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# Pluripotency re-centered around Esrrb

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**The orphan nuclear receptor estrogen-related receptor b (Esrrb) is a vital component of the core pluripotency network in embryonic stem cells (ESCs). However, its function is not clear and the identity of potential upstream regulators has remained elusive. Three elegant reports (Festuccia *et al*, 2012; Martello *et al*, 2012; Percharde *et al*, 2012) have now elucidated the role of Esrrb in ESC self-renewal and reprogramming.**

Mouse ESCs can be readily expanded and maintained *in vitro* in basal media that includes at least two of the three following molecules: (i) an inhibitor of mitogen-activated protein kinase (ERK) signalling; (ii) an inhibitor of the glycogen synthase kinase-3 (Gsk3i), a negative regulator of the Wnt signalling pathway; and (iii) the leukaemia inhibitory factor (Lif) activating the Jak/Stat pathway. Only two of these three molecules are required to support cultured ESCs, suggesting that the pluripotency gene regulatory network is sufficiently flexible to respond to independent signalling cues. Thus, it is of interest to discern which pluripotency regulators are engaged by these distinct signalling pathways and how these factors might be functioning.

Much attention is paid to Wnt signalling and its downstream effectors in regulating ESC self-renewal. It is clear that inhibition of Gsk3 (Gsk3i) supports mouse ESC self-renewal by stabilizing β-catenin and counteracting the activity of the transcriptional repressor Tcf3. However, targets that are activated upon Tcf3 abrogation, which are also critical for relaying the Gsk3i signal, are unknown. Smith and collaborators have now identified that Esrrb is the pivotal target of Tcf3, which is derepressed both by Gsk3 inhibition and the induction of the Wnt pathway in wild-type ESCs (Martello *et al*, 2012). Strikingly, depletion of Esrrb results in loss of self-renewing cells and expression of pluripotency genes in response to Gsk3i, and its overexpression reproduces the effect of Gsk3i, thereby allowing the maintenance of self-renewal in the absence of Lif and Gsk3i (Martello *et al*, 2012). The latter observation is also supported by the report from the Chambers group (Festuccia *et al*, 2012). These findings establish Esrrb as a potent regulator that is necessary and sufficient to mediate self-renewal downstream of the Wnt/Gsk3/Tcf3 signalling axis (Figure 1A).

The Smith and Chambers studies also found that Esrrb functions in parallel to the Lif signalling pathway (Figure 1A). Remarkably, Lif addition can enable self-renewal of ESCs in the complete absence of Esrrb (Martello *et al*, 2012; Festuccia *et al*, 2012). Esrrb −/− ESCs, grown in the presence of Lif, give rise to differentiated teratomas and to chimeric embryos upon blastocyst injection (Martello *et al*, 2012), demonstrating that these cells are pluripotent. Given that previous analyses suggested that removal of Esrrb would cause differentiation, which led to the original identification of Esrrb as pluripotency factor (Ivanova *et al*, 2006; Loh *et al*, 2006), this finding is somewhat surprising. To explain the difference, Festuccia *et al* show that the permanent absence of Esrrb results in loss of colony forming potential even in the presence of Lif, but that undifferentiated cells can persist. This is reminiscent of the phenotype observed upon Nanog deletion in ESCs. Therefore, the authors conclude that Esrrb is essential for ESC renewal under Gsk3i-dependent culture conditions, but dispensable in the presence of Lif. Furthermore, both studies demonstrate that a combination of Lif stimulation and Esrrb overexpression can cooperatively enhance self-renewal (Martello *et al*, 2012; Festuccia *et al*, 2012), supporting a model in which the pluripotency network can accept distinct, but parallel inputs to support a robust transcription factor network. Notably, another pluripotency factor, Klf4 (a well-known Yamanaka reprogramming factor), is activated by the Lif/Jak/Stat pathway (Niwa *et al*, 2009), indicating that independent signalling pathways can target different transcriptional regulators (Figure 1A). Interestingly in this context, Esrrb expression can replace the function of ectopic Klf4 during the induction of pluripotency (Feng *et al*, 2009; Figure 1C).

Esrrb expression can confer Lif-independent self-renewal (Zhang *et al*, 2008; Martello *et al*, 2012; Festuccia *et al*, 2012). This can also be replicated by expression of Nanog, another pluripotency transcription factor. Linking the two observations, the Chambers group demonstrates that Esrrb is a direct target of Nanog; that the ability of Nanog expression to confer Lif-independent self-renewal depends on Esrrb expression; and that Esrrb overexpression can maintain Lif-independent self-renewal in the absence of Nanog (Festuccia *et al*, 2012). As an extension of these findings, the Chambers group also uncovered an important role for Esrrb in reprogramming. They observed that overexpression of Esrrb can reprogram somatic cells, partially reprogrammed cells (pre-iPSCs), and EpiSCs to a

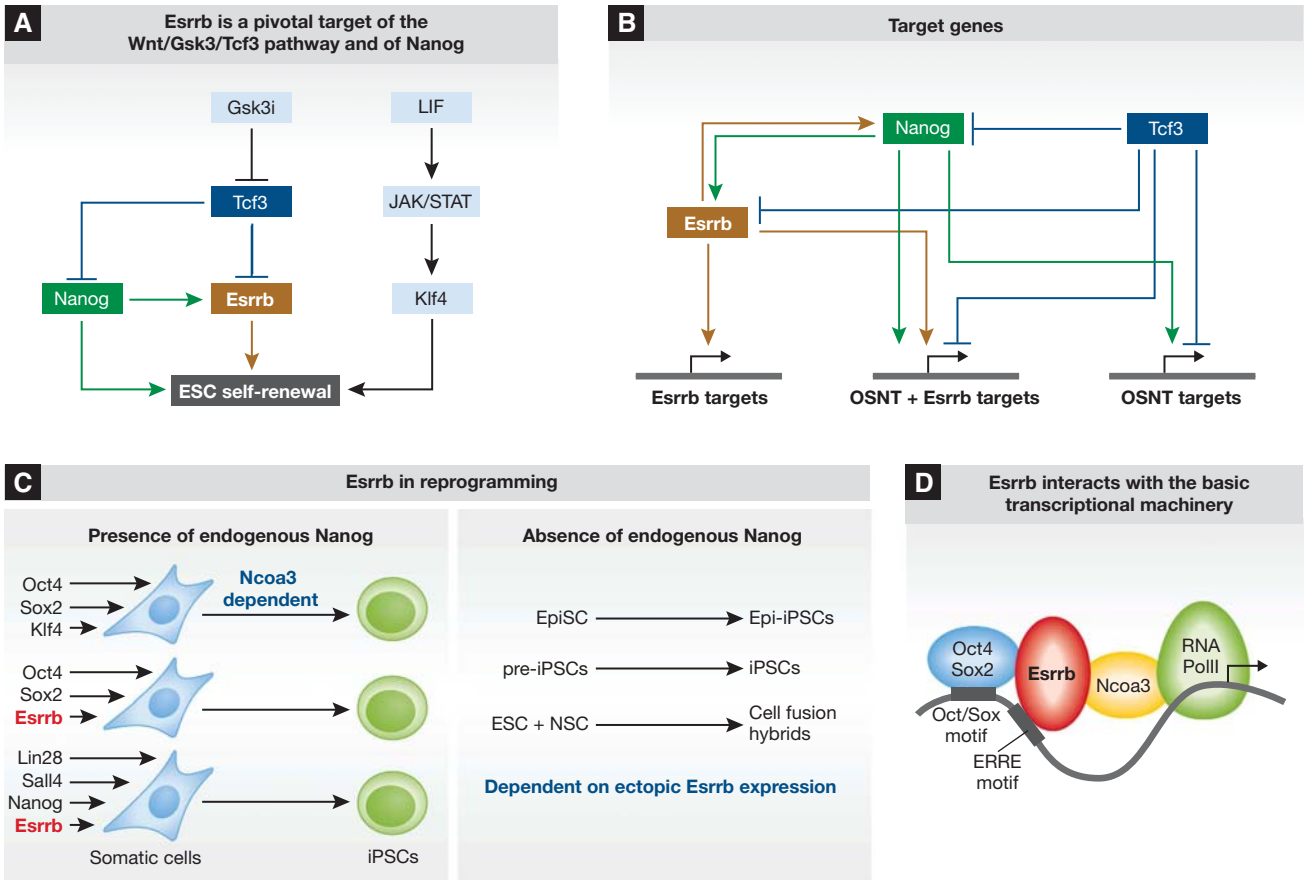


**Figure 1** Role of Esrrb in ESC self-renewal and reprogramming. (**A**) Self-renewal of ESCs is maintained by parallel signalling pathways. The primary role of the Wnt/Gsk3 signalling axis is the activation of Esrrb, by abrogating the activity of its repressor, Tcf3. Gsk3i indicates small molecule inhibition of Gsk3. Esrrb is also a key target of Nanog, which itself is also regulated by the Wnt/Gsk3 pathway. Overexpression of Esrrb or Nanog can confer Lif-independent growth; however, Nanog's effect is dependent on the presence of Esrrb. Klf4 is an important target of the largely parallel acting Lif/Jak/Stat pathway. (**B**) Cross-regulation of targets by the pluripotency factors Esrrb (E), Oct4 (O), Sox2 (S), Nanog (N), and Tcf3 (T). Many pluripotency loci are bound by combinations of Nanog, Oct4, Sox2, Tcf3, with or without Esrrb. However, Esrrb also has a large number of targets that are not occupied by the other four transcription factors. (**C**) Esrrb and reprogramming. In wild-type cells ('presence of Nanog'), Esrrb expression can replace the function of Klf4 in the context of the Oct4-Sox2 reprogramming factor combination. Ncoa3, a co-activator of Esrrb, is essential for reprogramming mediated by Oct4, Sox2, and Klf4. Esrrb is also part of a recently identified non-canonical reprogramming mixture. In the absence of endogenous Nanog, Esrrb overexpression allows the conversion of EpiSCs and pre-iPSCs (generated by Oct4-Sox2-cMyc-Klf4 expression) to the iPSC state, and reprogramming of NSCs by fusion with ESCs. (**D**) Esrrb interacts with the basal transcriptional machinery through Ncoa3. Esrrb, Oct4, and Sox2 often bind at enhancers of genes, where they are recruited through their respective DNA motifs. The interaction of Esrrb with Ncoa3 appears essential to engage the transcriptional machinery at the promoter.

pluripotent state, even in the absence of Nanog (Figure 1C). This finding is particularly striking given that Nanog is essential for the establishment of pluripotency by reprogramming (Silva *et al*, 2009). Thus, a critical function of Nanog is to activate Esrrb, and Esrrb expression can recapitulate the activity of Nanog in ESCs and during reprogramming (Festuccia *et al*, 2012). Notably, Nanog is not required to activate Esrrb under Gsk3i culturing conditions (Martello *et al*, 2012), supporting a model in which parallel pathways can activate Esrrb (Figure 1A).

Having established the position of Esrrb in these novel transcriptional hierarchies, it will now be interesting to reveal the binding and transcriptional targets of Esrrb. Notably, even though Esrrb can maintain ESC self-renewal in the absence of Nanog, Nanog enhances the maximal effectiveness of Esrrb in reprogramming (Festuccia *et al*, 2012), suggesting that Nanog has additional important targets. Interestingly, Esrrb, Tcf3, and Nanog bind many highly expressed genes in ESCs, and co-occupy a large number of them along with Oct4 and Sox2 (Figure 1B). Esrrb requires Oct4 for cooperative binding and transcriptional activation on some of these target genes, but the extent of cooperative binding genome-wide is unknown. The extensive overlap between Esrrb and Nanog binding may explain why Esrrb can replace Nanog. Importantly, since half of Esrrb's target genes are not bound by Oct4, Sox2, Nanog, nor by Tcf3 (Martello *et al*, 2012; Figure 1B), it is possible that some of these genes may function downstream of Gsk3i, where Nanog expression cannot rescue self-renewal in the absence of both Gsk3i and Lif, but Esrrb can (Martello *et al*, 2012). Interestingly, a recent study from the Jaenisch group showed that Esrrb induces reprogramming in combination with non-canonical reprogramming factors (Buganim *et al*, 2012). This observation highlights versatility in Esrrb function. Future experiments will reveal how Esrrb's function intersects with that of other pluripotency transcription factors at the target gene level, and how these interactions are modulated by various culturing conditions.

Esrrb is unique among pluripotency factors in that it interacts with a large number of chromatin remodelling factors, subunits of the Mediator complex, co-activators, and even RNA Polymerase II (van den Berg *et al*, 2010). Importantly, the Azuara group now reveals that one of these Esrrb interacting proteins, the co-activator Ncoa3, links Esrrb to RNA polymerase II (Figure 1D; Percharde *et al*, 2012). Importantly, Ncoa3 is essential for both the induction and maintenance of pluripotency (Figure 1C), and co-occupies many enhancer regions alongside Esrrb, Oct4, and Sox2 (Figure 1D; Percharde *et al*, 2012).

Taken together, these three new studies demonstrate that Esrrb can functionally replace Nanog, integrate the Wnt pathway into the pluripotency factor network, and connect the activity of the pluripotency factors with the basal transcriptional machinery, providing an exciting new link between extrinsic signals and the transcriptional output of target genes.

## Conflict of interest

The authors declare that they have no conflict of interest.

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