Maintenance of murine naive pluripotency drived by Esrrb and Nr5a2

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Abstract

Two transcription factors, Esrrb and Nr5a2, Orphan nuclear receptors, were identified to play a decisive function in development alongside Oct4 and Sox2.
In mouse Embryonic Stem Cells (ESCs) culture Their loss is characterized by disappearance of self-renewal capacity. In the Zygote it is linked with developmental gene expression disruption and apparition of protracted 2-cell like specific genes expression. To the 8-cell stages mitotic defects lead to a full developmental stop. This embryos are failing at upregulation key pluripotency TFs. The work presented here report that Esrrb and Nr5a2 are necessary for ESCs pluripotency maintenance suggesting their importance of the passage of the 2-cell zygotic gene activation. The exact molecular mechanism behind Esrrb and Nr5a2 is still unknown. The internship project will be to identify specific DNA targets of Esrrb and Nr5a2 as well as their impact on chromatin structure thus gene regulation.

Murine naïve pluripotency :

In ESCs Oct4 and Sox2 are the only known transcription factors (TFs) strictly necessary for self-renewal & pluripotency maintenance. Their alteration leads to overt differentiation while they are implicated in epiblast differentiation.
Oct4 and Sox2 are linked to TFs forming a pluripotency network whom depletion do not lead to such marked events of differentiation.

Thus, pluripotency is drived by a **Core** network and **supportive** network of TFs.

Supportive TFs : Esrrb

Esrrb, an Orphan Nuclear Receptors, is a prominent TFs part of the thought supportive network, downstream of the WNT pathway. It is known to mediates self renewal signals and was shown to bypass LIF cytokine mechanism : it is a pluripotency gatekeeper.

The loss of Esrrb in ESC lines is characterized by a no self renewal phenotype. However, this loss is bypassed by more stringent culture conditions suggestion the compensatory role of other TFs.

Supportive TFs : Nr5a2

Nr5a2 is also part of the supportive network and an Orphan Nuclear Receptors. It has a high sequence and structure homology with Esrrb. Both have overlapping developmental function concording with imbricated sets of DNA targets.

Both, Esrrb and Nr5a2 carry close DNA binding Domain and classical zinc finger patterns and Bind to an overlapping set of elements with a canonical binding preference for Erssb and a motif different at the 7th position

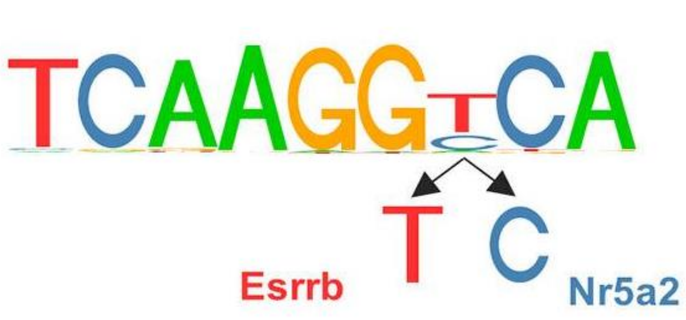

Figure 1 : DNA sequence identified by de novo motif discovery at all regions bound by Esrrb/Nr5a2 in FCS/LIF; note the seventh base can either be a T or a C.

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Festuccia N, Owens N, Papadopoulou T, Gonzalez I, Tachtsidi A, Vandoermel-Pournin S, Gallego E, Gutierrez N, Dubois A, Cohen-Tannoudji M, Navarro P. Transcription factor activity and nucleosome organization in mitosis. Genome Res. 2019 Feb;29(2):250-260. doi: 10.1101/gr.243048.118. Epub 2019 Jan 17. PMID: 30655337; PMCID: PMC6360816.

Esrrb & Nr5a2

Esrrb & Nr5a2 are coexpressed in ESCs

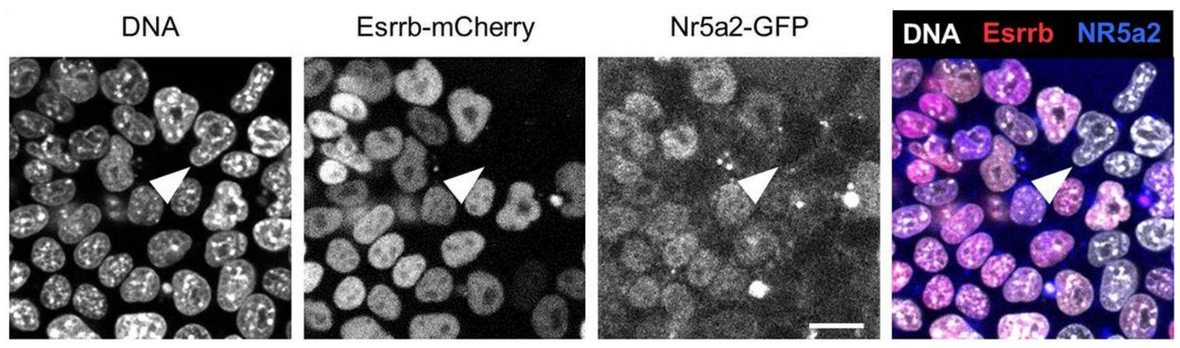

Figure 2 : Confocal microscopy images showing Esrrb-mCherry and Nr5a2-GFP expression in double knock-in ESCs cultured in FCS/LIF. Note that double-negative cells (white arrowheads) exist only in FCS/LIF.

Endogenous Esrrb KO cell lines rescued by a doxycyclyne (Dox) inducible transgene (EKOi) and Nr5a2 KO (EKOiE NrKO) showed that Nr5a2 is not stricly required for self renewal in contrary to Esrrb KO but worsen its effect.

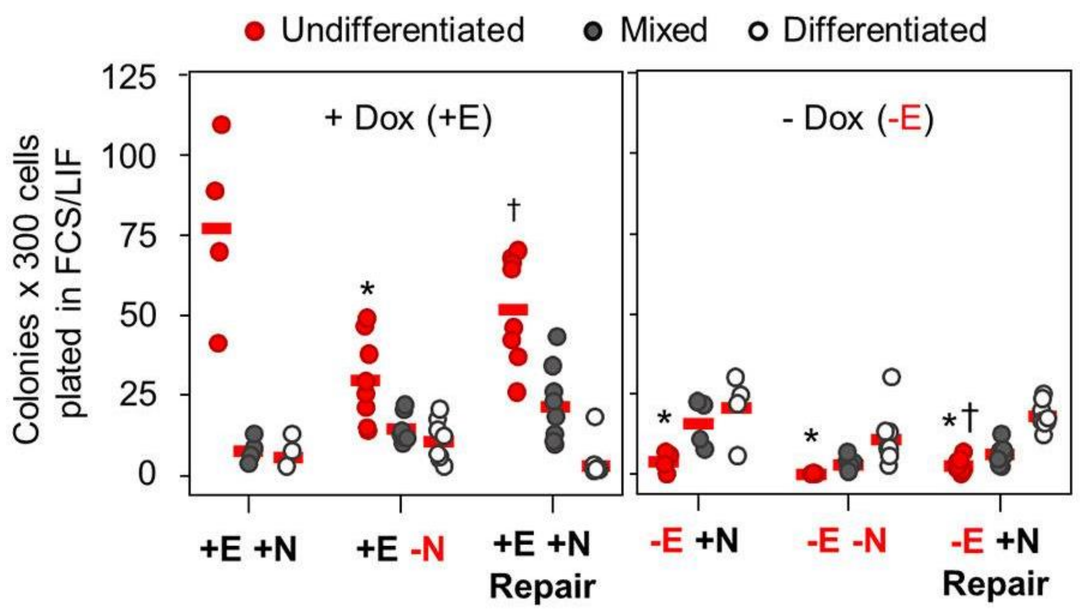

figure 3 : Quantification of the number of undifferentiated, mixed and differentiated colonies. Each circle represents an independent experiment (EKOiE n=4; other conditions n=8); the mean is marked by a red horizontal bar. Asterisks indicate P≤0.05 (Mann–Whitney) for the comparison of each condition to +E+N. Daggers indicate P≤0.05 (Mann–Whitney) for the comparison of +E−N to +E+N Repair or of −E−N to −E+N Repair. Depletion of Esrrb (−E), Nr5a2 (−N), or both genes (−E−N)

Esrrb & Nr5a2 control Oct4, Sox2 and Nanog binding…

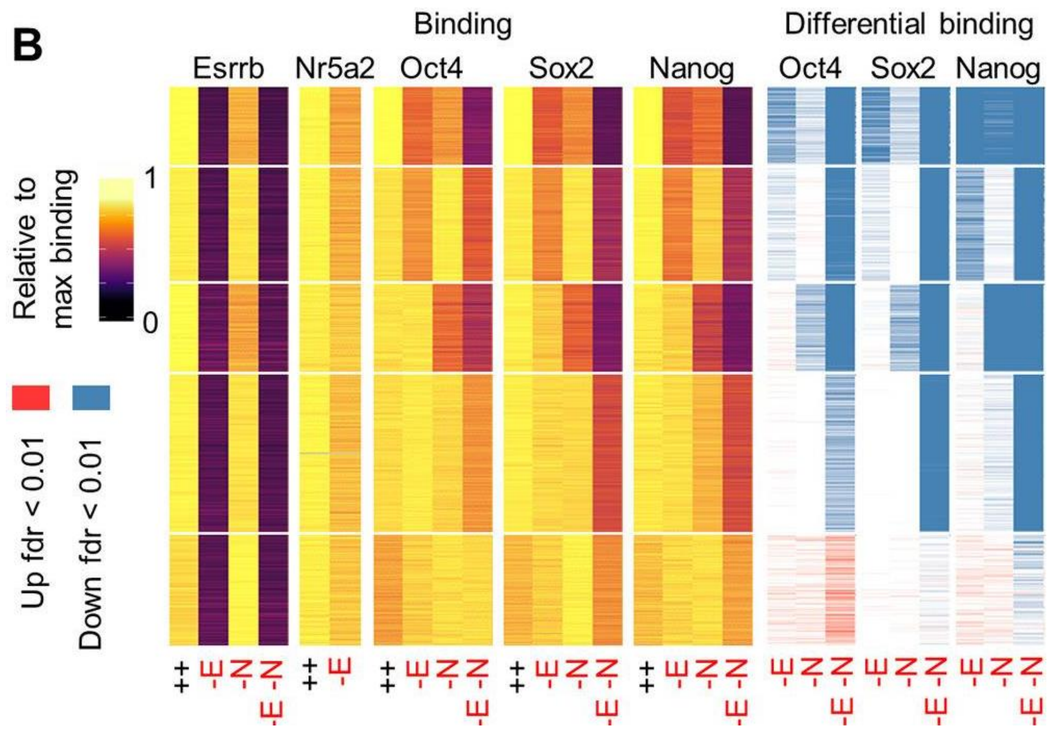

Figure 4 : Heatmap showing normalised TF binding levels at regions called as bound by Oct4, Sox2 and Nanog conjunctly with Esrrb or Nr5a2 (for each TF, the condition displaying maximal binding is set to one) in EKOiE (HA-Nr5a2) or EKOiE NrKO ESCs cultured in the presence (++, −N) or absence of doxycycline (−E, −E−N, respectively).

… In order to restrain differentiation and support naive pluirpotency. *Data not shown, sorry :'(*