Ritika Gurung, Ph.D.

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Education

- Ph.D. "Signal transduction pathways involved in lamin dynamics", 2011
  at the Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India
  Ph.D. supervisor: Dr. Veena K. Parnaik

- M.Sc. (Molecular and Human Genetics), 2004, Banaras Hindu University (BHU), India

- B.Sc. (Microbiology Honors), 2002, Delhi University (DU), India

Research Experience

- Postdoctoral Fellow with Prof. PW Ingham (Institute for Molecular and Cellular Biology, ASTAR, Singapore, 2013-2016)

- Summer training with Dr. L.S. Shashidhara (CCMB, May-July, 2003)

- Masters project for six months with Prof. S.C. Lakhotia (BHU, 2003-2004)

Work experience

- Senior Domain Expert (Science), with project MANAV - Human Atlas Initiative (Indian Institute of Science Education and Research (IISER), Pune, March 2021- Feb 2022)
  Bio-curation and Machine learning, developing prototype of a crowd sourcing platform
  [*Supervised project Domain (Science), Coordinate project administrative activities, did outreach activity to involve professionals from academia and industry for insight and perspective*]

- Domain Expert (Science), with project MANAV - Human Atlas Initiative, (IISER-Pune, Sept 2019-Feb 2021)
  [*Provided science inputs to develop use cases that determine scope of prototype platform, coordinated an external Proof-of-Concept activity for trial of the prototype platform*]

Honors and Awards

- Prof. B.R. Sheshacharya Memorial award for best paper presentation in poster session during the XXXII All India Cell Biology Conference and Symposium (2008) on "Stem Cells and Pattern Formation" at MACS-Agharkar Research Institute, Pune, India.

- Senior Research Fellowship (2006-2010) Council of Scientific and Industrial Research (CSIR), India

- Junior Research Fellowship (2004-2006) Council of Scientific and Industrial Research (CSIR), India

- National Eligibility Test (NET) for lectureship (2004) jointly conducted by Council of Scientific and Industrial Research (CSIR) and University Grants Commission (UGC), India

- Graduate Aptitude Test (GATE) Fellowship (2004) in Life Sciences

Research Interests

- Adult (muscle) stem cell biology and gene regulation in myogenic development
- Molecular basis of myofibrillar myopathy
- Signal transduction pathways and nuclear reorganization involved in cellular differentiation
- Mechanism of transcription and alternative splicing
- Cell cycle modulation

Technical Expertise

Cell biology

- Mammalian cell culture
- Maintenance and differentiation of adult stem cell from skeletal muscle (C2C12)
- Conventional fluorescence, confocal and bright field microscopy
- FACS for cell cycle analysis in myoblast and isolation of fast-twitch fibre specific myocytes from dissociated zebrafish embryo for transcriptome analysis
- Immunofluorescent labelling of cells and zebrafish whole embryo and tissue cryo-sections
- Whole mount RNA in-situ of zebrafish and drosophila embryos

Molecular biology and biochemistry

- Morpholino, TALEN and CRISPR/Cas9 based targeted genome editing in zebrafish
- Microarray(15k and 7.4k mouse cDNA array), Real-time PCR (ABI model-HT7900 Sequence Detector) based analysis
- Promoter-reporter analysis
- Multisite gateway cloning technology in zebrafish, and cloning for tagged-proteins expression for localization study and antibody production
- Invitro transcription for microinjection of zebrafish embryo
- pAd-Easy adenoviral based efficient gene delivery system for myoblast
- Immunoprecipitation and Chromatin Immuno Precipitation (ChIP), western and southern blotting
- DNA synthesis assay and Flow-cytometry (FACS Caliber Cytometer, Becton and Dickinson)

Publications

1. Gurung R, Ono Y, Baxendale S, Lee SL, Moore S, Calvert M, Ingham PW (2017) The zebrafish frozen mutant is a model for human myopathy associated with mutation of the unconventional myosin MYO18B. Genetics, 205(2):725-735

2. Gurung R. and Parnaik V.K. (2012) Cyclin D3 promotes myogenic differentiation and Pax7 transcription *J. Cell. Biochem.,113, 209-219.*

3. Mariappan I., Gurung R., Thanumalayan S. and Parnaik V.K. (2007) Identification of cyclin D3 as a new interaction partner of lamin A/C. *Biochem. Biophys. Res. Comm.*, 355, 981–985

Meetings and workshops

1. R. Gurung, Samantha Lin Chiou Lee, Sarah Baxendale Stephen Moore, Meredith Calvert, Fiona Chia, Xue Zhi Ouyang, Tom Carney and Philip W. Ingham (2015) "Unconventional myosin XVIIIB is required for skeletal and cardiac muscle myofibril assembly in zebrafish" at the American Society of Cell Biology (ASCB) meeting, San Diego, USA. (poster presentation)

2. Weixin Niah, R. Gurung, Reshma Taneja, Philip W. Ingham (2013) "Functional Analysis of Euchromatic Histone Lysine N-Methyltransferase 2 (G9a/EHMT2) in Zebrafish Skeletal Muscle" at the Indian Ocean Rim Muscle Colloquium, (IORM) Singapore. (poster presentation)

3. R. Gurung and V.K. Parnaik (2009) "Cyclin D3 expression primes myoblasts for differentiation" at the XXXIII All India Cell Biology Conference and International Workshop on Cell Cycle Regulation, School of Life Sciences, University of Hyderabad, Hyderabad, India. (poster presentation)

4. Ziess Apotome workshop (2008), held at CCMB, Hyderabad. (workshop attended)

5. R. Gurung and V.K. Parnaik (2008) "Signal transduction pathways involved in lamin A/C speckle dynamics" at the XXXII All India Cell Biology Conference and Symposium on "Stem Cells and Pattern Formation" at MACS-Agharkar Research Institute, Pune, India. (poster presentation)

Background and Research Experience

Pre-doctoral research work (2002-2004)

a. *Enhancer/suppressor screen for identifying the interacting partners of drosophila Ubx gene*
Summer Research Project (May-July, 2003)
(with Dr. L.S. Shashidhara, CCMB, Hyderabad)
My work involved a deficiency screening to identify the molecular interactors, enhancers/suppressors, of the homeobox gene, *Ultrabithorax* (*Ubx*) involved in wing development of the fruit-fly, *Drosophila melanogaster*. The UAS-Gal4 system was utilized for driving the tissue-specific expression of *Ubx* gene in the haltere of adult flies. In this preliminary screening a deficiency line was identified as an enhancer of the *Ubx*-overexpression phenotype.

b. *Investigation of the genetic interaction between the body pigment gene, ebony and yellow, and hsr-ω (93D) gene in D. melanogaster*
Masters Project (2003-2004)
(with Prof. S.C. Lakhotia, Department of Molecular and Human Genetics, BHU)
For this study I generated a line of ebony body coloured flies that overexpressed the non-coding RNA, hsr-*ω*. The expression of *hsr-ω* in the ebony-*05421* overexpression line, and its accumulation at the 93D locus, was verified by RNA *in-situ* hybridization (RISH) of whole-mount and polytene chromosome preparation from salivary glands under control and heat-shock conditions. Further, through Lac-Z staining of testis and single-fly PCR the male sterility phenotype in the ebony-*05421* was confirmed.

Doctoral research work (2004-2011)

In my doctoral research with Dr. V.K. Parniak, I studied the regulation of eukaryotic gene expression during myogenic differentiation. The subject of my inquiry, in particular, was the signal transduction pathways affected by cyclin D3 during the muscle differentiation. It is known that upon differentiation cyclin D3 is upregulated in muscle lineage cells. It has been suggested that this protein also regulates the signaling associated with redistribution of nucleoplasmic lamin by a process that exclusively occurs when proliferating myoblasts enter terminal differentiation. I applied a variety of molecular biology techniques to identify the signaling molecules induced by cyclin D3 with the overall aim of identifying pathways that could be involved in nucleoplasmic lamin redistribution. For my study I used C2C12 mouse myoblasts that ectopically express cyclin D3. The relevance of these muscle specific markers in myogenic development was analyzed by studying their expression under differentiation conditions. I used indirect immunofluorescence labeling, immunoprecipitation and western blotting analysis to understand the association of cyclin D3 to the nuclear matrix and verified it's *in vivo* interaction with lamin A/C. Based on a preliminary microarray study, I identified some candidate genes that were expressed differentially in cyclin D3 expressing myoblasts. Some of these, I further validated by real-time PCR analysis. For my studies I have optimized and applied the highly efficient Ad Easy adenoviral gene delivery system for transient transfection of myoblasts.

Lamins are distributed throughout the nucleoplasmic space as speckles in addition to the network at the nuclear periphery, called the nuclear lamina. My study was based on an earlier observation in the lab, where a dynamic reorganization of lamin speckles into an antigenically masked, dispersed network, was observed to occur exclusively in differentiating myocytes. Reorganization of these lamin speckles coincides with the onset of myogenic differentiation when expression of cyclin D3 is induced in myoblasts. Importantly, cyclin D3 directly mediates the reorganization of lamin speckles and this process requires active retinoblastoma protein (pRb). Other forms of nuclear remodeling are also known to occur during myogenesis. For instance, the reorganization of peripheral lamina and NuMAs (nuclear mitotic apparatus) occur in myotube nuclei as late differentiation events. Nucleoplasmic lamin speckles co-localize with splicing factor compartments and are involved in the spatial coordination of transcription and splicing. The regulatory function of lamin A during myogenesis is reflected from the fact that a majority of lamin mutations manifest as muscle wasting disorders. My observations suggest that lamins could be involved in events associated with early myogenic differentiation.

(i) A comparative study of the association of cyclin D3 with nuclear matrix and lamin A/C.

Myogenic cell lines, C2C12 and the non-myogenic cell lines, like HeLa transfected with cyclin D3 expression vector were analyzed for the expression of nuclear-matrix components by immunostaining and western blotting. Although the nuclear matrix anchorage of cyclin D3 was maintained in both transfected cell types, lamin speckles reorganization occurred specifically in myogenic cells. Lamin speckles reorganized prematurely in myoblasts upon stabilization of cyclin D3, by inhibiting the degradative phosphorylation on cyclin D3 mediated by GSK-3β signaling. Also, a distinct expression pattern of pRb phosphorylation was exhibited by myogenic cells different from that in HeLa cells, suggesting a lineage-restricted role of the nuclear matrix in myogenic development. Furthermore, ectopic HA-tagged cyclin D3 co-immuno- precipitates lamin A/C in transfected myoblasts, similar to endogenous cyclin D3 which was found to interact with lamins in myotubes. These observations suggest that the direct interaction of cyclin D3 with lamin A/C present in the nuclear matrix has a significant functional role in myogenic differentiation.

(ii) Optimization of the Ad Easy adenoviral transient transfection system for cyclin D3 expression in myoblasts.

To develop a transfection method with high transfection efficiency, I standardized the adenoviral system for gene delivery of the HA-tagged cyclin D3 into myoblasts which shows high transfection efficiency (~90%). Optimum expression of HA-tagged cyclin D3 was confirmed in myoblasts by 24 hours post-infection with adenoviruses. Expression level of HA-tagged cyclin D3 in myoblasts was twice as much as the levels of endogenous cyclin D3 expressed in 48hour myotubes. Further, I have confirmed that virally expressed cyclin D3 retains its ability to reorganize lamin speckles and nuclear matrix anchorage.

(iii) Effects of cyclin D3 on the expression of cell cycle markers and myogenic regulators

The effect on the phosphorylation status of pRb was studied using phospho-specific antibodies by western blot analysis. Cyclin D3 expression in myoblasts did not induce differentiation under serum-rich conditions which support proliferation, and did not alter levels of most G1-phase cell

cycle regulators, but it did induce expression of the cdk inhibitor p21.

(iv)Cell-cycle parameters of cyclin D3 expressing myoblasts

An interesting observation from the FACS analysis was that the expression of cyclin D3 in myoblasts induced cell cycle stalling in the G2/M-phase. A higher number of mitotic cells were observed in the cyclin D3 expressing myoblasts than control, suggesting that these cells were unable to pass through mitosis. Thus, an intermediate state was attained by cyclin D3 expressing myoblasts wherein they seem to be '*primed*' for, rather than induced into, differentiation.

(v) Study of the effects of cyclin D3 expression on early myogenic events

Elevated level of cyclin D3 expression in myocytes affected initial stages of differentiation, particularly the expression of early myogenic markers, myogenin and p21. The G2/M-phase stalled myoblasts expressing cyclin D3 were competent to differentiate. Infact, they expressed higher levels of myogenin earlier than control and had earlier induction of the cdk inhibitor, p21. Therefore, cyclin D3 upregulation under growth promoting conditions was sufficient to cause changes in nucleoplasminc organization but seemed to be assisted by additional factors to induce differentiation markers. It is known that serum rich conditions prevent pRb hypophosphorylation. Based on these observations we proposed that a switch to differentiation promoting conditions relieves such repression on pRb activity. Cyclin D3 expressing myoblasts seem poised for myogenesis as they respond more efficiently to serum withdrawal than control myocytes. They have better efficiency of myogenic gene expression that results in accelerated rate of cell cycle exit and entry into differentiation.

(vi)Study of the global effects of ectopic cyclin D3 expression in myoblasts

The pathways identified through transcriptional profiling of cyclin D3 expressing myoblasts were helpful in defining the distinct actions of cyclin D3 in muscle cells. Some of the primary genes identified in microarray analysis were validated by real- time PCR. These were broadly classified as cell cycle, myogenic and other candidate target genes. A number of the genes that were significantly upregulated in cyclin D3 expressing myoblasts were chromatin modifiers as well as those associated with splicing factors. In general, I observed marginal changes in gene expression due to cyclin D3 ectopic expression under uninduced (myoblast) conditions. This was consistent with the comparable level of expression of the G1 cell cycle markers and myogenin in transfected myoblasts. Significant differential gene expression occurred within 16h of differentiation in adenoviral-cyclin D3HA transfected myocytes. There was increased induction of all myogenic genes tested, such as myogenin, MyoD, Myf5 and MEF2C, in myocytes formed by adeno-cyclin D3HA myoblasts, which confirmed the "*myogenically primed*" status of cyclin D3 expressing myoblasts.

An unexpectedly high level of expression of the paired-box transcription factor, Pax-7, in adeno-cyclin D3HA myoblasts was also revealed from the real time PCR analysis. Further, a histone methyl transferase, RbBP5, known to interact with Pax-7 was upregulated in cyclin D3-overexpressing myocytes.

(vii)Study of the effects of Cyclin D3 on Pax-7 transcription in myoblasts

CyclinD3 expressing myoblasts expressed higher levels of Pax-7 and were competent to differentiate better than the control cells. A promoter-reporter assay showed ~2-fold activation of Pax-7 by cyclin D3 expression in myoblasts. Adeno-cyclin D3HA myoblasts had higher expression of Pax-7 family transcripts (Pax-7, Pax-3, Ash2L, MLL2, RbBP5 and Wdr5), as quantified by real-time PCR and most of these genes remained upregulated (~2-fold) after differentiation (in 16h myocytes). However, a temporally separate upregulation of Pax-7 and its interacting histone methyl transferase (HMT) complex was observed, as Pax-7 itself is efficiently downregulated upon differentiation. These results assume relevance in terminal differentiation processes, as Pax-7 expression is known to regulate chromatin changes that lead to active and tissue specific gene expression. Chromatin modifications and an increase in the levels of myogenic genes are probably the downstream effects of higher Pax7 expression.

Based on these studies we proposed that cyclin D3 primes myoblasts for differentiation and controls a transient stage within the muscle development process which is associated with irreversible exit from mitosis. The stalling of the cell-cycle at G2/M phase occurred due to sequestrated cyclins and cdks in myoblasts poised for differentiation. These events affect the nucleoplasmic lamin scaffold which possibly gets reorganized to bind myogenic factors that control various aspects of lineage restricted gene activation. The cyclin D3 mediated upregulation of promyogenic factor, Pax-7, is probably responsible for the earlier differentiation of myoblasts as Pax-7 is known to recruit histone methyl transferases during activation of myogenic genes. Our observations suggested that components of the pax-family interact with or are a part of, the cyclin D3-lamin scaffold that is necessary for proper myogenic gene expression.

Brief postdoctoral research work (February-August,2012)

During the brief period of postdoctoral training in Dr. Geyer's lab, at the University of Iowa, I had the opportunity to learn a number of techniques using the *Drosophila* model system. At that time her group was focused on understanding the characteristics of the genomic binding sites of the multi-zinc finger protein, Suppressor of hairy-wing [Su(Hw)]. Working towards this end I cloned the regulatory regions of selected direct target genes of Su(Hw) into a series of vectors that would be used for making reporter constructs for creating transgenic flies. Using some of the mutant genotypes previously generated in the lab, I performed Chromatin Immunoprecipitation to compare the chromatin landscape of some of the target genes between Su(Hw) null and wild-type condition. In addition, I have used confocal microscopy for immunofluorescence imaging, investigating the effects of loss of Su(Hw) on the nuclear architecture in fixed *Drosophila* embryos. In addition, through Fluorescent In-Situ Hybridization (FISH) based assay I sought to get some insights into the homologous chromosomal pairing in *Drosophila* and studied pairing at various chromosomal locations in the fly genome in a Su(Hw) null and wild type background.

Post-Doctoral research work (2013 - 2016)

During my postdoctoral research in Prof. Ingham's lab, at IMCB, I pursued my interest in muscle wasting diseases. I worked with the Zebrafish *in vivo* model system, ideal for studying muscle development. I investigated aspects of myopathy focusing on defects in myofibrillar assembly involving unconventional myosin. We characterized the zebrafish motility mutants, *frozen*. Myopathy in these

mutants is attributed to myofibrillar assembly defects due to lack of unconventional myosin XVIIIB (myo18b) expression in fast-twitch skeletal muscles. This work importantly resulted in establishing zebrafish mutants of Myo18b gene that model Klippel-Feil anomaly (KFA) in humans. One of the benefits of working with zebrafish as a model system is the feasibility of using gene editing and imaging techniques. Imaging the zebrafish samples for immune fluorescence and ALP assay was an essential tool for my investigation and I have developed good expertise in it. I have successfully utilized the CRISPR/Cas9 gene editing system to establish mutants of Myo18b gene to verify the *frozen* mutation. This postdoctoral research program not only increased the scope of my knowledge in muscle biology to a tissue developmental context but it also armed me with a very useful genetic model system to work with. This has great relevance while pursuing research in muscle diseases to understand their molecular mechanisms in a very holistic way.

Gap in Employment (2016-2019)

I have taken a maternity break from 2016 to 2019. During this period, I relocated from abroad back to India.

Work experience on Project MANAV - Human Atlas Initiative (2019 - 2022)

Currently, I am working with an IT company-Academia allied venture project aimed at building a bio-curation platform. This has been a three-year project to create a skin model from data-mining of open-source scientific literature by using ML/NLP based computation approaches, aided by intelligent crowd sourcing. It is a government funded project (Department of Bio-Technology, Govt. of India), based in Pune, India and we recently opened a prototype of the platform at the National level for beta testing.

In this project, I have held the position of a Domain Expert and was later promoted to Senior Domain Expert as a science team lead. I have actively created scientific content contributing towards the platform database, provided useful insights through data generated from *Proof-of-Concept* studies within my team and moderated Webinars to bring together amateur students and experts from academia working in varied biological fields. As a domain support to the platform development team, I have developed wireframes to depict ways to represent scientific content on the user interface (UI)/platform. I have also been involved with resource management and coordination between IT and research professionals/experts.

Specific areas of expertise

Cell biology, Developmental biology and Genetics
Biochemistry, Molecular biology and Microscopy

Future interests

I am very connected to science and translational research as my expertise have been focused largely on studying muscle wasting disorders. This is a huge advantage as it opens a wider perspective to molecular and cellular biology research by providing the much needed in-vivo context. However, having worked in Singapore for three years in the academia, exposed me to the novel and enjoyable ways that Science could be inculcated in young children. They involved scientists to help develop a natural tendency to ASK QUESTIONS, among young students to make them learn rather than

memorize science.

So, I am open to finding a well-matched teaching position as well, where my wide range of expertise and knowledge would be useful. At the basic level, I can train students in laboratory molecular biology and teach the biology courses in the curriculum. With a thorough experience in working with three genetic model systems I am well equipped to be an instructor for advanced courses at the B.Sc and MSc level (like cell biology, developmental biology and genetics). I have some experience with guiding school students and undergraduate students attached with the lab for summer training during my postdoctoral training at US and Singapore. Other than that, working at multidisciplinary institutes throughout my career I have had the advantage of being exposed to highly interactive niche of students and mentors. We have learnt to encourage each other's curiosity through regular seminars and scientific meetings. This experience has been useful towards gaining a wider perspective and better analytical ability for my research pursuit. I believe that my scientific acumen would serve the purpose well in teaching positions too. With the constant collaborative environment in which I have developed as a scientist I am sure I will be very comfortable in communicating with young minds.

Additionally, since I have substantial experience in research in both national and international environments, I would be a good fit for administrative positions that require people experienced in academia. In general, my expertise could be advantageous in positions that oversee organizational committee for educational institutions.