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Phylogenetic and comparative analysis of *Drosophila melanogaster* ecdysone receptor

Gagan Rani, Neha Gupta, Neeru Singh Redhu and Sudhir Kumar

Abstract
Ecdysone receptor (EcR), a heterodimer of the EcR and Ultraspiracle (USP) nuclear receptors; helps in regulation, reproduction, larval molting, and metamorphosis. In insects, EcR is activated by ecdysteroids. USP nuclear hormone receptor of the insects orthologs to mammalian Retinoid X receptor (RXR) protein. Ecdysone receptor is target for a wide range of pesticides and insecticides. These insecticides binds to their respective target sites in turn hinder the activity of ecdysone and retard the growth of insects. The study was focused on phylogenetic and comparative study of Drosophila ecdysone receptor with its orthologs. Physiochemical properties such as molecular weight, theoretical isoelectric point, extinction coefficient, aliphatic index, instability index, total number of negatively and positively charged residues and grand average of hydropathicity were computed. Along with these physiochemical properties cellular localilization, no. of transmembrane helices, other proteins with which this protein interact and gene ontology were also depicted using various tools.

Keywords: Ecdysone receptor, nuclear receptor, comparative study

Introduction
Insect development, namely metamorphosis, is regulated by the steroid hormone ecdysone (Thummel, 1995, 1996) [10, 11] and its counteragent juvenile hormone. Ecdysone receptor was a type of nuclear receptors (NRs), are a well-characterized superfamily of proteins containing over 150 members. The nuclear receptors are modular proteins, containing conserved domains for DNA-binding, ligand-binding (LDB) and other functions (Gronemeyer & Laudet, 1995). The physiological process of molting in insects is governed by hormones. The ecdysteroid hormones coordinate the major stages of insect development by binding to the ecdysone receptor (EcR). Ecdysone acts through the ecdysone receptor, a heterodimer of the EcR and USP nuclear receptors, to regulate reproduction, larval molting, and metamorphosis in insects, binds to and is activated by ecdysteroids. Ecdysteroids are the steroid hormones of arthropods, where they regulate moulting, metamorphosis, reproduction and diapause (Koolman 1989). These nuclear hormone receptor proteins are the insect orthologs of the mammalian retinoid X receptor (RXR) protein. The RXR is a type of nuclear receptor that is activated by 9-cis retinoic acid. The ecdysone receptor ECR, a nuclear transcription factor controlling insect development, is a novel target for Insecticides. Nuclear receptors (NRs) are a well-characterized superfamily of proteins containing over 150 members. The nuclear receptors are modular proteins, containing conserved domains for DNA-binding, ligand-binding (LDB) and other functions. Ecdysone acts in the form of its active metabolite 20–hydroxyecdysone (20E) by binding to the ecdysone receptor (ECR). RXR is capable of binding and being activated by different types of ligands, such as the potent retinoid 9-cis retinoic acid (9cRA) (Heyman *et al*., 1992; Levin *et al*., 1992) [5, 8], unsaturated fatty acids (de Urquiza *et al*., 2000; Kitareewan *et al*., 1996) [3, 7] and various synthetic ligands (Szanto *et al*., 2004) [12]. The DNA binding Usp, mediates its function. A peak of ecdysone in late 3rd instar larvae (Ashburner, 1972) [1] activates transcription of 'early' genes in salivary glands (Huet *et al*., 1995) [6], including *Broad Complex* (*BR-C*) and two homologs of mammalian Rev-Erb, *E75A* and *E75B*, which in turn activate a set of 'late' genes. When the level of ecdysone diminishes at the prepupal stage, the unliganded EcR/Usp complex is thought to directly repress these and other ecdysone-inducible genes. While the role of EcR/ Usp in ecdysone-dependent activation is well established, its ability to repress genes in the absence of ecdysone is less so. EcR/Usp, like mammalian NRs, recruits co-regulators. Once recruited, co-regulators modify histones, resulting in altered chromatin

structure affects access by transcription factors. Upon ligand binding, the helix H12 that bears the ligand-dependent activation function adopts the canonical agonist conformation, thereby allowing the recruitment of coactivators.

Methodology

Sequence retrieval

The amino acid sequences of Ecdysone receptor of Drosophila melanogaster and Homo sapiens were retrieved from NCBI having the accession number P34021, NP_ Q15406.2 respectively. The characterization of various isoforms was done by using bioinformatics tools *in silico.*

Characterization of target sequence

The physiochemical property of the protein was determined by using Protparam tool such as molecular weight, theoretical pI, total number of negatively and positively charged amino acids, amino acid composition of the protein, extinction coefficient, aliphatic index and GRAVY index. The subcellular localization of the protein was found out using Cello v2.5. The trans membrane helices were predicted using TMHMM tool of Expasy. Signal P 4.1 server was used to check if the protein is a signal peptide or not. STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database was used to identify proteins interacting with the EcR protein. Secondary structure prediction was done using SOPMA. Phylogenetic relations was done by MEGA7.

Result and Discussion

Protparam

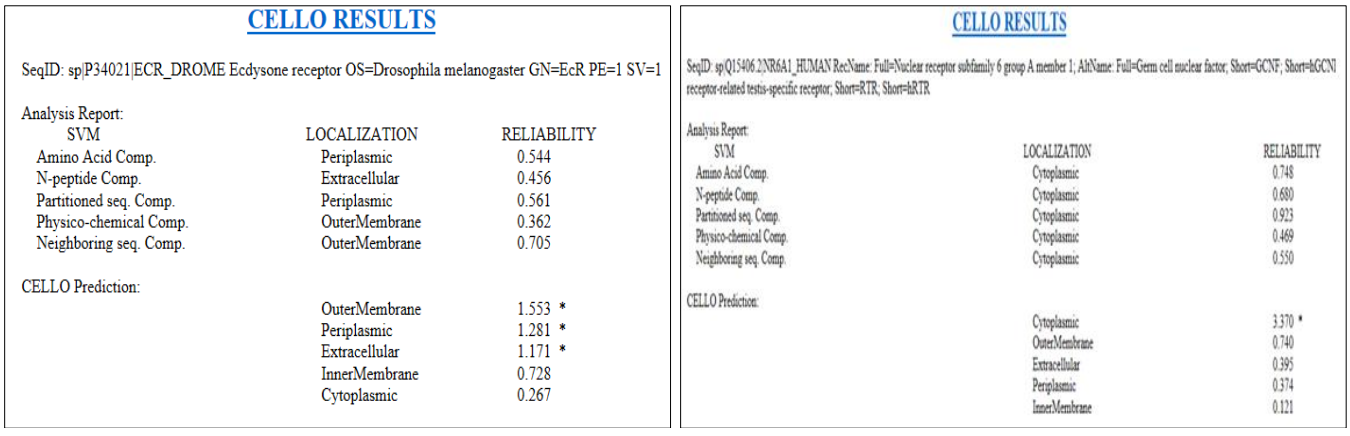
The results of Protparam tool show that the molecular weight of EcR protein of Drosophiila melanogaster and Homo sapiens were different (93853.02 and 54382.93 Daltons). Theoretical pI value for EcR protein were different for both with value of 6.43 and 5.87. Total no. of negatively charged (Asp+Glu) residues in EcR protein is 60 and total number of positively charged (Lys+Arg) residues is 51 leaving them with total charge of -1. Extinction coefficient (in M^{-1} cm^{-1} & at 280 nm measured in water) for the EcR protein assuming all pairs of Cys residues form cystines is 52925 and under same conditions extinction coefficient assuming all Cys residues are reduced was found to be 51800 EcR of *Drosophila melanogaster*. Extinction coefficient can be used to separate the protein from the solution. The instability index (II) determines the stability of the protein in a test tube and is computed to be 60.72, 59.01 for EcR of both. Since instability index for both the isoforms of protein is more than 40, it indicates that EcR protein for both may be unstable. A value 75.57 and 76.21 for aliphatic index for EcR of Drosophila melanogaster and Homo sapiens resecpectively, that indicates the relative volume of a protein that is occupied by aliphatic side chains, which in turn contributes to the increased thermo stability of protein. Positive Grand average of hydropathicity (GRAVY) index for EcR of Human, indicates that the isoforms are in hydrophobic in nature. Negative Grand average of hydropathicity (GRAVY) index for EcR of Drosophila melanogaster, indicates that the isoforms are in hydrophilic in nature.

Table 1: Various physiochemical properties by protparm

Ecdysone receptor Protein	Molecular weight	Theortical pi	Total charge	Instability index	Aliphatic index	Gravy index
Drosophila melanogaster	93853.02	6.43	-6	60.72	75.57	-0.330
Homo sapiens	54382.93	5.87	-9	59.01	76.21	0.438

Cello v2.5

The sub-cellular localization of the protein was predicted by using the tool Cello v2.5 which shows that EcR protein in drosophila melanogaster was cytoplasmic located and for homo sapiens that was located in inner membrane. EcR protein of drosophila melanogaster have the highest reliability (0.267).



CELLO RESULTS

SeqID: sp|P34021|ECR_DROME Ecdysone receptor OS=Drosophila melanogaster GN=EcR PE=1 SV=1

Analysis Report:

SVM	LOCALIZATION	RELIABILITY
Amino Acid Comp.	Periplasmic	0.544
N-peptide Comp.	Extracellular	0.456
Partitioned seq. Comp.	Periplasmic	0.561
Physico-chemical Comp.	OuterMembrane	0.362
Neighboring seq. Comp.	OuterMembrane	0.705

CELLO Prediction:

	OuterMembrane	1.553 *
	Periplasmic	1.281 *
	Extracellular	1.171 *
	InnerMembrane	0.728
	Cytoplasmic	0.267

CELLO RESULTS

SeqID: sp|Q15406.2|NR6A1_HUMAN RecName: Full=Nuclear receptor subfamily 6 group A member 1; AltName: Full=Germ cell nuclear factor; Short=GCNF; Short=hGCNF receptor-related testis-specific receptor; Short=RTR; Short=hRTR

Analysis Report:

SVM	LOCALIZATION	RELIABILITY
Amino Acid Comp.	Cytoplasmic	0.748
N-peptide Comp.	Cytoplasmic	0.680
Partitioned seq. Comp.	Cytoplasmic	0.923
Physico-chemical Comp.	Cytoplasmic	0.469
Neighboring seq. Comp.	Cytoplasmic	0.550

CELLO Prediction:

	Cytoplasmic	3.370 *
	OuterMembrane	0.740
	Extracellular	0.395
	Periplasmic	0.374
	InnerMembrane	0.121

Fig 1: Cello v2.5 results showing the cellular localization of the EcR (field marked as * indicates the cellular localization with the reliability)

SignalP

The SignalP tool predicts whether the protein is a signal peptide or not. Fig. 2a and 2b shows the results of signal P for the EcR protein of Drosophila melanogaster and Homo sapiens. Results clearly shows that protein was not a signal peptide since the D-score (discrimination score) is lesser than the cutoff in each case. So the protein in both case are non-secretory.

Fig 2a: Result of SignalP for EcR protein of *Drosophila melanogaster*

Measure	Position	Value	Cutoff	Signal peptide
Max C	21	0.133		
Max Y	21	0.125		
Max S	3	0.162		
Mean S	1-20	0.114		
D	1-20	0.119	0.450	No

Name=sp_P34021_ECR_DROME SP='NO' D=0.119 D-cutoff=0.450 Networks=Signal P-no TM

Fig 2b: Result of SignalP for EcR protein of *Homo sapiens*.

Measure	Position	Value	Cutoff	Signal peptide
Max C	25	0.110		
Max Y	11	0.127		
Max S	1	0.184		
Mean S	1-10	0.133		
D	1-10	0.130	0.450	No

Name=sp_Q15406.2_NR6A1_HUMAN SP='NO' D=0.130 D cutoff=0.450 Networks=SignalP-no TM

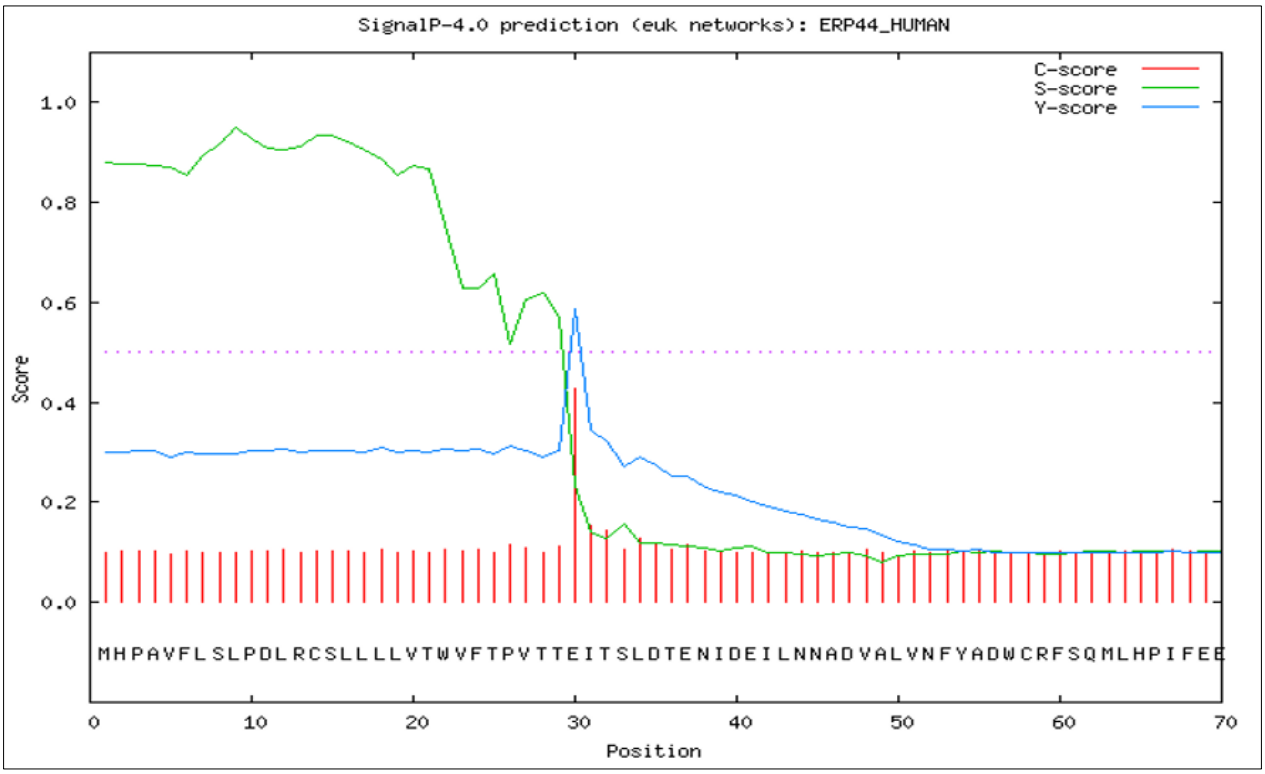


Fig. 2: SignalP graphical output showing C-score (raw cleavage site score), S-score (signal peptide score), Y-score (combined cleavage site score) EcR of Human

Post translational modification

Netnglyc server

The sequence may not contain signal-peptide and the protein that lack signal peptide are unlikely to be exposed to N-glycosylation. 10 sites have been predicted as N-glycosylated in Drosophila melanogaster. Out of the eight results, the sequence "NESG" at position 57, with highest potential of 0.6842, jury agreement of (9/9) and N-glyc result as ++ has the highest possibility to be N-glycosylated than the other predicted sites. Similarly, 1 site have been predicted as N-glycosylated in Homo sapiens. The site was on sequence "NKSI" at position 140, with highest potential of 0.6849, jury agreement of (9/9) and N-glyc result as ++ has the highest possibility to be N-glycosylated than the other predicted sites.

Table 3a: N glycosylation site for EcR protein of Drosophila melanogaster.

Seq Name	Position	Potential Jury	N-Glyc	Agreement	Result
sp_P34021_	ECR_DROME	36 NMSP	0.1568	(9/9)	---
sp_P34021_	ECR_DROME	57 NESG	0.7308	(9/9)	++
sp_P34021_	ECR_DROME	147 NSTT	0.6842	(9/9)	++
sp_P34021_	ECR_DROME	182 NGTP	0.1851	(9/9)	---
sp_P34021_	ECR_DROME	243 NESC	0.4011	(6/9)	-
sp_P34021_	ECR_DROME	367 NGSL	0.6057	(8/9)	+
sp_P34021_	ECR_DROME	452 NESQ	0.5802	(6/9)	+
sp_P34021_	ECR_DROME	524 NRSY	0.6792	(9/9)	++
sp_P34021_	ECR_DROME	723 NDSQ	0.4812	(6/9)	-
sp_P34021_	ECR_DROME	839 NVSM	0.4959	(4/9)	-

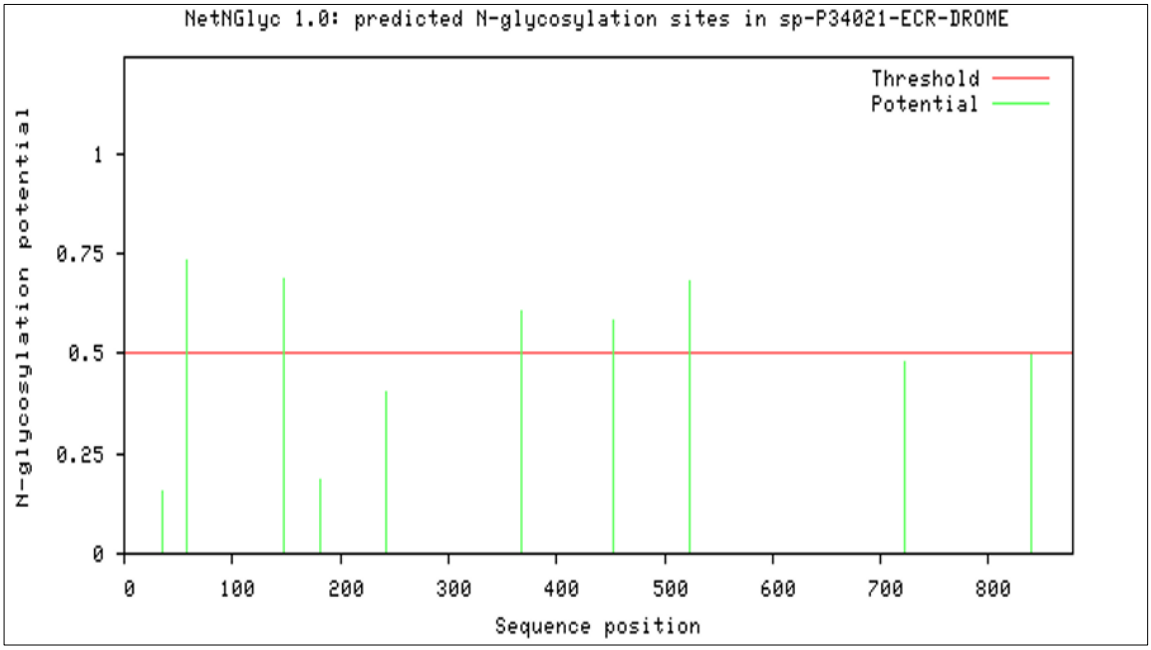


Fig 3a: represents N glycosylated sites with respect to Threshold=0.5(DROME)

Table 3b: N glycosylation site for EcR protein of *Homo sapiens*

Seq Name	Position	Potential Jury	N-Glyc	Agreement	result
sp_Q15406.2	NR6A1_HUMAN	140 NKSI	0.6849	(9/9)	++

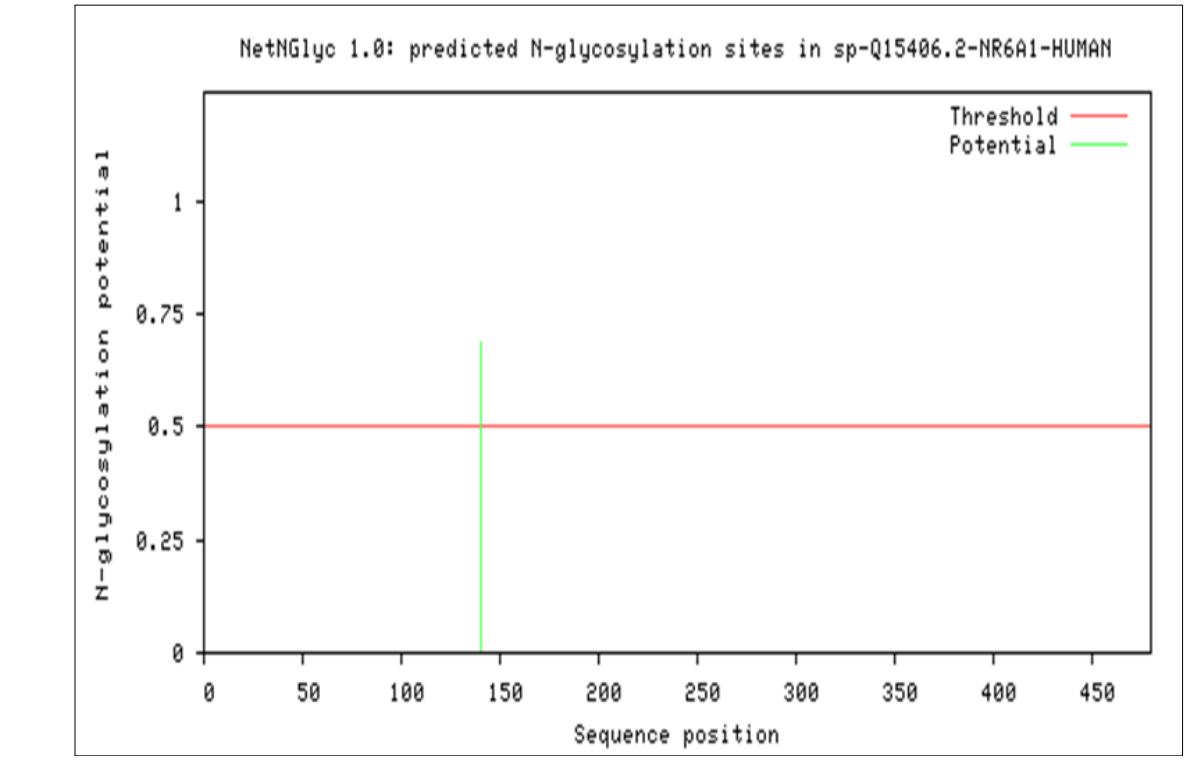


Fig 3b: represents N glycosylated sites with respect to Threshold=0.5 (HUMAN)

Prop 1.0 server:
In Drosophila melanogaster there was one signal peptide cleavage site was predicted and the cleavage site was in between 3&4 amino acids (Arg(R)/Lys(K):1). But in Homo sapiens there was none signal peptide cleavage site was predicted.

Sulfinator
There was 3 sulfated tyrosine detected in drosophila at position 47,430, 434 with sequence DSH-DYCD-QDV—W, KLIW-YQDGYEQ, QDG-YEQPSE. But in homo sapiens none site was predicted.

Fig 4: Sulfated tyrosine detected in Drosophila melanogaster.

Scan prosite result

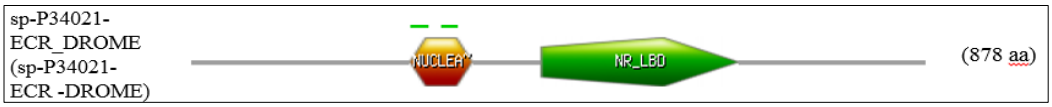
Scan prosite finds motifs that matches given sequences, in DROME that have sequence of 878 aa length that show 3 hits i.e [2 hits (by 2 distinct profiles) on 1 sequence] with 261-336 and 419-654 Amino acid number having score 19.155, 58.912 respectively. Third hit was [1 hit (by 1 pattern) on 1 sequence] for DROME. The same three hits was found in human but out of three 2 hits was found at position of 57-132 and 249-480 with a score of 20.412 and 43.237 respectively.

Found: 3 hits in 1 sequence

sp-P34021-ECR_DROME (878 aa)

MKRRWSNNGGFMRLPEESSSEVTSSSNGLVLPSGVNM
SPSSLDSHDYCDQDLWLCGNESGSFGGSNGHGLSQQQ
QSVITLAMHGCSSTLPAQTTIIPINGNANGNGGSTNGQY
VPGATNLGALANGMLNGGFNGMQQQIQNGHGLINSTT
PSTPTTPLHLQQNLGGAGGGGIGGMGILHHANGTPNGL
IGVVGGGGGVGLGVGGGGVGGLGMQHTPRSDSVNSIS
SGRDDLSPSSSLNGYSANESCDAKKSKKGPAPRVQEEL
CLVCGDRASGYHYNALTCEGCKGFFRRSVTKSAVYCC
KFGRACEMDMYMRRKCQECRLKKCLAVGMRPECVV
PENQCAMKRREKKAQKEKDKMTTSPSSQHGGNGSLA
SGGGQDFVKKEILDLMTCEPPQHATIPLLPDEILAKCQA
RNIPSLTYNQLAVIYKLIWYQDGYEQPSEEDLRRIMSQP
DENESQTDVSFRHITEITILTVQLIVEFAKGLPAFTKIPQE
DQITLLKACSSEVMMLRMARRYDHSSDSIFFANNRSYT
RDSYKMAGMADNIEDLLHFCRQMFSMKVDNVEYALL
TAIVIFSDRPGLEKAQLVEAIQSYYIDTLRIYILNRHCGD
SMSLVFYAKLLSILTELRTLGNQNAEMCFSLKLKNRKL
PKFLEEIWDVHAIPPSVQSHLQITQEENERLERAERMRA
SVGGAITAGIDCDSASTSAAAAAAQHQPQPQPQPQPSS
LTQNDSQHQTQPQLQPQLPPQLQGQLQPQLQPQLQTQ
LQPQIQPQPQLLPVSAPVPASVTAPGSLSAVSTSSEYMG
GSAAIGPITPATTSSITAAVTASSTTSAVPMGNGVGVGV
GVGGNVSMYANAQTAMALMGVALHSHQEQLIGGVA
VKSEHSTTA

Hits by profiles: [2 hits (by 2 distinct profiles) on 1 sequence]



PS51030 **NUCLEAR_REC_DBD_2** *Nuclear hormone receptors DNA-binding domain profile*

261 - 336:	**score** = 19.155

EELCLVCGDRASGYHYNALTCEGCKGFFRRSVTKSAV
YCCKFGRACEMDMYMRRKCQECRLKKCLAVGMRPE
CVVP

Predicted features

ZN_FING	264	284	NR C4-type
ZN_FING	300	319	NR C4-type

PS51843 **NR_LBD** *Nuclear receptor (NR) ligand-binding (LBD) domain profile*:

ZN_FING	264	284	NR C4-type
ZN_FING	300	319	NR C4-type

NQLAVIYKLIWYQDGYEqPSEEDLRRIMSQPDeNESQT
DVSFRHITEITILTVQLIVEFA
KGLPAFTKIPQEDQITLLKACSSEVMMLRMARRYDHSS
-DSIFFANNR-SYTRDSYKMA-
GMADNIEDLLHFCRQMFSMKVDNVEYALLTAIVIFS--
DRPGLEKAQLVEAIQSYYIDTL
RIYILNRHcGDSMsLVFYAKLLSILTELRTLGNQNAEMC
FSLKLKNRKLPKFLEEIWDVH
A

Predicted feature

DOMAIN	419	654	NR LBD

PS00031 **NUCLEAR_REC_DBD_1** *Nuclear hormones receptors DNA-binding region* **264 - 290**

Fig 5a: Fig represents various motif and domain for EcR of DROME.

Scan prosite results: Human

Found: 3 hits in 1 sequence

Sp-Q15406-2-NR6A1_HUMAN (480 aa)

MERDEPPPSGGGGGGGSAGFLEPPAALPPPPRNGFCQD
ELAELDPGTISVSDDRAEQRTCLICGDR
ATGLHYGIISCEGCKGFFKRSICNKRVYRCSRDKNCVM
SRKQRNRCQYCRLLKCLQMGMNRKAIREDGMPGGRN
KSIGPVQISEEEIERIMSGQEFEEEANHWSNHGDSDHSS

PGNRASESNQPSPGSTLSSSRSVELNGFMAFREQYMGM
SVPPHYQYIPHLFSYSGHSPLLPQQARSLDPQSYSLIHQ
LLSAEDLEPLGTPMLIEDGYAVTQAELFALLCRLADEL
LFRQIAWIKKLPFFCELSIKDYTCLLSSTWQELILLSSLT
VYSKQIFGELADVTAKYSPSDEELHRFSDEGMEVIERLI
YLYHKFHQLKVSNEEYACMKAINFLNQDIRGLTSASQL
EQLNKRYWYICQDFTEYKYTHQPNRFPDLMMCLPEIR
YIAGKMVNVPLEQLPLLFKVVLHSCKTSVGKE

Hits by profiles: [2 hits (by 2 distinct profiles) on 1 sequence]

PS51030 **Nuclear_Rec_Dbd_2** *Nuclear hormone receptors DNA-binding domain profile*:

57 - 132:	**score** = 20.412

QRTCLICGDRATGLHYGIISCEGCKGFFKRSICNKRVYR
CSRDKNCVMSRKQRNRCQYCR
LLKCLQMGMNRKAIRE

Predicted features

ZN_FING	60	80	NR C4-type
ZN_FING	96	115	NR C4-type

PS51843 **NR_LBD** *Nuclear receptor (NR) ligand-binding (LBD) domain profile*

249 - 480:	score = 43.237

QSYSLIHQLLSAEDLEPlgtPMLIEDGYAVT-------
QAELFALLCRLADELLFRQIAW
IKKLPFFCELSIKDYTCLLSSTWQELILLSSLTVYSKQ----
---IFGEladvtakYSPS
DEELHRFsDEGMEVIERLIYLYHKFHQLKVSNEEYACM
KAINFLN-QDIRGLTSASQLEQ

LNKRYWYICQDFTEYKYTHQP-
NRFPDLMMCLPEIRYIAGKMVNVPLEQLPLLFKVVLHS
CKTSVGKE

Predicted feature

DOMAIN	249	480	NR LBD

PS00031 **NUCLEAR_REC_DBD_1** *Nuclear hormones receptors DNA-binding region signature* :

Fig 5b: Fig represents various motif and domain for EcR of HUMAN.

Interpro scan

Gene ontology was done by Interpro scan. Resut of Interpro scan shows EcR protein of both fruit fly and human belongs to same family that was nuclear receptor type family. Cellular component for EcR protein of both were nucleus. Molecular activity that describes the function of protein. Molecualr activity of EcR protein for both Drosophila melanogaster and Homo sapiens were DNA binding, transcription factor activity, steroid hormone recptor activity, sequence-specific DNA binding and ZINC ion binding.

Table 4: Various biological and molecular function of EcR protein

Protein	Family	Biological process	Molecular activity	Cellular component
Drosophila melanogaster	Nuclear hormone receptor type, nuclear hormone receptor ligand binding domain type	Regulation of transcription, DNA-templated, ecdysone receptor-mediated signaling pathway, steroid hormone mediated signaling pathway	DNA binding, DNA binding transcription factor activity, steroid hormone receptor activity, ecdysteroid hormone receptor activity, steroid binding, zinc ion binding, sequence-specific DNA binding	Nucleus
Homo sapiens	Nuclear hormone receptor type,	regulation of transcription, DNA-templated, steroid hormone mediated signaling pathway	DNA binding, DNA binding transcription factor activity, steroid hormone receptor activity, ecdysteroid hormone receptor activity, steroid binding, zinc ion binding, sequence-specific DNA binding	Nucleus

Secondary structure prediction

Sopma

Secondary structure prediction was done using SOPMA. The results of secondary structure prediction are shown in fig. 6a and 6b. Different secondary structures are colour coded with different colors in the sequence, alpha helix(h) with blue, extended strand(e) with red, beta turn (b) with green and random coil(c) with yellow color in SOPMA. Predicted secondary structure in SOPMA shows that in case of EcR protein of DROME % of alpha helix is less with a value of 29.73 as compared to human that value is 40.62 %. But percentage of extended strand and random coil is higher in DROME. Percentage of Beta turn is almost same in both cases as shown in fig 6a and 6b. Higher number of helices makes the protein more flexible for folding that might increase interactions. There are no 3_{10} helix, Pi helix, Beta Bridge, bend region or ambiguous states in either of the case.

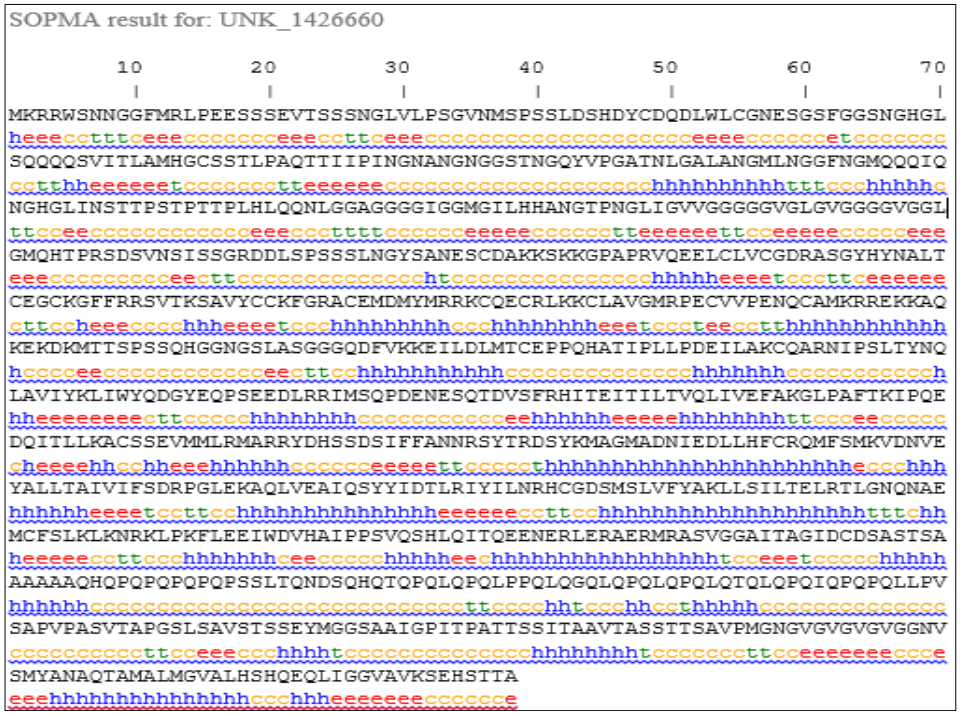


Fig 6a: SOPMA result showing secondary structure for EcR of Drome. (Helix represented by 'h' in blue, random coil by 'c' in yellow, extended strand by 'e' in red and beta turn by 't' in green)) colour.

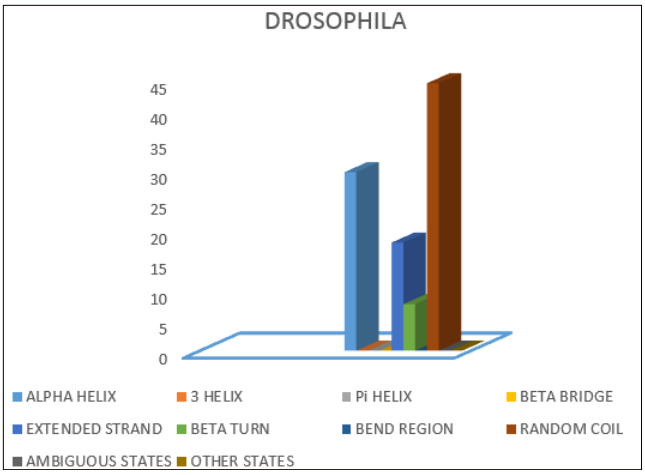


Fig 6b: Graph represents percentage Alpha helix, Extended strand, beta turn, random coil for EcR of Drosophila melanogaster.

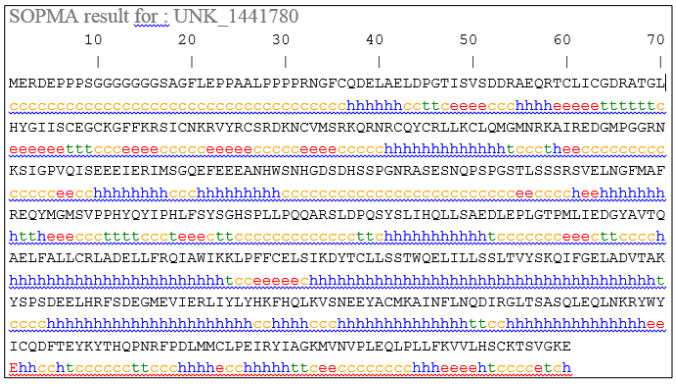


Fig 7a: SOPMA result showing secondary structure for EcR of Human. (Helix represented by 'h' in blue, random coil by 'c' in yellow, extended strand by 'e' in red and beta turn by 't' in green)) colour.

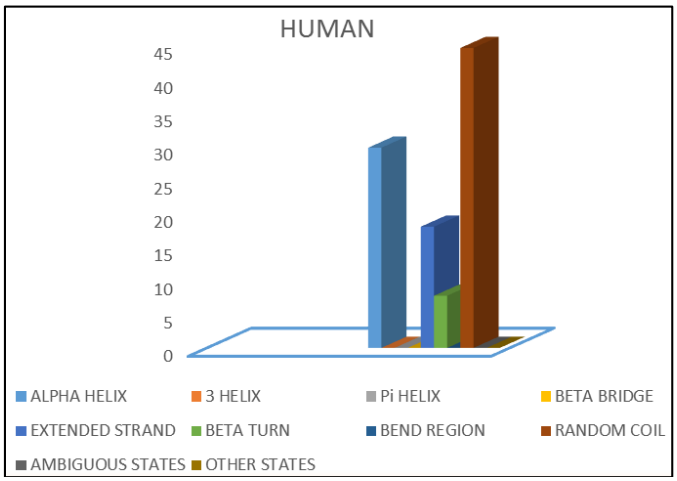


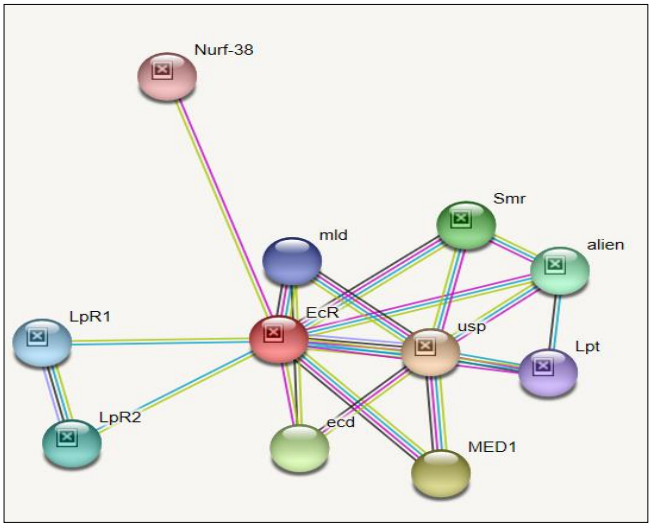
Fig 7b: Graph represents percentage Alpha helix, Extended strand, beta turn, random coil for EcR of Homo sapiens.

Protein interaction by string

STRING

Predicted that EcR protein of Homo sapiens interact with *Ultraspiracle, Mediator complex subunit, Ecdysoneless, Smrter, Alien; Component of the COP9, Lipophorin receptor* 1 etc.

In Homo sapiens string predicted that EcR protein interact with *Mitochondrial E3 ubiquitin protein Nuclear receptor corepressor Excision repair cross-complementing rodent repair deficiency, ATPase, RMI1, RecQ mediated genome instability 1, Nuclear receptor coactivator 1, Nuclear receptor corepressor 2 POU class 5 homeobox 1, Nuclear receptor coactivator 2;* etc.



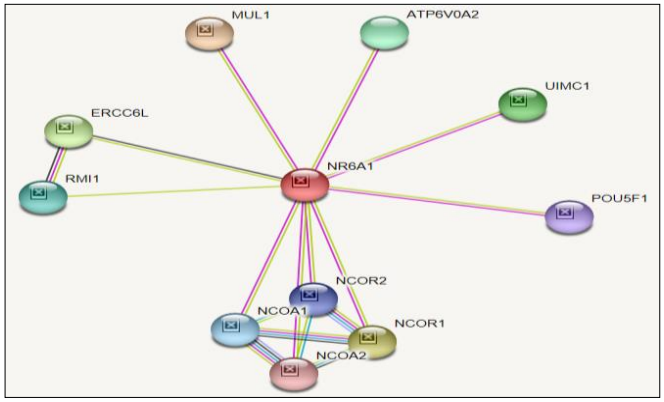
Your Input:

		Neighborhood	Gene Fusion	Cooccurence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
EcR	*Ecdysone receptor; Receptor for ecdysone. Binds to ecdysone response elements (ECRES) following ecdysone-binding, and recruitment of a complex containing the histone methyltransferase trr, leads to activate transcription of target genes (878 aa)*									

Predicted Functional Partners:

		Score
usp	*Ultraspiracle; Receptor for ecdysone. May be an important modulator of insect metamorphosis. Plays an important part in em…*	0.999
MED1	*Mediator complex subunit 1; Component of the Mediator complex, a coactivator involved in the regulated transcription of nearl…*	0.975
ecd	*Ecdysoneless; Required in both the follicle cells and the germline for oocyte development (684 aa)*	0.971
Smr	*Smrter (3607 aa)*	0.965
alien	*Alien; Component of the COP9 signalosome complex (CSN), a complex involved in various cellular and developmental proces…*	0.925
LpR2	*Lipophorin receptor 2 (1064 aa)*	0.908
LpR1	*Lipophorin receptor 1 (1076 aa)*	0.900
mld	*Molting defective (1965 aa)*	0.864
Lpt	*Lost PHDs of trr (1482 aa)*	0.855
Nurf-38	*Inorganic diphosphatase activity; Component of NURF (nucleosome remodeling factor), a complex which catalyzes ATP-depe…*	0.848

Fig 8a: STRING results showing the proteins with which EcR of *Drosophila melanogaster* interact.



Your Input:

		Neighborhood	Gene Fusion	Cooccurence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
NR6A1	*Nuclear receptor subfamily 6, group A, member 1; Orphan nuclear receptor. Binds to a response element containing the sequence 5'-TCAAGGTCA-3'. May be involved in the regulation of gene expression in germ cell development during gametogenesis (By similarity) (480 aa)*									

Predicted Functional Partners:

		Score
MUL1	*Mitochondrial E3 ubiquitin protein ligase 1; Exhibits weak E3 ubiquitin-protein ligase activity. E3 ubiquitin ligases accept ub…*	0.819
NCOR1	*Nuclear receptor corepressor 1; Mediates transcriptional repression by certain nuclear receptors. Part of a complex which…*	0.776
ERCC6L	*Excision repair cross-complementing rodent repair deficiency, complementation group 6-like; DNA helicase that acts as an…*	0.731
UIMC1	*Ubiquitin interaction motif containing 1; Ubiquitin-binding protein (PubMed-24627472). Specifically recognizes and binds 'L…*	0.680
ATP6V0A2	*ATPase, H+ transporting, lysosomal V0 subunit a2; Part of the proton channel of V-ATPases. Essential component of the…*	0.663
RMI1	*RMI1, RecQ mediated genome instability 1, homolog (S. cerevisiae); Essential component of the RMI complex, a complex…*	0.627
NCOA1	*Nuclear receptor coactivator 1; Nuclear receptor coactivator that directly binds nuclear receptors and stimulates the transc…*	0.619
NCOR2	*Nuclear receptor corepressor 2; Transcriptional corepressor. Mediates the transcriptional repression activity of some nucle…*	0.600
POU5F1	*POU class 5 homeobox 1; Transcription factor that binds to the octamer motif (5'-ATTTGCAT-3'). Forms a trimeric comple…*	0.593
NCOA2	*Nuclear receptor coactivator 2; Transcriptional coactivator for steroid receptors and nuclear receptors. Coactivator of the st…*	0.587

Fig. 8b STRING results showing the proteins with which EcR of *Homo sapiens* interact.

Mega7

The phylogenetic analysis result shows that the Ecdysone receptor of Drosophila *melanogasters* has a close evolutionary relation with the Ecdysone receptor of other group of insect like Schistosoma *mansoni*, Bombyx *mori*, Aedes *aegypti* which form one clade in the phylogeny tree and these insect group also show close connection with the Mammals which form another clade in the phylogeny tree.

Conclusion

From the above study it has been concluded that ecdysone receptor proteins of Drosophila melanogaster are unstable with hydropathy index >40 but Homo sapiens ecdysone receptor proteins of Drosophila melanogaster are stable with hydropathy index <40. Prop 1.0 and Sulfinator predicted one polypeptide cleavage site and 3 sulfated tyrosine in drosophila respectively but none in human. Sumoplot predicted 3 somolyation sites in drosophila but 1 in human. In Drosophila melanogaster cellular lolization of protein was cytoplasmicbut in mammals that was in inner membrane. EcR proteins in both species does not contain any signal peptide. Phylogenetic analysis dof 100 sequences by MEGA7 suggested a distant relationship between *Drosophila melanogaster* and Human. Conserverd domains are same in ecdysone receptor of both Drosophila melanogaster and Homo sapiens.

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