Current Trends in Biotechnology and Pharmacy
Vol. 12 (4) 349-354, October 2018, ISSN 0973-8916 (Print), 2230-7303 (Online)

Insilico Studies of FOR20 - A Centrosomal Protein

A. RanganadhaReddy[1*], N. MadhanSai[1], S. Krupanidhi[1], P. Sudhakar[2], and T.C. Venkateswarulu[1]
1*. Department of Biotechnology, VFSTR (Deemed to be University) Guntur - 522213, India
2. Department of Biotechnology, Acharya Nagarjuna University Guntur - 522 510, India
Corresponding author : rangaaluri@gmail.com

Abstract

There is enough scientific evidence on microtubule playing important role in various functions, one such is chromosomal segregation, which made them as primary targets in the cancer treatment. Centrosomal proteins plays vital role in maintaining microtubule dynamics that having significant effect on various functions involving microtubule such as chromosomal segregation, intracellular transport and cell polarity. There are diverse centrosomal proteins that are involved in this biological functions inside the cell system, to name a few EB1, EB2, tau, FOR20.We aim to characterize the regulation of microtubule dynamics by FOR20 and its implication in cellular processes like mitotic cell division, apoptosis, and cell migration. We worked on FOR20 specifically with its enough facts about involvement in maintaining microtubule dynamics and in cell migration and to find whether FOR20 regulate dynamic properties of microtubules by performing computational studies. The evidence of interaction of FOR20 with microtubule is poorly understood at molecular level.

FOR20 protein structure was modeled by using SWISS MODEL and the model was validated by using PROCHECK.

Keywords: FOR20 protein, Centrosomal protein, SWISS MODEL, PROCHECK.

Introduction

This report depicts the result of various interactions between FOR20 with tubulin. Instead of concentrating on a single biological process it uses computer simulations to understand the effects of these interactions at molecular level. Both prokaryotic and eukaryotic cells have cytoskeleton. It helps in maintenance of internal organization and shape of cells by providing mechanical support. It also plays important roles in muscle contractions, cell motility, axonal growth, cell migration, cell division and platelet formation and also helps in providing transport and communication within the cell [1]. Cytoskeleton is built up with three components microfilaments (actin Filaments), microtubules and intermediate filaments. Networks are formed by filamentous components. In prokaryotes microtubules and microtubules are present in cytoskeleton and only in animal cells intermediate filaments are found [2]. Microtubules are hollow rigid rods that are 25nm in diameter. Microtubules are vital formations it has repeated assemblage and breakdown [3]. Cell locomotion, intracellular transport and separation of chromosomes during mitosis all these require cytoskeleton and also determination of the shape of cells [4]. Microtubules unlike intermediate filaments that contain different types of fibrous proteins, it has a single tubulin protein. The protein is a dimer protein and it contains two 55-kd polypeptides they are á-tubulin and â-tubulin. Small families of similar genes encode á- and â-tubulin similar to actin.Another type of tubulin is ã- tubulin its present in the centrosome and plays an important role in initiation of microtubule assembly [5]. Polymerization of tubulin results in the formation of microtubules that contains 13 linear protofilaments that are assembled to a hollow core

[6]. Parallely head to tail arrays of tubulins make up protofilaments. Microtubules are polar structures it has two ends a positive end that grows fast and a minus end that has a slow growth rate. Direction along microtubules is determined by polarity, similar to how direction of myosin is determined by polarity of actin filaments. The tubulin protein dimers can both polymerize and depolymerize. Microtubules go through repeated phases of assembly and disassembly. á- and â-tubulin bind GTP that is analogous to ATP bound to actin to regulate polymerization. During or after polymerization GTP that is bound only to â-tubulin is hydrolyzed to GDP. Binding affinity of tubulin is weakened by hydrolysis of GTP and depolymerization takes place and results in the vital behaviour of microtubules [7]. Similar to actin microtubules also undergo tread milling that makes tubulin molecules to bind to GDP are lost from minus end and are replaced by the molecules that are bound to the plus end of the same microtubule [8]. Dynamic instability is caused due to GTP hydrolysis in which microtubules alternate between cycles of growth and shrinkage [9]. Rate of tubulin addition determines the growth or shrinkage of microtubule with respect to hydrolysis of GTP. GTP is hydrolyzed and the microtubules are retained only when new tubulin molecules that are GTP bound are added fastly to the positive end and the growth of microtubule continues. When the rate of polymerization slows down the GTP that has bound to tubulin on the positive end will be hydrolyzed to GDP. If this process takes place then dissociation of tubulin takes place that results in fast depolymerization and the microtubule shrinks. Continuous and fast turnover of the microtubules is caused due to dynamic instability that has been described by Tim Mitchison and Marc Kirschner in 1984 [10] it has half life of minutes in the cell. This turnover is important for the remodeling of cytoplasm that takes place during the mitosis process. As microtubules play an important role in mitosis the drugs that affect the microtubule assembly can also be used for treating cancer. Some of the frequently used drugs that bind to the protein tubulin which causes polymerization of microtubules and this causes blockage of mitosis process. [11]. Vincristine and Vinblastin are the two drugs that are used for cancer treatment cause the stop the cells division. Taxol instead of inhibiting the assembly of microtubules helps in stabilizing it [12]. Cell division gets blocked due to stabilization and hence its used as anticancerous drug and also as an experimental tool. Katanin, motor protein kinesin and dynein, and other centrosomal proteins are basically molecular motor proteins that interacts with microtubules and it changes the dynamics of microtubules like FOR20 [13]. Actin protein makes microfilaments or actin filaments and these filaments are 5 nm diameter and they are particular filaments. One of the most dynamic and smallest elements of cytoskeleton is actin. The filaments are type of polymers that are flexible, linear and can hold the resistance to multi-pico newton whereas they unravel upon nano newton tensile forces [14]. These filaments are either bundled up or are in the form of networks. These actin filaments can be seen in two orientations either in polar or non polar filamentous arrays that are bristly ends that pinpoint to the same ending of the bundle whereas in non polar filaments some of the bristle ends are pointed at one side and some are pointed to the other end [15]. Intermediate filaments are 10 nm in size and built up of tetrameric units [16]. They are found in cell of animals. They are formed from proteins that have alike features and structure and are said to be family protein.

Methodology

The primary sequence of FOR20 (Acc.ID. NP_653201.1) was retrieved from Swiss Prot. BLAST software was used for homology search of FOR20.

FOR20 crystal structure was modeled by using the SWISS MODEL [17] server with (PDB ID: 4BTJ) as structural template.

Stereo-chemical quality of all the chains in a protein within the given PDB structure can be done by PROCHECK analysis [18]. The regions that have unusual geometry are highlighted and the overall structural estimation is also provided.

Homology modeling

The steps to creating a homology model are as follows:

- Identify homologous proteins and determine the extent of their sequence similarity with one another and the unknown.
- Align the sequences.
- Identify structurally conserved and structurally variable regions
- Generate coordinates for core (structurally conserved) residues of the unknown structure from those of the known structure(s).
- Generate conformations for the loops (structurally variable) in the unknown structure.
- Build the side-chain conformations.
- Refine and evaluate the unknown structure.

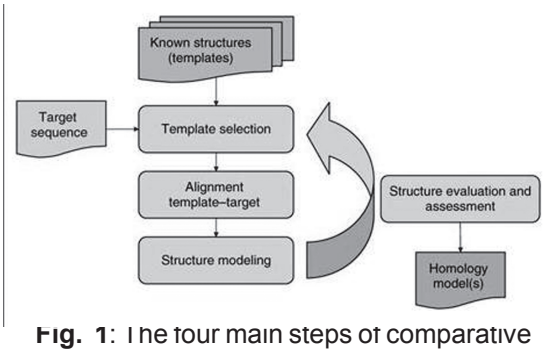

Fig. 1: The four main steps of comparative protein structure modeling: template selection, target–template alignment, model building and model quality evaluation [19-21].

Results and Discussion

Sequence Retrieval and Data Collection By using UniProtKB/Swiss-Prot database the details of FOR20 (Acc No. NP_653201.1**) the information such as** helix lengths, N-and C-terminus amino acid lengths etc., was retrieved. The sequence information is provided below.

Query sequence of FOR20

>sp|Q96NB1|FOPNL_HUMAN LisH domain-containing protein FOPNL OS=Homo sapiens OX=9606MATVAELKAVLKDTLEKKG VLGHLKARIR AEVFNAL DDDRE PRPSLSHE NLLINE LIREY LEFNKYKYTAS VLIAESG QPVVPLDRQFLIHELNAFEESKDNTIPLL YGILAHFLRGT KDGIQNAFL KGPSLQPSDP SLGRQPSRRKPMD DHLRKEEQKST NIEDLHV SQAVNR

BLAST tool helps to search the regions of similarity between nucleotide or protein sequences by using local alignment algorithm. Statistical significance of the matches is calculated by comparing the protein or nucleotide query to the sequences in the database. By using the BLAST tool the target sequence i.e., FOR20 (UniProt ID: NP_653201.1) was searched against the sequences of protein in the database. By analyzing the BLAST results it can be seen that maximum identity with target sequences are shown by three different proteins (PDB IDs: 1T11.1.A, 1Q1V.1.A, 2D68.1.B).For further use out of these three sequences 1T11.1.A is selected (Figure 2.).

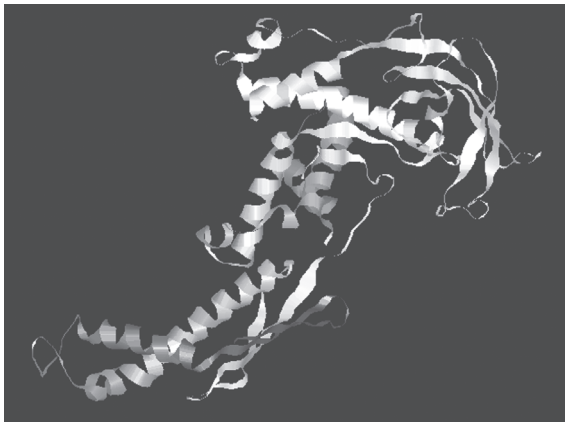

Fig. 2. Crystal structure of ribosomal chaperone trigger factor (PDB ID: 1T11) as structural template

Three-Dimensional Structure Prediction by SWISS MODEL

1T11 was retrieved through BLAST results and was taken as template, NP_653201.1 was taken as query sequence and Protein BLAST was performed, the 3D structure of FOR20 (NP_653201.1) was predicted by using the tool

SWISS MODEL and can be seen in Figure 3. Taking 1T11_1_A chain as templates that are homologous to query sequence tau-protein kinase the 3D structure of the FOR20 (NP_653201.1) was predicted. A model with RMS deviation is 0.75 after superimposition of FOR20 structure with templates 1T11 was obtained.

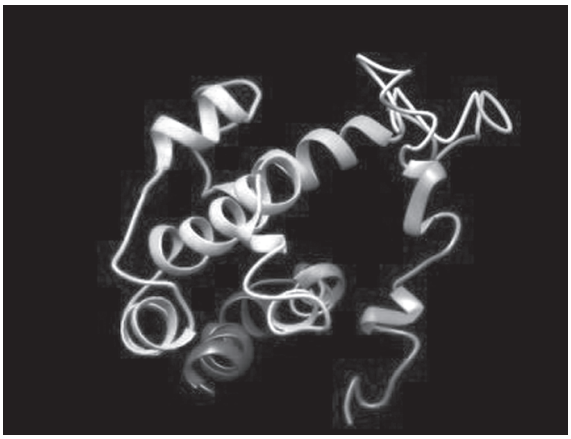

Fig. 3. Homology model of FOR20 protein structure built by SWISS MODEL server

Ramachandran Plot

PROCHECK checks the stereochemical quality of a protein structure, producing a number of PostScript plots analysing its overall and residue-by-residue geometry. It includes PROCHECK-NMR for checking the quality of structures solved by NMR and the results of procheck for protein structure after energy minimization is as follows (Figure 4).

PROCHECK is used for stereochemical assessment of the model. Ramachandran plot was developed by Gopalasamudram Narayana Ramachandran and Viswanathan Sasisekharan. Iit is used to visualize dihedral angles Psi and Phi of amino acid residues in protein structure. It is commonly known as Ramachandran map or a Ramachandran diagram or a [Psi and Phi] plot. It depicts the possible conformations of Psi and Phi angles for a polypeptide. The model developed by

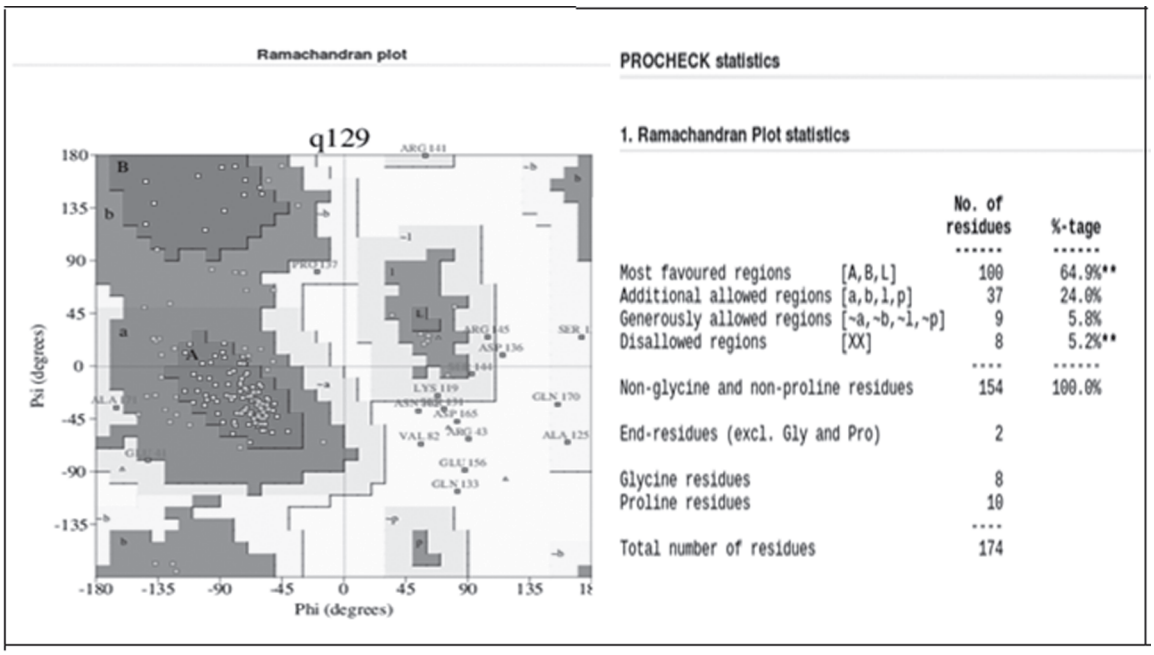

Fig. 4. Ramachandran plot analysis of the generated model

PROCHECK was checked with the Ramachandran plot, FOR20 protein had 100 (64.9 %) residues in the most favored region, 37 (24.0 %) residues in allowed region, 9 (5.8 %) generously allowed region and 8 (5.2 %) residues in disallowed region and it can be seen in the Figure 4.

Conclusion

In the current study we have modeled FOR20, an important centrosomal protein aids to investigating in different cellular functions like cell signaling. By using BLASTP the template 1T11 was found. SWISS MODEL was used for building the homology model of FOR20; by using PROCHECK the protein structure validation was done.

References

1. Bray, D. Cell movements from molecules to motility. Garland Science (2001).
2. Alberts, B. Molecular Biology of the Cell. Garland Science (2008).
3. Howard, J., Hyman, A.A. Microtubule polymerases and depolymerases. Current opinion in cell biology. 19, 31-35 (2007).
4. Kirschner, M., Mitchison, T. Beyond self-assembly: From microtubules to morphogenesis. Cell. 45, 329-342 (1986).
5. Vandecandelaere,A., Brune,M., Webb,M.R., Martin,S.R., Bayley, P.M.: Phosphate release during microtubule assembly: What stabilizes growing microtubules. Biochemistry. 38, 8179-8188 (1999).
6. Tilney, W.G., Bryan, J., Bush, D.J., Fujjiwara, K., Mooseker, M.S., Murphy, D.B., Snyder. D.H. Microtubules: evidence for 13 protofilaments. Journal of Cell Biology. 59, 267–275(1973).
7. Stewart, R.J., Farrell, K.W., Wilson, L. Role of GTP hydrolysis in microtubule polymerization: evidence for a coupled hydrolysis mechanism. Biochemistry. 29, 6489-6498 (1990).
8. O'Brien, E.T., Voter, W.A., Erickson, H.P. GTP hydrolysis during microtubule assembly. Biochemistry. 26, 4148-4156 (1987).
9. Simon, J.R., Salmon, E.D. The structure of microtubule ends during the elongation and shortening phases of dynamic instability examined by negative-stain electron microscopy. *Journal* of *Cell Science*. 96, 571-582 (1990).
10. Mitchison, T., Kirschner, N. Dynamic instability of microtubule growth 123. Nature. 312, 237-242 (1984).
11. Akhmanova,A., Hoogenraad,C. C.,Drabek, K., Stepanova,T.,D ortland,B.,Verkerk,T., Vermeulen, W., Burgering, B.M., De Zeeuw, C.I., Grosveld, F., Galjart, N. Clasps are CLIP-115 and -170 associating proteins involved in the regional regulation of microtubule dynamics in motile ftbroblasts. Cell. 104, 923-935 (2001).
12. Giannakakou, P., Sackett, D., Fojo, T.: Tubulin/microtubules: still a promising target for new chemotherapeuticagents *Journal* of the *National Cancer Institute.* 92, 182–183 (2000).
13. Ligon, L. A., Shelly, S. S., Tokito, M., Holzbaur, E. L.: The microtubule plus-end proteins EB1 and dynactin have differential effects on microtubule polymerization. *Molecular Biology* of the *Cell.* 14, 1405–1417 (2003).
14. Letourneau, P. C., Shattuck, T. A. Ressler, A.H. 'Pull' and 'push' in neurite elongation: observations on the effects of different concentrations of cytochalasin B and taxol. *Cell Motility* and the Cytoskeleton 8, 193–209 (1987).
15. Lin, C. H., Forscher, P. Cytoskeletal remodeling during growth cone-target interactions. Journal of Cell Biology. 121, 1369–1383 (1993).

16. Franke, W.W. Differentiation-related patterns of expression of proteins of intermediate-size filaments in tissues and cultured cells. Cold Spring Harbor Symposia on Quantitative Biology. Vol. 46. Cold Spring Harbor Laboratory Press, 1982.

17. Benkert, P., Biasini, M., Schwede, T. Toward the estimation of the absolute quality of individual protein structure models. Bioinformatics. 27, 343-350 (2011).

18. Laskowski, R.A., Malcolm, W.M., David, S.M., Janet, M.T. PROCHECK: a program to check the stereochemical quality of protein structures. Journal of applied crystallography. 26, 283-291 (1993).

19. Bordoli, L., Kiefer, F., Arnold, K., Benkert, P., Battey, J., Schwede, T. Protein structure homology modelling using SWISS-MODEL workspace. Nature Protocols. 4, 1-13 (2009).

20. Kopp, j., Schwede, T. Automated protein structure homology modeling: a progress report. Pharmacogenomics. 5, 405-416 (2004).

21. Marti-Renom, M.A., Stuart, A.C., Fiser, A., Sánchez, R., Melo, F., Sali, A. Comparative protein modeling of genes and genomes. Annual review of biophysics and biomolecular structure. 29, 291-325 (2000).