April 2021 **Roni Rak**

Part I: CURRICULUM VITAE

Personal .1

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Dates	Description
1984	Born in Israel

University Education and Additional Training .2

Dates	Description
2005 – 2008	B.Sc. in Life Sciences at Tel Aviv University.
2008 – 2009	M.Sc. in Neurobiochemistry, at Tel Aviv University. Rotation year of the Dean's list honors program for outstanding students. Supervision by: Prof Yoel Kloog
2009 – 2013	Ph.D. in Neurobiochemistry, at Tel Aviv University. Title of thesis: Design and development of a novel LIMK inhibitor as a potential cancer drug. Supervision by: Prof. Yoel Kloog
2013 – 2015	Postdoctoral position at Tel Aviv University with Prof. Oded Rechavi Research subject: Linking translation to protein misfolding
2015 – 2016	Postdoctoral position at Weizmann Institute with Prof. YitzhakPilpel Research subject: regulation of translation in proliferating and differentiated mammalian cells
2016 – 2020	Senior intern position at Weizmann Institute with Prof. YitzhakPilpel Research subject: regulation of translation in proliferating and differentiated mammalian cells

Positions Held and Academic Status .3

Teaching Experience / Guiding Students .4

Academic Contribution: .A

Guidance of M.Sc. Students (or B. Sci. internship) .B

Graduation date	Name	Title of thesis	Guidance with
2015	Mr. Idan Adir*	New tool for tracking individual tRNA species expression in Caenorhabditis elegans	Prof. Oded Rechavi
2019	Ms. Alisa Greenberg*	Systematic detection of cellular factors affectin translation accuracy upon physiological and genetic perturbations.	Prof. Yitzhak Pilpel

* under my direct guidance, informal.

Guidance of Ph.D. Students: .C

Graduation date	Name	Title of thesis	Guidance with
2021 (Exp)	Ms. Noa Hefetz Aharon*	Deciphering the role of translation supply and demand in human physiology.	Prof. Yitzhak Pilpel

* under my direct guidance, informal.

Post-Docs and Visiting Scientists: .D

Organization of Courses .E

Activity in Scientific and Agricultural Committees .5

International: .A

National: .B

Institutional: .C

Contribution to the Scientific Community .6

International: .A

National: .B

Institutional: .C

Outreach: .D

Editorial responsibilities: .E

Dates	Description
2016-2020	Reviewer (ad-hoc) of manuscripts for: Nucleic Acid Research, RNA(journal)

Active Participation in Meetings .7

International: .A

Date	Title of the Meeting	Place	Role
2019	RNA modifications: Form, function and mechanism	WIS, Israel	Poster
2018	One2Many Conference	WIS , Israel	Selected for oral presentation
2018	tRNA Meeting	Strasbourg, France	Selected for oral presentation
2018	38th Blankenese Conference	Hambourg, Germany	Poster
2017	42nd FEBS Conference: From molecules to cells and back	Jerusalem, Israel	Poster

* Lecture / poster presented by an active student

National: .B

Date	Title of the Meeting	Role
2019	The Annual Meeting of the Israeli Society of Evolutionary	Selected for oral presentation
2017	ILANIT/FISEB Conference	Poster
2015	Proteostasis Meeting	Selected for oral presentation

Institutional: .C

Research Grants .8

Internationally Peer Reviewed Grants: .A

Year	Granting Source	Duration (years)	Role *	Title (short)	Budget	
					Total (US $ / year)	Researcher (US $ / year)

Nationally Peer Reviewed Grants: .B

Year	Granting Source	Duration (years)	Role *	Title (short)	Budget	
					Total (US $ / year)	Researcher (US $ / year)
2018	ICRF	2	CI	Characterizing the spectrum and mechanisms of phenotypic errors in cancer	200,000	100,000

*PI = Principal Investigator; CI = Cooperating Investigator

Dates	Description
2016	Recipient of the Dean award, Wiezmann institute .

Part II: LIST OF PUBLICATIONS

	Marks:
X *	Equal contribution as the first author
Corresponding Author *(in cases where the researcher is the Corresponding Author)*	X **
	Marks (only for the first author):
X^S	Student under my supervision
X^T	Technician or research engineer working in my research team
X^PD, X^VS	Post-Doc or Visiting Scientist working in my research team

Articles in Reviewed Journals .1

.1 **Hefetz** N, Dahan O, Frumkin I, Pilpel Y, **Rak R**.** (2020). Manipulation of the human tRNA pool reveals distinct tRNA sets that act in cellular proliferation or cell cycle arrest. Elife, in revision. bioRxiv 2020.04.30.070789; doi: https://doi.org/10.1101/2020.04.30.070789

.2 **Rak R,** Polonsky M, Eizenberg I, Dahan O, Friedman N, Pilpel Y. (2020). Dynamic changes in tRNA modifications and abundance during T-cell activation. PNAS, under review. bioRxiv 2020.03.14.991901; doi: https://doi.org/10.1101/2020.03.14.991901

.3 Mordret E, Dahan O, Asraf O, **Rak R,** Yehonadav, A., Barnabas G.D, Cox J, Geiger T, Lindner A.B, Pilpel Y. (2019) .Systematic detection of amino acid substitutions in proteome reveals a mechanistic basis of ribosome errors and selection for translation fidelity. Molecular Cell, Volume 75, Issue 3.

IF 15.5; Category: Biochemistry and molecular biology ; Rank: 4/297, Cell Biology; Rank :11/195

.4 Kaminski Strauss S, Schirman D, Jona G, Brooks AN, Kunjapur AM, **Rak R,** et al. (2019) Evolthon: A community endeavor to evolve lab evolution. PLOS Biology 17(3): e3000182.

IF 7.0; Category: Biochemistry and molecular biology ; Rank: 34/297, Biology; Rank :6/93

.5 Sagi D*/**, **Rak R*/**,** Gingold H*, Adir I, Maayan G, Dahan O, Broday L, Pilpel Y, Rechavi O**. (2016) Tissue- and Time-Specific Expression of Otherwise Identical tRNA Genes. PLOS Genetics 12(8): e1006264.

IF 5.17 (6.1); Category: genetics & heredity; Rank 22/171 (15/166)

.6 **Rak R,** Elad-Sfadia G, Haklai R, Carmeli S, Wolfson H, Kloog Y. A novel LIMK2 inhibitor blocks pancreatic cancer growth in mice xenograft model, Oncoscience, 2014 Volume 1 issue 1.

IF 3.53 (3.16); Category: Oncology (Q2); Cancer Research (Q3)

.7 Mashiach Farkash E*, **Rak R*,** Elad-Sfadia G, Haklai R, Carmeli S, Kloog Y, & Wolfson H. Computer-Based Identification of a Novel LIMK1/2 Inhibitor that Synergizes with Salirasib to Destabilize the Actin Cytoskeleton. Oncotarget, 2012, Volume 3 issue 6.

IF 5.1 (6.0); Category: oncology; Rank 41/217 (21/197), cell biology; rank: 48/190 (33/185)

* equal contributor **corresponding author

Since previous promotion

Books, Reviews and Opinion Articles .2

1. **Rak R*,** Dahan O*, Pilpel Y. (2018). Repertoires of tRNAs: The Couplers of Genomics and Proteomics. Annual Review of Cell and Developmental Biology, 34(1).

IF 14.6(10.8); Category: cell biology; Rank 13/195, developmental biology; Rank 1/41

2. **Rak R,** Kloog Y. Targeting LIM kinase in cancer and neurofibromatosis (2014). Cell Cycle, Volume 13 issue 9.

IF 3.6(4.5).; Category: Cell Biology; Rank 95/195

* equal contributor

.1 Provisional application for patent in the US, Title: LIM KINASE INHIBITORS; Rak R, Mashiach Farkash E, Elad-Sfadia G, Haklai R, Carmeli S, Kloog Y, & Wolfson H.; Ramot file: 2012054-00; RCIP file:2161798 (2012)

Part III: DESCRIPTION OF MAJOR ACHIEVEMENTS

Ph.D period under Prof. Yoel Kloog at Tel-Aviv University

metastasis drug development

My Ph.D. focused on novel **drug development for cancer and Neurofibromatosis**. We hypothesize that targeting LIM kinase, an enzyme that regulates cell cytoskeleton formation, will reduce cell motility and cancer metastasis. LIM kinase phosphorylates and inhibits cofilin- actin depolymerization factor, and is hyper activated by Rho and Rac-1 proteins, in neurofibromin (*NF1*) depleted cells. In collaboration with Efrat Mashiach-Farkash, we used an **innovative computational screen** that utilized the 3D structure of the enzyme to search for proteins that carry a resembling active site. Based on this approach we could **repurpose an existing molecule to target LIMK and reduce its activity** (1-6). We found that the drug **reduces cell cytoskeleton formation and cell motility** in cells deficient in *NF1*, and in several cancer types. Furthermore, treatment of mice with the drug **blocks cancer formation in pancreatic cancer** (1-7). Based on our success in animal model, we issued a US patent.

Postdoctoral period under Prof. oded Rechavi at Tel-Aviv University

tRNA regulation in development and aging

My first Postdoctoral project was devoted to exploring the link between translation fidelity/efficiency and protein misfolding (1-5). The availability of mature tRNA affects translation rates, proper protein folding, and even mRNA stability (see review (Rak et al. 2018)). The saptio-temporal- regulation of tRNA is crucial for governing the integrity of the proteome. Yet exploring tRNA expression in specific tissues within the intact organism by using reporter proteins (such as GFP) is a challenging task. tRNAs are transcribed by Pol III, which can only transcribe short sequences (much shorter than a typical fluorescent reporter), and depends on tRNA-intrinsic sequences for expression.

During my Postdoctoral, In collaboration with Dr. Dror Sagi, I have **designed and built a novel reporter system that allows to track the expression of individual tRNA** genes in live *C. elegans* nematodes using cell specific fluorescent signal. Although the dogma states that tRNA expression is exclusively regulated by intrinsic control elements (A- and B- box sequences), our data revealed that six Trp-tRNA genes, 100% identical in sequence, are expressed in different tissues and **dynamically** change their **expression during development and aging**. Furthermore, expression

levels of one of these tRNA genes at young adulthood were predictive to the animal's lifespan. We further found that the differential expression of tRNAs that reside within introns of protein-coding genes is dependent on the host gene's promoter.

Postdoctoral and scientist period under Prof. Yitzhak Pilpel at Weizmann Institute

In my second postdoctoral research and as a senior scientist, I took a systemic approach to characterize the translation machinery and tRNA pool changes across evolution, across development and in changing physiological state.

translation in proliferation and differentiation

My main project was aimed towards understanding the role played by tRNAs in **mammalian cells** differs in proliferation and differentiation status. Previous work from the Pilpel lab uncovered a dual translational program in proliferating cancer cells and differentiated cells (Gingold et al. cell, 2014). Building on this work that focused mainly on cancer cells and artificial model systems, I wanted to study the **changes in the tRNA pool and demand for codon, dictated by mRNA expression, in natural physiologically relevant proliferation programs** (1-2). The immune system, in particular T-cells, provides an ideal system to study this question. T-cells undergo vigorous proliferation followed by differentiation step upon triggering of the T cell antigen receptor. **We developed a new method to deep-sequence tRNA molecules and used it in order to follow the tRNA pool during this process.** We found that mRNA demand for codons change in a coordinated manner with tRNA availability in the early activation state of the cells. In addition, we **developed a bioinformatic pipeline enabling us to deduce RNA modifications on tRNA molecules** from tRNA deep sequencing results. We found dynamic changes in tRNA modifications that are likely to affect translation rate and fidelity, and cause aberrant protein translation in highly proliferating T-cells.

Next, to explore the **causality between tRNA availability and cellular proliferation** we developed a **CRISPR-iCas9 system to target tRNA genes. We then used it to target two different sets of tRNAs differentially expressed during either proliferation or differentiation** (1-1). We measured the essentiality of those tRNA in several **cultured cell lines** differing in their proliferation rate and cancerous status. This work revealed that tRNA that are induced in proliferation are more essential in rapidly growing cell lines compared to tRNA induced in differentiation. We further study the effect of tRNA depletion in cellular arrest and found a different subset of tRNA that are involved in entering quiescence and senescence.

translation fidelity

translation errors are frequent, and can affect physiology and protein evolution. We developed the first methodology for systematic detection and quantification of errors through the proteome (1-3). Applying our pipeline on yeast and *E.coli* proteomes revealed that translation mistakes are not random but rather tend to occur at positions that are less evolutionarily conserved, minimally affect protein stability and are fastly translated. Furthermore, we found that most substitutions result from codon-anticodon mispairing rather than tRNA mis-charging.

experimental evolution

To assess different approaches scientists use in experimental evolution, we organized a **community challenge- Evolthon** (1-4). With scientists from multiple labs across the world, we evolved *E. coli* and *S. cerevisiae* in parallel using diverse environmental and genetic regimes toward

dealing with stress of low temperature. My team used an approach we termed "caching cold RNA" in which we reverse transcribed the mRNAs of yeast cultured under cold conditions and integrated it back to untreated yeast genome to create duplicated genes in the genome. After the evolution period, strains from all teams were evaluated based on their successful growth in low temperature as well as other challenges. We were able to recognize strategies that were most successful for the desired challenge and strategies that improved cell growth in various conditions.