Dorsal Ventral Limb Polarity: Generation of novel forms

Our goal is to elucidate the mechanisms underlying dorso-ventral (DV) limb patterning, exploring its evolutionary history and potential role in mammalian regeneration. The limb is anatomically divided into distinct dorsal and ventral compartments, essential for locomotion and manipulation. Lmx1b is the key regulator of DV limb patterning as it is both necessary and sufficient to specify dorsal limb pattern. However, how *Lmx1b* expression is regulated and how it controls the transcription of its direct target genes to instruct DV pattern remain poorly defined.

Over the past few years, our research has focused on exploring the regulation of *Lmx1b*, the key determinant gene for limb dorsality. Through chromatin profiling and functional analysis, we have identified the limb-specific cis-regulatory enhancer elements of *Lmx1b* and outlined a complex regulatory hub (Haro et al., 2021; PMID: 34545091). We have also gathered compelling evidence indicating the importance of this axis in both the fin-to-limb transition and regeneration (Castilla-Ibeas et al., 2023; PMID: 36640311). Building on our previous work, we now delve deeper into all these aspects to gain a comprehensive understanding of this locus.

The project offers a highly multidisciplinary and integrative experience where the student will gain expertise in advanced techniques and methodologies including genomic editing, mouse genetics, genomic analyses. Working with two embryonic models—the mouse and the chick—the student will explore the fundamentals of developmental biology, effectively combining both wet and dry lab approaches to extract the maximum biological significance from the research.

The Ros's lab is part of the Institute of Biomedicine and Biotechnology of Cantabria (https://web.unican.es/ibbtec/en-us/about-ibbtec/team/members/member-detail?d=MarianRosLab). The IBBTEC is a modern research institute of the CSIC, University of Cantabria and Government of Cantabria located in the Scientific and Technologic Park of Santander (Cantabria, Spain). It provides an enthusiastic and supportive environment with state-of-the-art research facilities.

Selection of 10 publications, categorized by theme, that demonstrate how the group's research has made significant contributions to advancing knowledge in the field:

Dorso-ventral patterning and Lmx1b function

1. Castilla-Ibeas A, Zdral S, Galán L, Haro E, Allou L, Campa VM, Icardo JM, Mundlos S, Oberg KC, **Ros MA*** (2023). Failure of digit tip regeneration in the absence of Lmx1b suggests Lmx1b functions disparate from dorsoventral polarity. ***Cell Rep***. Jan 31;42(1):111975. Significance: *We discovered persistent Lmx1b expression into adulthood and within the regeneration blastema suggesting novel roles for Lmx1b that go beyond dorsal-ventral patterning.* https://pubmed.ncbi.nlm.nih.gov/36641754/ *PMID*: 36641754
2. Haro E, Petit F, Pira CU, Spady CD, Ivey LA, Gray AL, Escande F, Jourdain A-F, Nguyen A, Fellmann F, Good J-M, Francannet C, Manouvrier-Hanu S, **Ros MA***, Oberg KC* (2021) Identification of limb-specific *Lmx1b* auto-regulatory modules with Nail-Patella Syndrome pathogenicity. ***Nat Commun***. *Sep 20;12(1):5533.* Significance: *This work was pioner in discovering Lmx1b transcriptional regulation and forms the basis for our continued work on limb dorso-ventral specificaiton.* https://pubmed.ncbi.nlm.nih.gov/34545091/
3. Fernandez-Guerrero M, Yakushiji-Kaminatsui N, Lopez-Delisle L, Zdral S, Darbellay F, Perez-Gomez E, Bolt CC, Sanchez-Martin MA, Duboule D,* and **Ros MA*** (2020) Specific ectodermal enhancers control the expression of *Hoxc* genes in developing mammalina integuments. ***Proc Natl Acad Sci U S A*** 117(48):30509-30519 Significance: *In collaboration with the Duboule's group, we discovered the enhancers controlling HoxC gene cluster expression in the ectoderm which jointly regulate hair and nail organ development.* https://doi.org/10.1073/pnas.2011078117
4. Pérez-Gómez, Fernández-Guerrero M, Campa V, Lopez-Gimenez JF, Rada-Iglesias A and **Ros MA*** (2020) Sp8 regulatory function in the limb bud ectoderm. submitted. Significance: *We unveiled the dual mechanism of Sp8 in activating Fgf8 and DV patterning genes: direct DNA binding at Sp consensus sequences and indirect engagement through Dlx5 interaction. bioRxiv 2020.02.26.965178;* https://doi.org/10.1101/2020.02.26.965178
5. Haro E, Delgado I, Junco M, Yamada Y, Mansouri A, Oberg KC, **Ros MA*** (2014) Sp6 and Sp8 transcription factors control aer formation and dorsoventral patterning in limb development. ***PLoS Genet***. 2014 Aug 28;10(8):e1004468. Significance: *We discovered the critical role of Sp6 and Sp8 as key regulators of Fgf8 and En1 in limb development.* https://doi.org/10.1371/journal.pgen.1004468

Role of *Hox* genes in digit formation:

6. Bastida MF, Pérez-Gómez R, Trofka A, Zhu J, Rada-Iglesias A, Sheth R, Stadler HS, Mackem S, **Ros MA*** (2020) The formation of the thumb requires direct modulation of *Gli3* transcription by Hoxa13. ***Proc Natl Acad Sci U S A***. 117(2):1090-1096. Significance: *We found that the mutual antagonism between Gli3 and Hox13 paralogs controls handplate asymmetry and triggers thumb formation.* https://doi.org/10.1073/pnas.1919470117
7. Sheth R, Marcon L, Bastida MF, Junco M, Quintana L, Dahn R, Kmita M*, Sharpe J*, **Ros MA*** (2012) Hox genes regulate digit patterning by controlling the wavelength of a Turing-type mechanism. ***Science*** 338: 1476-1480 Significance: *First to demonstrate a Turing-type self-organizing mechanism modulated by Hox genes as the basis for digit patterning.* https://doi.org/10.1126/science.1226804
8. González-Martín MC, Mallo M, **Ros MA*** *(2014)* Long bone development requires a threshold of Hox function. ***Dev Biol.*** 392(2):454-65. Significance: *The dose of 5'Hoxd genes determines long versus short bone development. doi:* https://doi.org/10.1016/j.ydbio.2014.06.004

Models for limb proximo-distal specification

9. Pickering J, Rich CA, Stainton H, Aceituno C, Chinnaiya K, Saiz-Lopez P, **Ros MA***, Towers M* (2018) An intrinsic cell cycle timer terminates limb bud outgrowth. ***Elife***. Sep 3;7. pii: e37429. *Significance: We indentified that a progressive intrinsic increase in Bmp expression is responsible for termination of the limb bud outgrowth. doi: https://doi.org/10.7554/eLife.37429.001*

10. Saiz-Lopez P, Chinnaiya K, Campa VM, Delgado I, **Ros MA***, Towers M* (2015) An intrinsic timer specifies distal structures of the vertebrate limb. ***Nat Commun***. 6:8108. *Significance: We proposed a complete model for limb proximo-distal specification that involves the switch from extrinsic signals to intrinsic timing. doi: https://doi.org/10.1038/ncomms9108*