I J C B
International Journal of Computational Biology
ISSN: 2229–6700, E-ISSN: 2229–6719, Vol. 2, Issue 1, 2011, pp-32-34
Available online at http://www.bioinfo.in/contents.php?id=96

ANALYSES OF ARGONAUTE– MICRORNA INTERACTIONS IN *ZEA MAYS*

SAYAK GANGULI[1]*, MOUMITA DE[2] AND ABHIJIT DATTA[1]
[1]DBT-Centre for Bioinformatics, Presidency University, Kolkata
[2]Post Graduate Department of Botany, Bethune College, Kolkata
*Corresponding author. E-mail: sayakbif@yahoo.com

Received: May 13, 2011; Accepted: May 20, 2011

Abstract- RNA interference (RNAi) is a naturally occurring phenomenon of post transcriptional gene silencing and has been found to be highly conserved among multicellular organisms. 21mer effector RNAs, named small or short interfering RNAs (siRNAs) or microRNAs are produced from a precursor hairpin structure by cleavage using a ribonuclease class III enzyme – DICER and are incorporated into a multimeric protein complex, known as the RNA-induced silencing complex (RISC). One of the two small RNA strands serves as the guide and tows the RISC to a complementary RNA. After hybridization the endonucleolytic "slicer" activity of RISC cleaves the target RNA brought about this time by the cytoplasmic variant of DICER, thus preventing its translation. miRNAs, however, are capable of inhibiting translation of the targeted mRNA without bringing about its degradation (at least in mammalian cells). Another important component of the RISC complex are the Argonaute proteins which play dual roles of stabilizing the complex and also regulate the formation of the RNA – RNA duplex. The need for *in silico* analysis of the components of the RNA interference pathway arises from the fact that very little is known about the structural and interacting properties of these components.
Keywords- siRNA, miRNA, Argonaute, Interaction, RISC, Interface residue

INTRODUCTION:

The phenomenon which was initially discovered in *Petunia* sp. and was then nomenclatured as co suppression (2) has moved on since to establish itself as one of the lynchpins in molecular biology research as "RNA Interference" (1). The process involves the production of 21 – 22 nucleotide long non coding RNA molecules in a coordinated step wise manner where two stages of processing are required – one nuclear and the other cytoplasmic.

The cytoplasmic processing event results in the silencing of the target mRNA leading to its degradation in plants or translational repression in animals. This event is regulated by a catalytic complex referred to as the RNA induced silencing complex (RISC) which involves the interfering RNA (mi/si/tasi etc.), the target mRNA, the ribonuclease III – DICER, and the Argonaute protein (8). Apart from these four key components, the catalytic engine also includes RNA binding proteins (invertebrate R2D2 and Loquacious, vertebrate specific TAR – RNA binding protein etc.) Amongst these members of the RISC, Dicer and Argonaute have been the most studied (3, 6-7).

The key roles of the nuclear ribonuclease III [Drosha and Pasha] is the processing of the pre miRNA transcript within the nucleus. These processed transcripts are then transported out of the nucleus via exportin 5 to the cytoplasm where it associates with Argonaute to form the pre – RISC. Prokaryotic Argonautes have been the most studied of all the proteins due to their abundance and feasibility in isolation. Ma et.al. (2004) have elucidated the function of Argonaute domains – PAZ and PIWI. Parker (2010) observes that prokaryotic argonautes require a guide strand for functioning. This is an essential difference between prokaryotic and eukaryotic RNA silencing. He further iterates that two Arginine residues (R 172 AND R548) are important in stabilizing the quasi helical nucleotide strand of the guide between the 11th and the 12th nucleotide. Another striking feature of such a complex is that there are no hydrogen bonding contacts from the Argonaute and the guide strand. This is a very interesting feature since the complex then is totally dependent on Van der Waal's contact or other electrostatic forces. This work focuses on the characterization of the interactions between the Argonaute - miRNA complexes in *Zea mays* – an essential crop plant around the world.

MATERIALS AND METHOD:

Sequences of Argonaute proteins were collected from the GENPEPT resource of NCBI and were then matched with those of SWISS – PROT to remove redundancies. Domains in the sequences were then identified using CDART at the NCBI Conserved Domain Database. The sequences were then

subjected to a STRUCTURE BLAST which provided the template for Homology modelling. To obtain the proper models a three pronged approach was adopted, which included server based and software based modelling for homologous sequences and Ab Initio modelling for sequences which exhibited <40% homology.

MODEL REFINEMENT AND SELECTION:

Out of the total 128 structures that were obtained by homology modelling, umbrella sampling was performed to select the model with the least free energy. This method scores over other methods since it can be used both with Monte Carlo and Molecular Dynamics simulations and the modifications of the potential function can be written as a perturbation:

$$\grave{V}'(r^N) = \grave{V}(r^N) + W(r^N)$$

Where $W(r^N)$ is a weighting function, which can be expressed as a quadratic form:

$$W(r^N) = kw(r^N - r_0^N)^2$$

The application of umbrella sampling brought down the number of models from 128 to 4. In case of Ab initio approach, the Car – Parrinello method was used for generating the structures. This method uses an alternative to the matrix diagonalisation methods, where it integrates molecular dynamics and simulated annealing to search for values of the basic set coefficients that minimize the electronic energy. The obtained models were then analyzed to detect the Accessible surface area (ASA) of the individual residues using the ASA view database and VADAR web servers. A list of the hydrophobic and hydrophilic residues was obtained. Following this the CASTp server was used to detect potential pockets in the obtained models. The results of ASA and CASTp were matched to formulate the potential nucleic acid binding sites.

DETECTION OF INTERFACE RESIDUES:

An amino acid can be defined as an interface residue if it loses >1A°^{2} of accessible surface area (ASA) when it passes from the uncomplexed state i.e. protein only to complexed state i.e. protein – RNA. The total number of interface residues in a single protein defines its nucleic acid binding site. The parameters calculated for each binding site included the size, polarity, interface sequence segmentation and the number of intermolecular hydrogen bonds. Interface residue propensity was calculated using the following formula:

$$AA_j = \frac{\sum_{i=1}^{N_i} ASA_{[AAj\,(i)]} \Big/ \sum_{i=1}^{N_i} ASA_{(j)}}{\sum_{i=1}^{N_s} ASA_{AAj\,(s)} \Big/ \sum_{i=1}^{N_s} ASA_{(s)}}$$

- Where $ASA_{AAJ(i)}$ = Sum of the ASA (in the protein) of the amino acid residues of type j in the interface.
- ASA_j = Sum of ASA in the protein of all amino acid residues of all types in the interface.
- $ASA_{AAJ(s)}$ = Sum of the ASA of the amino acid residues of type j on the protein surface (the surface being defined as those residues with > 5% relative ASA on isolation
- $ASA_{(s)}$ = Sum of the ASA in the protein of all amino acid residues of all types in the protein surface.
- N_i= Number of residues making up the interface
- N_s = Number of residues on the protein surface excluding the interface residue.

MOLECULAR DYNAMIC SIMULATIONS:

Two approaches were undertaken for simulating the protein – RNA interactions – blind docking was performed using the HEX 5.1 software and the results were then validated using AUTODOCK VINA.

RESULTS AND DISCUSSION

A total of 588 complexes were analyzed and From all the observations it can be concluded that neutral but slightly polar amino acids like cysteine and tryptophan are completely absent from the interacting surface of Argonaute proteins, while asparagine which is polar and neutral is absent from the groove of the proteins. Neutral and non polar amino acids like proline, methionine and phenylalanine are the most abundant and conserved residues. Future endeavours using SDM's etc can be used at these specific residues to cripple and exert a level of control over the RNAi machinery of the cell.

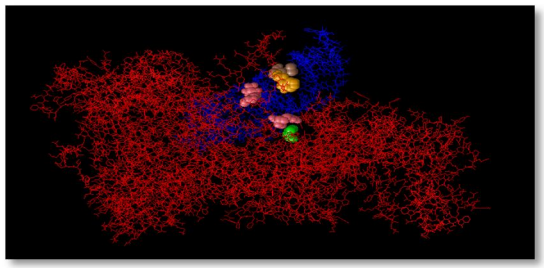

Fig. 1a- Different AGO –miRNA complexes

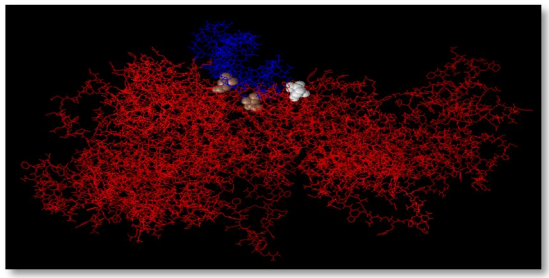

Fig. 1b- Different AGO –miRNA complexes

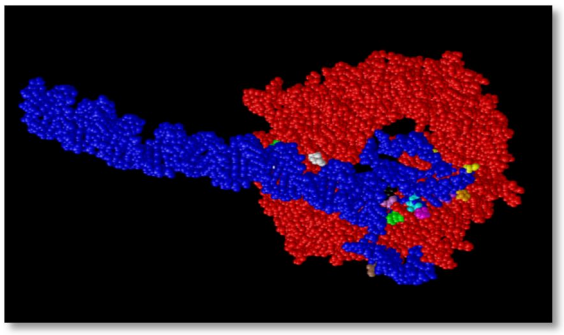

Fig. 1c- Different AGO –miRNA complexes

Table 1- Interacting residues common in all studied AGO 1 – miRNA and AGO 2 – miRNA complexes.

S.No	AGO 1	AGO 2
1	KIKALRG	MAYRGGGRGGR GGEQRPPY
2	QREQSI	DRDRVKIKA
3	PVDDNG	LPEVS
4	VRSTN	ARST
5	MAYRGGGRGGR GGEQRPPYSGRG	SPGV
6	GGAPPY	APAGEA
7	PSPGVPVI	GRRDMTD
8	TIRAPPPSHSSA	GSYG
9	RAFIR	IENA

The interacting residues were selected on the basis of their differences in the accessible surface areas prior to complex formation and following complex formation. Those residues that showed a change in the accessible surface area of 0.2 or more were treated as interface residues.

References

[1] Fire A., Xu S., Montgomery M.K., Kostas S.A., Driver S.E. and Mello C.C. *(*1998) *Nature,* 391*,* 806-*811*.

[2] Jorgensen R.A. (2003) *Sense cosuppression in plants: Past, present, and future. In RNAi: A guide to gene silencing (ed. G.J. Hannon), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY*, 5-22.

[3] Kim V.N. (2005) *Molecules and Cells,* 19,1-15.

[4] Ma J.B., Ye K., Patel D.J. (2004) *Nature,* 429:318–322.

[5] Parker J.S. (2010) *How to slice: snapshots of Argonaute in action Silence* doi: 10.1186/1758-907X-1-3.

[6] Sashital G.D., Doudna A.J. (2009) *Current Opinions in Structural Biology*, 20, 1-8.

[7] Winter J., Jung S., Keller S., Gregory R.I., Diedrichs S. (2009) *Nature, Cell Biology*, 11, 228-234.

[8] Ganguli S. and Datta A. (2011) *RNA Interference—Interactomics and Therapeutics in RNAi Technology, Edited by V. K. Gupta, CRC Press*, 347–356.