Sunaina Singh Rajput

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EDUCATION:

Degree/ Course	Year	Institution	CGPA / %
Ph.D. Biological Sciences	2013-19	Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bengaluru	-
Master of Science in Biological Sciences	2010-13	Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bengaluru	6.58 of 8.00
Bachelor of Sciences Microbiology, Genetics and Chemistry	2006-09	Osmania University, Hyderabad	73.1%

ADDITIONAL COURSE WORK:

ASCB-KGI Biotech course 2018 - Managing science in the biotech industry: An intensive course for students and postdocs, Keck Graduate Institute, USA

EXPERIENCE:

Company name	Period	Designation	Details
Institute of Genomics and integrative Biology (CSIR-IGIB)	February 2021-present	Project Scientist- I	Working towards applications of CRISPR-Cas9 in therapeutics
Institute of Genomics and integrative Biology (CSIR-IGIB)	June 2020-January 2021	Research Associate	Worked as a part of the RNA Biology Laboratory in developing and validating the FELUDA paper strip test for detection of COVID-19 from patient samples which has been launched as TATA MDcheck
Genemod, Inc. Seattle U.S.A	Jan 2020-present	Freelance consultant	• Validation and feedback on Genemod inventory and project management software for research labs • Literature search, support in design and development of software modules, and manuscript writing
Indian Institute of Technology (IIT-Delhi)	Sep 2019-April 2020	Research Associate	Worked as a part of the Enzymology and Microbial Biochemistry Laboratory • Identifying putative serine protease from an extremophile bacterial species using bioinformatics and cloned it for expression in *E. coli* • Anti-microbial resistance project to identify secondary metabolites of Marine actinomycetes for their activity against antibiotic resistant bacteria and understand the mechanisms of action

ROLES AND RESPONSIBILITIES:

Ph.D. Project

JNCASR
2013-2019

Investigating the role of Exocyst - a multisubunit tethering complex in autophagosome biogenesis

- Generated deletion and overexpression strains of *Saccharomyces cerevisiae*, and plasmids for fluorescent protein expression in *S. cerevisiae*
- Designed and performed experiments using techniques such as fluorescent and confocal microscopy, live cell imaging, Western blotting, size exclusion chromatography, and other Molecular Biology methods
- Data analysis, image analysis using Softworxs (G.E) and ImageJ softwares, statistical analysis using Graphpad Prism, preparation of manuscripts
- Purchase and procurement, laboratory management and maintenance of lab equipment

	• Practical laboratory course for batch of 8 M.S students, trained 5 summer interns and 3 M.S students • Participated in Student Buddy program - a STEM outreach activity of JNCASR
Master's thesis **JNCASR** 2012-2013	Identification of genes involved in autophagy and autophagy related pathways in the yeast, *Saccharomyces cerevisiae* • Generated about 150 mutants (knockout and temperature sensitive) of *S. cerevisiae* and screened them for defects in selective autophagy process using western blotting and fluorescence microscopy
Laboratory rotations **JNCASR** Jan-June 2012	Worked with Dr. Ravi Manjithaya, Autophagy Laboratory *1) Project title:* Studies on functional complementation of Slt2 in SLT2 knockout *Saccharomyces cerevisiae* • Established and standardized selective and general autophagy assays using fluorescence microscopy and western blotting methods. • Generated *S. cerevisiae* strains by genomic integration of a gene SLT2 in the genome of deletion strain *(slt2Δ)* at three different loci and studied the functional complementation. *2) Project title:* Development of luciferase based assay for unconventional protein secretion in yeast • Designed and generated plasmid constructs to express fusion protein of ACB1 and Nanoluciferase in *S. cerevisiae* • Developed an assay to detect levels of Acb1 secreted using luminometer
Laboratory rotation **JNCASR** May-Dec 2011	Worked with Prof. Namita Surolia, Molecular Parasitology Laboratory *1) Project title:* Cloning, expression and purification of *Plasmodium falciparum* Histidine Rich Protein-2 *(Pf*HRP2*)* • Cloned the *Plasmodium falciparum* Histidine Rich Protein2 (*Pf*HRP2) gene in pET28a vector for 6X-HIS tagged protein overexpression and purification using affinity chromatography

SUMMER INTERNSHIPS:

- May-July 2009: Late Prof. Obaid Siddiqi's Laboratory, National Centre for Biological Sciences (NCBS), TIFR, Bangalore
  - Project title: Electroshock conditioning of *Drosophila* Third Instar larvae
    Maintenance of *Drosophila* lines and collection of larvae
    Performed behavioural experiments to understand the effect of electroshock conditioning on learning and memory of *Drosophila* third instar larvae
- June-July 2008: Prof. Musti J. Swamy's Laboratory, School of Chemistry, University of Hyderabad
  - Project title: Purification of chito-oligosaccharide specific phloem lectins of snake gourds (*Trichosanthes Anguina*)
    Purified lectin proteins from the snake guards using affinity chromatography method

PUBLICATIONS:

Research articles:

- Mohd. Azhar, Rhythm Phutela, et al "Rapid, accurate, nucleobase detection using FnCas9", *MedRxiv* 2020
- **Sunaina Singh**, Sarika Chinchwadkar, Amol Aher, Saravanan Matheshwaran, Ravi Manjithaya, "Exocyst subcomplex functions in autophagosome biogenesis by regulating Atg9 trafficking" *Journal of Molecular Biology* 2019
- Gaurav Barve, Shreyas Sridhar, Amol Aher, Mayurbhai H. Sahani, Sarika Chinchwadkar, **Sunaina Singh**, K. N. Lakshmeesha, Michael A. McMurray, Ravi Manjithaya, "Septins are involved at the early stages of macroautophagy in *S. cerevisiae*", *Journal of Cell Science* 2018
- Sarika Chinchwadkar, Sreedevi Padmanabhan, Piyush Mishra, **Sunaina Singh**, S. N. Suresh, Somya Vats, Gaurav Barve, Veena Ammanathan, Ravi Manjithaya, "Multifaceted Housekeeping Functions of Autophagy", *Journal of Indian Institute of Science* 2017

Book chapters:

- **Sunaina Singh** and Ravi Manjithaya, "Role of golgi complex and autophagosome biogenesis in unconventional protein secretion"; *Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection, and Aging, volume 2: 1st edition, Ed: M A. Hayat* (2013)
- Huma Fatima, **Sunaina Singh**, Shivani Chaturvedi, Jasneet Grewal, Sunil K khare, "Technological advances in environment-friendly production of Succinic acid by utilization of agricultural wastes"; Circular Bioeconomy (2020) (revision submitted)

- Huma Fatima, **Sunaina Singh**, Jasneet Grewal, Sumit kumar, Sunil K Khare, "Biorefining lignocellulosic feedstocks for microbial production of organic acids"; Waste Biorefinery, volume IV (submitted)

AWARDS/FELLOWSHIPS/MEMBERSHIPS:

- Member of American Society of Biochemistry and Molecular Biology
- Department of Science and Technology - Science and Engineering Research Board (DST-SERB) travel award; July 2018
- Best poster award, In-house symposium, JNCASR - 2013
- Young investigator Award in Biology (YIAB) - 2nd prize for poster presentation in Graduate students meet, ACTREC, Mumbai; December 2010
- University Grants Commission (UGC), Govt. of India fellowship for summer internship; June-July 2008

REFERENCES:

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Exocyst Subcomplex Functions in Autophagosome Biogenesis by Regulating Atg9 Trafficking

Sunaina Singh [1], Ruchika Kumari [1], Sarika Chinchwadkar [1], Amol Aher [1], Saravanan Matheshwaran [2] and Ravi Manjithaya [1]

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https://doi.org/10.1016/j.jmb.2019.04.048

Abstract

During autophagy, double-membrane vesicles called autophagosomes capture and degrade the intracellular cargo. The *de novo* formation of autophagosomes requires several vesicle transport and membrane fusion events which are not completely understood. We studied the involvement of exocyst, an octameric tethering complex, which has a primary function in tethering post-Golgi secretory vesicles to plasma membrane, in autophagy. Our findings indicate that not all subunits of exocyst are involved in selective and general autophagy. We show that in the absence of autophagy specific subunits, autophagy arrest is accompanied by accumulation of incomplete autophagosome-like structures. In these mutants, impaired Atg9 trafficking leads to decreased delivery of membrane to the site of autophagosome biogenesis thereby impeding the elongation and completion of the autophagosomes. The subunits of exocyst, which are dispensable for autophagic function, do not associate with the autophagy specific subcomplex of exocyst.
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Introduction

Autophagy is an intracellular catabolic process involving capture of cytosolic cargo by double-membrane vesicular structures called autophagosomes. These autophagosomes then fuse with lysosomes (vacuoles in yeast) leading to degradation of the cargo [1]. Studies by various groups have led to the identification of more than 40 ATG proteins (*Au*tophagy Related *G*enes) and several accessory components. The molecular mechanisms of how these proteins function in the process of autophagy have been deciphered to a substantial extent [2]. Autophagosome biogenesis is a complex process that begins with the assembly of autophagy initiation complex (Atg1 complex) at PAS (Pre-autophagosomal structure, a perivacuolar autophagosome biogenesis site in yeast) followed by activation of VPS34 complex at this site to produce PI3P locally, thereby leading to the recruitment of other core autophagy proteins and nucleation of precursor autophagosome membrane. This nascent structure known as the phagophore further expands into double-membrane vesicle by addition of membrane derived from various sources mediated by Atg9 vesicles [3,4].

Atg9 is an integral membrane protein that appears as multiple puncta in cytoplasm [5]. These puncta represent the peripheral pool of Atg9 containing vesicles, which deliver membrane to PAS allowing for autophagosome expansion. These Atg9 vesicles are known to be derived from various membrane sources including Golgi associated secretory pathway [6–11].

Various secretory pathway proteins were shown to be important for the process of autophagosome

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Rapid, accurate, nucleobase detection using FnCas9

Single sentence summary

A method to identify nucleotide sequence or nucleobase identity using FnCas9 and its implementation in the rapid and accurate diagnosis of SARS-CoV-2

Authors

Mohd. Azhar[1,2,8], Rhythm Phutela[1,2,8], Manoj Kumar[1,2,8], Asgar Hussain Ansari[1,2,8], Riya Rauthan[1,2], Sneha Gulati[1], Namrata Sharma[1], Dipanjali Sinha[1,2], Saumya Sharma[1,2], Sunaina Singh[1], Sundaram Acharya[1,2], Deepanjan Paul[1], Poorti Kathpalia[1], Meghali Aich[1,2], Paras Sehgal[1,2], Gyan Ranjan[1,2], Rahul C. Bhoyar[1], Indian CoV2 Genomics & Genetic Epidemiology (IndiCovGEN) Consortium[1], Khushboo Singhal[1,2], Harsha Lad[3], Pradeep Kumar Patra[3], Govind Makharia[4], Giriraj Ratan Chandak[5], Bala Pesala[7], Debojyoti Chakraborty[1,2,8*], Souvik Maiti[1,2,6,8*]

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NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.

CHAPTER

6

Role of the Golgi Complex and Autophagosome Biogenesis in Unconventional Protein Secretion

Sunaina Singh and Ravi Manjithaya

OUTLINE

Abstract

Various proteins are now known to be transported to the cell surface or are secreted independently of the canonical ER–Golgi pathway. Some examples of such cargoes that are transported in the vesicular mode are acyl-CoA binding protein, cystic fibrosis transmembrane conductance regulator (CFTR), and some of the interleukins. For this form of conserved mechanism of unconventional secretion, there is an emerging role for autophagy and the Golgi-associated protein GRASP. Biogenesis of such unconventional vesicles, as shown in a recent study in yeast, occurs at unique regions near ER exit sites known as CUPS (compartments for unconventional protein secretion). CUPS are devoid of Golgi or endosomal proteins but harbor

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REVIEW ARTICLE

Multifaceted Housekeeping Functions of Autophagy

*Sarika Chinchwadkar, Sreedevi Padmanabhan, Piyush Mishra, Sunaina Singh, S. N. Suresh, Somya Vats, Gaurav Barve, Veena Ammanathan and Ravi Manjithaya**

Abstract | Autophagy is an evolutionarily conserved intracellular degradation process in which cytoplasmic components are captured in double membrane vesicles called autophagosomes and delivered to lysosomes for degradation. This process has an indispensable role in maintaining cellular homeostasis. The rate at which the dynamic turnover of cellular components takes place via the process of autophagy is called autophagic flux. In this review, we discuss about the orchestrated events in the autophagy process, transcriptional regulation, role of autophagy in some major human diseases like cancer, neurodegeneration (aggrephagy), and pathogenesis (xenophagy). In addition, autophagy has non-canonical roles in protein secretion, thus demonstrating the multifaceted role of autophagy in intracellular processes.

1 Introduction

Autophagy, an intracellular evolutionarily conserved process, involves engulfment of unwanted proteins and organelles by double-membrane vesicles, called autophagosomes, which then fuse with the lysosomes/vacuole, and the engulfed cargo is subsequently degraded. It is a cell survival mechanism under stress conditions and it also play important roles in many other intra-cellular processes like protein and organelle turnover and transport of some of the vacuolar enzymes. This process can be divided into various steps, including autophagy induction, nucleation, autophagosome formation, maturation, fusion with the lysosomes/vacuole, degradation of the cargo, and recycling of the precursor molecules, such as amino acids, lipids, and nucleotides, back to the cytoplasm. Autophagy is a tightly regulated cellular mechanism and its flux varies depending on the cell type(s) of an organism. Autophagy is involved in various physiological roles, such as cellular homeostasis, embryonic development, antigen presentation, protein quality control, and maintenance of the amino-acid pool during starvation conditions. It is also implicated in various pathophysiological diseases, such as infection, cancer, diabetes, and neurodegeneration.

Although autophagy is predominantly a cytosolic event, the nucleus exerts a considerable control in the extent of autophagy response, especially during adverse conditions, such as starvation. Depending on the cargo it captures, autophagy is broadly classified as general and selective autophagy. For example, as a response to nutrient deprivation, general autophagy is triggered where it captures random portion of cytosol. In contrast, selective autophagy ensures specific capture of cytosolic cargo, such as damaged or superfluous organelles. When selective autophagy captures and degrades mitochondria, the process is termed as mitophagy. Similarly, autophagic degradation of peroxisomes (pexophagy), Golgi (golgiphagy), ER (ER-phagy), ribosomes (ribophagy), etc., have been documented.[1] The genes comprising the autophagy machinery are named as ATG (AuTophaGy related gene).[1]

2 Process of Autophagy

2.1 Autophagy Induction

The initial characterization of autophagy flux with respect to involvement of molecular players was carried out in yeast extensively. Although recycling of the cytoplasmic contents happens at

Autophagosomes: The "Pac-Man" like double membrane vesicles involved in macroautophagy.

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RESEARCH ARTICLE

Septins are involved at the early stages of macroautophagy in *S. cerevisiae*

Gaurav Barve[1], Shreyas Sridhar[1], Amol Aher[1], Mayurbhai H. Sahani[1], Sarika Chinchwadkar[1], Sunaina Singh[1], Lakshmeesha K. N.[1], Michael A. McMurray[2] and Ravi Manjithaya[1,*]

ABSTRACT

Autophagy is a conserved cellular degradation pathway wherein double-membrane vesicles called autophagosomes capture long-lived proteins, and damaged or superfluous organelles, and deliver them to the lysosome for degradation. Septins are conserved GTP-binding proteins involved in many cellular processes, including phagocytosis and the autophagy of intracellular bacteria, but no role in general autophagy was known. In budding yeast, septins polymerize into ring-shaped arrays of filaments required for cytokinesis. In an unbiased genetic screen and in subsequent targeted analysis, we found autophagy defects in septin mutants. Upon autophagy induction, pre-assembled septin complexes relocalized to the pre-autophagosomal structure (PAS) where they formed non-canonical septin rings at PAS. Septins also colocalized with autophagosomes, where they physically interacted with the autophagy proteins Atg8 and Atg9. When autophagosome degradation was blocked in septin-mutant cells, fewer autophagic structures accumulated, and an autophagy mutant defective in early stages of autophagosome biogenesis (*atg1Δ*), displayed decreased septin localization to the PAS. Our findings support a role for septins in the early stages of budding yeast autophagy, during autophagosome formation.

This article has an associated First Person interview with the first author of the paper.

KEY WORDS: Autophagy, Noncanonical ring, Septin, Autophagosome biogenesis, Pre-autophagosomal structure, PAS, Atg9 trafficking

INTRODUCTION

Macroautophagy (herein autophagy) is an evolutionarily conserved intracellular waste disposal and recycling process that is critical for normal cellular and organismal homeostasis. Autophagy involves the formation of double-membrane vesicles called autophagosomes that engulf intracellular material destined for degradation. Autophagosomes eventually fuse with vacuoles or lysosomes, resulting in cargo degradation and recycling of cellular building blocks, such as amino acids, back to the cytoplasm. The biogenesis of autophagosomes remains incompletely understood.

In budding yeast cells, the site of autophagosome formation is known as the pre-autophagosomal structure (PAS) and is perivacuolarly located. Recent work has shown that the PAS is tethered to endoplasmic reticulum (ER) exit sites where multiple autophagy proteins colocalize in a hierarchical sequence (Graef et al., 2013; Suzuki et al., 2007). The membrane source for the developing autophagosome is contributed by the trafficking of Atg9 along with its transport complex (Atg1–Atg11–Atg13–Atg23–Atg27–Atg2–Atg18–TRAPIII) to help build the initial cup-shaped structure, the phagophore (Legakis et al., 2007; Reggiori et al., 2004; Tucker et al., 2003). Additional recruitment of the Atg5–Atg12–Atg16 complex as well as Atg8 allows the completion of the autophagosome (Feng et al., 2014).

Septin proteins bind guanine nucleotides and co-assemble in hetero-oligomers capable of polymerizing into cytoskeletal filaments (Mostowy and Cossart, 2012). Septin filaments associate directly with membranes in a curvature-dependent manner (Bridges et al., 2016) and regulate membrane dynamics, including vesicle fusion events (Mostowy and Cossart, 2012). In immune cells, septins also localize transiently to the phagocytic cup and are functionally involved in phagocytosis (Huang et al., 2008). Septins have been implicated in autophagy in mammalian cells infected by intracellular bacteria, where they form cage-like structures around the bacterial cells that colocalize with the autophagosome marker autophagosome marker MAP1LC3A, the homolog of yeast Atg8. It is believed that these structures entrap bacteria, restricting their motility and targeting them for autophagy-mediated degradation (Mostowy et al., 2009, 2010). During *Shigella* infection, assembly of septin cages and the autophagosome in the host mammalian cells are interdependent (Mostowy et al., 2010, 2011; Sirianni et al., 2016). Despite these findings, it remains unclear to what extent septins contribute to autophagy outside the context of bacterial infection (Torraca and Mostowy, 2016).

In *S. cerevisiae* cells undergoing mitotic proliferation, five septin proteins – Cdc3, Cdc10, Cdc11, Cdc12 and Shs1 – comprise an array of filaments that is directly associated with the plasma membrane at the mother–bud neck, and controls cell polarity, bud morphogenesis and cytokinesis (Glomb and Gronemeyer, 2016; Oh and Bi, 2011). Upon nitrogen starvation, diploid yeast cells undergo meiosis and sporulation, during which a cup-shaped double-membrane structure, the prospore membrane (PSM), engulfs haploid nuclei and other organelles to form stress-resistant spores (Neiman, 2005, 2011). Yeast septins are required for proper PSM biogenesis (Heasley and McMurray, 2016), but there was no known role for septins in yeast autophagy. Here, we describe autophagy defects in septin-mutant strains and physical interactions between septins and established autophagy factors that support a functional role for septins in yeast autophagy.

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