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Methods of Protein-Protein Interaction Detection

Abstract

In order to predict the protein function of a target protein, study of protein-protein interactions play a vital role. Many in-vitroandin-vivomethods are there for predicting protein-protein interactionslike AffinityPurification, TAP (tandem affinity purification)Y2H (yeast2hybrid) but these methods are associated with many drawbacks likecost,time and morefalsepositivesare obtained by using these methods. In order to overcome these drawbacks, in silico methods were developedlike sequence-based approaches, structure-basedapproaches, chromosome proximity, genefusion, in silico2hybrid, phylogenetictree, phylogenetic profile and gene expression-based methods. Networksare constructed havingalltheprotein-protein interactions by using computation altoolsforsignallingpathway identification and protein complex identification in specific diseases.

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Introduction

Each cellular procedure, for instance replication of DNA, control of cell cycle, transcription, translation and signal transductionrelies upon protein-protein collaborations in the body. Cell structure and functions are dependent on protein- protein co-operations. It has been reported over 80% of proteins don't work alone however in complexes. Breaking down these interactions can assist us with understanding cell association, procedure and function.

Due to the significance of protein in all living organisms, these interactions are considered one of the most important biological relationships [1]. It has been observed that unless there is some association between some components of protein complex with other components of the complex, the protein does not function [2]. PPI detection serves various perspectives in biology, for example in drug designing [3], signalling pathways in the cells so that we can have a better understanding of signal transduction[4], prediction of PPIs between species for therapeutic strategies [5] and protein function prediction[6]. In order to have a better understanding of molecular mechanisms in cells, PPI network reconstruction should be done.[7]

Control ofa large number of cellular processes is also done by transient protein-protein interactions, but difficulty in understanding these transient complexes are there as the proteins or conditions responsible for the transient reactions have to be identified first.Number of effects can be there of protein-protein interactions.

1. The kinetic properties of proteins can be changed.
2. Allows for substrate channelling.
3. Construction of a new binding site for small effector molecules can be done.
4. Protein can be inactivated or suppressed.
5. Serves as a regulatory role in upstream or downstreamlevel.

Protein-protein interactionscan help us in identifying a number of drug targets[8].In recent years, PPI data have been improved by using high throughput experimental methods like mass spectrometry. [9]

We are writing this review to give a summary on both classical and recent methods for identification of protein-protein interactions methods.

Aim of the Study

The aim of the study is to give a summary of various methods for detection of protein-protein interaction which includes various in-vivo as well as variousin vitro methods.This study also gives are view of the various databases to detect protein-protein interaction.

Predicting Protein-Protein Interactionby In-Vitro Techniques-

Tandemaffinitypurification-Mass spectroscopy (TAP-MS)-

PPI under intrinsic conditions of the cell are identified by this technique [10]. Purification process is done in two steps for the protein of interest which is double tagged on its chromosomal locus [11] . Proteins of interest can be identified through SDS-PAGE followed by mass spectrometry analysis [12].

Two essential components are there: TAP and MS. TAP is used to isolate protein complexes from cellsand thenthese complexes are digested by proteases into peptides. MS is used to identify these peptides. There are two types of proteins involved- proteins that are tagged are called as baits, and the proteins interacting with the bait proteins are called as preys. MS help us to reveal the identity and amount of these peptides present.

Affinity Chromatography

A matrixis there to which a ligand protein is covalently coupled under controlled conditions and this ligand protein can be used to select counter ligand proteins that bind and are retained. Most proteins are washed off under low-salt conditions; proteins that are retainedcan be eluted out by using high-salt solutions, cofactors or sodium dodecyl sulfate (SDS). Example of ligand and counter ligand interaction can be Enzyme-Substrate Reaction, Sugar- Lectins Reaction. Affinity Chromatography is based on specific biological reactions.

This method was first used to detect interaction of phage and host proteins with different forms of E. coli RNA polymerase [13] . Weakest interactions in proteins can also detected by this technique.Main drawback of this technique is that many false positive results arise in the column. Therefore, affinity chromatography results must be cross checked with MS in order to generate high-throughput data.Affinity purification combined with mass spectrometry (AP-MS) has emerged as an attractive technique for PPI detection [14] . Importantly, AP-MS technique can also assess PPIsin mammaliancelllinesortissues. Another advantage of using this technique is that they can provide quantitative information (q-AP-MS).

Affinity Blotting-

Fractionation of proteins is done first by PAGE and then transferred to a nitrocellulose membrane and are identified by their ability to bind a protein, or peptide. This method is similar to immunoblotting (Western blotting), which uses an antibody asthe probe.

This technique can analyse complex mixture of proteins without any need of purification. Therefore, this method has beenused for detection of membrane proteins, such as cell surface receptors[15]. Fractionation of cell lysates before gel electrophoresis can be done to increase the sensitivity of the method for detecting interaction with rare proteins.

Following factors must be taken into account for affinity blotting like the biological activity of the proteins on the membrane, the preparation of the protein probe, and the method of detection used.

Co-immunoprecipitation (Co- IP)-

For successful interactions, a whole cell extract is required where proteins are present in their native form in a complex mixture of cellular components. Cell lysates are generated, to which antibody is added and the antigen is precipitated and washed, and bound proteins are eluted and analysed. The antigen used can be purified protein or synthetic peptide coupled to carrier, and the antibody can be polyclonal or monoclonal antibody.

This technique has been classically used for detection of PPI in vitro. In this technique, antigen–antibody complex is used as bait and the prey can be the target interacting protein. Cell lysate is reacted with the bait-specific antibody. Along with the bait protein and any bait-associated proteins, the prey protein will be co-precipitated. The bait protein interacts with its specific antibody, which is bound to micro-beads, such as agarose, sepharose, or magnetic beads[16]. The prey protein binds to bait protein in the sample, resulting in abait–preycomplex, which willbeco-precipitated. Thepreyproteincanbe detected by downstream processing, such as the western blot. Mass spectrometric analysis can also be done for the prey protein identification[17]. Whether thetwotargetproteinsaredirectlyboundtoeachother,can not be proved by Co-IP becausethepresence of a third protein cannot be ruled out.

Protein Microarrays

Protein microarrays can detect the proteins and alsomonitor their expression levels. Protein-protein interactions and their functions can also be detected by this technique. It can be a piece of glass on which at separate locations, various protein moleculeshave been fixed in an ordered manner [18]. High-throughput protein analysis can be achieved by using protein microarray.Major advantage of microarray is that large numbers of samples can be run parallelbyautomatedprocess.

Achip is there onto which expressed and purified proteins are printedusing a micro array as discrete spots. A solution of labelled proteins is incubated with the chip. Washing is done to remove unbound proteins, and the position of the labels indicates the interaction between proteins (protein on the chip and protein from the solution)[19]. Many advantages are there of this technique over other techniques like high signal to noise ratio, higher sensitivity and efficiency and the relatively small quantity of sample requirement, but the proteins attached to the chip can disrupt protein interactions.

X-ray crystallography-

It is a form of high resolution microscopy [20]. By X-ray crystallography, we can visualise protein structures at atomic level, specifically we can observe how proteins interact with other molecules. X-ray crystallography gives a complete, high-resolution analysis of the three-dimensional structure of proteins.

Nuclear Magnetic Resonance (NMR)

The basis for the NMR spectroscopy is that magnetically active nuclei oriented by a strong magnetic field absorb electromagnetic radiation at characteristic frequencies governed by their chemical environment [21, 22].

This technique is a powerful tool to investigate weak protein-target interactions at physiological conditions [23], [24], and it is effective for investigating the weak PPIs at atomic levels [25]. Nuclear magnetic resonance effect is observed when magnetic nuclei take in and diffuse electromagnetic energy in a magnetic field. It was first described by Isidor Rabi in 1938 [26].

Paramagnetic relaxation enhancement (**PRE**) is one of the NMR approaches. It maintains a method for directlyinvestigating the presence and the nature of low population, transient intermediates under equilibrium conditions [27]. Data on complexes in the fast exchange regime, obtained from PRE, supplies useful information about intermediates. These observations reveal both the structural features and the presence of intermediate states. Another NMR procedure is 2D transferred nuclear Overhauser effect spectroscopy (TRNOESY), which is also a quick assay for identifying weak PPIs.

Predicting Protein-Protein Interaction By In-Vivo Techniques

Y2H method-

PPIs can be detected in-vivo by Y2H method [28] .There are twoproteindomains having two specific functions: (i) a DNA binding domain (DBD) to which DNA binds (ii) an activationdomain(AD)that leads to activation oftranscription ofDNA. Both of these domains are needed for transcription of a reporter gene [29] . PPIbetween protein pairs can be directly detected by Y2H analysis.Many disadvantages are there of this technique

1. Many false positive results may be reported.
2. Many true interactions may not be reported.
3. Interacting proteins must be locatedwithin the nucleus.
4. Proteins that need posttranslational modifications can't be detected by Y2H experiment.
5. Proteins which are not in their naturalphysiological environment may not be detected by this technique [30] .Duringthelastdecade,Y2H has been improved by designing new yeast strains containing multiple reporter genes and new expression vectors to facilitate the transformation of yeast cells with hybrid proteins [31] .

Synthetic lethality-

It is animportanttypeofin-vivo genetic screening. This methodology produces mutations or deletions in two or more genes which are viable alone but cause lethality when combined together under certain conditions [32].

Protein Fragment Complementary Assay (PCA)-

An YFP-based protein fragment complementation assay (PCA) was first applied to secretory pathway of living cells for identifying PPIs [33] . Secretory pathway is a challenging focus due to the transient nature of the interactions it contains. Detection of low-affinity interactions was achieved by fixing the complex by the reconstituted YFP. Yellow fluorescent protein PCA could visualize weak, transient protein interactions that may escape interest by co-immunoprecipitation and chemical cross-linking.

Fluorescence Resonance Energy Transfer(FRET)

FRET is a phenomenon frequently used for detection of PPI[34]. Two different fluorophores—the donor and the acceptor—are attached to two proteins of interest. The emission wavelength of the donor overlaps with the excitation wavelength of the acceptor. Complex formation results in the proximity of the donor and the acceptor, thus fluorescent emission at the acceptor wavelength occurs upon donor excitation (resulting also in quenched emission at the donor wavelength). CyPET (donor) and YPET (acceptor) proteins can be used, which is recognised as highly efficient FRET pair[35]. Protein purification must be done before PPI can be detected.

Recently, a FRET-based method termed FRETex has been developed, for fast and high-throughput PPI detection without the need for any protein purification. Interactions of multiple mutants of three different protein–protein complexes were detected by using FRETex: TEM1-b-lactamase binding BLIP [36] and ornithine decarboxylase (ODC) binding antizyme (Az) [37]. FRETexcan detect interactions ranging between nM to mM affinity.

LANCE (Lanthanide Chelate Excitation) is a TR-FRET technology. LANCE and LANCE Ultra assays require two fluorophores, a donor and an acceptor, and energy may be transferred between. A flash lamp or a laser at a wavelength of either 320 or 340 nm is used to excite the donor (a chelated Lanthanide Europium), which will cause an emission at 615 nm. This Europium emission can then excites the acceptor dye (ULightor APC) if it's in close proximity (should be 10 nm or less), which results in emission of light at 665 nm.Many advantages are there of LANCE technology

1. It is a rapid, reliable and reproducible assay platform.
2. It is a homogeneous assay format, thus requiring no wash steps.
3. The assay background is low due to its time-resolved nature.
4. The assay signal is stable for many days.

BRET

By using BRET, one can overcome some of the practical problems associated with FRET-based systems such as photobleaching, autofluorescence and simultaneous excitation of both donor and acceptor fluorophores. BRET is superior to FRET when studying light-sensitive tissues such as the retina and the plant tissues (which exhibit high autofluorescence due to photosynthetic pigments) [38] . It requires: (1) Proteins of interest should be labelled with either a donor or acceptor molecule (2) placement of these labelled proteins in the desired environment for assessing their potential interaction (3) Detection instrumentation to monitor resultant energy transfer.

Proteins of interest can be genetically fused to the bioluminescent donor or fluorescent acceptor (the 'BRET tags'). Energy transfer occurs when the protein of interest brings the donor and acceptor into close proximity, a distance generally indicative of interaction between proteins of interest. The resultant

acceptor energy emission can then be detected relative to the donor emission. A disadvantage of BRET is that it requires at least the donor to be part of a fusion protein.

Current BRET systems emitlight mostly in the green to yellow region of the visibles pectrum (510–570nm), rendering them suboptimal for imagingin living subjects. Examples of such systems include BRET1 38 and **BRET2** 39 . Red-shifted BRET system (**BRET3**) is developed with improved spectral properties [40] ,**BRET3** use an improved RLuc variant, RLuc8 [41] (λem=480nm forCLZ substrate), as the BRET donor and morange (λex/λem = 548/564 nm) as the BRET acceptor protein.

NanoBRET

With the recent development of Nluc, which emits bright, stable, and spectrally narrow luminescence, a BRET platform was designed with substantially improved capabilities. This was achieved by pairing Nluc with a spectrally well separated acceptor, thus effectively reducing the background caused by "bleed through" of the donor signal into the acceptor channel. Protein− protein interactions at low expression levels in living cells can also be analysed using NanoBRET platform.

Split Ubiquitin Assay

Ubiquitin is a highly conserved regulatory protein. Ubiquitin-specific proteases can recognise ubiquitin and cleaves the C-terminal covalent linkage between ubiquitin and the protein to which it is attached [41] . The C-terminal and N-terminal regions of ubiquitin (Cub and Nub) are split and each part is then fused to different proteins of interests, functional ubiquitin is formed upon interaction of both fusion proteins. To prevent spontaneous re-association of ubiquitin, amino acid 13 was converted from isoleucine to glycine (NubG). In the original design, the bait consisted, from the N-terminal to the C-terminal end, of the homodimerization domain of Gcn4 (protein of interest), Cub, mDHFR, and a hemagglutinin (HA) epitope tag. The prey was obtained by fusing the homodimerization do main of Gcn4 with NubG.Upondimerization of Gcn4, ubiquitin was reconstituted and mDHFR-HA was cleaved off by ubiquitin-specific proteases, and this was detected as a shift in a Western blot assay using anti-HA antibodies.

Later, this readout was replaced by reporter gene activation. The reporter mDHFR was replaced bythe hybrid transcription factor LexA-VP16.LexA-VP16 is cut off and it moves to the nucleus for activation of the reportergenes HIS3 and lacZ, after the interaction of bait and prey. This new reporter strategy allows for screening of a library for novel interactors.

Using the concept of the N-end rule, an alternative version was created. In Saccharomyces cerevisiae, protein stability depends on the nature of the N-terminal aminoacid42. Protein is stabilised when amino acids such as glycine, methionine, threonine, alanine and cysteine are present at its N-terminal end. In contrast, N-terminal basic (e.g., arginine) or bulky hydrophobicaminoacidstendtopromoteproteindegradationinanubiquitin-dependent manner 43 . The LexA-VP16 construct in the baitisreplacedbythe Reporter protein Ura3 for PPI analysis, with an arginineresi due (R-Ura3) betweenUra3 and Cub 44. When two proteins of interest interact, the reassembly of ubiquitin recruits the ubiquitin-specific proteases that cleave off Ura3. As a result, free Ura3 is quickly degraded due to the exposed N-terminal arginine residue. Consequently, the cells become resistant to5-FOA.

Fluorescence Correlation Spectroscopy (FCS)-

Conformational changes of fluorescently labelled molecules in a small interrogated volume, typically created by a confocal microscope results in fluctuations in fluorescence intensity which is measured by **FCS** [45] . This technique can measure several properties of a labelled molecule which includes the number of molecules in the interrogated volume, their diffusion rate, flow rate, and rotational dynamics (with polarized light) [46] . In a typical application, a burst of photons begins due to multiple cycles of excitation and emission when a diffusing fluorophore moves into the interrogated volume, and ends when the fluorophore leaves the interrogated volume. The duration of bursts is correlated with the diffusion rate.

To detect interactions that result in small changes in mass, an alternative methodology is opted that isfluorescencecross-correlationspectroscopy (FCCS; [46]). In **FCCS,** different fluorophores are labelled onto the interacting partners and the intensity fluctuations of the two species are cross-correlate. Their intensities will tend to fluctuate together if the two molecules interact.

SPARK (Specific Protein Association tool giving transcriptional Readout with rapid Kinetics)-

Specific protein pairs of interest (proteins A and B suppose) present in living cells can be detected by SPARK, and transcription of gene is resulted. In contrast to previous tools, SPARK is gated by externallyapplied blue light in contrast to previous tools. Hence, activation of transcription requires both protein A-protein B interaction and light. This generic and non-invasive form of temporal gating enables SPARK to capture PPI dynamics to some extent, and reduces background signal overall, while preserving the tremendous benefits of transcriptional readout. This technique was first used in characterisation of living mammalian cells, and was then applied to a range of PPIs, including eight different GPCRs.

12 different PPIs in mammalian cells were detected using SPARK, with 5 min temporal resolution and signal ratios up to 37. Combined with FACS, SPARK enabled 51 fold enrichment of PPI-positive over PPI-negative cells. SPARK has the potential to advance PPI analysis and discovery owing to its high specificity and sensitivity.

Protein-dimerization footprinting(PdF)

By directly transcoding the signal from physical PPIs into DNA sequences, quantitative measurement of physical PPIs in vivo can be done. In the PdF strategy, a DNA-binding domain is fused onto the target protein that can specifically recognize a defined DNA sequence but with negligible affinity.

However, when the interaction between target proteins in living cells becomes strong enough to allow the formation of a stable dimer, the DNA-binding domain binds to the specific DNA sequence with substantially higher affinity, thereby protecting the DNA sequence against subsequent DNase I digestion. Hereby, a physical PPI is transcoded into a specific DNA sequence that can be detected by quantitative PCR or other nucleic acid-based techniques, thus using the copy number to quantify physical interaction intensity

Predicting Protein-Protein Interactions By In-Silico Methods

Structure-Based Prediction Approaches

We can predict protein-protein interaction between two proteins,if both of the proteins have a similar structure. But sometimes, the structure of not all the proteins is known; structure prediction is done based on its sequence. PDB database can be used to build structure of a query protein. [47]

Recently, a new algorithm to infer protein-protein interactionwas developed 48 . The Coev2Net algorithm works on three steps- (i) Predicting the binding interface (ii) Checking the compatibility of the interface with an interface coevolution based model (iii) Evaluation of the confidence scores for the interaction [47].

Sequence-Based Prediction Approaches

Interaction found in one species can be used to infer the interaction in other species. Recently, a threading-based approach was developed to predict protein-protein interactions which take sequences as input. The algorithm, iWARP (Interface Weighted RAPtor), combinesanovel linear programming approach for interface alignment with a boosting classifier [48] for interaction prediction.

A new method called Universal In-Silico Predictor of Protein-Protein Interactions (UNISPPI) was introduced by Guilherme Valente et al. in which protein pairs based on primary sequence information were classified as interacting or non-interacting proteins[49]. Kernel methods come under hybrid methods as it uses a combination of properties like protein sequences, gene ontologies[50]. However, there are two different methods under sequence-based criterion.

Ortholog Based Approach- Annotationfromafunctionally definedproteinsequenceis transferred tothetargetsequencebasedonthe similarity. Annotation by similarity depends on the homologous nature of the query protein in the annotated protein databases using pairwise local sequence algorithm [51]
.

Domain-Pairs-Based Approach- Distinct regions in the protein sequence that are highly conserved during evolution are called as domains. It has been observed that domains are directly involved in the intermolecular interaction and hence are fundamental to protein-protein interaction. Many experiments have shown that domain-domain interactions (DDIs) aremore consistent than PPIs [52] . Use of the domains and their interactions can be used for predicting the protein-protein interactions and vice versa [53].

Chromosome Proximity/Gene Neighbourhood

If the gene neighbourhood is conserved across multiple genomes, and then there is a potential possibility of the functional linkage among the proteins encoded by the related genes. One of the pitfalls of thismethodisthat itisdirectlysuitableforbacterialgenome sincegeneneighbouringisconservedinthebacteria.

Gene Fusion

This technique can also be called as Rosetta stone method. It is based on the concept that some of the single-domain containing proteins in one organism can fuse to form a multi-domain protein in other organisms[54]. This domain fusion phenomenon indicates the functional association for those separate proteins, which are likely to form a protein complex. Proteins participating in the metabolic pathway generally show these fusion events [55]. However, it can be applied only to those proteins in which the domain arrangement exists.

In silico two hybrid (I2h)

To keep the protein function reliable, interacting proteins must undergo coevolution is the assumption behind this technique.In other words, if some of the key amino acids in one protein get changed, the related amino acids in the other protein which interacts with the mutated counter partner should also under go the compulsory mutations as well. Since I2h analysis is based on the prediction of physical closeness between residue pairs of the two individual proteins, the result from this method automatically indicates the possible physical interaction between the proteins.

Phylogenetic Tree

Phylogenetic tree is another method to detect interaction between the proteins. The phylogenetic tree is used to determine the evolution history of the protein. The mirror tree method predicts protein-protein interactions under the assumption that the interacting proteins show similarity in molecular phylogenetic tree because of the coevolution through the interaction[56]. The underlying principle behind the method is that the coevolution between the interacting proteins can be reflected from the degree of similarity from the distance matrices of corresponding phylogenetic trees of the interacting proteins. [57]

Phylogenetic Profile

Functionally linked proteins tend to coexist during evolution of an organism[58]. In other words, if two proteins are functionally related, there is a chance of them to be inherited together during evolution process [55]. Thus, their corresponding orthologs in other genome will be preserved or removed. Therefore, phylogenetic profile can help us in detecting the presence or absence (co-occurrence) of proteins. An occurrence of a certain protein in a set of genomes can be described by phylogenetic profile.

Many genomic events canlead to disturbances in the phylogenetic profile and contribute to noise like gene duplication or loss of gene functions during evolution. Satisfactory results were obtained only on prokaryotesbutnotoneukaryotes by using phylogenetic profile based methods[59].

Gene Expression

In order to detect the transcription level of a whole genein a cell or a tissue, gene expression method can be employed. Under different experimental conditions and time intervals, the level at which a particular gene is expressed in a cell can be quantified. Different expression genes can be grouped together based on their expression levels, by using clustering algorithm. Investigating the relationship between gene co-expression and protein interaction is the ongoing research [60].

Recently, a novel method called PPI prediction by integration of Co-expression, Codon usage and Conservation data (PPIccc) is developed, in which PPIsprediction is based on integration of three descriptors (gene expression data, codon usage analysis and conserved regions of protein surface residues). PPI prediction is done using similarity between two genes based on their gene co-expression values, codon usage and identifying mutually-constrained surface residues between protein products of those two genes.

Protein-Protein Interaction Databases

In order to organize and process the massive quantity of data generated, construction of computer databases is done. Thebiomolecularinteraction network database (BIND) is created on a specification system that provides an elaborate description of the manner in which the PPI data was derived experimentally [61] .The database of interacting proteins (DIP) is another database of studying protein-protein binary interactions experimentally determined. [62] The biological general repository for interaction datasets (BioGRID) is a database that shows protein and genetic interactions among thirteen different species[63]. Menthaarchives evidence collected different sources, which offers eight interactomes. The total number of interactions in a species for a protein can be obtained from Hit Predict[64]. STRING is a database of protein-protein interactions, including direct (physical) and indirect (functional) associations. The Molecular Interaction (MINT) database is another database of experimentally derived PPI data extracted from the literature, with the added element of providing the weight of evidence for each interaction[65]. The Human Protein Interaction Database (HPID) was developed to provide human protein interaction information obtained from existing structural and experimental data[66]. To identify previously reported interactions in PubMed for a protein of interest, The Information Hyperlinked Over Proteins (iHOP) can be searched[67]. Interactome3Dprovides the structural annotation of PPI networks. IntAct[68] is an open source database for the storage, presentation, and analysis of protein interactions. The web server called APID (Agile Protein Interaction Data Analyzer) allows exploration and analysis of currently known information about protein-proteininter actions integrated an dunified in a common and comparative platform 69 . The Protein Interaction Network Analysis (PINA2.0) platform is a comprehensive webresource, which includes a data base of unified protein-proteininteraction data integrated from sixmanually curated public databases and a setofbuilt-intoolsfornet work construction, filtering, analysis, and visualization70. Pathway commons is a web resource for collecting and disseminating biological pathway and interaction data.

Conclusion

Predicting protein-protein interactions using conventional methods does not guarantee 100% accuracy. Therefore, computational toolsare applied to study these interactions. These interactions can then serve as a starting point for further lab experiments. Networks are constructed having alltheprotein-proteininteractions by using computational tools for signalling pathway identification and protein complex identificationins pecificdiseases.

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