Curriculum Vitae

PURANJOY KAR

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Positions/ Employment (in chronological order)

August, 2006 - July, 2009: Graduate Student in Microbiology Honors Course, CU
August, 2009 - June, 2011: Post Graduate Student in Microbiology, WBSU
September, 2011 - December, 2013: Project Fellow (JRF), Dept. of Biochemistry, CU
December, 2013 - December 2015: Junior Research Fellow (NET), Dept. of Biochemistry, CU
December 2015 - 14/08/2018: Senior Research Fellow (NET), Dept. of Biochemistry, CU
17th August 2018 - 18th December 2018: Guest teacher in the Dept. of Microbiology, Vidyasagar University
27th August 2018 - 18th December 2018: Guest teacher in the Dept. of Molecular Biology and Biotechnology, Kanchrapara College
19th December 2018 - Till Date: Public Health Manager under NUHM at Habra Municipality, WBSH&FWS

Education

Examination	Year	Subjects Taken	% marks / Class	Name of the Institution
Master of Science (M.Sc.)	2011	**Microbiology**	73.41 (1st class)	Barrackpore Rastraguru Surendranath College (West Bengal State University)
Bachelor of Science (B.Sc.)	2009	**Microbiology** (Honors), Chemistry(Gen.) and Zoology (Gen.)	64 (1st class)	Dum Dum Motijheel College (University of Calcutta)
AISSCE (CBSE Std. 12)	2006	English, Mathematics, Physics, Chemistry and Biology	77.4	Kendriya Vidyalaya BKP
AISSE (CBSE Std. 10)	2004	English, Bengali, Mathematics, Science and Social Science	83.6	Kalyani Central Model School

Doctor of Philosophy (PhD)

Subject: Biochemistry (Dept. of Biochemistry, University of Calcutta)
Specialization: Molecular Biology & Biochemistry
Title of the thesis: Characterizing the role of Sty1 and Pap1 in *Schizosaccharomyces pombe* under nitrosative stress
Guide: Professor Sanjay Ghosh
Status: Awarded on 29/05/2019

Achievements / Awards

- Qualified in **Graduate Aptitude Test in Engineering- GATE** (2011) in Life Sciences - 15/03/2011

- Qualified in the Joint CSIR- UGC Test for **Junior Research Fellowship** (2013) and **Lectureship (NET)** in Life Sciences under University Grants Commission (UGC), held on 26/06/2013

Professional Membership of Scientific Society

- Life Member, **Society of Biological Chemists**, India. (**Membership No.- 2897**)

Peer Reviewed Publications

First Authorship

- **Transcription factors Atf1 and Sty1 promote stress tolerance under nitrosative stress in *Schizosaccharomyces pombe.***
  Puranjoy Kar, Pranjal Biswas, Sourav Kumar Patra, Sanjay Ghosh.
  Microbiological Research. 2018 Jan; 206: 82-90
  PMID: 29146263

- **Multimodal control of transcription factor Pap1 in *Schizosaccharomyces pombe* under nitrosative stress.**
  Puranjoy Kar, Pranjal Biswas and Sanjay Ghosh.
  BBRC. 2017 Jul 15; 489(1): 42-47
  PMID: 28528978

Co- Authorship

- **Nitric oxide sensing by chlorophyll a.**
  Abhishek Bhattacharya, Pranjal Biswas, **Puranjoy Kar**, Piya Roychoudhury, Sankar Basu, Souradipta Ganguly, Sanjay Ghosh, Koustubh Panda, Ruma Pal, Anjan Kr. Dasgupta
  Analytica Chimica Acta. http://dx.doi.org/10.1016/j.aca.2017.07.026
  PMID: 28864180

- **Nitrosative stress induces a novel intra S checkpoint pathway in *Schizosaccharomyces pombe* involving phosphorylation of Cdc2 by Wee1.**
  Pranjal Biswas, **Puranjoy Kar** and Sanjay Ghosh.
  Free Radical Biology and Medicine. 2015 May 22; 86:145-155.
  PMID: 26006103

Technical Skill and Expertise

- **Microbiological Techniques:** Isolation and identification of bacterial cultures from air, soil and water, Culture of bacteria, yeast and fungal cells, Gram staining, Endospore staining and Negative Staining of bacterial cultures, Antibiotic sensitivity tests, Transformation in bacteria and yeasts, Growth kinetics of bacteria and yeast cells, Cell viability assay using spot tests, Trypan Blue inclusion staining, Calcofluor staining of yeast cells.

- **Biochemical Techniques:** Assay of Redox active enzymes, Estimation of cellular thiols, Quantitative estimation of protein, DNA and RNA.

- **Molecular Biology techniques:** All standard molecular biology techniques, Isolation of Genomic DNA, Isolation of Plasmid DNA, Isolation of RNA, Preparation of cDNA, Semi quantitative PCR and Real Time PCR method.

- **Cell biology techniques:** Fluorescence Microscopy, Flow Cytometry (FACS).

- **Immunological Techniques:** Western Blotting, Immunoprecipitation, ELISA, Ouchterlony Double Immunodiffusion, Blood Grouping.

- **Proteomic Techniques:** Yeast Cell lysate preparation using glass bead lysis method and 2D gel electrophoresis

Seminars/Workshops attended

- Presented a poster entitled " **Multimodal control of Pap1 under nitrosative stress in *Schizosaccharomyces pombe***" at the One day Symposium on "Emerging Trends in Biology "held at Dept. of Biochemistry, University of Calcutta on 17/03/2017

- Presented a poster entitled "**The Sty1, Pap1 and Atf1 play distinct roles in *Schizosaccharomyces pombe* under nitrosative stress**" at 9th International Conference on Yeast Biology held between 9-12 December, 2015 at Kolkata, India

- Participated in a workshop on "**Real Time PCR & It's Applications**" held between 11-12March, 2015 held at Centre for Research in Nanoscience and Nanotechnology, University of Calcutta.

- Participated in an international symposium "**Biotica 2015**" on the 4th of January, 2015held at the Centre for Research in Nanoscience and Nanotechnology, University of Calcutta, Kolkata.

- Participated in a national seminar on the "**National Perspective of Microbiology Research in India**" held during 14-15 March, 2013 at the Department of Microbiology, University of Calcutta, Kolkata.

- Participated in a workshop on "**Intellectual Property and Innovation Management in Knowledge Era**" held on the 4th of June, 2012 at the Department of Biochemistry, University of Calcutta, Kolkata.

References

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Personal Data

Date of Birth	27th May, 1988
Nationality	Indian
Sex	Male
Marital Status	Married
Father's Name	Rabindra Nath Kar
Mother's Name	Madhumita Kar
Spouse	Dipanwita

Declaration

I hereby declare that all the statements made in this Curriculum- Vitae are true, complete and correct to the best of my knowledge and belief.

PURANJOY KAR

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Transcription factors Atf1 and Sty1 promote stress tolerance under nitrosative stress in *Schizosaccharomyces pombe*

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ABSTRACT

Nitric Oxide (NO) and its associated reactive nitrogen species (RNS) produce nitrosative stress under various pathophysiological conditions in eukaryotes. The fission yeast *Schizosaccharomyces pombe* regulates stress response mainly through the Sty1-Atf1 MAP Kinase pathway. The present study deals with the role of transcription factor Atf1 and Sty1 in *S. pombe* under nitrosative stress. In this study, exposure to an NO donor resulted in S-phase slowdown with associated mitotic block in *S. pombe*. Deletion of *sty1* and *atf1* in *S. pombe* had differential growth sensitivity towards NO donor. Both Sty1 and Atf1 were involved in regulating mitotic slowdown in *S. pombe* under nitrosative stress. Experimental data obtained in this study reveals a novel role of Atf1 in initiating the replication slowdown in *S. pombe* under nitrosative stress. Both Sty1 and Atf1 were accumulated in the nucleus in *S. pombe* under nitrosative stress in a concentration and time dependent manner. Atf1 is also found to be nuclear delocalized under longer nitrosative stress.

1. Introduction

Schizosaccharomyces pombe activate a variety of stress responsive pathways under various stress conditions. The core environmental stress response (CESR) is mediated by the Sty1 MAPK module with Sty1/Spc1 (homologous to mammalian p38) being the key player. Sty1 plays a key role in cell cycle regulation with a prolonged G2 phase being observed under *sty1* deletion. In presence of stress conditions like H_2O_2, glucose deprivation, osmotic stress and heat/cold shock Sty1 gets activated by dual phosphorylation at Thr171 and Tyr173 within the −TGY- motif (Perez and Cansado, 2010). Studies showed that activated Sty1 translocate to the nucleus and regulates its downstream effector protein, the bZIP transcription factor Atf1 (homologous to mammalian ATF2). Transcriptional regulation of CESR genes under stress has been found to be regulated differentially by both Sty1 and Atf1 either singly or in a concerted manner (Chen et al., 2003; Chen et al., 2008). Atf1 is found to control gene regulation either singly or by forming a hetero dimeric complex with Pcr1 (Wilkinson et al., 1996; Sanso et al., 2008). Atf1 of *S. pombe* was found to promote sexual differentiation and mitotic arrest under adverse conditions (Takeda et al., 1995). Atf1 has also been reported to be involved in conjugation, meiosis and osmotic stress response in *S. pombe* (Shiozaki and Russell, 1996). Phosphorylation of Atf1 is however reported not to directly activate the transcription factor but to modulate its stability either at the RNA level or by modulating its degradation by the ubiquitin-proteosomal pathway (Lawrence et al., 2007; Lawrence et al., 2009). Apart from being a transcriptional regulator, Atf1 is also reported to have a non DNA binding role in controlling cell cycle progression by protein–protein interaction with APC/C ubiquitin ligase (Ors et al., 2009). Although the role of MAP kinase Sty1, and its downstream transcription factor Atf1 has been extensively studied in *S. pombe* under oxidative stress but their role in stress tolerance against NO and reactive nitrogen species still remains to be elucidated.

Nitric oxide (NO), a membrane permeable diatomic gaseous molecule has diverse physiological roles in cellular system (Tousoulis et al., 2012; Omer et al., 2012). NO is synthesized by a family of enzymes, known as Nitric Oxide Synthases (NOSs) (NOSs, EC 1.14.13.39) that catalyze the conversion of L-Arginine to L-Citrulline and NO. NO functions as a signaling molecule at low concentration but it create stress at high concentrations (Murad, 2004; Gow et al., 1998; Nagy et al., 2010). When produced in excess amount, NO forms various reactive nitrogen species (RNS) which include nitrous oxide (N_2O), nitrogen dioxide (NO_2), di nitrogen tri oxide (N_2O_3), di nitrosyl iron complexes (DNIC), peroxynitrite ($ONOO^-$) and S- nitrosoglutathione (GSNO) (Patel et al., 1999). These RNS produce many adverse biological effects on cellular lipid, DNA and protein like, lipid peroxidation, DNA damage, abrogation of ATP synthesis, thiol oxidation, S-nitrosylation of proteins, protein tyrosine nitration etc. which gives rise to

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Multimodal control of transcription factor Pap1 in *Schizosaccharomyces pombe* under nitrosative stress

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ABSTRACT

Schizosaccharomyces pombe Pap1, a bZIP transcription factor, is highly homologous to the mammalian c-Jun protein that belongs to the AP1 family of transcriptional regulators. The role of transcription factor Pap1 has been extensively studied under oxidative stress. Two cysteine residues in Pap1p namely, C278 and C501 form disulfide linkage under oxidative stress resulting in nuclear accumulation. We first time showed the involvement of Pap1 in the protection against nitrosative stress. In the present study we show that *pap1* deletion makes growth of *S. pombe* sensitive to nitrosative stress. *pap1* deletion also causes delayed recovery in terms of mitotic index under nitrosative stress. Our flow cytometry data shows that *pap1* deletion causes slower recovery from the slowdown of DNA replication under nitrosative stress. This is the first report where we show that Pap1 transcription factor is localized in the nucleus under nitrosative stress. From our study it is evident that nuclear localization of Pap1 under nitrosative stress was not due to reactive oxygen species formation.

1. Introduction

Induction of stress responsive genetic responses helps living cells to adapt to fluctuations in stressful environment. *Schizosaccharomyces pombe* is an excellent model system to study how cells adapt to stressful situations [1–6]. There are two distinct pathways named as Sty1/Spc1 and Pap1 pathways which respond to various stresses in *S. pombe*. Diverse forms of stress including oxidative stress, osmotic stress, genotoxic stress, nutrient deprivation, high temperature etc. activate mitogen activated protein kinase Sty1 [7–9]. *S. pombe* Pap1, a bZIP transcription factor is highly homologous to the mammalian c-Jun protein that belongs to the AP1 family of transcriptional regulators [10]. Pap1 is known to activate multiple oxidative stress responsive genes in response to low concentrations of hydrogen peroxide (H_2O_2) [11]. Sty1 is absolutely necessary for cell survival following exposure to high concentrations of H_2O_2 [12]. Pap1 consists of two clusters of cysteine residues, one located at the center of the protein and the other at the carboxy terminal nuclear export signal region. Two cysteine residues in Pap1 namely, C278 and C501 form disulfide linkage under oxidative stress and thus makes it unable to interact with exportin Crm1 resulting in nuclear accumulation [13]. Nuclear accumulation of Pap1 is also triggered by cysteine alkylating agent diethylmaleate [14] or methylglyoxal (MG), a glycolytic metabolite [15]. Both diethylmaleate and MG seems to modify irreversibly at least two cysteine residues located at or close to the nuclear export signal of Pap1. MG does not affect the redox state of Pap1, unlike H_2O_2. Thus nuclear accumulation of Pap1 in *S. pombe* is triggered by both H_2O_2 dependent and H_2O_2 independent pathway.

We first time showed the involvement of Pap1 in the protection against nitrosative stress [1]. Kim et al. demonstrated that transcription of the *Schizosaccharomyces pombe* pap1(+) gene is positively regulated by nitrosative and nutritional stress in a Pap1 dependent manner. Spy1, a histidine-containing phosphotransfer (HPt) protein, in the fission yeast *Schizosaccharomyces pombe* is transcriptionally up-regulated by nitrosative and nutritional stresses in a Pap1-dependent manner [16]. The transcriptional response to NO in a *pap1* deleted *S. pombe* strain identified 45 genes that seem to be controlled by Pap1. Interestingly, Pap1 regulated genes in *S. pombe* were distinctly different under nitrosative stress than those reported under oxidative stress [3]. There was little overlap between the effects of NO and the responses to other stress agents in fission yeast, suggesting that different stress response mechanisms may be involved in *S. pombe* under nitrosative stress. In the present study *pap1* deletion made growth of *S. pombe* cells

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Nitric oxide sensing by chlorophyll *a*

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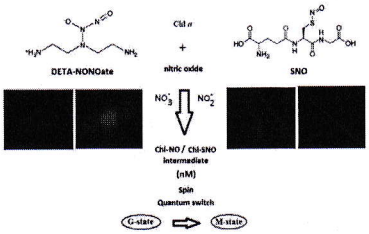
HIGHLIGHTS

- Green sensing of nitric oxide.
- Nitric oxide detection by chlorophyll *a* (chl *a*) at room temperatures.
- Reactant specific molecular recognition effects of chl *a*.
- SNO mapping by membrane bound chl *a* of cyanobacteria and identification of nitrogen fixing organisms.
- Novel methods of photosynthetic NO/SNO detection.

GRAPHICAL ABSTRACT



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Cyanobacteria

ABSTRACT

Nitric oxide (NO) acts as a signalling molecule that has direct and indirect regulatory roles in various functional processes in biology, though in plant kingdom its role is relatively unexplored. One reason for this is the fact that sensing of NO is always challenging. There are very few probes that can classify the different NO species. The present paper proposes a simple but straightforward way for sensing different NO species using chlorophyll, the source of inspiration being hemoglobin that serves as NO sink in mammalian systems. The proposed method is able to classify NO from DETA-NONOate or (Z)-1-[*N*-(2-aminoethyl)-*N*-(2-ammonioethyl) amino] diazen-1-ium-1,2-diolate, nitrite, nitrate and S-nitrosothiol or SNO. This discrimination is carried out by chlorophyll *a* (chl *a*) at nano molar (nM) order of sensitivity and at 293 K–310 K. Molecular docking reveals the differential binding effects of NO and SNO with chlorophyll, the predicted binding affinity matching with the experimental observation. Additional experiments with a diverse range of cyanobacteria reveal that apart from the spectroscopic approach the proposed sensing module can be used in microscopic inspection of NO species. Binding of NO is sensitive to temperature and static magnetic field. This provides additional support for the involvement of the porphyrin ring structures to the NO sensing process. This also, broadens the scope of the sensing methods as hinted in the text.

1. Introduction

The importance of NO as a reactive free radical in biological environment is well documented [1]. However, the role of NO in plant systems is relatively unexplored. A detailed understanding of the interaction mechanisms and knowledge regarding the possible roles of NO may be of some interest [2]. A number of extensive studies establish the significance of NO-driven interferences in plant systems [3]. NO influences growth parameters, metabolism, cell

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Nitrosative stress induces a novel intra-S checkpoint pathway in *Schizosaccharomyces pombe* involving phosphorylation of Cdc2 by Wee1

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ABSTRACT

Excess production of nitric oxide and reactive nitrogen intermediates causes nitrosative stress on cells. *Schizosaccharomyces pombe* was used as a model to study the cell cycle regulation under nitrosative stress response. We discovered a novel intra-S-phase checkpoint that is activated in *S. pombe* under nitrosative stress. The mechanism for this intra-S-phase checkpoint activation is distinctly different than previously reported for genotoxic stress in *S. pombe* by methyl methane sulfonate. Our flow cytometry data established the fact that Wee1 phosphorylates Cdc2 Tyr15 which leads to replication slowdown in the fission yeast under nitrosative stress. We checked the roles of Rad3, Rad17, Rad26, Swi1, Swi3, Cds1, and Chk1 under nitrosative stress but those were not involved in the activation of the DNA replication checkpoint. Rad24 was found to be involved in intra-S-phase checkpoint activation in *S. pombe* under nitrosative stress but that was independent of Cdc25.

1. Introduction

Nitric oxide (NO), a versatile diatomic gaseous molecule, has been known to play a signaling role in many physiological processes, at low concentrations [1]. NO is a membrane-permeable free radical with a very short half-life, that is biologically produced by the nitric oxide synthase (NOS) family of enzymes [2]. NO reacts in a concentration- and environment-dependent manner, leading to the formation of reactive nitrogen intermediates (RNIs). Excess or deregulated production of NO and resulting RNIs have been shown to have both cytostatic and cytotoxic activity due to the inhibition of ATP production [3–5], altered iron metabolism [5–7], direct inhibition of enzymes [8], and DNA damage [6,9,10].

The fission yeast *Schizosaccharomyces pombe* has been used previously as an excellent model system for investigation of the fundamental regulation and control mechanisms of various cellular processes including nitrosative stress [11,12]. Eukaryotic cells slow their progression through S phase when their DNA is damaged [13]. The biological importance of this response, which is called the intra-S-phase checkpoint, is that this checkpoint provides cells with time to repair the damaged DNA before its replication. Interest in the intra-S-phase checkpoint is enhanced by the strong correlation in several human genetic diseases between the loss of the intra-S-phase checkpoint and the susceptibility to cancer [14]. In all eukaryotic organisms the intra-S-phase checkpoint depends on the function of one or two members of the ATR family of protein kinases, ATR and ATM in vertebrate cells [15–17], Mec1 and Tel1 in the budding yeast, *S. cerevisiae* [18–20], and Rad3 and Tel1 in the fission yeast, *S. pombe* [21,22]. Most if not all of the intra-S-phase checkpoint pathways downstream of the ATR family depend on one or another member of the Chk family of protein kinases (Chk1 and Chk2 in vertebrates, Chk1 and Rad53 in budding yeast, Chk1 and Cds1 in fission yeast). The Chk kinases become active when they are phosphorylated by an ATR-family kinase [15,16]. One of the principal targets of the Chk kinases is the cyclin-dependent kinase, Cdk2, but Cdk2 is not directly regulated by the Chk kinases. Instead, Cdk2 is inhibited by phosphorylation of its tyrosine 15 (Tyr-15) and activated by removal of the phosphate at Tyr-15. Inactivation of Cdk2 could slow S phase by reducing the rate of initiation at origins. It has been shown that methyl methane sulfonate (MMS) could induce an intra-S-phase checkpoint in *S. pombe* that depends on Rad3 (similar to vertebrate ATR), Rad26 (similar to vertebrate ATRIP), and the group of proteins that presumably loads a checkpoint-specific PCNA-like structure onto damaged DNA (Rad17, Rad9, Rad1, and Hus1)

Abbreviations: NO, nitric oxide; NOS, nitric oxide synthase; RNIs, reactive nitrogen intermediates; MMS, methyl methane sulfonate; ATR, ataxia telangiectasia and Rad3 related; ATM, ataxia telangiectasia mutated; ATRIP, ATR interacting protein; PCNA, proliferating cell nuclear antigen; PI, propidium iodide; PBS, phosphate-buffered saline; FACS, fluorescence-activated cell sorting; HU, hydroxyurea; GR, glutathione reductase; GSH, reduced glutathione

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