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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/14689
DOI URL: http://dx.doi.org/10.21474/IJAR01/14689

RESEARCH ARTICLE

IDENTIFICATION OF *WEE* GENES IN *DICTYOSTELIUM DISCOIDEUM*

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Manuscript Info

Manuscript History
Received: 05 March 2022
Final Accepted: 08 April 2022
Published: May 2022

Key words:-
Cell Cycle, Wee1, Kinase, Dictyostelium Discoideum

Abstract

Cell cycle is a basic cellular mechanism that allows for cellular growth and development, regeneration or repair, and wear of tissues or even regeneration of the whole organism in certain cases. A failure during the cell cycle process leads to various dire consequences. Various proteins and kinases play a vital role in the regulation of the cell cycle to meet cellular demands and needs. Amongst the kinases, Wee1 is a key regulator of cell cycle progression which inhibits mitotic entry by phosphorylating the tyrosine residue on the M-phase-inducing kinase, Cdk1, at the G2/M transition. In *Dictyostelium discoideum*, *wee1* genes that regulate the cell cycle progression at the G2/M transition have not been identified and studied yet. Our findings support the existence of the Wee1 homolog in *D. discoideum*, which could play a role in cell division at G2/M.

Introduction:-

Wee1 was first identified in the fission yeast, *Saccharomyces pombe*, in 1978 (Thuriaux et al., 1978). It was part of mutant screen that affects the cell cycle in fission yeast. It was observed that mutants defined by the gene *wee1* would divide half the cell length of wildtype. It was also observed that while the loss of function of *wee1* would lead to small cell size cells, its overexpression would lead to elongated cells suggesting, a decreased G2 phase and an increased G2 phase, respectively (Csikász-Nagy et al., 2006). Later, it was shown that Wee1 acts as a negative regulator of the maturation-promoting factor (MPF) complex, which includes Cdk1 and mitotic cyclin (Russell and Nurse 1987). Cdk1 is encoded by the gene *cdc2* and Wee1 negatively regulates Cdk1 by phosphorylating its tyrosine residue. In the absence of Wee1 protein, the MPF shows high activity, leading to faster cell division. An excess of Wee1 leads to blocking the activity of MPF, leading to a longer time of mitosis (Aguda 1999). Thus, Wee1 has been identified as a key regulator of the cell cycle.

In later work, genes similar to *wee1* have been identified in several organisms, including humans (Igarashi et al., 1991), *Trypanosoma brucei* (Boynak et al., 2013), *Schizosaccharomyces cerevisiae* (Booher et. al., 1993), *Arabidopsis thaliana* (Sorrell et al., 2002), *Drosophila* (Stumpff et al., 2004), *Xenopus* (Mueller et. al., 1995). Interestingly, it has been observed that the humans Wee1 can efficiently rescue the *wee1* mutation in *S. pombe*, highlighting its functional conservation.

The current study aims to identify *wee1* genes in the cellular slime mould *D. discoideum*. The *D. discoideum* is an interesting model system because it exists as a unicellular organism in the form of amoeba as well as a multicellular form with only two types of cells, spore and stalk (Wang et al., 2021). Multicellularity in *D. discoideum* is achieved

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by aggregation of the amoebic cells under conditions of starvation and does not involve any cell division to achieve this.

In *D. discoideum*, it has been shown that the stage of the cell cycle may influence the fate of the cells into a prespore or prestalk pathway (Weijer and Zimmerman 1993). This offers a unique opportunity to study the role of the Wee1 protein in determining cell fate. As a first step, the *wee1* gene of *Dictyostelium* needs to be identified, which is presented in this work.

Methods:-

A blast search was performed using the BLAST server at the dictybase site (http://dictybase.org) using the known sequence of *S. pombe* Wee1, which was collected using BLASTp at NCBI. The similarity of the proteins encoded by the identified *D. discoideum wee1* genes to those encoded by other organisms was checked using CLUSTAL OMEGA. The sequences of *D.discoideum* Wee1 proteins were analysed for the presence of a kinase domain by using the online software SMART with the Pfam database (http://smart.embl-heidelberg.de/). Protein structures were predicted using I-TASSER (https://zhanggroup.org/I-TASSER/) and viewed using PyMOL software. A phylogenetic tree was created using the Neighbor-Joining method.

Results:-

Wee1 homolog in *D. discoideum* was identified by carrying out a blast search using the *S. pombe* Wee1 protein sequence (Accession No. NP_587933.1) as a query sequence. The BLAST program was used for this search. This resulted in identifying a few similar proteins in *Dictyostelium* of which three of the genes, i.e., DDB_G0277539, DDB_G0291133, and DDB_G0291842 showed similarity with the *S. pombe* Wee1 protein sequence. These three genes have been designated as *weeA*, *weeB*, and *weeC* respectively, in the current study. It was also observed that these genes were also identified when querying the *Dictyostelium* genome database (http://dictybase.org).

The similarity between the protein encoded by the above three genes to the known Wee1 protein from *S. pombe, S. cerevisiae, T. brucei*, and humans was analysed. The percentage similarity between these proteins is summarised in Table No. 1, which ranges from 21 to 31 %. Further, the three proteins share 28 to 32 % similarity amongst them.

Although the overall similarity between *D. discoideum* Wee proteins with other organisms was low, the analysis of the catalytic domains of the entire three genes showed the presence of a distinctive signature of the Wee protein. The catalytic domain of WeeA spans from 508 to 846 amino acids, WeeB from 177 to 462 amino acids, and WeeC from 77 to 331 amino acids. The protein sequence alignment has been presented in Fig 1, and the signature amino acids and sub- sequences are highlighted.

The kinase catalytic domains have eleven subdomains, which have been reported earlier with a high degree of conservation (Hanks and Diego 1994; Hanks et al., 1987). The Glycine-rich loop, GxGxxG, and Lys residues that are involved in ATP binding should be present in the catalytic domain. The calatytic segment contains the consensus sequence IVHxDLKPxNIx and the invariant residues Asp and Asn, which are involved in the phosphotransfer process, are found in this motif. The activation loop containing the EGD motif (Glu-Gly-Asp) is found only in the subdmain VIII of the Wee1 protein kinase family. In subdomain IV, a Trp and in subdomain X, a Trp and Arg are the conserved residues, which are another diagnostic trait present in all the members of the Wee1 kinase family (Mueller, Coleman, and Dunphy 1995). *Dictyostelium* WeeA, WeeB, and WeeC also contain all the eleven subdomains and the required residues which are shared by the members of the Wee1 kinase family.

A comparative analysis between the proteins also showed that among the three WeeA proteins from *D. discoideum*, the catalytic domain for WeeA is split into two parts (Fig 1). This was also confirmed by analysing the domain architecture using online software SMART with the Pfam database (Fig 2 A). It was observed that the proteins encoded by WeeB and WeeC have a catalytic domain spanning 286 and 255 amino acids, respectively. In the case of WeeA, the catalytic domain was split into two parts, which were 154 amino acids (508 to 661) and 134 amino acids (713 to 846). This is also reflected by creating a structure using I-TASSER and viewing it using PyMOL (Fig 2 B).

In this study, we also analysed the phylogenetic position of the three Wee proteins of *Dictyostelium* using Neighbor-Joining method (Fig 3). This study also showed that the WeeA was diverged from the WeeB and WeeC and the WeeA showed some similarity to humans while WeeB and WeeC are closer to yeast proteins.

In summary, we have identified that *D. discoideum* carries three copies of the *wee* gene, which have variations among them. Given that multicellular *Dictyostelium* has only two cell-types, it would be interesting to study the role of three *wee* genes in its growth and development.

Table 1:- Percentage identity.

D. discoideum	Humans	*S. pombe*	*S. cerevisiae*	*T. brucei*
WeeA	31 %	23 %	23 %	21 %
WeeB	24 %	30 %	27 %	23 %
WeeC	26 %	31 %	26 %	22 %

Fig 1:- Multiple sequence alignment of the catalytic domains of the putative *D. discoideum* Wee1 proteins, DDB_G0277539, DDB_G0291133, and DDB_G029142 with other Wee1 protein kinases from other organism using

CLUSTAL OMEGA is shown. Asterisks sign below the sequences indicates the conserved amino acids. Two vertical dots indicate conserved substitutions, while a single dot indicates semi-conserved substitutions. Gaps are introduced for optimal alignment and are represented by dashes. Roman numerals are used to indicate the 11 preserved subdomains (Hanks and Diego 1994; Hanks et. al., 1987). A yellow box denotes the catalytic and a grey box denotes the activation segments. The conserved EGD motif is marked with a black box. Amino acids that are conserved in all known members of the Wee1 kinase family but not in other eukaryotic protein kinases are indicated by triangles. *D. discoideum* WeeA contains an additional stretch of 51 amino acids, which is marked with a red box. Sequences shown here are for Wee1 kinase protein of *T. brucei* (TbWee1, JN083854), humans (HsWee1, NP_003381.1), *S. pombe* (SpWee1, NP_587933.1), *S. cerevisiae* (ScSwe1, NP_012348.1).

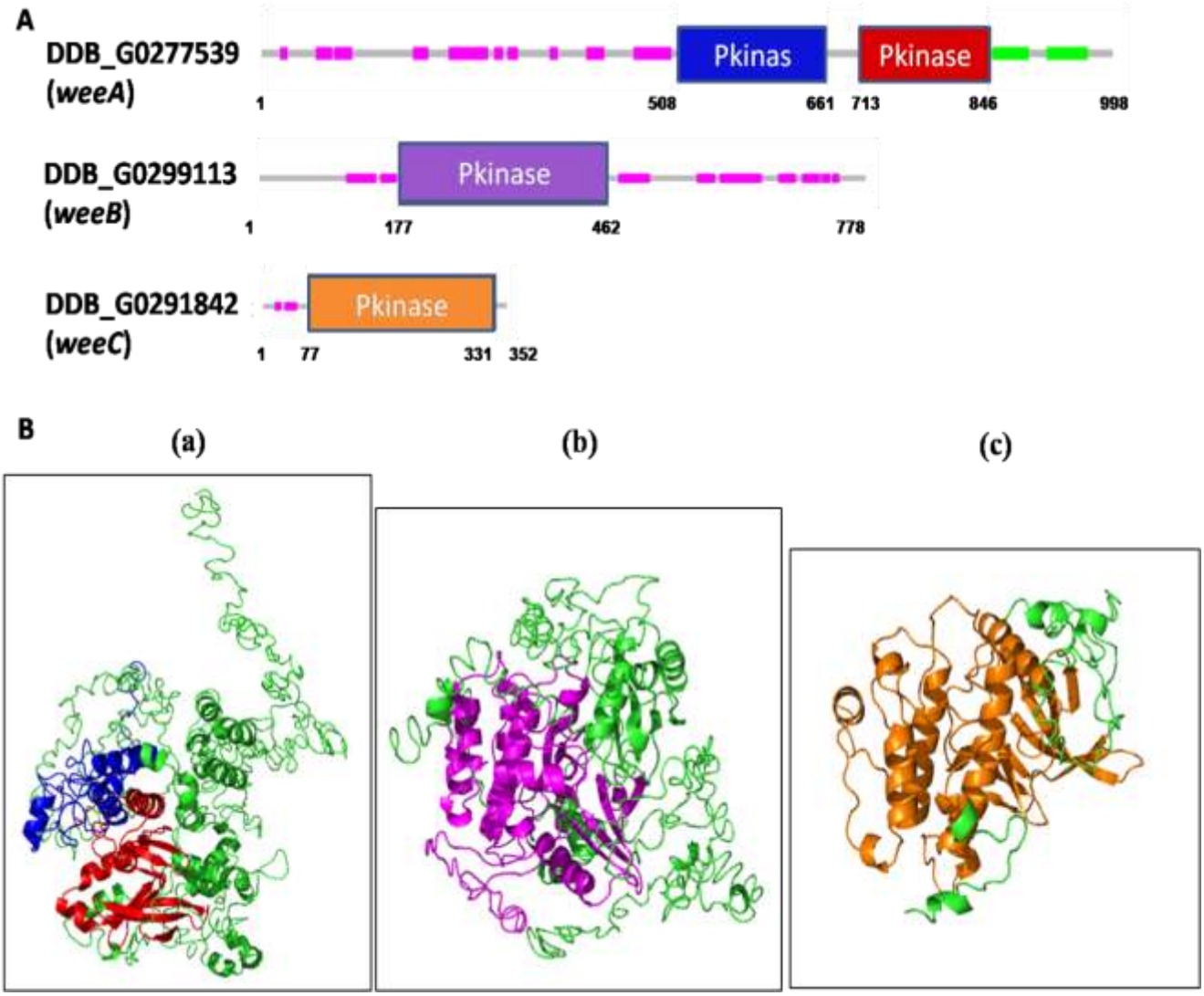

Fig 2:- A. Domain architecture analyses of DDB_G0277539, DDB_G0291133 and DDB_G0291842 proteins from *D. discoideum* are shown. These were derived using the online software SMART with Pfam database (http://smart.embl-heidelberg.de/). The domains present in each protein are shown with their respective genomic positions. Pkinase represents the catalytic domain. B. Predicted protein structures of (a) DDB_G0277539, (b) DDB_G0291133 and (c) DDB_G0291842 are shown. The structures were created using I-TASSER and viewed using PyMOL. The putative kinase domain of WeeA is highlighted in red (amino acid residue from 508 to 661) and blue (amino acid residue from 713 to 846); WeeB in purple (amino acid residue from 177 to 462); and WeeC in orange (amino acid residue from 77 to 331).

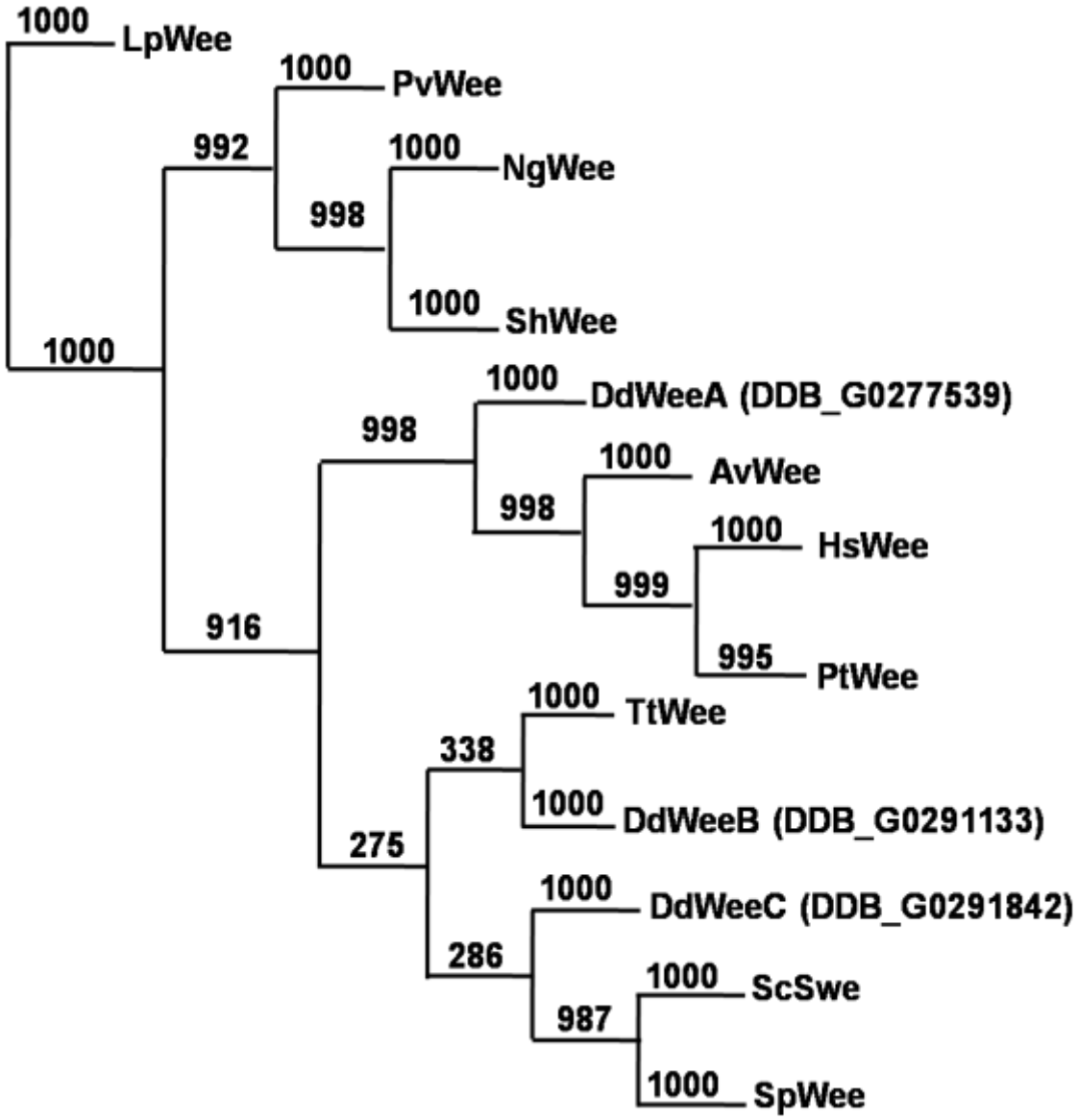

Fig 3:- Phylogenetic tree is created using Neighbor-Joining method. Protein sequences of *D. discoideum* WeeA, WeeB and WeeC and that of other organisms are used to create an unrooted phylogenetic neigbour- joining dendogram showing bootstrap values out of 1000.Dd, *D. discoideum*;LpWee, membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase-like isoform X1 [*Limulus polyphemus*]; PvWee, membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase [*Pogona vitticeps*]; NgWee, membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase isoform X1 [*Nannospalax galili*]; ShWee, membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase [*Sarcophilus harrisii*]; AvWee, Wee1-like protein kinase [*Araneus ventricosus*]; HsWee, WEE1 protein kinase [*Homo sapiens*]; PtWee, wee1-like protein kinase 2 [*Pseudonaja textilis*]; TtWee, WEE protein kinase [*Thecamonas trahens* ATCC 50062]; ScSwe, Swe1 protein kinase [*S. cerevisiae*]; SpWee, M phase inhibitor protein kinase Wee1 [*S. pombe*].

Acknowledgment:-

Thiyam Lakshmi Devi thanks the Department of Science and Technology (DST), New Delhi, India for providing the Senior Research Fellowship.

Conflict of Interest

The author declares no competing financial interest and no conflict of interests.

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