BIOGRAPHICAL SKETCH

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NAME: P Rocha, Pedro

eRA COMMONS USER NAME (credential, e.g., agency login): pereip02

POSITION TITLE: Tenure-Track Investigator

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)	END DATE MM/YYYY	FIELD OF STUDY
Lisbon University, Lisbon	BS	07/2005	Microbiology and Genetics
Max Planck Institute for Molecular Genetics, Berlin	PHD	02/2011	Biology
New York University School of Medicine, New York, New York	Postdoctoral Fellow	04/2018	Biology

A. Personal Statement

My graduate studies at the Max-Planck Institute for Molecular Genetics focused on gene regulation and the role of transcriptional co-regulators during mouse development (Rocha et al. Development 2010). During this time I realized the importance of the mechanism of DNA packaging in the eukaryotic nucleus and how chromatin contacts (or loops) between enhancers and promoters are essential components of gene regulation. I therefore devoted my postdoctoral training to explore the multiple ways in which nuclear organization and chromosomal interactions are important for regulation of cellular processes in development and disease. In the Skok lab I studied the process of Class Switch Recombination in B cells and showed that DNA interactions are important to orchestrate this recombinatorial event and avoid translocations that can lead to lymphomas (Rocha et al. Mol Cell 2012 and Cell Reports 2016). I also developed techniques that were lacking in the field such as a CRISPR/dCas9-based system for live visualization of multiple loci in different colors (Fu, Rocha et al. Nature Communications 2016) and contributed to the development of computational pipelines for analyses of DNA interactions (Raviram et al. PLOS Comp. Bio 2016 and Jiang et al. NAR 2016). As nuclear organization came to the forefront of gene regulation I was fortunate to be involved in several collaborations that elucidated different mechanisms by which nuclear organization impacts gene expression. Some of the most gratifying were collaborations with the Reinberg lab where we showed how chromosomal domains and boundaries are established in vivo (Narendra et al. Science 2015) and with the Aifantis lab where we demonstrated how transcriptional co-regulators help establish regulatory DNA loops (Aranda-Orgilles et al. Cell Stem Cell 2016) and finally how disruption of chromatin architectural proteins can lead to leukemia (Mullenders et al. JEM 2015). In my own lab our goal is to identify the mechanisms by which nuclear organization and chromosomal domains are established and define the mechanisms by which these regulate gene expression throughout development and during disease onset. To achieve this, I will build on my developmental biology background and use the early mouse embryo as a model system. Fertilization is the ultimate reprograming experiment where two highly differentiated cells (oocyte and sperm) fuse to form a zygote with totipotent potential. Within a few cell divisions the first cell-lineages will be established, and different gene-expression programs put in action. This is then a perfect system to study how chromosomal interactions, and insulation are established to control gene expression. I will additionally focus on the proteins that help establishing chromosomal domains and investigate how lack of chromatin insulation might be related to the phenomenon of stochastic gene expression. Finally, my background in genome-editing and live-imaging microscopy will allow me to not only validate my findings but also to translate them into disease-related models. Joining NICHD at NIH was a strategic decision. My goals perfectly aligned with the objectives of the Division of Intramural Research and the resources available to

me in the Division of Developmental Biology provide assurance that my research plan is fully supported. Finally, I integrated a very strong affinity group, with six other laboratories that study Genetics and Epigenetics of Development.

1. Fu Y, Rocha PP, Luo VM, Raviram R, Deng Y, Mazzoni EO, Skok JA. CRISPR-dCas9 and sgRNA scaffolds enable dual-colour live imaging of satellite sequences and repeat-enriched individual loci. Nat Commun. 2016 May 25;7:11707. PubMed Central PMCID: PMC4894952.
2. Narendra V, Rocha PP, An D, Raviram R, Skok JA, Mazzoni EO, Reinberg D. CTCF establishes discrete functional chromatin domains at the Hox clusters during differentiation. Science. 2015 Feb 27;347(6225):1017-21. PubMed Central PMCID: PMC4428148.
3. Rocha PP, Skok JA. The origin of recurrent translocations in recombining lymphocytes: a balance between break frequency and nuclear proximity. Curr Opin Cell Biol. 2013 Jun;25(3):365-71. PubMed Central PMCID: PMC3691303.
4. Rocha PP, Scholze M, Bleiss W, Schrewe H. Med12 is essential for early mouse development and for canonical Wnt and Wnt/PCP signaling. Development. 2010 Aug;137(16):2723-31. PubMed PMID: 20630950.

B. Positions and Honors

Positions and Employment

2011 - 2018	Postdoctoral Research Fellow, New York University School of Medicine, New York, NY
2018 -	Tenure-Track Investigator, National Institute of Child Health and Human Development, Bethesda, NY
2018 -	Adjunct Investigator, National Cancer Institute, Bethesda, NY

Other Experience and Professional Memberships

Honors

2004	Academic Merit Award, Lisbon University
2004 - 2005	Leonardo DaVinci Fellowship, European Comission
2007 - 2010	Marie Curie Early Stage Researcher, European Commission
2012 - 2014	Postdoctoral Fellowship, National Cancer Center
2014 - 2016	Scholar Award, American Society of Hematology
2016 - 2018	K99 Fellow, NIGMS

C. Contribution to Science

1. During my PhD I developed a mouse model of the MED12 subunit of the Mediator complex. Many functions have been attributed to this subunit but functional validation had been missing in an in vivo setting. My studies allowed this and showed the importance of MED12 in several developmental processes and in proper signal transduction through different signaling pathways. Moreover these mice carrying a conditional allele for MED12 are now distributed in many different labs and will be used to assess the functional relevance of MED12 and the Mediator in establishment of Super-Enhancers and also of human diseases where MED12 is known to be mutated such as uterine leiomyomas or the Opitz Kaveggia syndrome. This earlier work also allowed me to be in a good position for collaborative studies exploring the role of the Mediator subunit in nuclear organization and gene regulation

    a. Aranda-Orgilles B, Saldaña-Meyer R, Wang E, Trompouki E, Fassl A, Lau S, Mullenders J, Rocha PP, Raviram R, Guillamot M, Sánchez-Díaz M, Wang K, Kayembe C, Zhang N, Amoasii L, Choudhuri A, Skok JA, Schober M, Reinberg D, Sicinski P, Schrewe H, Tsirigos A, Zon LI, Aifantis I. MED12 Regulates HSC-Specific Enhancers Independently of Mediator Kinase Activity to Control

Hematopoiesis. Cell Stem Cell. 2016 Dec 1;19(6):784-799. PubMed Central PMCID: PMC5268820.

b. Thomas-Claudepierre AS, Robert I, Rocha PP, Raviram R, Schiavo E, Heyer V, Bonneau R, Luo VM, Reddy JK, Borggrefe T, Skok JA, Reina-San-Martin B. Mediator facilitates transcriptional activation and dynamic long-range contacts at the IgH locus during class switch recombination. J Exp Med. 2016 Mar 7;213(3):303-12. PubMed Central PMCID: PMC4813673.

c. Rocha PP, Scholze M, Bleiss W, Schrewe H. Med12 is essential for early mouse development and for canonical Wnt and Wnt/PCP signaling. Development. 2010 Aug;137(16):2723-31. PubMed PMID: 20630950.

d. Rocha PP, Bleiss W, Schrewe H. Mosaic expression of Med12 in female mice leads to exencephaly, spina bifida, and craniorachischisis. Birth Defects Res A Clin Mol Teratol. 2010 Aug;88(8):626-32. PubMed PMID: 20589884.

2. 95% of Lymphomas are of B cell origin and most of these cancers originate by off target activity of the enzyme AID. However the determinants of AID off-target activity haven't been completely characterized. Using 4C-seq to assess the chromosomal architecture of B cells I have demonstrated that physical proximity to Igh at the time of Class Switch Recombination (CSR) is a dangerous predisposition for the AID-caused mutations and translocations that are known to occur during lymphoma formation. Furthermore, I have continued to use tools of nuclear organization to uncover the mechanism of CSR as well as understanding how somatic recombination can lead to genomic instability.

   a. Campos-Sanchez E, Deleyto-Seldas N, Dominguez V, Carrillo-de-Santa-Pau E, Ura K, Rocha PP, Kim J, Aljoufi A, Esteve-Codina A, Dabad M, Gut M, Heyn H, Kaneda Y, Nimura K, Skok JA, Martinez-Frias ML, Cobaleda C. Wolf-Hirschhorn Syndrome Candidate 1 Is Necessary for Correct Hematopoietic and B Cell Development. Cell Rep. 2017 May 23;19(8):1586-1601. PubMed Central PMCID: PMC5510986.

   b. Rocha PP, Raviram R, Fu Y, Kim J, Luo VM, Aljoufi A, Swanzey E, Pasquarella A, Balestrini A, Miraldi ER, Bonneau R, Petrini J, Schotta G, Skok JA. A Damage-Independent Role for 53BP1 that Impacts Break Order and Igh Architecture during Class Switch Recombination. Cell Rep. 2016 Jun 28;16(1):48-55. PubMed Central PMCID: PMC4927351.

   c. Mullenders J, Aranda-Orgilles B, Lhoumaud P, Keller M, Pae J, Wang K, Kayembe C, Rocha PP, Raviram R, Gong Y, Premsrirut PK, Tsirigos A, Bonneau R, Skok JA, Cimmino L, Hoehn D, Aifantis I. Cohesin loss alters adult hematopoietic stem cell homeostasis, leading to myeloproliferative neoplasms. J Exp Med. 2015 Oct 19;212(11):1833-50. PubMed Central PMCID: PMC4612095.

   d. Rocha PP, Micsinai M, Kim JR, Hewitt SL, Souza PP, Trimarchi T, Strino F, Parisi F, Kluger Y, Skok JA. Close proximity to Igh is a contributing factor to AID-mediated translocations. Mol Cell. 2012 Sep 28;47(6):873-85. PubMed Central PMCID: PMC3571766.

3. Technological advances have always allowed for large leaps in scientific discovery. Recognizing this I have always dedicated a large part of my research to development of techniques and analytic tools in the field of nuclear organization.

   a. Raviram R, Rocha PP, Luo VM, Swanzey E, Miraldi ER, Chuong EB, Feschotte C, Bonneau R, Skok JA. Analysis of 3D genomic interactions identifies candidate host genes that transposable elements potentially regulate. Genome Biol. 2018 Dec 13;19(1):216. PubMed Central PMCID: PMC6292174.

   b. Jiang T, Raviram R, Snetkova V, Rocha PP, Proudhon C, Badri S, Bonneau R, Skok JA, Kluger Y. Identification of multi-loci hubs from 4C-seq demonstrates the functional importance of simultaneous interactions. Nucleic Acids Res. 2016 Oct 14;44(18):8714-8725. PubMed Central PMCID: PMC5062970.

c. Fu Y, Rocha PP, Luo VM, Raviram R, Deng Y, Mazzoni EO, Skok JA. CRISPR-dCas9 and sgRNA scaffolds enable dual-colour live imaging of satellite sequences and repeat-enriched individual loci. Nat Commun. 2016 May 25;7:11707. PubMed Central PMCID: PMC4894952.

d. Raviram R, Rocha PP, Müller CL, Miraldi ER, Badri S, Fu Y, Swanzey E, Proudhon C, Snetkova V, Bonneau R, Skok JA. 4C-ker: A Method to Reproducibly Identify Genome-Wide Interactions Captured by 4C-Seq Experiments. PLoS Comput Biol. 2016 Mar;12(3):e1004780. PubMed Central PMCID: PMC4777514.

4. The role that nuclear organization plays in control of gene expression is now easily recognized. As one of the earliest adopters of these techniques I have had the opportunity to contribute to several studies that described how nuclear organization and chromatin architecture play important roles in controlling gene expression

   a. Oksuz O, Narendra V, Lee CH, Descostes N, LeRoy G, Raviram R, Blumenberg L, Karch K, Rocha PP, Garcia BA, Skok JA, Reinberg D. Capturing the Onset of PRC2-Mediated Repressive Domain Formation. Mol Cell. 2018 Jun 21;70(6):1149-1162.e5. PubMed Central PMCID: PMC7700016.

   b. Modrek AS, Golub D, Khan T, Bready D, Prado J, Bowman C, Deng J, Zhang G, Rocha PP, Raviram R, Lazaris C, Stafford JM, LeRoy G, Kader M, Dhaliwal J, Bayin NS, Frenster JD, Serrano J, Chiriboga L, Baitalmal R, Nanjangud G, Chi AS, Golfinos JG, Wang J, Karajannis MA, Bonneau RA, Reinberg D, Tsirigos A, Zagzag D, Snuderl M, Skok JA, Neubert TA, Placantonakis DG. Low-Grade Astrocytoma Mutations in IDH1, P53, and ATRX Cooperate to Block Differentiation of Human Neural Stem Cells via Repression of SOX2. Cell Rep. 2017 Oct 31;21(5):1267-1280. PubMed Central PMCID: PMC5687844.

   c. Mullenders J, Aranda-Orgilles B, Lhoumaud P, Keller M, Pae J, Wang K, Kayembe C, Rocha PP, Raviram R, Gong Y, Premsrirut PK, Tsirigos A, Bonneau R, Skok JA, Cimmino L, Hoehn D, Aifantis I. Cohesin loss alters adult hematopoietic stem cell homeostasis, leading to myeloproliferative neoplasms. J Exp Med. 2015 Oct 19;212(11):1833-50. PubMed Central PMCID: PMC4612095.

   d. Narendra V, Rocha PP, An D, Raviram R, Skok JA, Mazzoni EO, Reinberg D. CTCF establishes discrete functional chromatin domains at the Hox clusters during differentiation. Science. 2015 Feb 27;347(6225):1017-21. PubMed Central PMCID: PMC4428148.

5. a. Tottone L, Lancho O, Loh JW, Singh A, Kimura S, Roels J, Kuchmiy A, Strubbe S, Lawlor MA, da Silva-Diz V, Luo S, Gachet S, García-Prieto CA, Hagelaar R, Esteller M, Meijerink JPP, Soulier J, Taghon T, Van Vlierberghe P, Mullighan CG, Khiabanian H, Rocha PP, Herranz D. A Tumor Suppressor Enhancer of *PTEN* in T-cell development and leukemia. Blood Cancer Discov. 2021 Jan;2(1):92-109. PubMed Central PMCID: PMC7810363.

   b. Beck DB, Basar MA, Asmar AJ, Thompson JJ, Oda H, Uehara DT, Saida K, Pajusalu S, Talvik I, D'Souza P, Bodurtha J, Mu W, Barañano KW, Miyake N, Wang R, Kempers M, Tamada T, Nishimura Y, Okada S, Kosho T, Dale R, Mitra A, Macnamara E, Matsumoto N, Inazawa J, Walkiewicz M, Õunap K, Tifft CJ, Aksentijevich I, Kastner DL, Rocha PP, Werner A. Linkage-specific deubiquitylation by OTUD5 defines an embryonic pathway intolerant to genomic variation. Sci Adv. 2021 Jan;7(4) PubMed Central PMCID: PMC7817106.

   c. Belver L, Yang AY, Albero R, Herranz D, Brundu FG, Quinn SA, Pérez-Durán P, Álvarez S, Gianni F, Rashkovan M, Gurung D, Rocha PP, Raviram R, Reglero C, Cortés JR, Cooke AJ, Wendorff AA, Cordó V, Meijerink JP, Rabadan R, Ferrando AA. GATA3-Controlled Nucleosome Eviction Drives *MYC* Enhancer Activity in T-cell Development and Leukemia. Cancer Discov. 2019 Dec;9(12):1774-1791. PubMed Central PMCID: PMC6891196.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1FQDt7qsIF5/bibliography/public/

D. Additional Information: Research Support and/or Scholastic Performance

Completed Research Support

K99 GM117302-01, National Institute of General Medical Sciences (NIGMS) 03/01/16-01/31/18
Pereira Da Rocha, Pedro (PI) A novel role for 53BP1 independent of damage that controls chromatin structure
Role: PI

NA, American Society of Hematology Skok, Jane A (PI) 07/01/14-07/01/16
Nuclear Organization and its effect on AID mediated targeting and repair pathway choice
Role: PDC

NA, National Cancer Center Skok, Jane A (PI) 01/07/12-01/07/14
The protective role of chromosome dynamics during Class Switch Recombination
The aim of this project was to characterize how chromosome dynamics are important to prevent and repair DNA damage in recombining lymphocytes
Role: Post-Doctoral Scholar