RESEARCH HIGHLIGHT

Transcription initiation by the ERRs: no ligand but two activation pathways

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Estrogen-related receptors (ERRα, β and γ) are orphan nuclear receptors that control the transcription of genes involved in the production of cellular energy in response to developmental and physiological cues. Nakadai and colleagues now demonstrate that ERR isoforms utilize two complementary mechanisms to induce distinct gene programs: one dependent on a direct interaction with the p52 subunit of the initiation factor TFIIH, the other contingent on the presence of cell-specific coactivators to recruit p300 and Mediator to ERR-bound promoters.

Nuclear receptors are site-specific DNA-binding transcription factors that transduce variations in the level of hormones and metabolites into precise gene programs. Decades of research has shown that positive control of gene transcription by nuclear receptors and other transcription factors requires the recruitment of coactivators. These proteins promote increased accessibility to chromatin and/or act as scaffolds to promote interactions between coactivators and the mediator complex (Mediator), leading to transcriptional initiation by RNA polymerase II and requisite general transcription factors (GTFs).[1] Estrogen-related receptors (ERRα, β and γ, also known as ESRRA, ESRRB and ESRRG) play key roles in the maintenance of energy homeostasis as they serve as metabolic regulatory hubs integrating diverse biological signals driving the response to changes in cellular energy demands.[2] In this context, the transcriptional activity of the ERRs has shown a reliance toward the presence of PGC-1α, a coactivator whose expression is induced by energy-demanding physiological states, such as acute exercise and exposure to cold. Although the three ERR isoforms regulate common gene targets, they also perform diverse tissue-specific functions. Notably, ERRα plays a key role in the transcriptional response to insulin signaling,[3] while ERRβ is considered an important factor sustaining pluripotent stem cell identity.[4,5] Mechanistically, PGC-1α contacts the ERRs via interaction with a domain referred to as activation function 2 (AF2), which can also be used to interact with members of the steroid receptor coactivator (NCOA1/2/3) family. While ERRα has a strict dependence on PGC-1α for inducing the expression of metabolic programs, ERRβ and γ are more versatile as they can function independently of PGC-1α in embryonic stem cells (ESCs) and muscle in vivo.[5,6] Taken together, these observations suggest the existence of mechanistically distinct ERR target gene activation pathways necessary to execute the full complement of their biological functions (Fig. 1).

In a study published in *Cell Research* by Nakadai et al.,[7] the authors sought to elucidate the mechanisms of ERR-dependent transcription activation using in vitro transcription systems reconstituted with purified factors and naked DNA or chromatin templates. The two systems, both designed to identify cofactor dependencies, can differentiate between mechanisms requiring chromatin modification and those functional on naked DNA. On DNA templates, ERRα efficiently enhanced transcription but unexpectedly, PGC-1α did not further stimulate transcription in this system. In contrast, ERRα is unable to activate transcription on chromatinized template unless PGC-1α, the histone acetylase transferase p300 and Mediator are all present. The authors then demonstrated that the function of PGC-1α is to act as an adaptor protein to recruit p300 and Mediator to DNA-bound ERRα, thus for ERRα-dependent transcription on a chromatin template. The results obtained in vitro could be replicated in mouse embryonic fibroblasts which express neither ERRα nor PGC-1α by reintroducing ectopic wild-type or mutated proteins. The question left to be answered was by which mechanism ERRα stimulates transcription on naked DNA in the absence of PGC-1α. Protein pull-down experiments combined with mutational analyses of ERRα functional domains showed that direct physical interactions between the p52 subunit of the GTF TFIIH and the DNA-binding domain of ERRα are required for transcriptional activation in this context. This mechanistic property is shared with the ERRβ and γ isoforms. The authors then cleverly used mouse ESC self-renewal, a process dependent on the presence of ERRβ and NCOA3 but independent of PGC-1α, as a model to validate the biological relevance of their findings. An intricate combination of functional genomics and mutational analyses not only showed that both AF2-bound cofactors and TFIIH interaction are indeed necessary for ERRβ-dependent ESC pluripotency, but also that the two mechanisms are involved in the regulation of both common and specific subsets of genes dictating different functions.

Taken together, these findings constitute a major conceptual advance in our understanding of ERR transactivation mechanisms. First, the work shows that, unlike the accepted mechanism that nuclear receptors directly recruit Mediator, the ERRs can also use the AF2-dependent PGC-1α and NCOA3 coactivators to promote interaction with Mediator and p300 on promoters. As such, the study offers a mechanism by which AF2-bound coactivators can act as protein ligands for the orphan ERRs. Interestingly, a control experiment showed a negligeable

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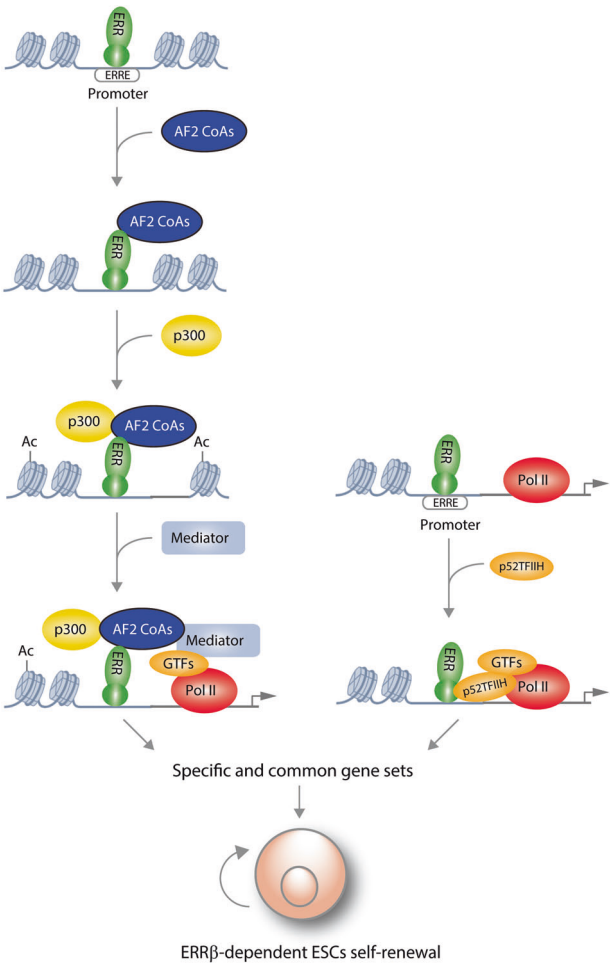

Fig. 1 Model depicting the two proposed mechanisms of transcriptional activation by members of the ERR subfamily of nuclear receptors. Left panel: AF2-bound coactivator-dependent activation pathway. In a stepwise manner, an ERR isoform (ERRα, β or γ) first binds its recognition site on the promoter, referred to as an ERR response element (ERRE). Second, the promoter-bound ERR recruits a coactivator via interaction with its AF2 domain. Transcriptional activation by ERRα is strongly dependent on PGC-1α while NCOA3 can serve as a functional analog of PGC-1α for ERRβ and γ in ESCs. Third, the coactivator enlists the histone acetylase transferase p300 to further promote chromatin opening (histones marked by Ac). Fourth, the coactivator recruits Mediator to facilitate the formation of the pre-initiation complex (GTFs plus Pol II). The strict requirement of PGC-1α interaction with the AF2 of ERRα and subsequent recruitment of p300 and Mediator parallels the mechanism employed by ligand-dependent nuclear receptors, providing further evidence that PGC-1α acts as a protein ligand for ERRα. Right panel: TFIIH-dependent activation pathway. The p52 subunit of TFIIH contacts the DNA-binding domain of the three ERRs resulting in transcriptional activation. The exact mechanism by which a direct interaction between an ERR isoform and TFIIH leads to gene activation remains to be investigated. Bottom panel: transcriptional activation by the two pathways results in the regulation of both specific and common gene sets. Both AF2- and TFIIH-dependent physical interactions reported in the study were found to be required by ERRβ to maintain the self-renewal potential of ESCs.

function for PGC-1α for the heterodimeric nuclear receptor RXRα/PPARγ, supporting the concept that the ERRs are the actual transducer of PGC-1α activity in most contexts. Second, a direct interaction between a GTF (TFIIH) and a transcription factor (ERRα) is shown for the first time to be required for target gene activation. It would be of great interest to investigate whether this mechanism can be extended to other nuclear receptors and transcription factors. Nuclear receptors arose more than 500 million years ago and the ERRs can be viewed as founding members of this family,[8] raising the intriguing question of whether the direct interaction between a GTF and ERR represents a more ancient mode of transcriptional initiation. Third, both pathways are required to promote distinct gene programs necessary to sustain ERRβ-dependent ESC pluripotency. ERRβ has also been reported to bind directly to Mediator to promote enhancer–promoter communication in ESCs,[9] again suggesting the existence of alternative initiation mechanisms employed by ERRβ in the absence of PGC-1α.

This work emphasizes ligand-independent mechanisms of transcription initiation by the orphan ERRs. Nonetheless, the ERRs possess a functional ligand-binding pocket.[10] How would the activities of the TFIIH- and AF2-dependent pathways be differentially influenced by a natural or synthetic agonist or inverse agonist and whether specific physiological signals dictate the activation of the TFIIH pathway remains important open questions, especially in the context of the ERRs as potential therapeutic targets.

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ADDITIONAL INFORMATION

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