BIOGRAPHICAL SKETCH

NAME:Giraldez, Antonio J

eRA COMMONS USER NAME: giraldeza

POSITION TITLE: Professor of Genetics

EDUCATION/TRAINING

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Cadiz (Spain)	B.S.	06/1996	Chemistry
University Autonoma of Madrid (Spain)	B.S.	06/1998	Molecular Biology
European Molecular Biology Laboratory (Germany)	Ph.D.	04/2002	Developmental Biology
Skirball Institute. New York University,	Postdoctoral	12/2005	Developmental Biology
Harvard University, MA.	Postdoctoral	12/2006	Developmental Biology

A. Personal Statement

My laboratory combines Genomics, Biochemical, Developmental and Computational approaches to investigate the molecular mechanisms of gene regulation and how they shape embryonic development after fertilization. My contribution to science has resulted in paradigm shifts within several areas: i) the identification of a novel miRNA family (miR-430) that regulates the maternal to zygotic transition, later shown to provide a conserved regulatory mechanism in drosophila (miR-309) and xenopus (miR-430/427), ii) the identification of deadenylation as one of the molecular mechanisms that mediate miRNA-mediated repression, iii) the discovery of alternative processing pathways for microRNAs independent of Dicer and required for blood development; iv) the translation of micropeptides encoded in genes thought to be non-coding RNAs; v) the identification of a global gene regulatory layer in vertebrates by translated uORFs (~50%) of genes; vi) Identification of a new layer in the genetic code in animals (identified also by the Coller lab in Yeast), where translation of specific triplet codons, mediate mRNA deadenylation, decay and translation efficiency to shape gene expression across animals (codon optimality code, under review); vii) Identification of a novel function of nanog/oct4 and soxb1 as key transcription factors to mediate activation of the zygotic genome after fertilization.

Initially focused on the function of microRNAs in development, more recently these findings have taken us into the fascinating regulatory codes that shape post-transcriptional gene regulation using massive parallel reporter assays of the 5', CDS and the 3'UTR for mRNA stability and translation in the context of the vertebrate embryo. We have implemented new approaches to monitor translation in the developing embryo, and defined targeting rules to increase the efficiency for CRISPR/Cas9 to mutagenize the genome. This has allowed us to begin uncovering the regulatory codes that shape embryogenesis from new coding regions in the genome to new transcriptional codes that regulate genome activation and cell fate identity during development which form the basis of this proposal. To tackle these avenues, my laboratory includes 14 scientists, with expertise in Computational Biology (2 post-docs, 1 PhD student), Biochemistry (2 postdocs), Developmental Biology (2 post-docs, 2 PhD students), Genomics (2 postdocs, 1 PhD Student) and two technicians. I am currently principal investigator on several Foundation-, University- and NIH funded grants, grants. I successfully administered the projects (e.g. staffing, research protections, budget), collaborated with other researchers, and produced several peer-reviewed publications as a senior author including papers in Science, Nature, Nature Genetics, Nature structural and Molecular Biology, PloS Genetics, Genes & Development and EMBO J.

My broader contribution to science beyond scientific discoveries can be divided in three fronts, **a)** In my laboratory, my trainees receive a multidisciplinary training and have now taken faculty positions in leading universities (D. Cifuentes, BU; M. Lee, U Pittsburg; A Bazzini, Stowers; Y Mishima, UTokyo; E. Hoffman, Yale University) and students have also taken positions within and outside academia (Stahlhut C Postdoc,CSHL;

Staton AA, Scientific writer Infusion Communications; Johnstone T, Juno pharmaceuticals Data scientist; Bonneau AR, Postdoc MIT). **b)** at Yale University, as Director of Graduate Studies in the Department of Genetics my contribution has been: i) shaping the curriculum to encourage students to focus on their scientific projects early on, reducing the time to graduation to 4.8 years from (6 years before I was DGS). ii) mentor and encourage students to get exposure to a wide range of different professional careers (within and outside academia) serving as advisor to the Biomedical Careers Science Committee and the annual career symposium, and more recently Chair of the Genetics Department and co-founder of the personalize medicine institute. **c)** Within the broader scientific community, I currently participate in multiple scientific review boards for young investigators and fellowships (Pew, Damon Runyon). I have been leading genomics efforts to analyze gene expression across tissues for over 20 laboratories in the zebrafish community as part of an effort to develop a mod-encode for zebrafish and make the data publicly available. We have also developed experimental approaches to define the targeting rules of Cas9 and CPF1, developing CRISPscan to identify the most active sgRNAs across species, which is now broadly used in the community (CRISPRscan.org).

In summary, I have a demonstrated record of successful and productive research projects, mentoring and have the necessary expertise in areas of high relevance for this project.

B. Positions and Honors

Positions and Employment

2017-	Chair. Genetics Department Yale University School of Medicine.
2014-	Professor. Yale University School of Medicine. New Haven, CT. USA
2013-2014	Associate Professor with tenure. Yale University School of Medicine. New Haven, CT.
2011-	Director of Graduate Studies in Genetics. Yale University School of Medicine.
2011-2012	Associate Professor. Yale University School of Medicine. New Haven, CT. USA
2007-2011	Assistant Professor. Yale University School of Medicine. New Haven, CT. USA

Other Experience and Professional Memberships

2017-2021	NIH Dev1 study section permanent member.
2016-2019	Pew Scholars Alumni Review Board
2016-2017	Damon Runyon Cancer Research Foundation Fellowship Award Committee
2016	NIH/NIAMS ad hoc reviewer Board of Scientific Counselors
2015	NIH Dev1 study section ad hoc reviewer
2014	NIH/SREA reviewer for a CSR study section, ad hoc reviewer
2012-	Member, executive committee of the Developmental Biology Training Grant
2012-	Member, executive committee for the Human Genetics Training Grant
2012-	Member, executive committee Molecular Cell Biology, Development and Genetics
2011-	Member, advisory committee for the Genetics Training Grant
2008	NIH reviewer for a Molecular Neurogenetics study section, ad hoc reviewer
2004-	Member, New York Academy of Sciences

Honors

2017	Blavatnik National Award for Young Scientists (Finalist)
2016	Whitman Center Research Fellow MBL (2016, 2017)
2016	HHMI Faculty Scholar 2016-2021
2016	Blavatnik Award for Young Scientists (National Finalist)
2016	Whitman Center Research Fellow MBL
2014	Vilcek Prize for Creative Promise in Biomedical Sciences
2009	Kavli Frontiers of Science Speaker
2008	Pew Scholar in Biomedical Sciences
2007	Lois E. and Franklin H. Top, Jr., Yale Scholar Award
2007	John Kendrew Young investigator Award EMBL, Heidelberg
2007	NYAS Blavatnik Young Investigator Award (Finalist)
2004	HFSP Postdoctoral Fellowship
2003	EMBO Postdoctoral Fellowship
1998	3rd National Prize in Chemistry Degree. Ministry of Science. Spain

C. Contribution to Science

1. Identification of key factors that activate the vertebrate genome after fertilization:

A central question in biology is how embryonic development is initiated in the fertilized egg. Upon fertilization the zygotic genome is silent, and the first steps of development are instructed by maternal mRNAs and proteins deposited in the egg. However, the factors that initiate gene expression in the silent vertebrate embryo to trigger embryonic development were unknown. We exploited ribosome footprinting in embryogenesis, to define the most highly translated maternal components during the maternal-to-zygotic transition (MZT). This opens the door for identifying the machinery that initiates every aspect of zygotic development, and lead us to the identification of Nanog, Oct4 and SoxB1 as long sought-after transcription factors key to activating the genome, and provide an entry point to understand how the embryo activates transcription. Nanog, Oct4 and SoxB1 maintain stem cell identity and function during reprogramming in vitro, while in vivo they initiate the zygotic program of development by reprogramming terminally differentiated cells, sperm and oocyte, to transient totipotency. These link stem cell biology, cellular reprograming and early embryonic development. I was senior author in these studies.

a. Lee MT, Bonneau AR, Takacs CM, Bazzini AA, DiVito KR, Fleming ES & **Giraldez AJ**‡. Nanog, SoxB1 and Pou5f1/Oct4 regulate widespread zygotic gene activation during the maternal-to-zygotic transition**. Nature,** 2013 Nov 21;503(7476):360-4.
b. Lee MT, Bonneau AR, **Giraldez AJ**‡. Zygotic genome activation during the maternal-to-zygotic transition. **Annu Rev Cell Dev Biol.** 2014;30:581-613.

2. <u>Regulation of the maternal program, and how the cells clear the past during cellular transitions:</u>
All animals inherit maternal information in the oocyte in the form of mRNAs and proteins needed to undergo the first developmental processes after fertilization. A large fraction of this information is degraded upon activation of the zygotic genome. This represents one of the most profound remodeling of gene expression in biology, but the effectors and the molecular mechanisms underlying this global regulation remained poorly understood. Our contribution is meaningful in two ways: First, we have uncovered a novel function for microRNAs (miR-430) in the clearance of a large fraction (~20-30%) of the maternal mRNAs during the maternal to zygotic transition in zebrafish. Indeed, this uncovered a conserved mechanism across several species through the same (Xenopus) or different (Drosophila) microRNAs. Second, we have identified that translation of specific codons induces mRNA stabilization or decay, and that this mechanism regulates differential mRNA decay during the maternal to zygotic transition across species (zebrafish, mouse, xenupus and drosophila). Together this provides two new paradigms of how embryos regulate the previous developmental program (microRNAs and coding sequence), with implications reaching from cellular transitions to fertility. I was leading or corresponding author in these studies.

a. **Giraldez AJ‡,** Mishima Y, Rihel J, Grocock R, Dongen S, Inoue, K, Enright A, and Schier AF‡. Zebrafish miR-430 promotes deadenylation and clearance of maternal mRNAs. **Science.** 2006.Apr 7;312(5770): 75-9. Epub 2006 Feb 16. **‡**Corresponding author.
b. **Giraldez AJ‡,** Cinalli RM, Glasner ME, Enright A, Thomson JM, Baskerville S, Hammond SM, Bartel D, and Schier AF‡. MicroRNAs regulate brain morphogenesis in zebrafish. **Science**. 2005 May 6;308(5723): 833-8.
c. Bazzini A‡, del Viso F, Moreno-Mateos MA, Johnstone TG, Vejnar CE, Qin Y, Yao J, Khokha MK, and **Giraldez AJ‡.** Codon identity regulates mRNA stability and translation efficiency during the maternal-to-zygotic transition. **EMBOJ.** 2016 Jul 19. pii: e201694699.
d. Beaudoin JD, Novoa EM, Vejnar CE, Yartseva V, Takacs CM, Kellis M, **Giraldez AJ‡**. Analyses of mRNA structure dynamics identify embryonic gene regulatory programs. **Nat Struct Mol Biol.** 2018 Aug;25(8): 677-686. doi: 10.1038/s41594-018-0091-z. Epub 2018 Jul 30.

<u>3. Uncovered novel mechanisms of microRNA processing and function:</u>
The discoveries of microRNAs lead to the identification of a new layer gene regulation, with far reaching implications in all aspects of biology, however the mechanism remained poorly understood. At a time when the filed was solely focussed on the regulation of translation, our contribution resulted in a paradigm shift with the discovery that microRNAs cause mRNA decay through deadenylation of their endogenous targets. The dynamic analysis of miRNA function in vivo combined with ribosome footprinting lead to the discovery that translation is repressed by reducing the initiation rate, before triggering deadenylation of the message. These findings have provided some of the basis to understand the molecular mechanisms of miRNA function.

a. Bazzini AA, Lee MT, **Giraldez AJ**‡. Ribosome Profiling Shows That miR-430 Reduces Translation Before Causing mRNA Decay in Zebrafish. **Science** 13 April 2012: 233-237.
b. Takacs C‡ and **Giraldez. AJ**‡. miR-430 regulates oriented cell division during neural tube development in zebrafish. **Developmental Biology,** 2015 Nov 30.

Over the last decade, Dicer was thought to be strictly required for the biogenesis of all miRNAs and siRNAs. Combining high-throughput sequencing of small RNAs in mutants of the miRNA-processing pathway, we identified a novel miRNA processing pathway independent of Dicer, but dependent on the catalytic activity of Argonaute2 (Ago2). We showed that this pathway is required for the processing of miR-451, a miRNA conserved in all vertebrates, expressed in the blood. This revealed important roles for this pathway in erythrocyte maturation across species. Furthermore, followup study, we uncovered PolyAribonuclease (Yoda et al., Cell Reports 2013) as the enzyme that acts downstream of Ago2 in the processing of miRNAs. This enzyme provides an entry point to study small RNA turnover, as it is likely involved in the trimming of other small regulatory RNAs.This work helped to pave the way for new avenues of research in the study of miRNAs, by challenging a dogma, and defining a novel cellular pathway to produce small RNAs.

c. Cifuentes D, Xue H, Taylor DW, Patnode H, Mishima Y, Cheloufi S, Ma E, Mane S, Hannon GJ, Lawson N, Wolfe S, **Giraldez AJ‡**. A novel miRNA processing pathway independent of Dicer requires Argonaute2. **Science.** 2010, Jun 25;328(5986):1694-8. Epub 2010 May 6
d. Yoda M, Cifuentes D, Izumi N, Sakaguchi Y, Suzuki T, **Giraldez AJ‡** and Tomari Y‡. PARN mediates 3′-end trimming of Argonaute2-cleaved precursor microRNAs. **Cell Reports**, 2013, 5, 1–12, Nov. 14.

4. Characterization of the coding potential of the vertebrate genome:

Analysis of the eukaryotic genome has identified a large class of transcripts known as long-noncoding RNAs that lack classical hallmarks of protein-coding genes. However, many of these transcripts harbor short open reading frames, but their coding potential has not been experimentally addressed. Conversely, short peptides have emerged as important regulators of development and physiology, but their identification has been limited by their size. We have pioneered the combination of high resolution ribosome footprinting, with mass spectrometry, conservation analysis and a novel computational method (ORFscore) to define the actively translated open reading frame in individual transcripts. This approach enabled us to identify several hundred novel micropeptide-encoding genes (20-100amino acids) in zebrafish and humans, within genes thought to lack coding potential. This study provides a conceptual shift in the study of predicted non-coding RNAs, uncovering previously unidentified small peptide-encoding genes with potential regulatory potential in vivo. This opens new areas of inquiry in genomics, cell biology and developmental biology for understanding the function of these micropeptide-encoding genes in the context of a whole organism.

a. Bazzini AA[‡#], Johnstone TG[#], Christiano R, Mackowiak SD, Obermayer B, Fleming ES,Vejnar CE , Lee MT , Rajewsky N[‡], Walther TC and **Giraldez AJ[‡]**. Identification of smallORFs in animals using ribosome footprinting and evolutionary conservation . **EMBO J.** 2014, May 2;33(9):981-93.
b. Johnstone TG, Bazzini AB and **Giraldez AJ[‡]**. Upstream ORFs act as prevalent translational repressors in vertebrates. **EMBO J**, 2016, Apr 1;35(7):706-23.

5. Identification of the features governing CRISPR/CAs9 mediated targeting:

CRISPR-Cas9 technology provides a powerful system for genome engineering. However, variable activity across different single guide RNAs (sgRNAs) remains a significant limitation. In this study, we have analyzed the molecular features that influence sgRNA stability, activity and loading into Cas9 in vivo. On the basis of these results, we created a predictive sgRNA-scoring algorithm, CRISPRscan.org, that effectively captures the sequence features affecting the activity of CRISPR-Cas9 in vivo. These results identify determinants that influence Cas9 activity and provide a framework for the design of highly efficient sgRNAs for genome targeting in vivo across different species.

a. Moreno-Mateos MA, Vejnar CE, Beaudoin JD, Fernandez JP, Mis EK, Khokha MK, **Giraldez AJ.** CRISPRscan: designing highly efficient sgRNAs for CRISPR-Cas9 targeting in vivo. **Nature Methods**. **2015** Oct;12(10):982-8. doi: 10.1038/nmeth.3543. Epub 2015 Aug 31.

b. Moreno-Mateos MA, Fernandez JP, Rouet R, Vejnar CE, Lane MA, Mis E, Khokha MK, Doudna JA, **Giraldez AJ**.CRISPR-Cpf1 mediates efficient homology-directed repair and temperature-controlled genome editing. **Nature Communications**. 2017 Dec 8;8(1):2024. doi: 10.1038/s41467-017-01836-2.

Complete List of Published Work in MyBibliography: http://www.ncbi.nlm.nih.gov/sites/myncbi/1fgGueqge_X5L/bibliography/42129772/public/?sort=date&direction=ascending

D. Research Support

Active

NIH/ NIGMS R35GM122580 (Giraldez, PI) 05/01/17-04/30/22
Molecular mechanisms of the maternal to zygotic transition
The goal of understanding basic conserved principles of how genes are regulated that can be applied to other systems, including reprogramming of the cellular fates and cancer.

NIH R35 HL135767 (Mani, PI)(Co-Investigator) 01/23/17-12/31/23
The Identification and characterization of genetic variants underlying cardiovascular diseases. The proposed studies are focuses on the in vivo genetic studies of Cardiovascular Diseases.

55108524 (Giraldez, PI) 10/01/16-09/30/21
HHMI-Gates Foundation Molecular analysis of the maternal to zygotic transition
This grant supports the investigation of the mechanisms of the maternal to zygotic transition and is not a full HHMI grant but a 100K scholarship for 5 Years.

Simons Foundation (Giraldez, PI) 09/01/17-08/31/20
Effect of Autism risk genes in neural cell identity using single cell seq
This pilot project allowed us to develop single cell seq and apply it to characterize cell diversity in CHD8 mutants

NIH (sub-University of Utah) (Giraldez, PI) 09/01/2017-08/31/19
Initial Formation of 3D Chromatin Domains in Early Vertebrate Embryos
Our central goal is to determine when and how higher-order 3D chromatin architecture is first established in early embryos, and its impact on transcription.

Completed

NIH R01HD074078-05 NCE (Giraldez, PI) 08/15/12-04/30/18
Functional analysis of the zebrafish genome through RNA-seq and ribosome profile
Major goals are to improve functional genomics in zebrafish and provide fundamental tools to the community.

NIH R01GM101108-04 NCE (Giraldez, PI) 05/01/12-02/28/17
Molecular Characterization of the mircoRNA Processing Pathways
Major goals of this project aim to investigate the molecular mechanisms underlying Dicer independent microRNA processing.

NIH R01GM081602-09 (Giraldez, PI) 09/01/13-08/31/17
The Role of MicroRNAs in Vertebrate Development
The major goal of this project was to understand how miRNAs function in embryogenesis

NIH R01GM103789 NCE (Giraldez, PI) 09/01/12-08/31/17
Analysis of the gene networks regulating the maternal to zygotic transition
Major goals are to understand how gene expression is regulated during maternal zygotic transition

NIH R01GM102251 NCE) (Giraldez, PI) 08/10/12-05/31/17
Molecular mechanisms of MircoRNA mediated regulation
The major goal of this project is to understand the mechanism by which these microRNAs regulate other genes in the cell, what might help us develop specific ways to modulate their activity during human disease.