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EVOLUTIONARY HISTORY OF DORSO-VENTRAL POLARITY IMPACT IN THE FIN TO LIMB TRANSITION

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PLAN DE FORMACIÓN

Background: Recently, we discovered the limb-specific enhancers of *Lmx1b*, the transcription factor responsible for the dorsalization of the limb (Haro et al., 2021; PMID: 34545091). We showed that these enhancers, termed *LARM1* and *LARM2*, control *Lmx1b* transcription in a complex, modular and autoregulatory mode. The CRISPR-Cas removal of *LARM1* and *LARM2* (*LARM1/2* mutants) recapitulates the *Lmx1b*-null phenotype exclusively in the limb bypassing the *Lmx1b* perinatal lethality due to *Lmx1b* pleiotropy. The observation that *LARM1/2* mutants cannot walk because their double ventral limbs are unable to lift the body weight led to the **hypothesis** that a progressive elaboration of dorsoventral polarity had to accompany the initial steps of the fin-to limb transition towards appendages capable of lifting the body weight, as observed in *Tiktaalik.* Considering the deep roots of regulatory networks, we propose to investigate whether the *LARM* sequences were involved in providing increasingly adaptive differentiation between dorsal and ventral sides.

Preliminary results: To test whether the *LARM* sequences were involved in the elaboration of the DV pattern along the fin to limb transition, we have searched for *LARM* orthologs in several fish species including chondrichthyans (**Ros**'s lab). In addition, enhancer reporter assays in zebra fish performed in the **Tena**'s lab show that all the orthologs have activity in the pectoral fin, indicating at least some degree of functional conservation.

1. TAREAS A REALIZAR

1)CRISPR-Cas substitution of the mouse *LARM* sequences (partially or totally) by a selected fish *LARM* cognate, to test the functional capacity of ancestral *LARM* enhancers in the mammalian genomic landscape. For these experiments we will start with the spotted gar enhancer because this species is most likely closest to the ancestral actinopterygian condition. Method: i) CRISPR-Cas genomic edition by electroporation of mouse zygotes

2)Phenotypic characterization at morphological, cellular and molecular levels of the limbs of the resulting mice with the exogenous enhancer substitutions. Methods: i) skeletal stainings, ii) histology, IF, IHC, iii) ISH, HCR, iv)RT-qPCR.
We expect that the ancestral fish enhancer configurations will result in incomplete/deficient DV patterning in the mouse limb.

Project specific competences to develop by the JAE-pre student:
Familiarization with the Developmental Biology principles and concepts.
Knowledge of the evolution of appendages and the fin to limb transition
Introduction to the mouse model and mouse genetics
Learning of the fundamental skills of molecular biology techniques
Learning (theoretical and practical) of the CRISPR-Cas technology
Attendance to seminars (lab meetings, seminars etc.)

Transversal competences to develop by the JAE-pre student:
Gathering and managing of scientific information
Acquisition of good laboratory practices and safety in the workplace
Knowing and implementing ethical and responsible research
Written and oral communication of results

2. RELEVANCIA DEL PROGRAMA DE INVESTIGACIÓN (ORIGIN, CO-EVOLUTION, DIVERSITY AND SYNTHESIS OF LIFE).

The fin to limb transition is a clear example of the power of morphological changes to alter the life of the organisms. However, how this transition occurred remains mostly unresolved. Based on our results we propose that the elaboration of the dorso-ventral pattern must have occurred concomitantly with the other critical adaptations that about 360 MYA transformed the fins into limbs allowing tetrapods to conquer the land.

In the last decades, several research groups have focused on how modifications of the antero-posterior and proximo distal patterning could have been modulated to produce the tetrapod limb pattern and digit formula. Therefore, several models and speculations were made regarding the evolution of these two axes along the fin-to-limb transition an the contribution to the current tetrapod limb morphology. However, the study of dorsal-ventral patterning has been rather neglected and it is only now that it has clearly emerged as a potential crucial factor in the fin to limb transition.

Our studies will provide molecular insights into the evolutionary changes and mechanisms behind the generation of dorsoventral tetrapod morphologies as well as their evolutionary origins and history. This interspecies transgenic approach will be

directed towards examining the evolutionary history of the *LARM* regulatory regions and their potential contribution to the fin morphology evolution.

This project is devised to combine the vast expertise of the Ros' group in limb development and patterning with the expertise of the Tena's team in evolution and transcriptional regulation. The student will be settled in the Ros' group and participate in the regular online meetings between the two groups. The Tena group will select the spotted gar sequences to be introduced in the mouse. Such sequences will be introduced in the mouse using CRISPR-Cas electroporation of zygotes in the Ros' lab. We will take advantage of D*LARM1/2* mutants that lack both enhancers. Our results will notably expand our knowledge in one of the crucial transitions in vertebrate evolution.

3. GRUPOS DE INVESTIGACIÓN

1.- Juan J. Tena (CABD-UPO-CSIC) Epigenomics
in disease and aging
El Árbol de la Vida. Entrelazando Genómica y Evolución
jjtenagu@upo.es

2.- Marian Ros (IBBTEC). **Tutor responsable de la ayuda**
Regulación de la Expresión Génica durante el Desarrollo
El Desarrollo del Fenotipo
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Destacamos que el proyecto propuesto en está EoI contribuirá sin duda a estrechar la colaboración interdisciplinar entre estos dos grupos, completamente dentro de la temática de la red LifeHUB.CSIC.

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BIOLOGÍA SINTÉTICA APLICADA AL ESTUDIO DE PROTEÍNAS ESPECÍFICAS DE TARDÍGRADOS CON PROPIEDADES PROTECTORAS DEL ADN

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