BIOGRAPHICAL SKETCH

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NAME: Carlos R. Escalante

eRA COMMONS USER NAME (credential, e.g., agency login): cescalante1

POSITION TITLE: Assistant 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
Instituto Tecnologico, Monterrey, Mexico	B.S	1981-1985	Chemistry
Georgetown University	Ph.D.	1986-1992	Chemistry
Columbia University	Post-Doc	1993-1997	Structural Biology

A. Personal Statement

My expertise is in structural biology and biochemistry using multiple techniques such as X-ray crystallography, Analytical Ultracentrifugation and SAXS. As a postdoctoral fellow and later as a principal investigator, I have determined the structure of several Protein-DNA complexes implicated in gene regulation and I have been a leader in structural studies of interferon regulatory factors (IRF) solving the structure of the first IRF factor in complex with DNA. We have developed an all-encompassing methodology that includes a hybrid structural biology approach with X-ray Crystallography, Small-Angle X-ray Scattering (SAXS) and Electron Microscopy complementing each other. In addition, we have the expertise to characterize and study all enzymatic properties of the system using biochemical and biophysical techniques such as analytical ultracentrifugation, fluorescent spectroscopy and isothermal titration calorimetry among others. I have been studying the AAV Rep proteins for several years and my group has been involved in the major developments related to structure-function studies of Rep68 and Rep40 proteins from AAV-2. I have determined the structure of many Protein-DNA complexes implicated in gene regulation such as interferon regulatory factors 1,3 and 4, NF-kB p65/p50 heterodimer and AAV2-Rep proteins. We have determined the structure of the first member of the eukaryotic transcription and replication terminator family Reb1 in complex with DNA, which is a ortholog of TTF-I. I have trained post-docts, graduate students, undergraduates and high school students during my scientific career being this an important part of my research laboratory.

Zarate-Perez F. Bardelli M, Burgner JW 2nd, Villamil-Jarauta M. Das K, Kekilli D, Mansilla-Soto J, Linden RM and **Escalante CR**. (2012) The interdomain linker of AAV-2 Rep68 is an integral part of its oligomerization domain:role of a conserved SF3 helicase residue in oligomerization. PloS Pathogens Jun;8(6)e 1002764. PMCID:PMC3375335

Mansilla-Soto, J., Yoon-Robarts, Rice,M.,W., Arya, S., **Escalante, CR.**, and Linden, R.M. (2009) DNA structure modulates the oligomerization properties of the AAV initiator protein Rep68. PLoS Pathogen, 5:e1000513. PMCID:PMC2702170

Yoon-Robarts M, Blouin, AG, Bleker, S., Kleinmschmidt, JA., Aggarwal, AK; **Escalant, CR**, Linden RM. (2004) Residues within the B' motif are critical for DNA binding by the superfamily 3 helicase Rep40 of adeno-associated virus type 2. J.Biol.Chem. 279, 50472-50481. PMID:15371437

Escalante, CR., Yie J., Thanos, D., Aggarwal, AK., (1998) Structure of IRF-1 with bound DNA reveals determinants of interferon regulation. Nature 391:103-106. PMID:9422515

B. Positions and Honors

1986	Chemistry Teacher, Universidad de Nuevo Leon, Mexico.
1986-92	Predoctoral Fellowship, Georgetown University
1993-94	Postdoctoral fellowship, Conacyt, Mexico.
1993-1997	Postdoctoral Scientist, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY
1997-01	Instructor, Department of Physiology and Biophysics Mount Sinai School of Medicine, New York, NY
2001-2008	Research Assistant Professor, Department of Structural and Chemical Biology Mount Sinai School of Medicine, New York, NY
2008-present	Assistant Professor, Department of Physiology and Biophysics VCU School of Medicine, Richmond, VA.
2015-2019	Blick Scholar Award

C. Contributions to Science

Project 1 - Mechanisms of Transcriptional Activation by IRF4: The achilles heel of multiple myeloma.
During my early scientific career in the Aggarwal laboratory I could determine the first structure of an interferon regulatory factor (IRF-1) providing insights into the regulation of the interferon-β gene. In subsequent research, I played a prominent role in the structural characterization of the interferon β-enhanceosome including the structure of NF-κB p50/p65 and IRF-3. Members of the IRF family are characterized by the presence of two conserved domains: An N-terminal DNA binding domain (DBD) that binds DNA specifically and a C-terminal domain known as interferon association/activation domain (IAD) involve in protein-protein interactions and formation of homo- and hetero-oligomers. I have continued the studies of IRF gene regulation in my research group focusing in understanding the astonishing plasticity of IRF4 in controlling a wide range of genes by interacting with a large and diverse number of other transcription factors. For instance, in the development of B-cells, it partners with the ETS transcription factor PU.1 to bind to multiple enhancer sites. Another important IRF4 partnership is its interaction with the leucine-zipper heterodimer BATF-JunB required for the development and differentiation of T, B and dendritic cells. Other partners include STAT6, Bcl6, E2A and other IRF proteins. These interactions not only are important in B-cell development but also genes that control key metabolic pathways such as glucose metabolism, lipid biosynthesis and cell-cycle progression. We have recently determined the X-ray structure of IRF4 IAD domain that provides insights into the some of the structural determinants of IRF4 unique properties. Moreover, using SAXS, we have also determined the first domain structure of a full-length IRF protein showing that the putative linker connecting the DBD and the IAD domains may fold into a domain structure.

Remesh, SG., Santosh,V., **Escalante, CR**. (2015) Structural Studies of IRF4 Reveal a Flexible Autoinhibitory Region and a compact linker domain. J.Biol.Chem. 290, 27779-90. PMCID:PMC4646024

Glasmacher E, Agrawal S, Chang AB, Murphy TL, Zeng W, Lugt BV, Khan AA, Ciofani M, Spooner C, Rutz S, Hackney J, Nurieva R, **Escalante CR**, Ouyang W, Littman DR, Murphy KM, Singh H. (2012) A genomic

regulatory element that directs assembly and function of immune-specific AP-1 IRF complexes. Science 338, 975-80. PMCID:22983707

Escalante, CR., Nistal-Villan, E., Shen,L., Garcia-Sastre,A. and Aggarwal, A. (2007) Structure of IRF-3 bound to the PRDIII-I Regulatory element of the Human Interferon-β Enhancer. Molecular Cell 26, 703-716. PMID:17560375

Escalante, CR., Brass AL., Pongubala JM., Shatova, E., Shen., L, Singh, H., Aggarwal AK.(2002) Crystal structure of PU.1/IRF-4/DNA ternary complex. Mol. Cell.10:1097-105. PMID:12453417

Project 2 - Mechanism of AAV mediated Site-Specific Integration

My research group has produced important insights to understand the DNA transactions that control AAV life cycle. Among them: 1) During my early independent research I played a critical role in the determination of the AAV-2 Rep40 protein; 2) We have completely characterized the oligomeric behavior of Rep68, a problem that had been intractable due to its tendency to aggregate. 3) We have determined that the linker region is critical for the oligomerization and function of Rep proteins; 4) We have solved the X-ray structure of AAV-2 OBD in its apo-form and bound to the AAVS1 site and currently working on improving the diffraction of AAV-2 OBD bound to the nicking site (two manuscripts under review); 5) Using EM single particle reconstruction, we have determined the structure of Rep68-AAVS1 complex showing that forms an heptameric ring providing insights not only into the initial step of the site-specific integration (Manuscript in preparation). In all, our research has contributed important insights towards our understanding of the assembly mechanism of AAV Rep proteins to DNA providing insights into the initial steps that lead to site-specific integration.

Bardelli M, Zarate-Perez F, Agundez L, Linden RM, **Escalante CR,** Henckaerts E. (2016) Identification of a functionally relevant AAV Rep68 oligomeric interface. J.Virol. May 11, pii: JVI.00356-16. [Epub ahead of print]**.** PMCID:PMC4944294

Musayev, FN., Zarate-Perez, F., Bishop, C., Burgner, J.W., **Escalante, CR.** (2015) Structural Insights into the assembly of the Adeno-Associated Virus Type 2 Rep68 protein on the Integration site AAVS1. J.Biol.Chem. 290, 27487-99. PMCID:PMC4646001.

Musayev FN, Zarate-Perez F, Bardelli MR, Bishop CM, Saniev EF, Linden RM, Henckaerts E,**Escalante CR**. (2015) Structural Studies of AAV2 Rep68 reveal a partially structured linker and compact domain conformation. Biochemistry, 54, 5907-19 PMCID:PMC4636433.

Nathalie Dutheil, Sarah Smith, Leticia Agúndez, Zoé Vincent-Mistiaen, **Carlos R. Escalante**, R. Linden, Els Henckaerts. (2014) Adeno-associated virus Rep represses the human integration site promoter by two pathways that are similar to those required for the regulation of the viral p5 promoter. J.Virol. 88, 8227-8241. PMID:PMC4135950

Project 3 - Structural and Functional Studies on the mechanisms of Eukaryotic Transcription and Replication Termination.

A third project in my research group focuses in determining the molecular mechanisms of eukaryotic transcription termination by RNA pol I and Replication termination occurring in rRNA genes. Chromosomal DNA is often transcribed and replicated at the same time especially within the S phase of the cell cycle. This lack of temporal and spatial separation of the two DNA transactions, subjects the genome to the risk of transcription invading DNA replication and *vice versa*. This can produce chromosome breakage and genome instability. In eukaryotes, physiologically programmed replication termination remains mostly unknown, however, there seems to be at least two kinds of fork arrests: (i) physiologically programmed polar arrest and (ii) non-polar arrest at sites that bind tightly to nonhistone proteins. In rDNA, Ter sites are found in the non-transcribed spacers region of rDNA repeats where Ter-terminator protein complexes coordinate transcription and replication approaching the complexes from opposite directions. The Reb1 class of terminator proteins that performs these functions contains Myb-like sequence-specific binding domains that are conserved from yeast to humans. In *S.pombe*, the

Reb1 protein binds to tandem pairs of terminator sites (Ter2 and Ter3) located on the spacer regions of rDNA repeats in chromosome III and to Ter sites located elsewhere in the remaining 2 chromosomes. The interaction of Reb1 with its cognate Ter sites promotes at least 4 functions, namely termination of DNA replication, activation of transcription, termination of transcription and long-range chromosome to chromosome interaction called "chromosome kissing". We have solved the structure of Reb1-Ter3 DNA complex at 2.7Å resolution. This is the first structure of an eukaryotic replication and transcription terminator protein. It reveals an unexpected arrangement of five α-helical domains, four of them forming a composite DNA binding domain (DBD) that wraps around DNA causing a bend of approximately 50°. Through yeast molecular genetics performed in the laboratory of our collaborator Dr. Bastia, we have determined that the C-terminal and N-terminal regions contain a transcription termination (TTD) and a transcription activator domain (TAD), respectively and that Reb1-Ter interaction is necessary but not sufficient for transcription termination and initiation. A preliminary structural analysis of this work has been published and the manuscript with our major findings is currently in preparation.

Rahul Jaiswal, Malay Choudhury, Shamsu Zaman, Samarendra K. Singh, Vishaka Santosh, Deepak Bastia and **Carlos R. Escalante** (2016) Functional Architecture of the Reb1-Ter Complex of Schizosaccharomyces pombe. Proc.Natl.Acad.Sci. Apr 19;113(16):E2267-76. doi: 10.1073/pnas.1525465113. Epub 2016 Mar 28. PMCID:PMC4843429

Rahul Jaiswal, Samarendra K. Singh, Deepak Bastia, **Carlos R. Escalante**. (2015) Crystallization and preliminary X-ray characterization of the eukaryotic replication terminator Reb1-Ter DNA complex. Acta Crystallographica Section F. 71, 414-418.PMCID:4388176.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/carlos.escalante.1/bibliography/40266968/public/?sort=date&direction=descending

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

Current
R01GM124204 (P.I. Escalante) 09/30/2017 – 07/31/2021
Structural and Mechanistics insights into AAV mediated Site-Specific Integration
The major goal of this project is to study the interactions and events of Rep68 with DNA that lead to site-specific integration.

VCU-Blick Scholar Award (P.I: Escalante) 07/01/2015 – 06/30/2019
University Research award

Completed
VCU-PRQF AWARD (P.I: Escalante) 06/01/2014 – 12/31/2015
Structural Studies of IRF4 as target of Multiple Myeloma.
The major goal of this project is the structural determination of IRF4 and IRF4 in complex with multiple transcription partners in order to generate targets for the development of novel therapeutics against multiple myeloma.
OVERLAP: None

R21CA179008-01 (PI: Escalante) 07/01/2013 – 06/30/2015
Structural Studies of epigenetic regulation of rRNA gene by TTF-I.
In this new R21 we proposed to develop a system to understand: 1) How TTF-I binds chromatin templates and the structural changes occurring after this initial binding and 2) How TTF-I recruits CSB and the changes that occur upon recruitment in the position of the nucleosomes.
OVERLAP: None

ACS-IRG GRANT (PI: Landry,J; Co-PI: Escalante, CR) 02/01/2014-01/31/2015
Structural Studies on the Chromatin Remodeling Complex NURF
The aim of this project is the structure characterization of NURF using both EM and SAXS techniques.
OVERLAP: None

1R01GM092854 (PI: Escalante) 9/302010 – 08/31/2014
AAV Rep-DNA Complexes Underlying Site-Specific Integration.
The major goal of this project is to study the interactions and events of Rep68 with DNA that lead to site-specific integration.
OVERLAP: None

R56A1046458 (PI: Pintel, Co-PI: Escalante) 09/15/2013 – 08/31/2014
Parvovirus RNA processing and gene expression strategies.
Role in this project is the structural and biophysical characterization of AAV5 Rep proteins. I am a Co-Pi in this project.
OVERLAP: None