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The Phylogenetic Analysis of Animal and Plant D-Type Cyclins

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Abstract: The phylogenetic relationships and divergence among different subgroups of plant and animal D-type cyclin sequences are studied. The orthologous plant and animal D-type cyclin translated sequences are extracted from NCBI GenBank database categorically identified as D, D1, D2, D3 and D5 types. Multiple sequence alignment was carried out using Clustal W tool of EBI and phylogram was constructed. Further, distinct nodes of phylogram were identified and their pattern was studied using cyclin signature from Prosite database. The results were discussed in terms of the relationships among different subgroups of D-type cyclins forming distinct nodes of phylogram and the conservation of their patterns at each node.

Key words: Clustal W, cyclin signature, D-type cyclins, multiple alignment, pattern, phylogram

INTRODUCTION

Self-reproduction of every cell is the most fundamental characteristic feature of all living organisms and it plays a crucial role during development. Cells must sense whether the division to continue or cease to allow differentiation into specialized organs (Francis, 1998). The progression of cell cycle in eukaryotes is under the control of a family of proteins called cyclins. Different classes of cyclins viz., A, B, D and E are present in many different groups of eukaryotes including plants, nematodes, marine organisms and vertebrates.

The expression pattern of cyclins is different during different stages of cell cycle and even in different organs of the same organism. A1-type and A2-type cyclins are expressed at the stage of S to M transition (Shaul *et al*., 1996). B-type cyclins are regulators of G2/M transition (Renaudin *et al*., 1998). E-type cyclins act as regulators for G1/S-checkpoint in the control of cell division. D-type cyclins are essential for the progression of cells through the G1 phase of the cell cycle. The G1 phase is central to the integration of signals that regulate both the exit from the cell cycle to differentiation and the reactivation of cell proliferation (Gutierrez *et al*., 2002). Originally, they were identified as proto-oncogenes activated by translocation to a thyroid promoter in parathyroid adenomas (Motokura *et al*., 1991). Increase in the levels of D-type cyclins was observed in human testicular cancer and thyroid cancer (Bartkova *et al*., 1999; Wang *et al*., 1998). The growth promoting effects of the D-type cyclins may manifest via their interactions with tumor suppressor genes (Peters, 1994). D-type cyclins have multiple functions - they preferentially associate with two closely related members of the cyclin-dependent kinase family, Cdk4 and Cdk6 and these complexes are capable of phosphorylating retinoblastoma gene product (pRb) leading to its inactivation. Juan *et al*. (1996) from the study on human tumor cell lines stated that the expression of cyclin D1 or D3 in normal cells is

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discontinuous, occurring transiently at G1 phase but these proteins are expressed persistently throughout the cell cycle in some tumor lines. In tomato three distinct CycD3s are involved playing different roles in the fruit development (Kvarnheden *et al*., 2000).

The present study is focused on D-type cyclins because they play a highly significant role both in the progression of cell cycle and in the expression of tumor cell lines. Genbank translated D-type cyclin sequences were collected from NCBI. Presence of different subgroups of D-type cyclins in various organisms provides insight for studying family relationships. Therefore, to perform the task, multiple sequence alignment was carried out on plant and animal D-type cyclins, as sequence analysis forms crucial step in molecular evolutionary studies (Martin *et al*., 2007). Further, pattern based studies are initiated to facilitate an understanding of residue variations that reflect evolutionary aspects of D-type cyclins.

MATERIALS AND METHODS

D-Type Cyclins

A preliminary survey was carried out in our laboratory on D-type cyclin genes deposited in GenBank database of NCBI release 163.0 as of December 2007 (www.ncbi.nlm.nih.gov/) (Benson *et al*., 2007). Forty three D-type cyclin translated sequences of plant and animal origins were extracted. The sequences were grouped into D, D1, D2, D3 and D5 types of cyclins. The number of sequences in each type of cyclin was as follows: 15 sequences of cyclin D, nine cyclin D1, eight cyclin D2, ten cyclin D3 and one cyclin D5, respectively.

Molecular Phylogeny

Multiple sequence alignment was carried out using default Gonnet matrix of ClustalW (Thompson *et al*., 1994) tool maintained at EBI (www.ebi.ac.uk). Phylogram was constructed using Neighbor-Joining method as implemented in ClustalW. A phylogram with five distinct nodes were observed. The number of sequences at each node was as follows: seven sequences at node-1, six at node-2, four at node-3, six at node-4 and seven at node-5, respectively. Out of 43 D-type cyclins, thirteen sequences were left without forming any node. These sequences showed maximum divergence and their similarity scores ranged from 8-49%.

Pattern Study

Further the common structural domain among the cyclins of individual nodes was studied using cyclin signature (PS00292) from prosite database (www.expasy.org/prosite). The signature sequence was found to be a domain of 32 amino acid residues as given below:
R - x(2) - [LIVMSA] - x(2) - [FYWS] - [LIVM] - x(8) - [LIVMFC] - x(4) - [LIVMFYA] - x(2) – [STAGC] - [LIVMFYQ] - x - [LIVMFYC] - [LIVMFY] - D - [RKH] - [LIVMFYW]

RESULTS

The phylogram can be conveniently divided into five major nodes, distinct nodes were identified for animal and plant sequences. Further, the subgroups among animal cyclin sequences formed individual nodes i.e D1, D2 and D3 sequences are represented as 1st, 2nd and 3rd nodes respectively of the phylogram. The plant cyclin sequences formed 4th and 5th nodes. The remaining thirteen left over sequences are representatives of both animal and plant D-type sequences (Fig. 1).

Multiple sequence alignment of each node revealed number of sites that are conserved and a few positional sites that are semi-conserved. This aspect was taken into consideration to study the variation of residues with respect to the sequences that diverged from each node.

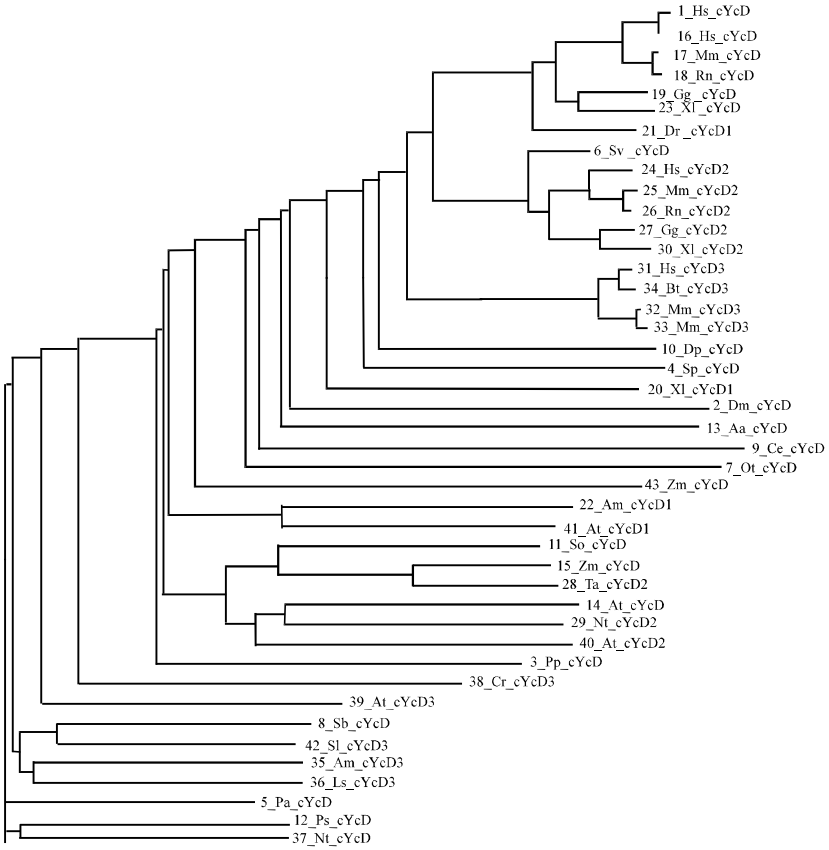

Fig. 1: Phylogenetic tree showing relationships among 43 D-type cyclin sequences. Hs_cycD: *Homo sapiens* (AAA52136); Hs_cycD1: *Homo sapiens* (M64349.1); Mm_cycD1: *Mus musculus* (NP_031657); Rn_cycD1: *Rattus norvegicus* (NP_741989); Gg_cycD1: *Gallus gallus* (NM205381) Xl_cycD1: *Xenopus laevis* (CAA61664); Dr_cycD1: *Danio rerio* (NM_131025); Sv_cycD: *Stizostedion vitreum* (AAC68476); Hs_cycD2: *Homo sapiens* (NP_001750); Mm_cycD2: *Mus musculus* (AAH49086); Rn_cycD2: *Rattus norvegicus* (NP_071603); Gg_cycD2: *Gallus gallus* (NM_204213); Xl_cycD2: *Xenopus laevis* (CAA61665); Hs_cycD3: *Homo sapiens* (AAH11616); Bt_cycD3: *Bos taurus* (AAI05237); Mm_cycD3: *Mus musculus* (AAH05605); Rn_cycD3: *Rattus norvegicus* (NP_036898); Dp_cycD: *Dreissena polymorpha* (AAM44813); Sp_cycD: *Strongylocentrotus purpuratus* (NP_999664); Xt_cycD1: *Xenopus tropicalis* (NM_001016108); Dm_cycD: *Drosophila melanogaster* (AAG13285); Aa_cycD: *Aedes aegypti* (EAT36255); Ce_cycD: Caenorhabditis elegans (AAC35273); Ot_cycD: *Ostreococcus tauri* (AAV68601); Zm_cyc5: *Zea mays* (AAX54698); Am_cycD1: *Antirrhinum majus* (CAB61221); At_cycD1: *Arabidopsis thaliana* (CAA58285); So_cycD: *Saccharum officinarum* (AAV28532); Zm_cycD: *Zea mays* (AAL83926); Ta_cycD: *Triticum aestivum* (AAQ08041); At_cycD: *Arabidopsis thaliana* (CAB41347); Nt_cycD2: *Nicotiana tabacum* (CAA09852); At_cycD2: *Arabidopsis thaliana* (CAA58286); Pp_cycD *Physcomitrella patens* (CAD21955); Cr_cycD3: *Chenopodium rubrum* (CAA09769); At_cycD3: *Arabidopsis thaliana* (CAA58287); Sb_cycD: *Scutellaria baicalensis* (BAE06272); Ls_cycD: *Lagenaria siceraria* (AAM77273); Am_cycD3: *Antirrhinum majus* (CAB61222); Pa_cycD: *Populus alba* (AAO72990) Ps_cycD: *Pisum sativum* (BAA33153); Nt_cycD3: *Nicotiana tabacum* (CAA09853)

As shown in Fig. 1, *Homo sapiens* cyclin D at node 1 was reported to have around 76-99% similarity with animal D1 cyclins. Cyclin D1 from *Drosophila melanogaster* has weak similarity with other cyclin D1 sequences. Therefore multiple sequence alignment was carefully studied to enumerate the residue variation. Such variations existed in one or more proteins at various positions but the interesting feature such as a complete residue to residue identity was observed for cyclin D pattern signature (Fig. 2).

Node 2 represented only cyclin D2 sequences with one cyclin D sequence *Stizostedion vitreum* being diverged from the remaining cyclin D2 sequences. Cyclin D of *Stizostedion vitreum* diverged from the group and it has 82-85% similarity with other sequences of the node. Patterns represent the regions of structural and functional similarity. Hence, the pattern region studied for this group showed a complete similarity except *Stizostedion vitreum* cyclin D which is devoid of 6 amino acid residues from the beginning (Fig. 3). Hence, *Stizostedion vitreum* cyclin D sequence showed divergence from cyclin D2.

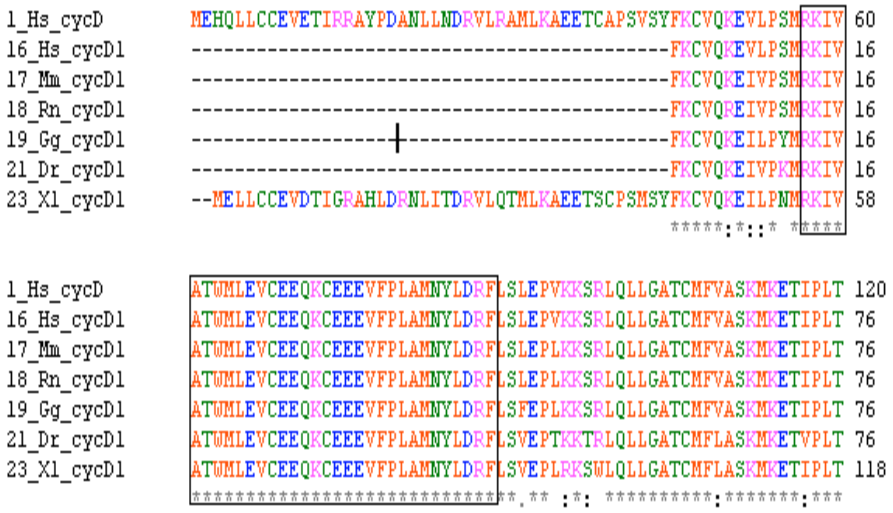

Fig. 2: Multiple alignments of D-type cyclins at node 1 showing cyclin signature pattern

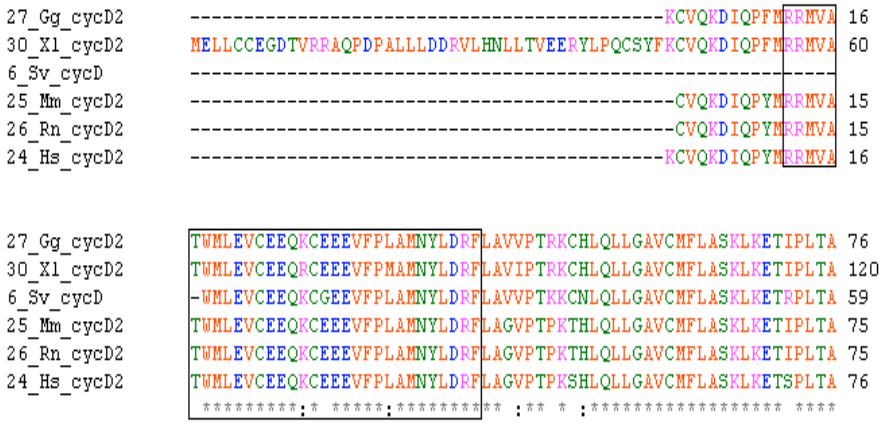

Fig. 3: Multiple alignments of D-type cyclins at node 2 showing cyclin signature pattern

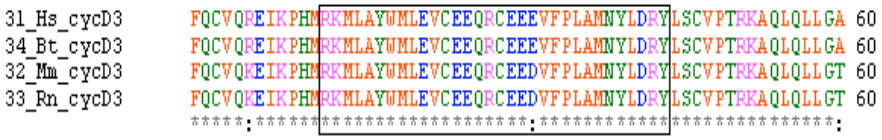

Fig. 4: Multiple alignments of D-type cyclins at node 3 showing cyclin signature pattern

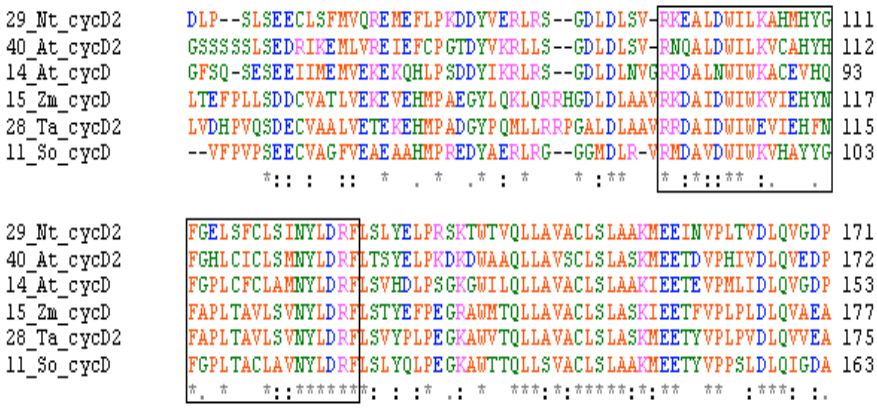

Fig. 5: Multiple alignments of D-type cyclins at node 4 showing cyclin signature pattern

Fig. 6: Multiple alignments of D-type cyclins at node 5 showing cyclin signature pattern

Similarly, analysis at node 3 with four cyclin D3 sequences (*Homo sapiens* cycD3, *Bos taurus* cycD3, *Mus musculus* cycD3, *Rattus norvegicus* cycD3) showed 92-97% similarity with *Homo sapiens* cyclin D3 and pattern residues are entirely similar (Fig. 4).

Node 4 represented six plant cyclin sequences of cyclin D and cyclin D2 type. *Saccharum officinarum* cyclin D is derived from *Zea mays* cyclin D and *Triticum aestivum* cyclin D2 sequences. The similarity score ranged from 37- 75%. Various semiconserved residue substitutions were observed within the pattern region for this group but the pattern completely satisfied cyclin signature (Fig. 5).

Node 5 contains seven plant cyclin sequences of cyclin D and D3 type. The similarity scores among these sequences were in the range of 49-56%. Variations were observed in *Populus alba* cyclin D with 50% similarity with other sequences of the group. In this sequence there are two amino acid residues which do not match with the pattern. They are F (104) which is out of [LIVM] and G (118) which is out of [LIVMFYA]. The remaining cyclins such as *Scutellaria baicalensis* cyclin D, *Solanum lycopersicum* cyclin D3, *Antirrhinum majus* cyclin D3, *Lagenaria siceraria* cyclin D3 as well as *Pisum sativum* cyclin D and *Nicotiana tabacum* cyclin D3 formed a group (Fig. 6).

Remaining different D-type cyclin sequences such as cyclin D of *Drosophila melanogaster*, *Strongylocentrotus purpuratus*, *Ostreococcus tauri*, *Caenorhabditis elegans*, *Dreissena polymorpha*, *Aedes aegypti* and cyclin D1 of *Xenopus tropicalis*, *Antirrhinum majus*, cyclin D1 and D3 of *Arabidopsis thaliana*, cyclin D5 of *Zea mays*, cyclin D of *Physcomitrella patens* and cyclin D3 of *Chenopodium rubrum* appeared between third and fifth nodes respectively. They exhibited a weak to moderate similarity (8-49%).

DISCUSSION

Though, each of the D-type cyclins was sufficient to drive oncogenic proliferation, the functions of individual subgroups are specific. Differential expression and abundance of cyclin subgroups was observed as studied from the immunohistochemical and immunochemical analysis of 32 human testicular tumors in relation to cell type, proliferation, differentiation and malignancy (Bartkova *et al*., 1999). The promoters of these individual D-type cyclins are specific, influencing their oncogenic cell proliferation. Concurrent results were observed from the studies of mouse fibroblasts, i.e., Ras oncogene signaled through cyclin D1, while Myc influenced the cell cycle machinery by transcriptionally upregulating cyclin D2 and the cells lacking all three D-cyclins show greatly reduced susceptibility to the oncogenic action of Ras and Myc (Yu *et al*., 2005). Global expression correlation analysis further supports distinct expression patterns for CycD subgroups (Menges, 2007).

Further, differences were identified in pRb-binding motif of cyclin D LxCxE, suggesting the functional differences between different subgroups of D-type cyclins (Baker *et al*., 2005). Plant D-type cyclins have higher homology to animal D-type cyclins than any other class of cyclins and the residues of identical positions is about 20-25% (Renaudin *et al*., 1996). The present study of phylogenetic relationship among different subgroups of D-type cyclins revealed near and far evolutionary relationship among plants and animals. The occurrence of the subgroups of cyclins as distinct nodes in the phylogram indicates the variations in the amino acid sequence within these subgroups. Several interesting features were observed in the analysis. In two instances animal cyclin D sequences showed similarity with cyclin D1 and D2 (1st and 2nd nodes) respectively. Further, the animal and plant sequences showed a clear divergence but in few cases, different cyclin D1, D2, D3 and D5 sequences showed little divergence from the clusters thereby forming specific subgroups of D-type cyclins.

To confirm such relationships a comparison was carried out between conserved residues pattern of D-type cyclin sequences present at each node. Individual nodes have shown a clear match with cyclin signature (PS00292). As the nodes (1st, 2nd and 3rd) represented individual subgroups of animal D-type cyclin sequences, residue to residue identity was observed among the subgroups. Although variations were observed across different nodes of the 43 D-type cyclin sequences, the residue conservation in the pattern region of various subgroups are important for specific functions of individual subgroups.

CONCLUSION

In this study, evolutionary relationships among various cyclins of plant and animal origin enunciated the importance of identifying various unique features such as the divergence of plant and animal D-type cyclins, as well as few clusters that formed specific D-type sub-groups. Overall, for all the multiple alignments carried out, few amino acid residue variations are observed at certain positions, however, in most of the cases, complete residue identity was observed for pattern signature, respectively. A few exceptions are reported by cyclin D and D3 type plant sequences where two amino acid residues did not match the pattern and hence represented divergence from the cluster. Similar observation can also be made for D-type cyclins at node 5. Finally present analysis suggest that the presence of clear match/identity within pattern region of a protein, cyclin in this case, suggest that phylogenetic trees reveal a near and far evolutionary relationships based on the residues that are more conserved or in other words, the rate of mutations for a particular protein influences the rate of divergence. Hence it should be noted that the pattern region represents residue conservation and such features should also be considered for phylogenetic tree construction.

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