

Study of the evolution of nuclear receptor ligand binding using *Amphioxus* as a model

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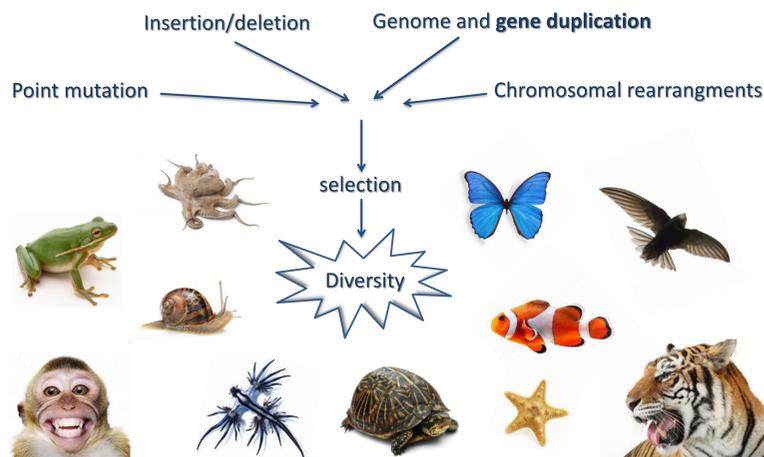
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Introduction and goals of the study

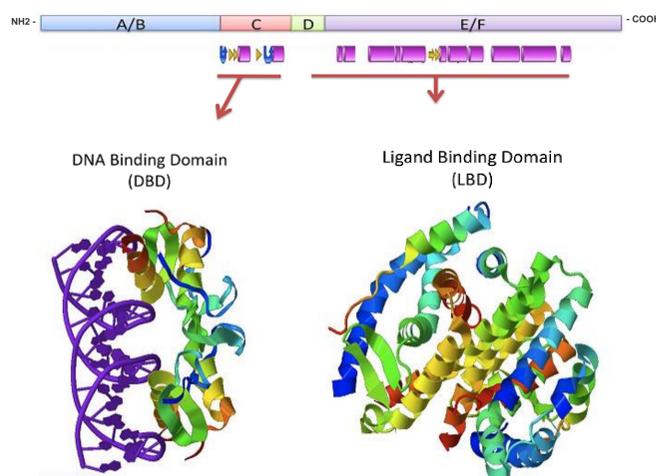
Nuclear receptors (NR) are ligand-activated transcription factors involved in the regulation of a wide variety of physiological processes. They bind to response elements present in the enhancer or promoter region of their target genes and recruit transcriptional co-regulators.



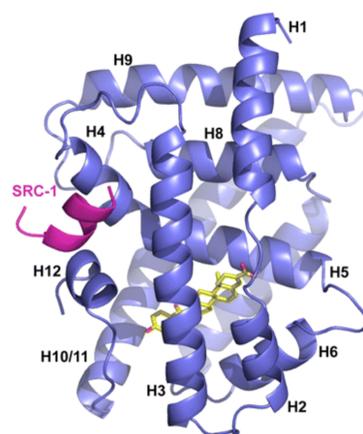
Phylogenetic analyses have shown that NRs arose very early in the metazoan lineage and diversified through complex series of gene duplication and gene loss events. The factors governing the evolution of ligand specificity of NRs in cases of sequence divergence are not well known. In fact, the ligand/receptor coevolution mechanism cannot operate in this case, because the ligands are not gene products, but are small molecules, derivatives of biochemical pathways or derive from an external source, such as food. Cephalochordate amphioxus (*Branchiostoma lanceolatum*) is widely considered as the best available stand-in for the chordate ancestor with a simple, vertebrate-like genome. The entire NR set is present in the amphioxus genome containing 33 NRs which is significantly more than expected. The high number of NRs in amphioxus is mainly due to a massive lineage-specific expansion of NR1H receptors. In addition an entirely new type of nuclear receptor was identified that define on its own a seventh subfamily of NRs called NR7.

In this study we address the question of the functional evolution of receptors for non peptidic ligands at the structural and functional levels by combining 3D-structure determination of the ligand binding domains of NR7 and the 10 NR1H paralogs resulting from a lineage specific expansion and monitoring their ligand binding properties and their impact on gene expression and development.

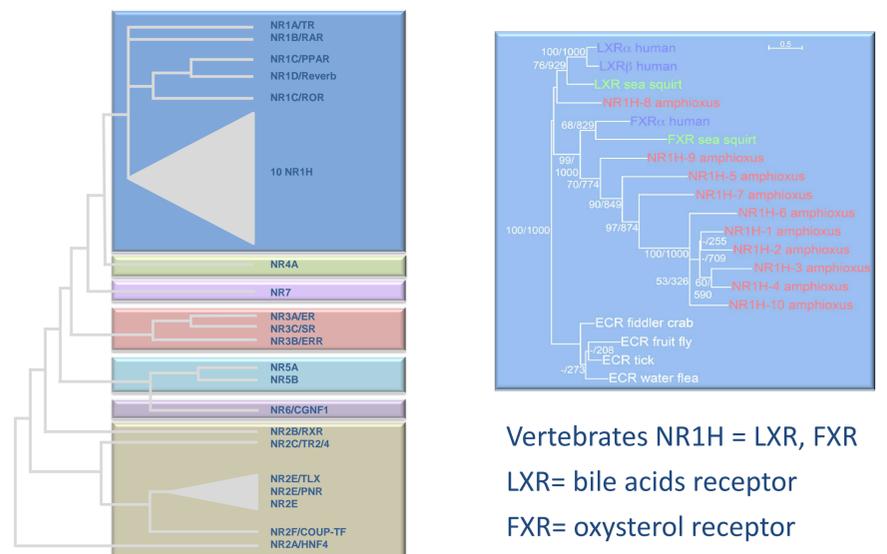
General domain structure of nuclear receptors



Overall structure of human FXR in complex with MFA-1 and an SRC-1 coactivator peptide



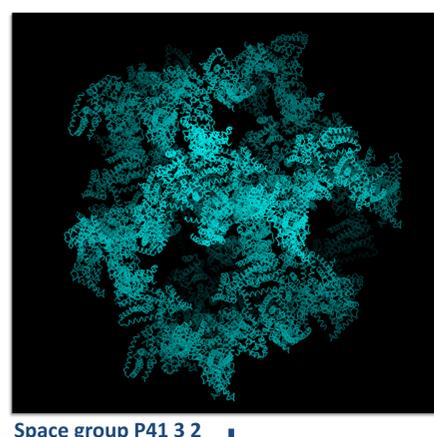
Nuclear receptors in Amphioxus and the lineage specific expansion of NR1H



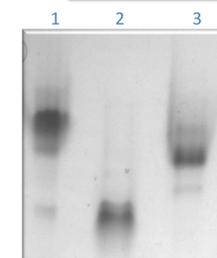
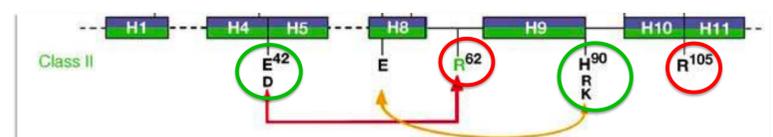
Results of ligand library scan to identify possible NR1H ligands

Level of affinity	antifungal				antipsychotic		synthetic FXR/LXR ligands		steroids						
	Tioconazole	Clotrimazole	Ketoconazole	Fluconazole	Reserpine	Clorophipos	GW4064	T0901317	GW9966	Cholestanol	Androstano	Elitrolone	Estro	Allopremedil	Pregnenolone Sulfate
EC50<10															
10<EC50<50															
50<EC50<100															
EC50>100															
	LXR														
	NR1H-8														
	FXR														
	NR1H-9														
	NR1H-5														
	NR1H-7														
	NR1H-6														
	NR1H-1														
	NR1H-2														
	NR1H-3														
	NR1H-4														
	NR1H-10														
	ECR														

Crystal packing of His-NR1H10 in complex with T0109317 ligand



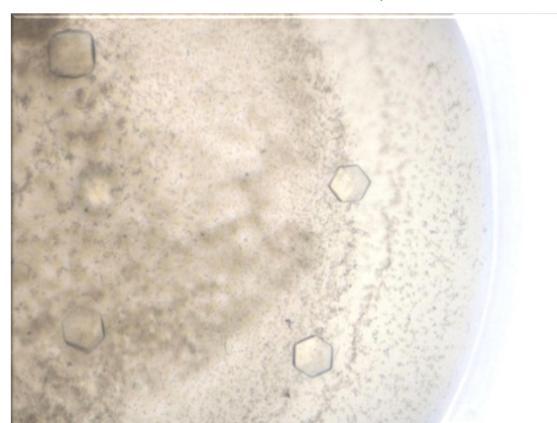
Two essential residues for heterodimerisation not conserved in Amphioxus



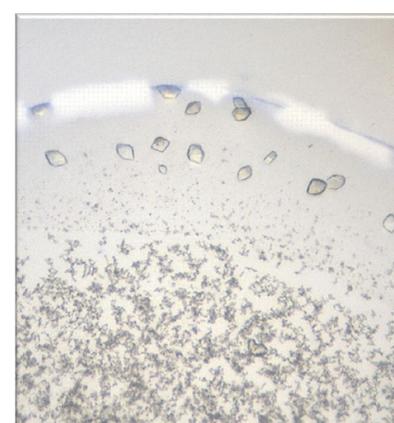
According to the native gel, NR1H10 forms complex with RXR



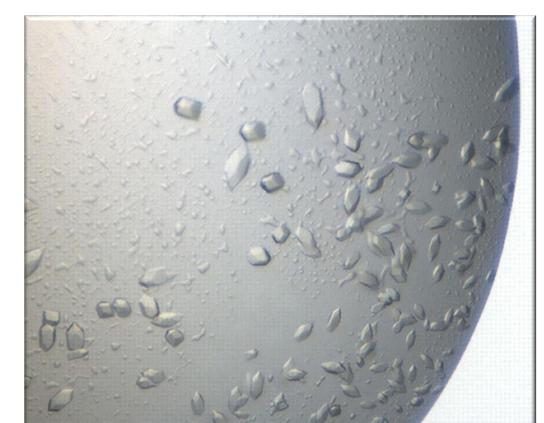
Crystals of His-NR1H10



Crystals of His-NR1H10_T-0901317



Crystals of NR1H10_T-0901317



Crystals of His-NR1H10_His-RXR in complex with T-0901317 ligand and SRC-1 peptide