Trichoplax, the simplest known animal, contains an estrogen-related receptor: Implications for the evolution of vertebrate and invertebrate estrogen receptors

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Abstract. Although, as their names imply, vertebrate and invertebrate estrogen receptors [ERs] and estrogen-related receptors [ERRs] are related transcription factors, their evolutionary relationships to each other are not fully understood. We searched recently sequenced genome of *Trichoplax*, the simplest known animal, and genomes from three lophotrochozoans: *Capitella*, a worm, *Helobdella robusta*, a leech, and *Lottia gigantea*, a snail, to elucidate the origins and evolution of ERs and ERRs. BLAST found an ERR in *Trichoplax*, but no ER. BLAST searches of the lophotrochozaons found ERRs in all three and invertebrate ERs in *Capitella* and *Lottia*, but not in *Helobdella*. These database searches and a phylogenetic analyses indicate that invertebrate ERs arose in a protostome, and vertebrate ERs arose later in deuterostome.

Key words: estrogen receptor evolution, invertebrate estrogen receptors; estrogen related receptor, Trichoplax

1. Introduction.

From the beginning, when estrogen-related receptor α [ERRα] and ERRβ were first cloned [1], the ERR has been an enigma [2,3]. As its name implies, the ERR sequence is similar to that of vertebrate estrogen receptors [ER]. The ligand-binding domain of human ER and ERRα and ERRβ have about 35% sequence identity and 60% positive matches, when conservative replacements such as arginine/lysine and glutamic acid/aspartic acid are considered. Yet these ERRs do not bind estradiol or other steroids [1-3]. Subsequently, ERRγ [4] was cloned and also found to lack steroid-binding activity. Indeed, a *bona fide* biological ligand for an ERR

has not yet been identified. As a result, the ERR belongs to the orphan receptor group [5,6] in the nuclear receptor family of transcription factors [7-10].

An explanation for the absence of steroid binding by ERRs came from analysis of the crystal structures of human ERRα [11,12] and ERRγ [13], which showed that the ligand binding site is too small to accommodate a steroid [11-14]. Unlike the ER, the ERR does not require a ligand to become transcriptionally active. The ERR is constitutively active in cell assays [1-5]. In the last few years, there has been progress in beginning to elucidate ERR functions, which include regulating bone formation [2,3,15,16] and mitochondrial biogenesis [16-18].

Complicating understanding the evolution of ERRs and vertebrate ERs was the cloning in the last five years of several invertebrate ERs from mollusks [19-23]. Invertebrate ERs have about 35% sequence identity and 55% positive matches with the estrogen-binding domain in human ERα. Similar to ERRs, invertebrate ERs do not bind estradiol with high affinity, in contrast to vertebrate ERs, which are activated by 0.2 nM estradiol [24]. Also, similar to ERRs, invertebrate ERs are constitutively active transcription factors in cell assays [19,21,22]. A biological function for invertebrate ERs has not been reported.

The phylogenetic relationships of vertebrate and invertebrate ERs to each other and to ERRs are still not fully understood [9,15,16,19,21]. When did the ancestral ER/ERR arise? Was this ancestor more like an ERR or an ER? How did the estrogen-binding vertebrate ER and the constitutively active invertebrate ERs evolve [25-27]? That is, did vertebrate and invertebrate ERs evolve from a gene duplication of an ancestral ER, or did the vertebrate and invertebrate ERs evolve from separate ancestral genes? An opportunity to address these questions comes from recent sequencing by the Joint Genome Initiative [http://genome.jgi-psf.org] of genomes of *Trichoplax*, which is considered to be the simplest metazoan [28,29] and of three lophotrochozoans: *Capitella*, a segmented worm, *Helobdella*, a leech and *Lottia*, a snail.

As reported here, BLAST [30] searches found an ERR, but no ER in *Trichoplax*, indicating that ERRs are more ancient than ERs. BLAST searches of the three recently sequenced lophotrochozoan genomes found ERRs in *Capitella*, *Helodbdella* and *Lottia*, and invertebrate ERs in *Capitella* and *Lottia*. The current genome release of *Helobdella* does not contain an invertebrate ER. ERRs, but not ERs, are found in ecdysozoa, the other main protostome group. Thus, invertebrate ERs are restricted to lophotrochozoans. Phylogenetic

analyses [31,32] of protostome and deuterostome ERRs and ERs place invertebrate ERs close to an ancestral protostome ERR. In contrast, steroid-binding ERs evolved later in a deuterostome.

2. Methods

BLAST [30] was used to collect ERR and ER sequences from the JGI server and GenBank. A multiple alignment of ERs and ERRs was done with Clustal X 2.0 [31] using the iteration option for each alignment step in the multiple alignment. This alignment was converted to a phylogenetic tree using the neighbor-joining algorithm [32] with a correction of branch lengths for rate heterogeneity between sites.

Accessions for the sequences are human ERRγ [GenBank:**AAQ93376**], human ERα [GenBank:**NP_000116**], *Octopus* ER [GenBank:**ABG00286**], *Aplysia* ER [GenBank:**AAQ95045**], *Thais* ER [GenBank:**BAC66480**] *Marisa* ER [GenBank:**ABI97119**], *Nucella* [GenBank: **ABQ96884],** oyster ER [GenBank:**BAF45381**]., Human ERβ [GenBank:**6166154**], *Xenopus tropicalis* ERα [GenBank:**NP_988866**] and ERβ [GenBank:**NP_001035101**], *Drosophila melanogaster* ERR [GenBank: **NP_729340**], *Apis mellifera* ERR [GenBank: **110756963**], *Daphnia* ERR [jgi|**Dappu1|46682**], *Capitella* ERR [jgi|**Capca1|108381**] and ER [jgi|**Capca1|170275**], *Lottia* ERR [**jgi|Lotgi1|168715]** and ER [jgi|**Lotgi1|132166**] and *Helobdella* ERR [jgi|**Helro1|106750]**.

3. Results and Discussion

3.1 Four Nuclear Receptors are present in a basal diploblast

We used the DNA and ligand-binding domains of human ERRγ, human ERα, octopus ER, *Aplysia* ER, *Thais* ER and oyster ER as queries for BLAST searches for orthologs in *Trichoplax*, *Capitella*, *Helobdella*, and *Lottia* on the JGI server. The BLAST search of *Trichoplex* with ERRγ yielded four high scoring nuclear receptors. Searches with human ERα and invertebrate ERs found the same genes in *Trichoplax*. To classify the four *Trichoplax* genes, we used their sequences as queries for BLAST searches of GenBank. This identified *Trichoplex* jgi|Triad1|16711|gw1.23.179.1 as an ortholog of ERR; the other *Trichoplax* genes were found to be orthologs of COUP, RXR or HNF4 [Table 1]. Thus, ERR, COUP, RXR and HNF4 have ancestors in a primitive multicellular animal belonging to the phylum Placozoa [28,29].

Table 1. Nuclear Receptor Genes in Trichoplax

Gene ID in JGI Databank	Homolog in GenBank	BLAST score	% Identity and %Positives, and Gaps
jgi|Triad1|16711|gw1.23.179.1 ligand-binding domain	Human ERRγ pdb|1KV6|A	6e-55	Identities: 99/222 (44%) Positives:155/222 (69%) Gaps: 0/222 (0%)
>jgi|Triad1|49897|fgeneshTA2_pm.C_scaffold_2000050	Human RXR AAH63827.1|	1e-124	Identities:216/330 (65%) Positives:260/330 (78%) Gaps:17/330 (5%)
jgi|Triad1|50786|fgeneshTA2_pm.C_scaffold_12000032	HNF4 NP_849180.1	1e-125	Identities: 223/324 (68%) Positives: 265/324 (81%) Gaps: 7/324 (2%)
>jgi|Triad1|9010|gw1.23.150.1 DNA-binding domain	Human ERRγ AAH64700.1	1e-28	Identities: 53/85 (62%) Positives: 67/85 (78%) Gaps: 0/85 (0%)
>jgi|Triad1|21656|e_gw1.2.1246.1	Human COUP ref|NP_005645.1	3e-73	Identities: 143/322 (44%) Positives; 205/322 (63%) Gaps; 12/322 (3%)

The Trichoplax genome at JGI was searched with the amino acid sequence for human ERα. Column 1 lists the five entries that were retrieved, two of which correspond to the ligand-binding and DNA-binding domains of human ERRγ.
Columns 2 and 3 show the highest scoring entry in GenBank and its BLAST score.
Column 4 shows the % identities, positives, which include identities and conservative replacements, and the gaps in the BLAST alignment.

We focused the rest of our analyses on the relationship of the *Trichoplex* ERR ancestor to invertebrate and vertebrate ERRs and ERs. BLAST searches of the JGI server retrieved ERRs from *Capitella*, *Helobdella* and *Lottia*, and an invertebrate ER from *Capitella* and *Lottia*. BLAST did not find an invertebrate ER in *Helobdella*. BLAST search of the JGI server retrieved an ERR from *Daphnia*, a water flea. BLAST did not find an invertebrate ER in *Daphnia*. We also used BLAST to retrieve invertebrate ER sequences from *Aplysia californica*, *Octopus vulgaris*, oyster, and two snails: *Thais clavigera* [22]and *Marisa cornuarietis* [23]and ERR sequences from *M. cornuarietis*, *Apis mellifera*, and *Drosophila melanogaster*.

3.2 Invertebrate ERs evolved in lophotrochozoans

To clarify the evolutionary relationships of various invertebrate and vertebrate ERRs and ERs, we constructed a phylogenetic tree of their ligand-binding domains as shown in Figure 1. The phylogeny places node B, the common ancestor of protostome and deuterostome ERRs, only 0.1 unit from node A, their common ancestor with *Trichoplax* ERR. Node C, the common ancestor of invertebrate and vertebrate ERs, is only 0.35 units from node A. The phylogeny

indicates that invertebrate ERs arose in a lophotrochozoan from an ERR-like ancestor. A practical application of this phylogeny is to suggest that invertebrate ERs are likely to have function(s) that resemble or overlap ERR functions [2,15-18].

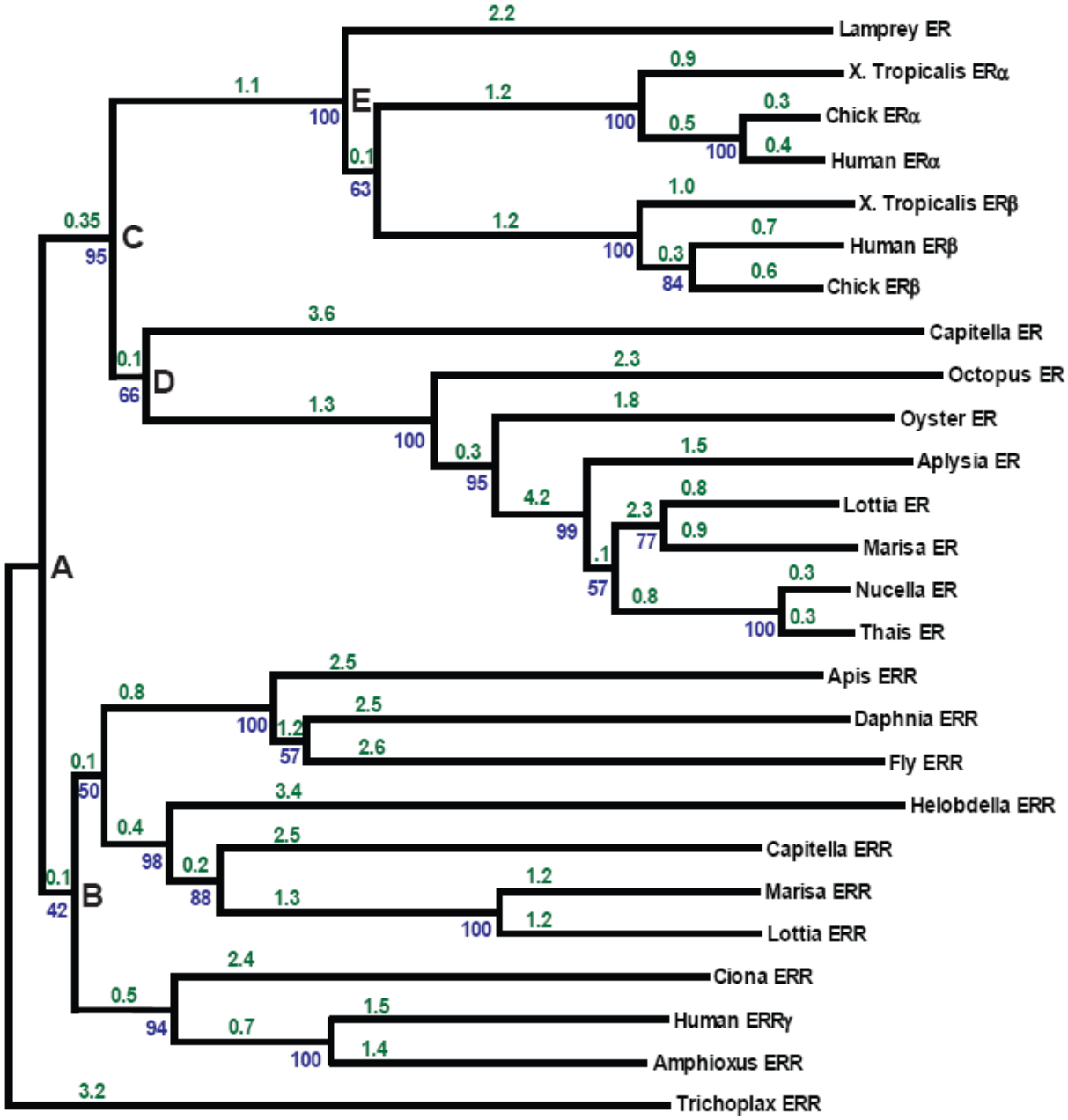

Figure 1. Phylogenetic analysis of invertebrate and vertebrate ERs and ERRs. ERs and ERRs were aligned with Clustal X 2.0 [31], using the iteration option for each alignment step in the multiple alignment. The phylogenetic tree was constructed with the

neighbor-joining algorithm [32] with a correction of branch lengths for rate heterogeneity between sites. Branch lengths are proportional to the distance between proteins. Shown at the nodes are bootstrap values for each branch of the tree, which is the percent this cluster was found in the 1,000 bootstrap trials. Branches with bootstrap values that are greater than fifty percent are significant. Node A is 0.1 unit from node B, the common ancestor of protostome and deuterostome ERRs, and 0.35 units from node C, the common ancestor of protostome and deuterostome ERs. Node D, the ancestral invertebrate ER, is 0.1 unit from node C, while node E, the ancestral vertebrate ER, is 1.1 units from node C.

The evolution of invertebrate ERs from an ERR-like ancestor is consistent with functional similarities between invertebrate ERs and vertebrate ERRs. Both vertebrate ERRs and invertebrate ERs are constitutively active and do not bind estradiol. The crystal structures of human ERRs [11-14] and a 3D model of octopus ER [33] indicates that their ligand-binding domains are too small to accommodate estradiol.

3.2 A steroid-regulated ER evolved in a deuterostome

Node E, the ancestral vertebrate ER, is 1.1 units from node C, which is 11X more longer than the distance between node D and node C. This larger evolutionary distance is consistent with differences between vertebrate and invertebrate ERs in ligand-binding and dependence on a ligand for transcriptional activity. The absence of an invertebrate ER outside of lophotrochozoans and the absence of an invertebrate ER in the recently completed sea urchin genome [34] indicate that a steroid-binding ER evolved in deuterostome [25,26], which would mean that vertebrate and invertebrate ERs have two distinct ancestors.

4. References

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