.

We study how the temporal behaviour of p53 and MDM2 are affected by stress inducing bioactive molecules NO (Nitric Oxide) in the p53-MDM2-NO regulatory network. In the single cell study, we have found three distinct types of temporal behaviour of p53, namely, fixed point oscillation (or oscillation death), damped oscillation and sustain oscillation, depending on the amount of stress induced by the NO concentration, indicating how p53 responses to the incoming stress. In coupled system with nitric oxide as diffusively coupling molecule, we found nitric oxide as strong coupling molecule within a certain range of coupling strength (ε) beyond which it become weak synchronizing agent. We also study the group of such identical systems arranged in a three dimensional array with nearest neighbour diffusive coupling and found that the systems at near and far distances interact almost instantaneously. The noise in stochastic

system is found to help to reach these states much faster as compared to deterministic case.

We construct a stress p53-MDM2-p300-HDAC1 regulatory network that is activated and stabilized by two regulatory proteins p300 and HDAC1. Different activation levels of p53 observed due to these regulators during stress condition have been investigated using deterministic as well as stochastic approach to understand how cell responds during stress condition. We found that these regulators help in adjusting p53 to different conditions as identified by various oscillatory states, namely fixed point oscillations, damped oscillations and sustain oscillations. On assessing the impact of p300 on p53-MDM2 network we identified three states: first stabilized or normal condition where the impact of p300 is negligible, second an interim region where p53 is activated due to interaction between p53 and p300 and finally the third regime where excess of p300 leads to cell stress condition. Similarly evaluation of HDAC1 on our model led to identification of the above three distinct states. Also we observe that noise in stochastic cellular system helps to reach each oscillatory state quicker than those in deterministic case. The constructed model validated different experimental findings qualitatively.

We investigate the activation of p53 and MDM2 steady state levels induced by a cellular protein MTBP (MDM2 binding protein) under different stress conditions, by using MTBP-MDM2-p53 regulatory network. We have modeled an integrated p53-MDM2 autoregulatory model (Proctor and Gray, 2008) including the effect of MTBP which is allowed to bind with MDM2 (Brady et al., 2005). Our simulation results in three approaches namely deterministic, Chemical Langevin equation and stochastic simulation of Master equation show a clear transition from damped limit cycle oscillation to fixed point oscillation during a certain time period with constant stress condition in the cell. This transition is the signature of transition of p53 and MDM2 levels from activated state to stabilized steady state levels. We present various phase diagrams to show the transition between unstable and stable states of p53 and MDM2 concentration levels and also their possible relations among critical value of the parameters at which the respective protein level reach stable steady states. In the stochastic approach, the dynamics of the proteins become noise induced process depending on the system size. We found that this noise enhances the stability of the p53 steady state level.

We present an integrated model of p53-MDM2-Glucose to explain the dynamics of the system induced by ARF (alternative reading frame) protein which is activated due to DNA damage caused by ROS (reactive oxygen species) via diffused glucose molecules in the system. The concentration level of glucose in the system triggers the system at different states: from fixed point oscillation to sustain oscillation, sustain oscillation to damped oscillation and damped oscillation to oscillation death regimes corresponding to various cellular states. We also found that the noise measured in terms of system size (V) in a stochastic system tries the system to stabilize more effectively. The phase diagram in (T_s-k) plane shows different regimes of system states induced by glucose level (k) in the system. Further, the phase diagram (A_s-k) indicates the p53 level induced by glucose level in the system and A_s follows power law behavior at the high *k* regime.