BIOGRAPHICAL SKETCH

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NAME: Pestov, Dimitri

eRA COMMONS USER NAME (credential, e.g., agency login): pestov

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Moscow State University, Moscow	MS	05/1988	Biochemistry
University of Illinois, Chicago, IL	PHD	12/1997	Molecular Genetics
University of Illinois, Chicago, IL	Postdoctoral	04/2001	Mammalian Cell Biology

A. Personal Statement

I have studied ribosomes for over two decades, with a particular emphasis on understanding the effects of stress on ribosome homeostasis. My studies were the first to document the p53-mediated response to defects in ribosome biogenesis in mammalian cells, pioneering the concept of nucleolar stress as a common denominator in the cellular sensing of environmental and metabolic perturbations. My work helped to establish a number of methods that are currently in use for analyzing rRNA processing in mammalian cells. In the proposed studies, we pursue a new research direction to seek answers to the longstanding question of how the oxidative environment affects ribosomes. Due to the field's still nascent state of knowledge about this fundamental aspect of ribosome biology, this is an exciting area to explore. I am particularly fascinated by the emerging links between ribosome oxidation and cancer cell metabolism, which came to light in our recent collaborative studies with Steven Zheng's group at Rutgers Cancer Institute. In this project, I also look forward to continuing my longstanding collaboration with Natalia Shcherbik, with whom we have published in the last several years on the damage caused to yeast ribosomes by oxidants and redox-active metals. The unique experience in the redox biology of ribosomes gained through these recent studies puts my research group in a solid position to tackle experimental challenges in the proposed study.

A selection of four publications highlighting relevant experience for this project:

1. Wang X, Zhang H, Sapio R, Yang J, Wong J, Zhang X, Guo JY, Pine S, Van Remmen H, Li H, White E, Liu C, Kiledjian M, Pestov DG, Zheng SXF. SOD1 regulates ribosome biogenesis in KRAS mutant non-small cell lung cancer. *Nat Commun.* 2021; 12(1):2259. PMCID: PMC8050259.

2. Sapio RT, Burns CJ, Pestov DG. Effects of hydrogen peroxide stress on the nucleolar redox environment and pre-rRNA maturation. *Front Mol Biosci.* 2021; 8:678488. PMCID: PMC8107432.

3. Trainor BM, Pestov DG, Shcherbik N. Development, validation, and application of the ribosome separation and reconstitution system for protein translation in vitro. *RNA.* 2021; 27(12):1602-1616. PMCID: PMC8594471.

4. Zinskie JA, Ghosh A, Trainor BM, Shedlovskiy D, Pestov DG, Shcherbik N. Iron-dependent cleavage of ribosomal RNA during oxidative stress in the yeast Saccharomyces cerevisiae. *J Biol Chem.* 2018; 293(37):14237-14248. PMCID: PMC6139556.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022	Ad hoc reviewer, NIH, NIH Director's Transformative Research Award
2022	Ad hoc reviewer, NIH, MG study section
2020-present	Full Member, Cancer Institute of New Jersey
2016	Ad hoc reviewer, NIH, Cell Biology, Developmental Biology and Bioengineering Review Panel
2013-present	Associate Professor, Rowan University, Stratford, NJ
2010-present	Member, American Society for Microbiology
2007-2013	Assistant Professor, University of Medicine and Dentistry of New Jersey, Stratford, NJ
2001-2007	Research Assistant Professor, University of Illinois at Chicago, Chicago, IL
2000-present	Member, RNA Society

Honors

2012	Excellence in Research Award, Foundation of UMDNJ
1995	University Fellowship, University of Illinois at Chicago
1994	Dean's Scholar Award, University of Illinois
1993	Dean's Scholar Award, University of Illinois

C. Contributions to Science

1. Genetic selection of growth-inhibitory genes in mammalian cells.

   My early work as a graduate student contributed to the development of an approach, termed the selection of genetic suppressor elements, to isolate inhibitors of a given phenotype without prior knowledge of the genes involved (a). Later, I developed a genetic system to identify mammalian genes that reversibly inhibit cell cycle progression (b). Building on my background in molecular genetics and cell culture, I conducted an unbiased search for proliferation-arresting sequences in a mouse cDNA library (c), which ultimately led to the discovery of p53 surveillance of the nucleolar function.

   a. Holzmayer TA, Pestov DG, Roninson IB. Isolation of dominant-negative mutants and inhibitory antisense RNA sequences by expression selection of random DNA fragments. *Nucleic Acids Res.* 1992 Feb 25;20(4):711-7. PMCID: PMC312009.
   b. Pestov DG, Lau LF. Genetic selection of growth-inhibitory sequences in mammalian cells. *Proc Natl Acad Sci U S A.* 1994 Dec 20;91(26):12549-53. PMCID: PMC45476.
   c. Pestov DG, Grzeszkiewicz TM, Lau LF. Isolation of growth suppressors from a cDNA expression library. *Oncogene.* 1998 Dec 17;17(24):3187-97. PubMed PMID: 9872334.

2. Discovery of the nucleolar stress response.

   Having identified the nucleolar protein Bop1 in my graduate work, I continued studying this protein and the role of its yeast ortholog Erb1 in ribosome biogenesis (a, b). My effort to understand the unusual growth-inhibitory effects of Bop1 mutants led to the first publication to show that defects in ribosome biogenesis can activate a p53-dependent cell cycle checkpoint in mammalian cells, a phenomenon we termed the "nucleolar stress response" (c). This work was extensively cited and influenced the field by changing our thinking about the roles of the nucleolus in stress signaling. These findings have also led to a better understanding of the etiology of ribosomopathies, human diseases caused by mutations in ribosomal proteins and ribosome biogenesis factors.

   a. Strezoska Z, Pestov DG, Lau LF. Bop1 is a mouse WD40 repeat nucleolar protein involved in 28S and 5. 8S rRNA processing and 60S ribosome biogenesis. *Mol Cell Biol.* 2000 Aug;20(15):5516-28. PMCID: PMC86002.

b. Pestov DG, Stockelman MG, Strezoska Z, Lau LF. ERB1, the yeast homolog of mammalian Bop1, is an essential gene required for maturation of the 25S and 5.8S ribosomal RNAs. *Nucleic Acids Res.* 2001 Sep 1;29(17):3621-30. PMCID: PMC55883.

c. Pestov DG, Strezoska Z, Lau LF. Evidence of p53-dependent cross-talk between ribosome biogenesis and the cell cycle: effects of nucleolar protein Bop1 on G(1)/S transition. *Mol Cell Biol.* 2001 Jul;21(13):4246-55. PMCID: PMC87085.

3. Mechanisms of mammalian ribosome biogenesis.

When I began studying mammalian ribosome biogenesis, the field was still in its infancy. My group identified several ribosome biogenesis factors and characterized their function in pre-ribosome maturation. In one of the first studies of its kind, we described the coordinated action of two mammalian nucleolar proteins during 60S ribosomal subunit maturation (a). Our work advanced the field by developing methods to study pre-rRNA processing in mammalian cells (b). A detailed analysis of mouse pre-rRNA processing led us to propose a unifying model for a key event during ribosome assembly: the split of pre-rRNA between the large and small ribosomal subunits. As part of this work, we developed a new technique, the ratio analysis of multiple precursors, which facilitates the analysis of the ribosome biogenesis pathway (c). We recently collaborated with our colleagues in Spain to develop a new method for the purification and tagging of human nucleolar pre-40S ribosomes at distinct maturation stages (d).

a. Lapik YR, Fernandes CJ, Lau LF, Pestov DG. Physical and functional interaction between Pes1 and Bop1 in mammalian ribosome biogenesis. *Mol Cell.* 2004 Jul 2;15(1):17-29. PubMed PMID: 15225545; NIHMSID: NIHMS35038.

b. Pestov DG, Lapik YR, Lau LF. Assays for ribosomal RNA processing and ribosome assembly. *Curr Protoc Cell Biol.* 2008 Jun; Chapter 22: Unit 22.11. PubMed PMID: 18551418; NIHMSID: NIHMS250088.

c. Wang M, Anikin L, Pestov DG. Two orthogonal cleavages separate subunit RNAs in mouse ribosome biogenesis. *Nucleic Acids Res.* 2014;42(17):11180-91. PMCID: PMC4176171.

d. Nieto B, Gaspar SG, Moriggi G, Pestov DG, Bustelo XR, Dosil M. Identification of distinct maturation steps involved in human 40S ribosomal subunit biosynthesis. *Nat Commun.* 2020 Jan 9;11(1):156. PMCID: PMC6952385.

4. Pathways and factors involved in the degradation of ribosomal RNA.

Our understanding of how cells degrade incorrectly assembled or damaged ribosomes is incomplete. Working in mammalian cells, our lab showed for the first time the role of polyadenylation by non-canonical poly(A) polymerases in eliminating abortive pre-rRNA transcripts (a). Our studies elucidated the quality control of ribosome assembly by the mammalian exonuclease Xrn2. This mechanism, in which Xrn2 probes 5' ends of pre-rRNAs during ribosome maturation, determines whether pre-rRNAs will be processed to mature forms or discarded, depending on whether the corresponding pre-ribosomal complexes have been correctly assembled (b). Our work in yeast implicated cytoplasmic nucleases in the regulation of ribosome turnover during nutritional shifts in this organism (c).

a. Shcherbik N, Wang M, Lapik YR, Srivastava L, Pestov DG. Polyadenylation and degradation of incomplete RNA polymerase I transcripts in mammalian cells. *EMBO Rep.* 2010 Feb;11(2):106-11. PMCID: PMC2828747.

b. Wang M, Pestov DG. 5'-end surveillance by Xrn2 acts as a shared mechanism for mammalian pre-rRNA maturation and decay. *Nucleic Acids Res.* 2011 Mar;39(5):1811-22. PMCID: PMC3061060.

c. Pestov DG, Shcherbik N. Rapid cytoplasmic turnover of yeast ribosomes in response to rapamycin inhibition of TOR. *Mol Cell Biol*. 2012 Jun;32(11):2135-44. PMCID: PMC3372233.

5. Understanding the impact of oxidative stress on ribosomes.

My laboratory has made pioneering contributions to understanding the complex relationship between oxidative stress, ribosome biogenesis, and ribosome maintenance in eukaryotic cells. In collaboration

with Natalia Shcherbik, we have shown that the binding of intracellular iron to rRNA acts as the principal mechanism of rRNA strand cleavage occurring in yeast under oxidative stress (a, b). In our recent studies, we developed several new tools to study the oxidation of nucleolar pre-rRNA in mammalian cells (c) and discovered a previously unknown nucleolar role of the mammalian superoxide dismutase SOD1, explaining a critical aspect of its requirement for viability in lung cancer cells (d).

a. Zinskie JA, Ghosh A, Trainor BM, Shedlovskiy D, Pestov DG, Shcherbik N. Iron-dependent cleavage of ribosomal RNA during oxidative stress in the yeast Saccharomyces cerevisiae. *J Biol Chem.* 2018 Sep 14;293(37):14237-14248. PMCID: PMC6139556.

b. Smethurst DGJ, Kovalev N, McKenzie ER, Pestov DG, Shcherbik N. Iron-mediated degradation of ribosomes under oxidative stress is attenuated by manganese. *J Biol Chem.* 2020 Dec 11;295(50):17200-17214. PMCID: PMC7863898.

c. Sapio RT, Burns CJ, Pestov DG. Effects of hydrogen peroxide stress on the nucleolar redox environment and pre-rRNA maturation. *Front Mol Biosci.* 2021; 8:678488. PMCID: PMC8107432.

d. Wang X, Zhang H, Sapio R, Yang J, Wong J, Zhang X, Guo JY, Pine S, Van Remmen H, Li H, White E, Liu C, Kiledjian M, Pestov DG, Zheng SXF. SOD1 regulates ribosome biogenesis in KRAS mutant non-small cell lung cancer. *Nat Commun.* 2021 Apr 15;12(1):2259. PMCID: PMC8050259.

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