BIOGRAPHICAL SKETCH

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NAME: Kiledjian, Megerditch

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

POSITION TITLE: Distinguished Professor and Chair

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
Cook College, Rutgers University	BA	05/1985	Biochemistry
University of Pennsylvania; PA	PhD	12/1990	Molecular Biology
University of Pennsylvania; PA		1990-1993	Postdoctoral Training – RNA Biology
University of Pennsylvania; PA		1994-1995	Postdoctoral Training – RNA Biology

A. Personal Statement

My lab has been studying the regulatory components involved in mRNA turnover since 1995. We have a track record of cutting-edge research that is transformative and consistently opens new areas of inquiry in the mRNA decay field with an emphasis on mRNA decapping. We utilize both mammalian and yeast systems with biochemical, structural and genetic approaches to advance the discovery of novel mechanistic avenues of regulated RNA-mediated gene expression. Moreover, many of the decapping enzymes we have identified (and do not patent) are available commercially for use by the scientific community. We initially established a cell free mRNA decay system which faithfully recapitulated regulated mRNA decay in vitro. We successfully utilized this system to decipher the molecular pathways and mechanisms of mRNA decay and the nucleases involved, specifically mRNA decapping enzymes. We identified and demonstrated Dcp2, Nudt16 and Nudt3 are mRNA decapping enzymes. We also identified the scavenger-decapping enzyme, DcpS which primarily functions on methyl-cap structure that is generated by the 3´end decay pathway and its role in cognitive disability. These findings enabled us, as well as many colleagues, to pursue the regulatory role of decapping enzymes in mRNA biology. Furthermore, in collaboration with Dr. Liang Tong's lab, we demonstrated a new class of noncanonical decapping enzymes consisting of yeast Rai1 and Dxo1 and mammalian DXO are involved in a novel 5´-end surveillance mechanism that detected and degraded incompletely capped RNAs. In addition, we demonstrated that a broad spectrum of Nudix hydrolases in mammals and yeast, previously unknown to have decapping activity, possess decapping activity. More recently, we reported the presence of NAD capped mRNA and noncoding RNAs in mammalian cells. Furthermore, we showed that the NAD cap can promote RNA decay through DXO- or Nudt12-directed mechanisms. In addition we recently demonstrated that the yeast Xrn1 exoribonuclease is also a deNADding enzyme and most significantly, it modulates mitochondrial NAD capped RNA decapping (deNADding) and appears to control intramitochondrial NAD levels. Lastly, the surprising finding that mitochondrial NAD-capped mRNAs can associate with translating ribosomes open a new area of metabolite cap function in mRNA biology. I look forward to continuing these studies focused on the regulation imparted by the 5¢end of mRNA and the correlation of RNA metabolism to cellular metabolism and function.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

1990 - 1993 HHMI Postdoctoral Fellow, Biochemistry & Biophysics, University of Pennsylvania, PA
1994 - 1995 HHMI Postdoctoral Fellow, Genetics, University of Pennsylvania, PA
1995 - 2001 Assistant Professor, Cell Biology and Neuroscience, Rutgers University, NJ
2001 - 2005 Associate Professor, Cell Biology and Neuroscience, Rutgers University, NJ
2002 - Present Member of the Cancer Institute of New Jersey, New Brunswick, NJ
2005 - 2015 Professor, Cell Biology and Neuroscience, Rutgers University, NJ
2007 - 2012 Vice Chair, Cell Biology and Neuroscience, Rutgers University, NJ
2012 - Present Chair, Cell Biology and Neuroscience, Rutgers University, NJ
2015 - Present Member, Human Genetics Institute of New Jersey
2015 - Present Distinguished Professor, Cell Biology and Neuroscience, Rutgers University, NJ
2017 – 2022 Senior Member, Brain Health Institute

Other Experience and Professional Memberships

1989 -Present Member, American Association for the Advancement of Science
1990- Present Member, American Society of Microbiology
1992- Present Member, RNA Society
1997- Present Member, American Society for Biochemistry and Molecular Biology
2006- Present Member, Society for Neuroscience
2011- 2017 Study section member, NIH/Genes, Genomes and Genetics IRG, MGB Study Section

Honors

1980 United States Army Science and Engineering Award
1980 United States Air Force Zoology Award
1980 Bausch and Lomb Science Award
1983-1985 George H. Cook Scholar
1985 Souren Ohanian Hamazkayin Fellowship
1990-1993 Howard Hughes Medical Institute Associate
1995 Cooley's Anemia Young Investigator Award
1999 Johnson & Johnson Discovery Award
2011 Weehawken Academic Hall of Fame
2015 Rutgers University Distinguished Professor
2018 Fellow, American Association for the Advancement of Science
2020 Rutgers University Board of Trustees Award for Excellence in Research

C. Contributions to Science

1. <u>Establish an *in vitro* mRNA decay system:</u> Early in my independent career, we set out to establish an *in vitro* mRNA decay system that recapitulated controlled mRNA decay using the stable α-globin mRNA. This allowed us to decipher the molecular mechanism by which the 3´UTR of this mRNA confered stability through an association with the poly(A)-binding protein on the 3´ poly(A) tail. This system also enabled us to expand our studies into broader regulation of mRNA decay with an emphasis on decapping enzymes.

a. Wang, Z, Day, N., Trifillis, P. and **Kiledjian, M.** (1999) An mRNA Stability Complex Functions with the Poly(A)-Binding Protein to Stabilize mRNA In Vitro. *Mol. Cell. Biol.* 19:4552-4560 PMCID: PMC84253
b. Wang, Z. and **Kiledjian, M.** (2000) Identification of an Erythroid-Enriched Endoribonuclease Activity Involved in Specific mRNA Cleavage. *EMBO J.* 2:295-305. PMCID: PMC305563
c. Wang, Z. and **Kiledjian, M.** (2000) The Poly(A)-Binding Protein and an mRNA Stability Protein Jointly Regulate an Endoribonuclease Activity. *Mol. Cell. Biol.* 20:6334-6341. PMCID: PMC86108
d. Rodgers, N., Wang, Z, and **Kiledjian, M.** (2002). Characterization and Purification of a Mammalian Endoribonuclease Specific for the a-Globin mRNA. *J. Biol. Chem.* 277:2597-2604.

2. **<u>Identification of the scavenger decapping enzyme, DcpS:</u>** In 2001 we reported the identification of a decapping activity and protein which hydrolyzes the residual cap structure following 3´-5´ exonucleolytic decay. This protein was DcpS, a Histidine Triad motif containing hydrolase that preferentially functions on cap structure. We subsequently demonstrated that the yeast ortholog of DcpS functions to enhance the 5´'3´ exonucleolytic

activity of Xrn1 to influence 5´ end decay. More recently, we demonstrated a role for DcpS in cognitive function. Individuals harboring homozygous mutations that disrupt DcpS decapping activity have severe cognitive defects. Moreover, by developing induced pluripotent stem cells (iPSCs) from the affected individual cells that were differentiated into induced neurons (iN), we showed these neurons exhibit defects in neural differentiation, neurite outgrowth abnormalities and growth cone projection aberrations as a function of defective DcpS decapping. We will pursue the molecular etiology of the impaired DcpS decapping on cognition within this proposal.

a. Wang, Z, and **Kiledjian, M.** (2001). Functional Link between the Mammalian Exosome and mRNA Decapping. *Cell*. 107:751-762.
b. Liu, H., Rodgers, N., Jiao, X, and **Kiledjian, M.** (2002). The Scavenger mRNA Decapping Enzyme, DcpS, is a member of the HIT Family of Pyrophosphatases. *EMBO J.* 21:4699-4708. PMCID: PMC126188
c. Ahmed, I., Buchert, R., Zhou, M., Mittal, K., Scheller, U., Rafiq, M.A., Brohi, M.Q., Mikhailov, A., Bhatti, A.,Sticht, H., Carter, M., Uebe, S., Reis, A., Ayub, M., John, P., **Kiledjian, M**.*, Vincent, J.B.*, Jamra, R.J.* (2015). Mutations in DCPS and EDC3 in Autosomal Recessive Intellectual Disability Indicate a Crucial Role for mRNA Decapping in Neurodevelopment. *Hum Mol Genet.,* 24:3172-3180. PMCID: PMC4424955 *co-corresponding authors
d. Salamon, I., Palsule, G., Luo, X., Roque, A., Tucai, S., Khosla, I., Volk, N., Liu, W., Cui, H., Dal Pozzo, V., Zalamea, P.,Jiao, X., D'Arcangelo, G., Hart, R.P. Rasin, M.R. and **Kiledjian, M.** (2021) mRNA-decapping associated DcpS enzyme controls critical steps of neuronal development. *Cerebral Cortex*. 32:1494-1507. PMCID: PMC8971079

3. <u>Identification and characterization of all currently reported mammalian mRNA decapping enzymes:</u> In 2002, we reported the identification of Dcp2 as an mRNA decapping enzyme. This was the first of three papers with this discovery. Dcp2 is a Nudix hydrolase motif-containing protein and the first decapping enzyme identified. Although at the time it was thought to be a default decapping enzyme that decapped and degraded all mRNAs, our insight that Dcp2 had RNA binding capacity led the way to subsequently demonstrate that it preferentially functions on only a subset of mRNAs. One major class consisted of mRNAs involved in innate immunity and further reinforced that additional mRNA decapping enzymes must also exist in mammals. Consistent with this premise, we subsequently reported that Nudt16 is a cytoplasmic decapping enzyme and more recently showed that Nudt3 is also an mRNA decapping enzyme that selectively functions on mRNAs encoding proteins involved in cytoskeletal architecture and cell migration. We've also identified additional Nudix proteins with *in vitro* decapping activity (Nudt-2, 12, 15, 17 and 19) and are currently determining their function in cells.

a. Wang, Z., Jiao, X., Carr-Schmid, A. and **Kiledjian, M.** (2002). Dcp2 Protein is a Regulated Mammalian mRNA Decapping Enzyme. *Proc. Natl Acad. Sci. USA*, 99:12663-12668. PMCID: PMC130517
b. Song, M., Li, Y. and **Kiledjian, M.** (2010). Multiple mRNA decapping enzymes in mammalian cells. *Mol. Cell*, 40:423-432. PMCID: PMC2982215
c. Grudzien-Nogalska, E., Jiao, X., Song, M.G., Hart, R.P. and **Kiledjian, M.** (2016). Nudt3 is a mRNA Decapping Enzyme That Modulates Cell Migration. *RNA,* 22:773-781. PMCID: PMC4836651
d. Mauer, J., Luo, X., Blanjoie, A., Jiao, X., Grozhik, A.V., Patil, D.P., Linder, B., Pickering, B.F., Vasseur, JJ., Chen, Q., Gross, S.S., Elemento, O., Debart, F., **Kiledjian, M.** and Jaffrey, S.R. (2017). Reversible methylation of m^6A_m in the 5´ cap controls mRNA stability. *Nature*, 541:371-375. PMCID: PMC5513158

4. <u>Identification of a novel 5' end quality control mechanism:</u> In a collaborative effort with Liang Tong's lab, we identified a heretofore-unknown 5´end capping quality control mechanism. Our studies showed that mRNA capping, long thought to be a default process that occurred on all RNA Polymerase II primary transcripts, is inaccurate. A quality control mechanism exists that clears incompletely capped transcripts. This was initially shown in yeast with our demonstration that the Rat1 interacting protein, Rai1, can selectively hydrolyze the 5´ end of mRNAs that either lack a cap or possess an incomplete cap. Exposure of the 5´ end subsequently enables the Rat1 exonuclease component of the Rai1-Rat1 heterodimer, to degrade the RNA. Moreover, we showed that under stress conditions, incompletely capped RNAs were normally generated and cleared by Rai1-Rat1 suggesting addition of a cap is a regulated process for the first time. We also identified a second yeast protein of previously unknown function, which exhibited preferential decapping activity on incompletely capped mRNAs. We termed this protein Decapping eXOnuclease 1 (Dxo1) due to the fact that it possesses intrinsic 5´-3´ exoribonuclease activity, which can singlehandedly decap and degrade mRNAs with incomplete caps. We also extended these studies into mammalian cells and demonstrated that the mammalian homolog of Rai1/Dxo1, we termed DXO, possessed biochemical properties of both the Rai1 and Dxo1 activities. Surprisingly, DXO functions

in pre-mRNA 5´ capping quality control with minimal effects in representative mature mRNAs tested. Our studies established a new paradigm in mRNA processing where incompletely capped pre-mRNAs are degraded by DXO and are not efficiently spliced and polyadenylated thus serving as a surveillance protein in mammalian 5´-end capping quality control at the pre-mRNA level.

a. Xiang, S., Cooper-Morgan, A., Jiao, X., **Kiledjian, M.**, Manley, J.L., Tong, L. (2009). Structure and function of the 5'→3' exoribonuclease Rat1 and its activating partner Rai1. *Nature*, 458:784-788. PMCID: PMC2739979
b. Jiao, X., Xiang, S., Oh, C., Martin, C.E., Tong, L. and **Kiledjian, M.** (2010). Identification of a quality control mechanism for mRNA 5´-end capping. *Nature*, 467:608-611. PMCID: PMC2948066
c. Chang, J.H., Jiao, X., Chiba, K., Oh, C., Martin, C.E., **Kiledjian, M.*** and Tong, L.* (2012). Dxo1, a novel eukaryotic enzyme with both decapping and 5´-3´ exoribonuclease activity. *Nat. Struc. Mol. Biol.*, 19:1011-1017. PMCID: PMC3711404 * co-corresponding authors
d. Jiao, X., Chang, J.H., Chiba, K., Kilic, T., Tong, L.* and **Kiledjian, M.*** (2013). A mammalian pre-mRNA 5´-end capping quality control mechanism and an unexpected link of capping to pre-mRNA processing. *Mol. Cell*, 50:104-115. PMCID: PMC3630477 * co-corresponding authors

5. <u>NAD capping in eukaryotic cells:</u> We recently demonstrated that human cells contain both NAD capped mRNAs as well as NAD-capped noncoding RNAs. Moreover, we identified the mammalian and yeast DXO proteins as potent NAD cap decapping (deNADding) enzymes (even more robust on NAD capped RNAs than the above- mentioned function on incompletely capped RNAs) that degrade NAD capped mRNAs. Importantly, we showed that an NAD cap on an mRNA does not support stability or translation, but rather promotes rapid mRNA decay. We subsequently showed that the Nudix hydrolase Nudt12 is also a deNADding enzyme that targets a distinct subset of NAD-capped RNAs than that of DXO and the stability of these mRNAs are differentially modulated by nutrient and environmental stress. Our findings demonstrate a novel mode of RNA metabolism that may be linked to cellular metabolism. Most recently we uncovered a surprising new role for the well characterized Xrn1 exonuclease as a deNADding enzyme that can localize to mitochondria in yeast and selectively modulate the stability of NAD-capped mitochondrial RNAs and in turn contribute to overall mitochondrial NAD under respiratory stress. These findings implicate mitochondrial NAD caps in the maintenance of mitochondrial homeostasis and will be further pursued in this application.

a. Jiao, X., Doamekpor, S., Bird, J.G., Nickels, B.E., Tong, L., Hart, R.P. and **Kiledjian, M**. (2017). 5´-end NAD^+ cap in human cells promotes RNA decay through DXO-mediated deNADding. *Cell*, 168:1015-1027. PMCID: PMC5371429
b. Grudzien-Nogalska, E., Wu, Y., Jiao, X., Cui, H., Mateyak, M. K., Hart, R. P., Tong, L., and Kiledjian, M. (2019). Structural and mechanistic basis of mammalian Nudt12 RNA deNADding. *Nat Chem Biol* **15**, 575-582 PMCID: PMC6527130
c. Sharma, S., Grudzien-Nogalska, E., Hamilton, K., Jiao, X., Yang, J., Tong, L., and **Kiledjian, M.** (2020). Mammalian Nudix proteins cleave nucleotide metabolite caps on RNAs. *Nucleic Acids Res* **48**, 6788-6798. PMCID: PMC7337524
d. Sharma S, Yang J, Grudzien-Nogalska E and **Kiledjian M**. (2022) Xrn1 is a deNADding Enzyme Modulating Mitochondrial NAD-capped RNA. *Nature Com*. **13,** 889 (2022). PMCID: PMC8850482

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