From the archives to the Future...
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The biology of Hyaluronan (HA) remains incomplete without the mention of hyaladherins, which influence its cellular functions. An important member of the hyaladherin family is HABP1, a glycoprotein that binds specifically to HA, among glycosaminoglycans. It was purified and characterised from rat tissues and shown to be localized on cell surface. The involvement of HABP1 in HA mediated cellular signalling, cell adhesion and tumour formation has already been reported from our laboratory. The gene encoding this protein was identified, sequenced and overexpressed in E. coli. The recombinant protein was purified protein and found to bind HA, like the tissue protein. The gene encoding this protein was localized on human chromosome 17p12-p13 with known STS marker. In addition, numerous pseudogenes were reported for the protein on chromosomes 4, 11, 15 and 21. HABP1 was shown to be a multifunctional protein as its sequence was identical with p32, a protein co-purified with the alternate splicing factor SF2 and the receptor of gC1q, the globular head of the complement component 1q. Biochemical analysis and sequence search established HABP1 to be evolutionarily conserved and detected it in species ranging from yeast to mammals. HABP1 is synthesised as a 282 amino acid long pro-protein, from which the first 73 amino acids are proteolytically cleaved off to yield the mature protein of 209 amino acids. Though only a mitochondrial localization signal is well characterized in the precursor form of this evolutionarily conserved protein, studies with the tagged mature protein revealed that it mainly gets localized to the nucleus. In addition to binding HA, HABP1 also interacts with numerous cellular proteins like the transcription factor II B, the lamin B receptor, vitronectin, high molecular weight kininogen and factor XII. Apart from its cellular ligands, HABP1 also interacts with a number of viral proteins like Adenovirus core protein V, EBNA-1 of Epstein Barr virus, HIV 1 Rev, HIV 1 Tat and Hepatitis C virus core protein. These interactions have given new dimensions to the actual
functional role of HABP1. The crystal structure of the protein divulged it as a doughnut shaped trimer, with each monomer possessing seven consecutive β-strands (β1 to β7) forming a highly twisted anti-parallel β-sheet. The β-strands are flanked by one N-terminal (αA) and two C-terminal (αB and αC) α-helices. All the three helices are located on the same side of the β-sheet. The N-terminal α-helix (αA) forms a coiled-coil structure with the C-terminal α-helix (αC) of the adjacent sub-unit. This interaction is essential for the maintenance of the trimeric assembly. The HA-binding motif in this trimeric assemblage is solvent exposed. In summary, the overall architecture of the trimer can be visualised as if the β-sheet forms a hyperboloid shaped spool with α-helices wrapped around it. The HA binding motif of HABP1 consists of $^{119}$KLVRKVAGEK$^{128}$, with an extra, evolutionarily conserved glutamic acid residue (E$^{127}$) and is slightly different from the canonical B-(X)7-B motif. The additional acidic residue in the HA-binding motif makes this molecule an interesting one to study. The crystal structure however reveals that this glutamic acid residue (E$^{127}$) participates in a salt bridge formation with arginine (A$^{246}$) of the same sub-unit. This salt bridge formation prevents the E$^{127}$ residue from being exposed at the binding motif, thus restoring the B-(X)7-B motif at that position.

Despite significant work carried out on HABP1/p32/gC1qR by different groups including ours, no study has been initiated in understanding its evolutionary significance, cellular dynamics and its structure-function relationship. With the above mentioned information in hand, this study was initiated to perceive on one hand, the evolutionary significance HABP1, while on the other, the structure-function relationship and cellular dynamics of this protein in understanding its biological functions in a convincing manner.
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The present findings...

This study has established the evolutionary significance of HABP1, using its pseudogene as a molecular marker. The presence of a pseudogene like sequence was established in numerous eukaryotic species, while a related sequence was detected in Methanosarcina barkeri, an archaea. This sequence was G+C rich in nature and showed around 45% homology with the HABP1 pseudogene sequence located on chromosome 21. Analysis of rat liver and kidney sections documented the specific localization pattern of HABP1 in these tissues and established its secretory nature. HABP1 has been postulated to play a role in hyaluronan uptake for degradation in the liver. The distribution pattern suggested a role for this protein in tissue function and architecture. Sub-cellular localization profile of HABP1 was extended to ER and golgi complex, apart from its previously reported localization in the nucleus, cytoplasm and cell membrane. During mitosis, the dynamics of HABP1 corroborated with the distribution pattern of representative proteins of nuclear, ER and golgi complex. Localization of HABP1 in cell membrane of live cells substantiates its role in cell adhesion and de-adhesion.

Analysis of the N- and C-terminal truncated variants of HABP1 ascertained the role of the terminal helices in maintaining the oligomeric assembly and their ability to bind HA. Studies reveal that these variants, though structurally different from the parent protein, can bind HA with equal efficiency. However, studies with mutated variants differing in their HA-binding motif indicated that those amino acid residues in the motif were critical for binding. Mutations at K^{119} and E^{127} residues in the HA-binding motif of HABP1 decreased the HA-binding ability of the resultant mutants. Transient expression of HABP1/truncated variants in COS-1 cells resulted in the disruption of the cytoskeletal network in the cell by modulating f-actin polymerization. These expressions also led to the formation of vacuoles in the cells, which altered the morphology of the...
cells. The vacuoles were characterized to be heterogeneous in nature, but their cellular manifestations resembled that of autophagic vacuoles. It was thus postulated that the autophagic nature of the vacuoles might be due to stress conditions originating prior to programmed cell death, either via autophagy or apoptosis. Taken together, these data elucidate the structure-function relationship of HABP1 in cellular functions, validating its role as a multifunctional protein.

**From facts to the future...**

This work has opened new avenues for future work that needs to be pursued to ascertain the role of HABP1 in cellular functions. Nuclear localization of glycoproteins has always remained a puzzle for scientists. The significance of this localization, coupled to the functional probabilities is an interesting area to investigate. Being a glycoprotein and getting localized to the nucleus, HABP1 presents us with an interesting molecule to pursue research in this direction. The dynamics of this protein during mitosis is speculative of its role in cell division and warrants subsequent work in this direction to strengthen the postulate. Transient expression of the point mutants in mammalian cell-lines can shed some more light on the *in vivo* manifestations of cellular changes. The mammalian expression system used in this study provides us a tool, which can be used in surmise cellular and morphological changes owing to the expression of HABP1 and its variants. The structure-motif controversy, governing the binding of HA to HABP1 would be settled, if the crystal structure of the variants could be determined. This would also aid us in understanding the localized structure around the HA-binding motif. In the light of the recent findings and the present work, it is not preposterous to presume that *in vivo* experiments, coupled to structural analysis would probe successfully into the cellular functions of HABP1 and HA-HABP1 interactions.

*And the journey continues.......*