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Correlation in Evolution of Adrenergic Receptors with their Interacting Partner

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Abstract

The prediction of protein interacting partners with bioinformatics methods has become a topic of increasing interest in recent years. Studies of interacting proteins have found correlated evolution of the sequences of binding partners or coadaptation of interacting partners, such that as a result substitution or mutation of an amino acid residue in the interface of one protein will select for the coordinated mutation and subsequent substitution of an amino acid in the interface of its binding partner. We have analysed coevolution of receptors and ligands i.e interacting partners by using adrenergic receptors (alpha1,alpha2,beta1,beta2,beta3) as model. We show that there is correlation between the adrenergic receptor and ligand evolutionary distances by analyzing correlation coefficient values and p-values. As p-values validate the correlation results. Phylogenetic trees of receptor and ligand are also analysed and compared to further predict the protein coevolution results.This approach can be applied to a variety of ligand and receptor systems.

Keywords: *Adrenergic, coevolution , ligands, receptor,phylogenetic analysis,homologous.*

Introduction

Protein–protein interactions (PPIs) refer to intentional physical contacts established between two or more proteins as a result of biochemical events and electrostatic forces. In fact, proteins are vital macromolecules, at both cellular and systemic levels, but they rarely act alone. Physically interacting proteins can influence each other's rates of divergence (Pazos *et.al*1997).Although sequence conservation is a major mechanism maintaining protein interactions (Mintseris and Weng 2005), interaction interfaces diverge over time. Coevolution has been proposed to contribute to the rapid adaptive divergence of proteins mediating host–pathogen interactions and of reproductive proteins (Clark *et.al*2006,2009; Sawyer and Malik2006).Functionally related proteins could also evolve at correlated rates if their expression levels covary over time. With the advancement in avalaibility of sequences of various organisms genome,it has allow us to predict interactions among various proteins by using computational methods. By "coevolution" we are referring to the coadaptation of interacting proteins. Proteins and their interacting partners must co-evolve so that any divergent changes in one partner's binding surface are complemented at the interface by their interaction partner (Atwell *et al.,*1997,Jespers *et al*.,1999, Moyle et al 1994 and Pazos *et al*., 1997).We have developed a method to measure quantitatively the correlation between the phylogenetic tree of a ligands with the phylogenetic tree of a receptor family.We have considered that if evolutionary information, in the form of statistical comparisons between the phylogenetic trees of protein families that interact with one another, can be used to recognize these interactions and for this we used adrenergic receptors and its ligands, to develop a standard for measuring the co-evolution of interacting partners.

Adrenergic receptors are GPCR (G Protein coupled receptor) that belong to the large multigenic family of receptors coupled to GTP-binding proteins. Adrenergic receptors constitute, after rhodopsin, one of the best studied models for the other receptors coupled to G proteins that are likely to display similar structural and functional properties. Adrenergic receptors are expressed on virtually every cell type in the body.These receptors are also targets for therapeutically administered agonists and antagonists.Two pharmacologic types have been identified: alpha (alpha-1, alpha-2) and beta-adrenergic (beta-1,beta-2 and beta-3) receptors all of these have subtypes characterized by both structural and functional differences. The alpha-2 and beta receptors are coupled negatively and positively, respectively, to adenylyl cyclase via Gi(inbitory) or Gs(stimulatory) regulatory proteins, and the alpha-1 receptors modulate phospholipase C via

Table 1: Showing types, agonist and antagonist of adrenergic receptors

Receptor	Subtpye	Function	Ligand	G-type
Alpha Adrenergic receptors	Alpha1	Smooth muscle Vasoconstriction in many blood cells.	Norepinephrine, dopamine	Gq
	Alpha2	Inhibition of insulin release in pancreas	Norepinephrine, dopamine	Gi
Beta Adrenergic receptors	Beta1	Increase cardiac output by increase heart rate	PSD-95	Gs
	Beta2	Smooth muscle relaxation Inhibit insulin secretion	PSD-95	Gs
	Beta3	Enhancement of lipolysis in adipose tissue	c-Src	Gs

the Go protein.The beta-1 adrenergic receptor (beta-1 adrenoreceptor), also known as ADRB1 is associated with Gs heterotrimeric and is expressed predominantly in cardiac tissue. The beta-2 adrenergic receptor, also known as ADRB2, is a receptor within a cell membrane which reacts with adrenaline (epinephrine) as a hormone or neurotransmitter affecting muscles or organs.

The beta-3 adrenergic receptor (beta-3 adrenoreceptor), also known as ADRB3 is involved in the regulation of lipolysis and thermogenesis. Ligands of adrenergic receptors with which we are going to analyze coevolution are norepinephrine, dopamine, PSD-95 and c-Src.We have also analyzed evolution of non interacting partner i.e serotonin protein with adrenergic receptos. In the present study adrenergic receptors alpha-1, alpha-2, beta-1, beta-2, beta-3 homologous sequences have been retrieved by using PSI Blast, minimum 7 homologous protein sequences have been analyzed for each receptor sequence and its interacting partner. We have done multiple sequence alignment of each query protein sequence by using CLUSTALW and phylogenetic trees are constructed using PHYLIP of the receptors and their ligands. Correlation coefficient of adrenergic receptors with their interacting and non-interacting partners have been calculated by comparing evolutionary distances of ligand with their interacting receptors. Interacting partners gives positive result and non-interacting partners gives negative result which shows that interacting partners coevolve and this correlation is validated by predicting p-values.

Materials and methods

- Sequence retrieval using NCBI and BLAST:search for agrenergic receptors alpha1,2,3 and beta1,2 receptor sequences from homepage of NCBI (www.ncbi.nim.nih.gov).Retrieve FASTA sequence of each receptor and go for PSI BLAST(http://blast.ncbi.nlm.nih.gov/).Results shows 100 blast homologous proteins sequences with their alignment score of each receptor.Make file of each receptor with 10 sequences in fasta.Similarly make FASTA file of each ligand.
- To perform multiple sequence alignment in ClustalW: upload Fasta file of each receptor and ligand separately on clustalw(www.ebi.ac.uk/Tools/msa/clustalw).Download and save alignment file as.clustalw of each receptor and each ligand separately.
- Phylip: Open prodist option of phylip(http://mobyle.pasteur.fr/).Upload the .clustalw file of each receptor separately. Result appear on prodist phylip in form of matrix.Save the matrix file of each receptor in text format.Similarly make matrix file of each query ligand separately.Now make two common matix ,one of all receptors and one of all ligands.Select Neighbour-Joining method and again click run.Save the outtree file in text format.
- TreeViewX: Open TreeViewX home page.Open the phylip saved file of outtree of receptor and ligand separately.Choose option phylogram or cladogram.
- SPSS: Open matrices file of each receptors and ligands.Compare the matrices by corelation method.

Results

Receptors query sequence in fasta format were retrieved from NCBI(www.ncbi.nim.nih.gov)

Table 2: Accession number of retrieval sequence of adrenergic receptors

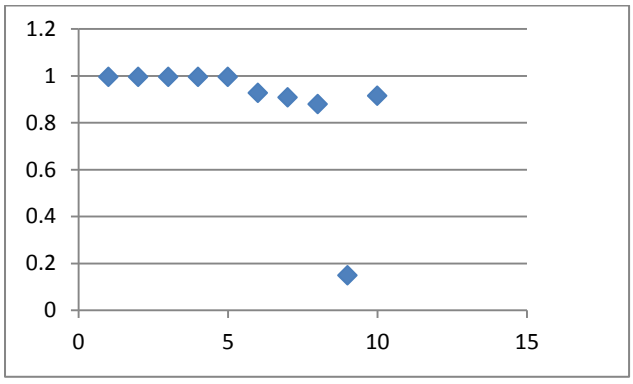
Species	Alpha1	Alpha2	Beta1	Beta2	Beta3
Nipponia nippon	XP 009471050.1	NP 000672.3	XP009470511.1	XP009470511.1	XP009467156.1
Caltype anna	KF099010.1	KF098074.1	XP008489618.1	XP008496996.1	XP008489618.1
Cuculus canoras	KF081512.1	KF081515.1	XP009569768.1	XP009566323.1	XP009569768.1
Colius striatus	KFP33920.1	KFP31818.1	XP01093503.1	XP10193503.1	XP010937503.1
Acanthistia chloris	KFP72118.1	KFP71034.1	XP009069256.1	XP009069256.1	XP009069256.1
Musmuscullus	AAC02658.1	NP 003378.1	AAA02929.1	NP 031446.2	CAA42966.1
Rattus norvegius	NP 058887.2	NP 036871.3	BAA00527.1	NP036624.2	AAA74470.1
Heterocephalus glaber	EHB14258.1	XP004839000.1	EHB09742.1	XP004885465.1	XP004855510.1
Bos tauras	NP 7700201	NP 776924.1	ABG56138.1	NP776656.1	NP 919242.1
Homo sapiens	AAH95512.1	KFQ2258.1	NP 000675.1	AAB82150.1	NP 000016.1

Table 3: Showing results of correlation of norepinephrine and alpha1

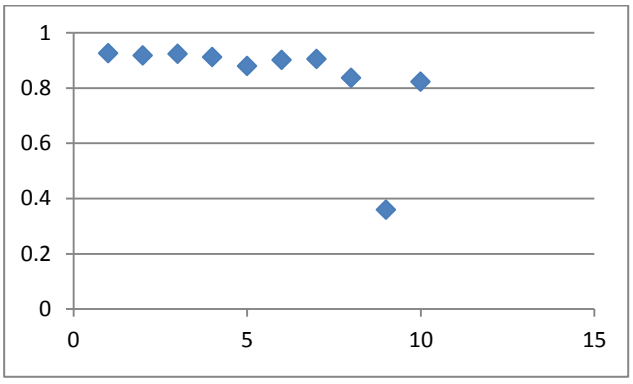
Species	r Value	p Value
Nipponia Nippon	0.994	0.000
Caltype anna	0.994	0.000
Cuculus canoras	0.995	0.000
Colius striatus	0.994	0.000
Acanthistia chloris	0.995	0.000
Musmuscullus	0.926	0.000
Rattus norvegius	0.907	0.000
Heterocephalus glaber	0.879	0.001
Bos tauras	0.148	0.684
Homo sapiens	0.914	0.000

Table 4: Showing results of alpha1 and dopamine

Species	r Value	p Value
Nipponia nippon	0.925	0.000
Caltyoe anna	0.917	0.000
Cuculus canoras	0.923	0.000
Colius striatus	0.912	0.000
Acanthistia chloris	0.879	0.001
Musmuscullus	0.901	0.000
Rattus norvegius	0.905	0.003
Heterocephalus glaber	0.836	0.000
Bos tauras	0.359	0.308
Homo sapiens	0.822	0.004



Graph 1: Showing r value between norepinephrine and alpha

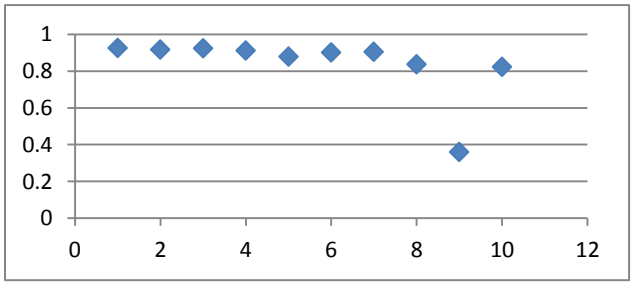


Graph 2: Showing r value between alpha1 and dopamine

Table3 and4 shows r (correlation coefficient) values of evolutionary distances of 10 homologous species (5 aves and 5 mammals) of dopamine with same 10 homologous species of alpha1 adrenergic receptor which are highly positive and out of 10 species, 9 have values of r greater than 0.85 and all 9 have p-values 0.00 i.e the results are significant as p- value less than or equal to 0.05 in two tailed test predict significant r-values. Similarly r-values among evolutionary distances of 10 homologous species of alpha1 adrenergic receptor were calculated with evolutionary distances of 10 homologous species of norepinephrine transporter. Out of 10 species 9 of the species show r-values greater than 0.82 showing high positive correlation in evolution and p-values vary from 0.00 to 0.004 which shows highly significant results. This quantify that adrenergic receptor alpha1 coevolved (highly correlated evolution) with its ligands (norepinephrine and dopamine).

Table 5: Showing results of alpha2 and dopamine

Species	r Value	p Value
Nipponia nippon	0.719	0.019
Caltype anna	0.854	0.021
Cuculus canoras	0.853	0.002
Colius striatus	0.852	0.002
Acanthistia chloris	0.823	0.003
Musmuscullus	-0.337	0.0341
Rattus norvegius	0.136	0.708
Heterocephalus glaber	0.512	0.130
Bos tauras	0.339	0.338
Homo sapiens	-0.239	0.505

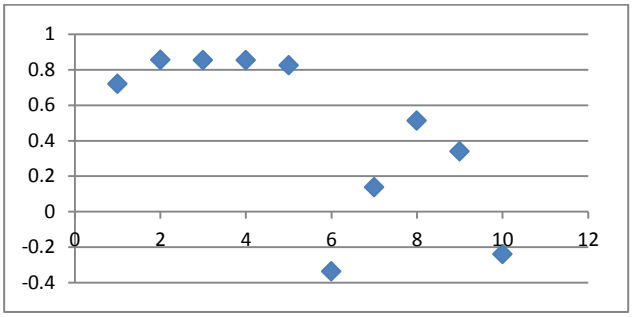


Graph 3: Showing r value between alpha2 and dopamine

Table 6: Showing results of alpha2 and norepinephrine

Species	r Value	p Value
Nipponia nippon	0.731	0.016
Caltype anna	0.837	0.003
Cuculus canoras	.831	0.003
Colius striatus	.843	0.002
Acanthistia chloris	.832	0.003
Musmuscullus	-0.160	0.658
Heterocephalus glaber	.425	0.221
Rattus norvegius	0.727	0.017
Bos tauras	0.406	0.244
Homo sapiens	0.104	0.775

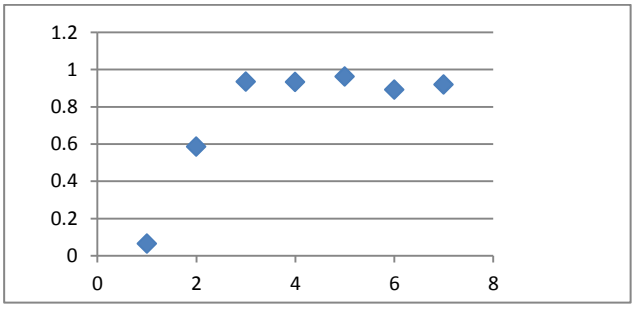
Similarly in table 5 and 6 we analysed r-values of evolutionary distance values of 10 homologous species of adrenergic receptor alpha2 with evolutionary distances of 10 homologous species of its ligands (norepinephrine and dopamine).In table 5 (showing r-values of evolutionary distances of alpha2 adrenergic receptor with dopamine) out of ten homologous species, 5 homologous species (*Nipponia Nippon, Calypte Anna, Cuculus Canoras, Colius Striatus and Acanthisttia Chloris*) all are Aves have r values greater than 0.71 and also have significant p-values showing positive and correlated evolution of dopamine with alpha2 in Aves. As other 5 species (*Musmusculus, Rattus, Homo sapiens, Bos taurus, Heterosaphalus glaber*) which are mammals have r-values 0.5 or less, 2 species (*Mus musculus and Homo sapiens*) also show negative r-values showing negative correlation in mammals. P-values are insignificant which validate that receptor and ligand evolution in mammals is correlated. Similar results were analyzed in table6 for alpha2 adrenergic receptor with norepinephrine ligand. These results show that binding affinity of adrenergic receptor alpha2 with its ligands varies between species (Aves and mammals) (Ying Li *et al.*, 2005).



Graph 4: Showing r value between alpha2 and norpenephrine

Table 7: Showing results of beta1 and PSD-95

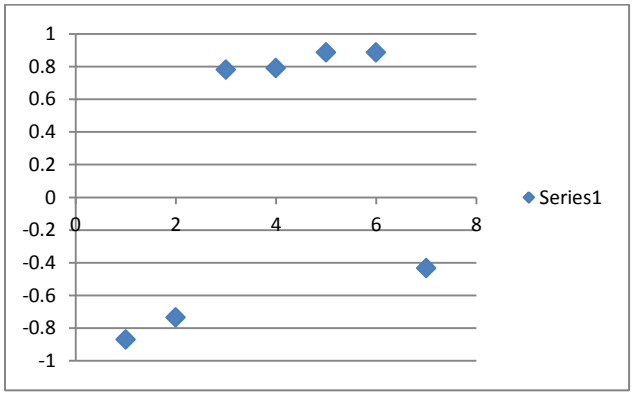
Species	r Value	p Value
Nipponia nippon	0.064	0.892
Caltype anna	0.586	0.167
Musmuscullus	0.934	0.002
Rattus norvegius	0.933	0.002
Heterocephalus glaber	0.961	0.001
Bos tauras	0.892	0.007
Homo sapiens	0.919	0.003



Graph 6: Showing r value between beta1 and PSD-95

Table 8: Showing results of beta2 and PSD-95

Species	r Value	p Value
Nipponia nippon	-0.870	.011
Caltype anna	-0.735	.060
Musmuscullus	0.781	.038
Rattus norvegius	0.791	.034
Heterocephalus glaber	0.886	.008
Bos tauras	0.887	.008
Homo sapiens	-0.433	.332



Graph 7: Showing r value of beta2 and PSD-95

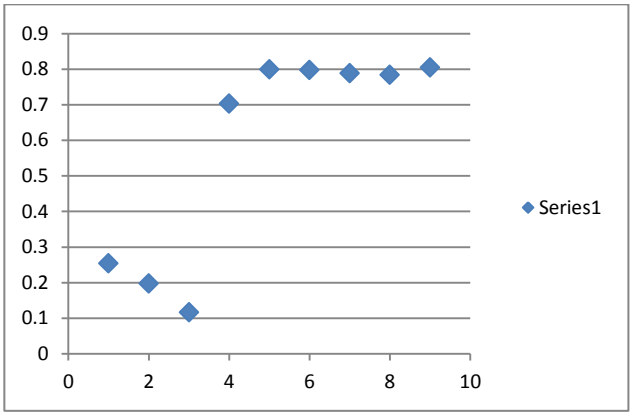
Table 7and table 8 shows r-values among evolutionary distance matrices of beta1 and bata2 adrenergic receptors species with its ligand PSD-95.In table 5 out of 7 species (common homologous species in both receptor and ligand detected by PSI-BLAST) are analysed in beta1 and PSD-95. Out of 7, 2 species which are Aves (*Nipponia nippon and Calypte anna)* shows less positive r values(0.064,0.58) and p-values are also greater than 0.05 which show insignificant correlation results in Aves.But in mammals(*Homo sapiens,Mus musculus, Rattus norvageicus, Bos taurus, Heterocephalus gabeler*) r-values of evolution of PSD-95 with beta1 adrenergic receptor are highly positive(0.89-0.96) with significant p-values i.e less than 0.05. Similar results of beta2 adrenergic receptor with PSD-95 ligand were analyzed in table8 showing highly positive result of r-values in mammals and significant p-values i.e less than 0.05 but in Aves correlation is not significant. Therefore in Aves PSD-95 does not show any correlation in evolution with adrenergic receptor beta2.But in mammals they are highly correlated. This shows that PSD-95 interaction with beta2 adrenergic receptor varies between species.

Table 9: Showing results of beta3 and c-Src

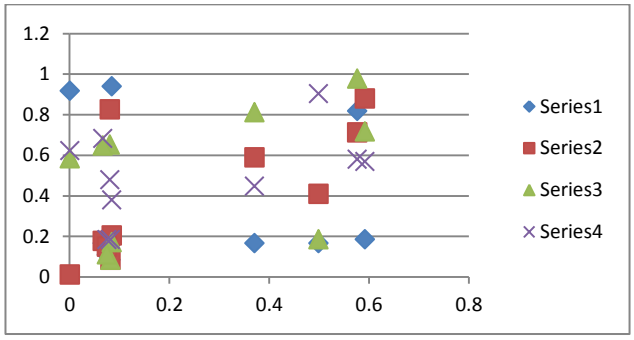
Species	r Value	p Value
Nipponia nippon	0.254	0.510
Caltype anna	0.1898	0.626
Cuculus canoras	0.117	0.765
Acanthistia chloris	0.703	0.035
Musmuscullus	0.799	0.010
Rattus norvegius	0.798	0.010
Heterocephalus glaber	0.789	0.011
Bos tauras	0.784	0.012
Homo sapiens	0.805	0.009

Table 10: Showing results of serotonin of alpha1, alpha2, beta1, beta2, beta3

Species	Alpha1		Alpha2		Beta1		Beta2		Beta3	
	R	p	r	p	r	p	r	P	R	P
Nipponia nippon	0.577	.081	0.1534	0.155	0.080	0.827	0.163	0.653	0.272	0.479
Caltype anna	0.600	.067	0.176	0.167	0.463	0.178	0.166	0.648	0.160	0.682
Cuculus canoras	0.575	.082	0.446	0.085	0.572	0.084	0.572	0.084	0.487	0.184
Colius striatus	0.571	.085	-0.42	0.940	0.440	0.204	0.470	0.171	0.334	0.380
Acanthistia chloris	0.618	.075	0.503	0.110	0.491	0.150	0.535	0.111	-0.486	0.184
Musmuscullus	0.318	0.371	0.119	0.167	0.195	0.590	0.087	0.812	0.291	0.447
Rattus norvegius	0.243	0.499	0.088	0.167	0.295	0.409	0.455	0.186	-0.047	0.904
Heterocephalus glaber	-0.193	0.592	0.455	0.185	0.055	0.880	-0.131	0.718	0.220	0.569
Bos tauras	-0.201	0.577	-0.131	0.819	0.134	0.712	-0.010	0.977	0.214	0.580
Homo sapiens	-0.882	0.001	-0.010	0.918	0.097	0.012	0.197	0.585	0.191	0.623



Graph 8: Showing r value beta3 and c-Src



Graph 9: Showing r value between serotonin and alpha1, alpha2, beta1, beta2, beta3.

Table 9 shows correlation values of evolutionary distances of beta3 adrenergic receptor (9 species) with its ligand evolutionary distances i.e c-Src (9 homologous species). The r-values show significant result in mammals with highly positive r-values (0.70 -0.80) and p-value(0.00-0.03)showing significant correlation in mammals as compare to Aves. As in Aves r-values are less positive and p-values greater than 0.05 showing insignificant correlation results of beta3 adrenergic receptor with c-Src ligand in Aves.

Similarly r-values of all adrenergic receptors were calculated with serotonin transporter which is also a neurotransmitter, most of the values are less than 0.5 as shown in table8 and all the p-values are greater than 0.05 which shows insignificant results of r values. So these values indicate that serotonin is a non-interacting partner and its evolution is not correlated with evolution of adrenergic receptors. This also show that proteins that are known to interact physically are more strongly coevolving than proteins that simply belong to the same biochemical pathway.

Phylogenetic trees

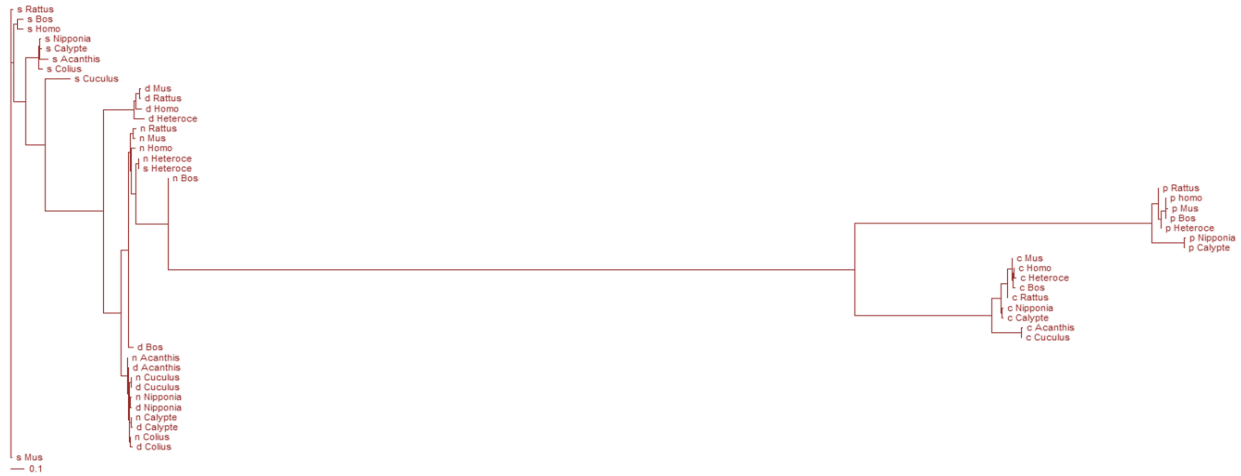


Figure 1: Phylogenetic tree of ligands (N for norepinephrine, d is for Dopamine, s for Serotonin, c for c-Src, p forPSD-95

Figure1 and 2 showing phylogenetic tree of adrenergic receptors(alpa1,alpha2,beta1,beta2,beta3) and its ligands visualised in TREE VIEWX tool from tree file which is predicted in PHYLIP software. Both the trees were compared and we analysed that norepinephrine and dopamine are closely related in evolution and belong to same group. As norepinephrine binds strongly to alpha adrenergic receptors, so dopamine which is closest neighbouring partner of norepinephrine also binds to same receptors (alpha1 and alpha2) (Chern-Sing Goh *et al.,*2000). PSD-95 which is the ligand of both beta1 and beta2 adrenergic receptors lies separately from norepinephrine and dopamine in different group but beta1 and beta2 adrenergic receptors are closely related in receptor phylogenetic tree which proves that closest neighbours have more chances of occurrence of binding to same ligand or receptor.

c-Src which binds to beta3 adrenergic receptor belongs to different group from PSD-95 as beta3 in receptor tree belongs to different group from beta1 and bet2.Serotonin which is non -interacting partner of adrenergic receptors does not coevolve with adrenergic receptors as it is far distant from interacting ligands.

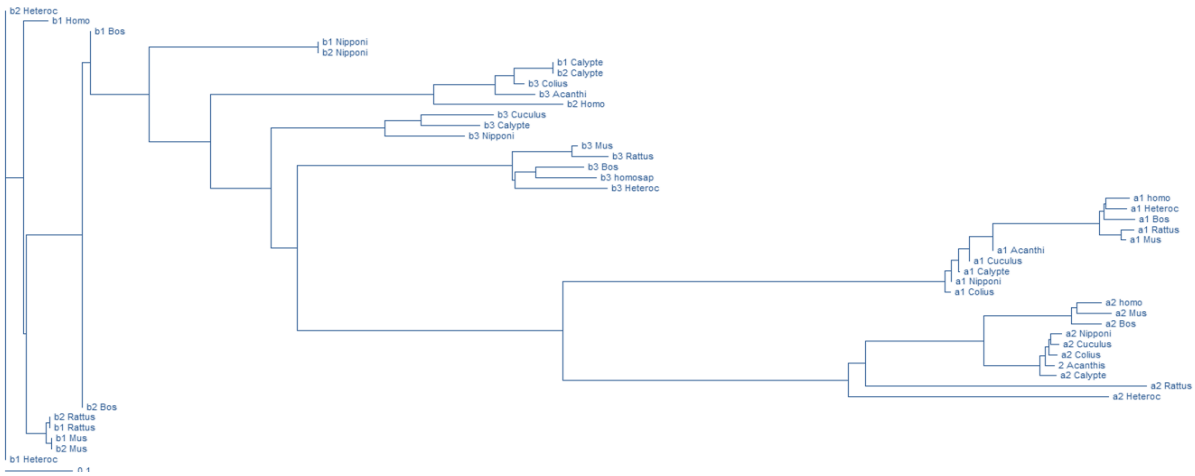


Figure 2: Phylogenetic tree for receptor (N for norepinephrine, d is for Dopamine, s for Serotonin, c for c-Src, p forPSD-95)

Discussion

The prediction of protein interaction partners with bioinformatics methods has become a topic of increasing interest in recent years. Different methods have emerged based on the study of conservation of gene order (Dandekar *et al*., 1998), the presence/absence of pairs of proteins in full genomes (Gaasterland and Ragan, 1998; Pellegrini *et al*.,1999) or the presence of proteins assembled in multi-domain proteins in other genomes (Enright *et al*., 1999; Marcotte *et al*., 1999).Co-evolution has an important function in the evolution of species. Our method was applied to ligands and receptors in the search for binding partners. Detecting molecular coevolution can expose functional interactions between molecules in the cell, generating insights into biological processes, pathways, and the networks of interactions important for cellular function. Prediction of interaction partners from different protein families exploits the property that interacting proteins can follow similar patterns and relative rates of evolution.

In the current study we used methods for detecting coevolution based on the similarity of phylogenetic trees or evolutionary distance matrices among adrenergic receptors and their ligands. Adrenergic receptors are responsible for the fight-or-flight response, which includes widening the pupils of the eye, mobilizing energy, and diverting blood flow from non-essential organs to skeletal muscle. Firstly to detect coevolution of adrenergic receptors with their interacting partners we analyzed and select minimum 7 homologous species by PSI-BLAST for each adrenergic receptor i.e alpha1, alpha2, beta1, beta2, beta3 and their corresponding ligands by. Then we make evolutionary distance matrices of adrenergic receptors, their interacting and non interacting partners separately by using Phylip. Then correlation(r) and p-value of each species of each receptor with its corresponding ligand species was calculated i.e homo sapiens species of alpha1 adrenergic receptor with homosapiens species of dopamine. R-values are also shown in graph in the form of scatterplot.Interacting proteins show positive correlation as they have maximum positive values of r. Similarly r-values of all adrenergic receptors were calculated with serotonin transporter which is also a neurotransmitter, most of the values are less than 0.5 and all the p-values are greater than 0.05 which shows insignificant results of r values. So these values indicate that serotonin is a non-interacting partner and its evolution is not correlated with evolution of adrenergic receptors. This also show that proteins that are known to interact physically are more strongly coevolving than proteins that simply belong to the same biochemical pathway.

This conclude that most strongly coevolving proteins suggest interactions that have been maintained over long periods of evolutionary time, and that are thus likely to be of fundamental importance to cellular function (Elisabeth R.M. Tillier et al.2009).An extreme of co-evolution i.e less correlation values or insignificant results of two interacting proteins would be those cases in which both proteins are simultaneously lost in the same species, probably because one of them cannot perform its function without the other **(**Florencio Pazos and Alfonso Valencia, 2001). The results indicate that it is indeed possible to distinguish statistically a few true interactions among many possible alternatives, opening up the possibility of searching for interaction partners in large collections of proteins and complete genomes.

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