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The Application of Modern Biotechnology in Protein Interaction Research-A Review

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Abstract

Intermolecular interaction is the material basis for cells to achieve their functions, and protein-protein interaction is an important approach to illuminate the regulation network of biological molecules and has important theoretical significance and potential application value for revealing the activity law of life in nature. This paper mainly summarizes and analyzes the new advances and applications of modern biotechnologies in the study of protein-protein interactions, including local surface plasmon resonance (LSPR), yeast two-hybrid, GST-Pull-down, bimolecular fluorescence complementation, and co-immunoprecipitation. At the same time, the principles of different research methods for protein-protein interaction and their other applications in the field of life sciences are also discussed, all of these will provide a reference value for the analysis of protein-protein interaction and the molecular regulation mechanism of biomacromolecules.

Keywords: Protein interaction; Biotechnology; Theory; Application; New progress.

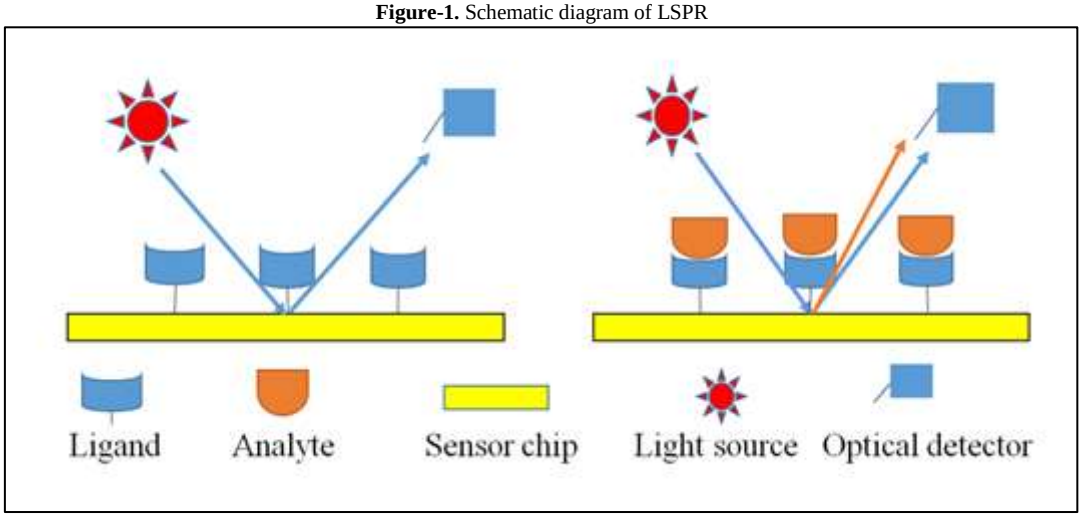
1. Introduction

With the development of life science, the study of molecular regulatory networks has gradually become a hot spot and focus of people's research, and the interaction between molecules is the material basis for the study of molecular regulatory networks [1]. Intermolecular interactions in the field of life sciences mainly include those between biomolecules such as protein-protein, protein-nucleic acid, protein-fat, etc [2]. In particular, protein-protein interactions have become a top priority in the study of molecular regulatory networks [3]. In the field of modern biotechnology, the commonly used methods to study and prove intermolecular interactions include yeast two-hybrid system, bimolecular fluorescence complementation, co-immunoprecipitation, GST-Pull-down, etc [4]. At present, the rapid development of these modern biotechnologies has led biotechnology to play an important role in the study of the interaction between biomacromolecules. For example, the Molecular Interaction Analyzer uses the principle of LSPR to detect the biomolecular interactions occurring on the surface of the sensor chip in real time. It has become a powerful tool to study the intermolecular interactions in vitro and reveal the regulatory network and mechanism of cell molecules [5]. LSPR technology has played an extremely important role in drug development, target protein determination, signal pathway research, genetic improvement of crop varieties and other fields [6]. Therefore, this paper summarizes the application of modern biotechnologies such as LSPR, yeast two-hybrid, GST-Pull-down, bimolecular fluorescence complementation and co-immunoprecipitation in protein interaction research, especially the new progress and application of these technologies in rice biomacromolecule interaction and its gene expression regulation, in order to provide new clues for revealing the molecular regulation mechanism of biomacromolecules by using modern biotechnology in the future.

2. Application of Local Surface Plasmon Resonance in Protein Interaction Research

When the incident photon frequency matches the overall vibration frequency of the conducting electrons of the nanogold particles, the nanogold particles will have a strong absorption effect on the photon energy and trigger the local surface plasmon resonance phenomenon(LSPR). In brief, LSPR is a resonance phenomenon. When light irradiates the metal surface, the free electrons in the metal outer layer are in a highly active state and collective oscillation occurs, while when the incident light frequency is consistent with the free electron resonance frequency, surface plasmon resonance occurs [7]. It is precisely because the electron resonance on the metal surface absorbs the energy of the incident light, so the energy of the reflected light will be greatly weakened to a certain extent. Because the propagation constant of surface plasma wave is very sensitive to the change of refractive index of metal surface, the refractive index of metal surface can be changed by certain means, and then the corresponding information of the material that changes the refractive index of metal surface can be obtained by the change of LSPR. For example, when the target in solution is connected with the LSPR sensor, the target is connected to the metal surface through the molecular recognition unit, which leads to the increase of the refractive index of its surface and the change of the propagation constant of the surface plasma wave. The change of the propagation constant will affect the effect of the surface plasma wave and the incident light, and ultimately lead to the change of the detection signal [8]. When a binding ligand is fixed on the surface of the sensor chip, and then the sample containing other binding molecules passes through the surface at a specific flow rate and time, when the molecules in the solution combine with the probe fixed on the sensor chip, the thickness of biomolecular layer on the surface of gold nanoparticles changes and the refractive index changes. This change causes the LSPR absorption peak to shift to a higher wavelength and is detected by a high sensitivity detector. These changes are proportional to the increased mass (bound biomolecules) on the sensor surface, so that various data and characteristics of the interaction can be determined, including affinity, dynamics (binding and dissociation rates) and specificity, etc. (Fig. 1). Therefore, the local surface plasmon resonance technology can be used to analyze the interaction between proteins and proteins.

Figure-1. Schematic diagram of LSPR



LSPR plays a very important role in the research of biomolecular interactions, such as pharmacology, proteomics and cell signal transduction. OpenSPR (LSPR) was used to detect the affinity of different surface nanoparticles with target protein TfR (transferrin receptor) in the absence/presence of protein crowns. The results showed that after the formation of protein crowns, the interaction of all nanoparticles with TfR decreased to the level of PEG-PN, and the ligands could be covered by protein crowns in vitro [9]. In order to investigate whether the inhibitory mechanism of TAD-1822-7-F2 and F5 is related to the interaction of JAK2 protein, the interaction between F2/F5 and JAK2 was detected by OpenSPR (LSPR). It was found that both F2/F5 and JAK2 have strong affinity. The anti-cancer targets and mechanisms of F2/F5 were quickly determined, which can effectively guide the drug use and how to eliminate some negative effects [10]. Some studies have found that melatonin, as a mitochondrial targeted antioxidant, promotes the accumulation of PINK1 on the mitochondrial membrane, enhances the accumulation and translocation of NRF2, and melatonin significantly improves the interaction between NRF2 and PINK1 [11]. The interaction of 11 polypeptides with CSFV YE2 protein was evaluated by Paecilomyces galactosus and FlexX/SYBYL programs, respectively, and the evaluation scores of all peptides were correlated with their KD values [12]. The results show that it is better to fully consider the flexibility of the peptide when performing peptide-protein molecular docking than to find more potential binding sites on the surface of the target protein. These validation data provide a reasonable reference for the molecular design of peptides and provide effective guidance for the functional localization of target proteins. Therefore, using OpenSPR (LSPR) can better detect the interaction between proteins and proteins, laying a solid foundation for the later study of protein function.

3. Application of Yeast Two-Hybrid Technology in Protein Interaction Research

Fields and Song established a yeast two-hybrid system as a classical and effective method for studying protein interactions in the study of transcriptional regulation of eukaryotic genes [13]. The basic principle is based on the characteristics of GAL4 transcription activator, that is, GAL4 transcription activator is a combination of two separate but functionally independent domains, namely DNA-binding domain (DNA-BD) and transcription activation structure (AD), which can reconstruct functional transcription factors and restore their original functions when they are close in space [14]. In the MATCHMAKER yeast two-hybrid system (Y2H), bait protein expression was fused to DNA-BD of GAL4, while prey protein expression was fused to GAL4 DNA-AD. When baits and prey proteins interact, DNA-BD forms functional transcription factors with AD and initiates the activation of downstream reporter gene expression. The interaction between the two proteins can be judged by detecting the expression products of the reporter gene [15, 16]. This method can be used to determine the interaction of new proteins, to analyze the interaction of two known proteins, and to analyze the protein domain of the interaction. On this basis, yeast one-hybrid, yeast three-hybrid and bacterial two-hybrid were extended [17].

The yeast two-hybrid technology is a relatively simple and fast method to study protein interaction, which is widely used to study protein-protein interaction. Liu *et al*. conducted a systematic protein-protein interaction analysis of 10 structural protein kinases and 9 abscisic acid-induced bZIPs using yeast two-hybrid technology, and identified 14 positive interactions, and SAPK10 may phosphorylate bZIP77 in vitro, providing a holistic view of the SAPK-bZIP interaction and providing new clues to the mechanism by which abscisic acid regulates flowering in rice [18]. OsFLN1 is localized in chloroplasts, and He et al. demonstrated that OsFLN1 and HSA1/OsFLN2 interact with thioredoxin to regulate chloroplast development by yeast two-hybrid experiments. The results showed that OsFLN1 and HSA1/OsFLN2 contribute to chloroplast biogenesis and plant growth [19]. Bello and Hou *et al*. reported the functional properties of the rice NF-Y heterotrimeric complex NF-YB1-YC12-bHLH144, which is composed of NF-YB1 and NF-YC12, and then sequentially combined with bHLH144, revealing a new pathway for grain quality regulation that has great potential for genetic improvement of rice by controlling the NF-YB1-YC12-bHLH144 complex [20]. Su et al. found that OsHAL3 acted as a positive regulator of flowering in rice. OsHAL3 overexpression lines exhibited an early flowering phenotype, while the downregulation of OsHAL3 expression caused by RNA interference delayed flowering under induced photoperiod. The change of flowering time was not

accompanied by the change of Hd1 expression, but with the decrease of Hd3a and MADS14 transcripts. OsHAL3 and Hd1 co-localized in the nucleus, andOsHAL3, a new light-responsive protein that plays an important role in photoperiod control, may be regulated by the formation of a complex with Hd1, while interacting in the dark and inhibited by white or blue light. Our findings open a new perspective for photoperiod control of flowering pathways [21]. Wang et al. screened GbVWR interacting proteins by yeast two-hybrid system. A total of 24 potential interacting proteins with GbVWR were screened, and the mechanism of cotton disease resistance mediated by GbVWR was preliminarily explored by analyzing the interaction relationship [22]. Xu et al. used yeast two-hybrid technology to study the protein interacting with the tumor suppressor gene LKB1. After yeast two-hybrid, 17 clones were obtained. After verification analysis, a non-repeat positive clone was finally determined. The obtained protein encoded by one gene may reveal a new mechanism of action of LKB1 [23]. Xu et al screened standard homogenized cDNA Library of human gastric mucosal epithelial tissues by yeast two-hybrid system, searched for proteins interacting with SHIP2, selected 39 positive clones, and verified by sequencing comparison analysis, reverse hybridization and immunoprecipitation test, finally identified a protein interacting with SHIP2, prohibitin1/PHB. All of those lay a foundation for further research on the molecular mechanism of SHIP2 molecule in gastric cancer to play a "tumor suppressor" role [24]. Therefore, by yeast two-hybrid technology, the relationship between proteins and proteins is analyzed, which provides important information for further revealing the function of proteins and their molecular regulatory mechanisms.

4. Application of GST Pull Down Technology in the Study of Protein-Protein Interaction

In recent years, glutathione S-transferase (GST) pull-down technique is commonly used to detect protein-protein interactions in vitro [25]. GST is used to establish GST gene fusion system, which is used to purify and detect proteins of interest. In a GST gene fusion system, GST sequences are incorporated into an expression vector next to the gene sequences encoding proteins of interest. By inducing the expression of fusion protein by vector promoter, the interested protein was fused with GST protein. The GST fusion protein can be purified from cells by high affinity with glutathione. Fusion protein provides an important biological detection of protein-protein interaction. For example, in order to prove that X (bait protein) binds to protein Y(target protein), a GST-X fusion protein can be constructed. The test beads coated with tripeptide glutathione strongly bind to GST fusion protein (GST-X), so if X can bind to Y, then GST-X can also bind Y, and Y will also exist on the bead. After that, GST-X was washed out by competitive binding with GST using eluent containing glutathione to capture the target protein Y. The interaction between proteins was detected by Western Blot、SDS-PAGE electrophoresis and mass spectrometry [26]. The pull-down technique is suitable for a wide range of interaction studies. Prey protein samples can be derived from purified protein and tissue lysates, or from protein samples obtained through expression system and in vitro translation system [27]. The purified protein sample was used for pull-down to remove the interference of other proteins, so the interaction relationship was more reliable and occurred directly. By fishing for prey protein or prey protein complex in mixed samples such as cell lysate, differential band proteins were separated by SDS-PAGE electrophoresis, and unknown interaction proteins could be identified by mass spectrometry [28]. It is worth noting that there are some limitations in pull-down Technology [29]. The structure and activity of decoy protein obtained by prokaryotic expression is not as good as that of eukaryotic expression system and natural protein. At the same time, some biological macromolecules that can interact in vivo may not be detected in the pull-down test in vitro.

At present, in vitro pull-down technology has been widely used in rice research, and has made a series of important progress. The hybrid sterility of Asian rice and African rice is mainly controlled by *S1* locus. Xie et al. found that three closely linked genes *(S1A4*, S*1TPR* and *S1A6*) in African *S1* allele (*S1-g*) constitute a killer protection system, eliminating gametes carrying Asian allele (*S1-s*) [30]. In the Asian African hybrid (S1-gS1-s), the interaction of S1TPR-S1A4-S1A6 in sporophyte tissue produces male and female gamete abortion signals. Knockout of any S1-g gene will eliminate interspecific sterility, while S1-s gametes will selectively abort due to lack of S1TPR. It is found that it will help to overcome the barriers of interspecific reproduction and increase the yield of rice through Asian African hybridization. Phosphate (Pi) is an important plant nutrient, and most phosphate starvation response genes are controlled by AtPHR1 and OsPHR2 proteins in Arabidopsis and rice, respectively. It has been reported that SPX domain is involved in the negative regulation of phosphate starvation response. Wang et al. found that SPX1 and SPX2 are phosphate dependent inhibitors of OsPHR2 activity in rice, and SPX1 and SPX2 inhibit their binding to P1BS through the interaction between SPX domain and PHR2, which provides evidence to support the participation of SPX1 and SPX2 in Pi sensing mechanism of plants [31]. GRAS protein family is a plant specific superfamily involved in plant growth and signal transduction. Zhang et al. found that a GRAS protein DHD1 delayed rice heading and increased rice yield. DHD1 interacts with OsHAP5cC/D in vitro and in vivo. DHD1 and OsHAP5C/D were located in the nucleus and showed rhythmic expression. Both DHD1 and OsHAP5C/D affect heading date by regulating the expression of Ehd1. Studies suggest that DHD1 interacts with OsHAP5C/D to delay heading stage by inhibiting Ehd1 expression [32]. Huang et al. validated the interaction between CIPK7 and CBL1 by GST pull-down test, and successfully constructed recombinant plasmids of CBL1 and CIPK7. Soluble GST-CIPK7 and CBL1-His fusion proteins were obtained by induced expression and purification. GST pull-down assay confirmed that CBL1 could bind to CIPK7, and there was a direct interaction between CIPK7 protein and CBL1 protein, which laid a foundation for further study of the function of protein kinase CIPK7 [33]. Hou et al. validated the interaction between influenza virus PB1-F2 protein and heat shock protein Hsp40 obtained by yeast two-hybrid screening using GST pull-down test in both positive and negative aspects in vitro. The in-depth study of the

interaction between PB1-F2 and Hsp40 protein will not only help to reveal the molecular mechanism of PB1-F2, but also laid a foundation for the study of the biological function of Hsp40 [34]. Hu et al. constructed recombinant prokaryotic expression vectors of NDV M gene and importinβ1 chicken gene, obtained the recombinant protein by prokaryotic expression system, and then verified the interaction between NDV M protein and importinβ1 chicken protein by GST pull-down technology [35], these studies laid a working foundation for further exploring the role of chicken importinβ1 protein in the nuclear localization of M protein and in the replication and pathogenesis of NDV. Therefore, pull-down technology is used to study the interaction between proteins and proteins, which provides important technical support for in-depth research in rice, chicken, virus and other fields.

5. Application of Bimolecular Fluorescence Complementation BiFC in the Study of Protein Interaction

Bimolecular Fluorescence Complementation (BiFC) is a new technology and strategy for studying protein interaction in living cells of plants in recent years, and has been widely used and studied in recent years. Bimolecular fluorescence complementation technology is to separate a complete fluorescent protein to form a C-terminal and an N-terminal, and then connect the two ends with the target protein to inject the carrier linking the target protein into the plant body. If the target protein interacts, it will be close to each other, thus forming a complete fluorescent protein, which re-fluoresces, demonstrating the interaction between the two proteins. Conversely, if there is no interaction between the two target proteins, the fluorescent signal will not be re-emitted. Baird et al. first proposed the concept of bimolecular fluorescence complementation, which refers to the phenomenon that when two proteins originally capable of interacting are connected with the N and C-terminal fragments of green fluorescent protein respectively to express the fusion protein in living cells, the N and C ends of the fluorescent protein will be close to each other, thus forming a complete fluorescent reaction of fluorescent protein [36]. A complete fluorescent protein was cut from the middle to form two polypeptide macromolecules, and then the two polypeptide molecules were linked with two leucine zippers that were known to interact with each other to form a pair of fusion expression vectors, which were successfully transformed into Escherichia coli. It was found that a yellow fluorescent protein fluorescent signal was found in the cell body, which proved that this interacted with the fusion expression vector. This technique was called bimolecular fluorescent complementation and could be used to verify the protein interaction [37]. In addition, it has been widely used by researchers because of its simple operation, simple principle, low experimental cost and less limitation. Walter et al. first applied the concept of bimolecular fluorescence complementation in plants to verify the interaction of two transcription factors [38]. Boruc et al. analyzed cyclin-dependent protein kinase complexes in Arabidopsis thaliana using bimolecular fluorescence complementation technology, and obtained 58 core cyclins and 357 interaction relationships [39]. Moreover, the characteristics of BiFC itself are very suitable for analyzing the interaction between viral proteins and viral proteins and between viral proteins and hosts [40]. Although many experimental methods have been discovered for protein-to-protein analysis, almost all of them need to destroy cells or indirectly to achieve detection purposes. BiFC technology can detect the interaction of target proteins and determine the location of target proteins very intuitively. Because when the activity of fluorescent protein is reconstructed, it can restore the characteristics of fluorescent protein and re-emit fluorescence, which can very intuitively reflect the interaction between proteins.

Bimolecular fluorescence complementation is a commonly used method to study protein interactions and is widely used in rice research.Hu et al. found a QTL locus *qTGW3* that controls grain size and weight in rice, and its encoded kinase OsGSK5/OsSK41 regulates the accumulation and transcriptional inhibitory activity of OsARF4 protein by interacting with and phosphorylating OsARF4 [41]. Inhibiting the function of OsSK41 or OsARF4 increases the grain size and weight of rice. Plant cytoplasmic male sterility (CMS) is the result of incompatibility between organelles and nuclear genomes, which prevents self-pollination and enables hybrid crop breeding to improve yield. Since the 1970s, Wild Abortive cytoplasmic male sterility (CMS-WA) has been utilized in most "three-line" hybrid rice production, but the molecular basis of this trait remains unknown. Luo et al. reported a new mitochondrial gene WA352, which confers CMS-WA to wild rice because its encoded protein interacts with the nuclear-encoded mitochondrial protein COX11 [42]. In CMS-WA, WA352 preferentially accumulates in the anther tapetum, thereby inhibiting COX11's role in peroxide metabolism and triggering premature tapetum cell death and pollen abortion, while WA352-induced sterility can be inhibited by two fertility restoration genes (Rf), which provide new insights into rice molecular design breeding. Hu et al. predicted the protein interacting with HspA1 in porcine Eperythrozoon by using bioinformatics database, and then used BiFC technologyto detect whether there was an interaction between HspA1 and DnaJ in 293T cells, laying a foundation for further in-depth study of the biological function and mechanism of HspA1 in porcine Eperythrozoon [43]. Using the Multisite Gateway complex vector construction technology, two fragments of the fluorescent protein molecule, N-terminal and C-terminal, were fused with Aspergillus oryzae Fus3 and Ste12 proteins, respectively, using the cut green fluorescent protein, and the obtained transformants were observed by fluorescence [44]. The interaction of proteins Fus3 and Ste12 in the asexually propagating strain Aspergillus oryzae was confirmed by BiFC technology, suggesting that they may participate in other cellular functions besides sexual reproduction through interaction, and providing a new detection technology and method for the study of protein interaction function of Aspergillus oryzae.

6. Application of Co-immunoprecipitation (Co-IP) in the Study of Protein Interaction

Co-immunoprecipitation (co-IP) is a technique that preserves bound proteins after complete cell lysis under non-denaturing conditions to detect whether two known proteins bind to each other in cells or to find new proteins that interact with known proteins [45]. Immunoprecipitation of intact protein complexes (antigens together with any protein or ligand bound to them) is called co-immunoprecipitation. By targeting known proteins with antibodies, Co-IP can pull the entire protein complex out of solution when proteins in the complex bind tightly to each other, thereby identifying unknown interacting proteins that bind to the complex. This pull-down of protein complexes from solution is sometimes referred to as a "pull-down". Co-IP is a direct and reliable technique used by molecular biologists to analyze protein-protein interactions. Co-IP can not only verify the interaction relationship between two known interacting proteins, but also screen the interacting proteins of the target proteins by mass spectrometry. The results of this method have the advantages of shorter time-consuming and reliable results than yeast two-hybrid, but also have higher requirements on the test technology [46]. Co-IP also has its limitations: different tags and vectors have an impact on protein expression levels. The protein expression level should be detected by Western before the formal test. The weak interaction between proteins may be destroyed during the experiment, and indirect interaction proteins can’t be detected.

Co-IP plays a very important role in studying protein interactions. Liu et al. found a transcription factor OsMADS1 encoded by qLGY3, a QTL locus controlling grain yield, in rice, and demonstrated by co-immunoprecipitation and yeast two-hybrid that two subunits of the βγ dimer of G protein, GS3 and DEP1, regulate the size and shape of rice grains by interacting with OsMADS1. The MAPK signaling pathway plays an important role in many processes of plant growth and development. Xu et al. discovered and revealed the OsMKKK10-OsMKK4-OsMAPK6 cascade-mediated signaling pathways controlling grain size and weight in Rice by studying the functions of OsMKK10 and OsMKK4 and their interactions [47]. Nucleotide binding sites are leucine-rich repeat (NLR) receptors that sense pathogen effectors and trigger plant immunity. Zhai et al. found that the PigmR gene locus encodes a group of NLRs, including PigmR, which confers broad-spectrum resistance to rice blast fungi, and discovered a RRM protein, PIBP1, which can specifically interact with PigmR and other similar NLRs to trigger resistance, and found that previously unappreciated RM transcription factors directly react with neutrophils to activate plant defens [48]. Chromatin immunoprecipitation (CHIP) assay was performed on transcriptional regulator Hac1 of Penicillium recumbens by optimizing the crosslinking time, ultrasound conditions, mycelial dosage and other conditions, and the specific DNA sequence bound by transcription factor Hac1 was successfully enriched, laying a foundation for the study of its regulation mechanism and biological function [49] .

7. Application of Bioinformatics in Protein Interaction

Bioinformatics is an interdisciplinary field, analyzing biological data by developing algorithms and software tools. It is an emerging interdisciplinary scientific field and a combination of science (biology, mathematics, computer science) and engineering to analyze and interpret biological data. Bioinformatics has been used for computer analysis of biological queries using mathematical and statistical techniques. Bioinformatics is an important part of its research direction mainly through the use of computer programming, in which the main areas of interest include: sequence analysis, gene and protein expression, cell tissue analysis, structural bioinformatics, and network and systems biology [50]. With the advent of the post-genomic era, the focus of bioinformatics research has shifted from genome sequencing to annotating the functions of sequenced genomes, that is, proteomics research, especially the interaction between proteins and proteins. Various life activities in cells are closely related to the interactions between proteins. Therefore, a deep understanding of protein interactions and elucidation of the whole proteome interaction network are prerequisites for revealing the mysteries of life activities [51].

With the rapid rise of bioinformatics, the application of bioinformatics technology in proteomics research has become more and more in-depth. Cheng et al. used homology modeling (Swiss-model) and fold recognition (Phyre2) to construct the structure of MBL2 protein and MASPs family proteins, respectively, and used STRING database and ZDOCK3.02 to predict and analyze the interaction of MBL2 with MASP1 and MASP2 proteins [52]. The results showed that MBL2 had direct interaction with MASP1 and MASP2, and formed interaction network with COLEEC11, COLEC10, FCN2, C4B, etc. Through bioinformatics analysis and reported research results, three proteins interacting with TaNRX1 were predicted [53]. Through Y2H and BiFC validation, it was finally determined that the interacting proteins of wheat nucleoredoxin TaN-RX1 were TaPDI, TaPP2Ac and TaTRX-h. The response of three interacting proteins to simulated drought stress was preliminarily analyzed by heterologous expression of yeast, and it was found that overexpression of TaPDI, TaPP2Ac and TaTRX-h contributed to alleviating the inhibitory effect of drought stress on yeast growth. Therefore, it is possible to preliminarily predict whether there is an interaction between proteins and proteins through bioinformatics strategies, providing important information for further validation of protein interactions.

8. Prospects

With the development of life science, the study of protein interaction has become the mainstream and hot spot in the study of biological macromolecule regulatory network. However, the technologies for studying protein interaction have their own advantages and disadvantages. Some technologies can complement each other, so they can combine multiple technologies to optimize the system, minimize interference, and then improve the accuracy of the results. With the development of bioinformatics and proteomics, the study of protein interaction has entered the

era of omics, and a large number of large-scale, multi-directional and short-time proteins have gradually become the targets of studying protein interaction. The rapid development of modern biotechnology, through the improvement of existing biotechnologies (such as local plasmon resonance, yeast two-hybrid, GST-Pull-down, bimolecular fluorescence complementation and co-immunoprecipitation), and the combination of different biotechnologies to accelerate the innovation of new technologies, will make the study of protein interaction more and more rapid and accurate.

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