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The Proteomics Research in Bovine

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Abstract: Proteomics is an important supplement for genomics research, every process of life is controlled by interactions between proteins. Proteomics is an objective, complicated, interlaced and precision controlled response network and is widely used in the study of animal development, physiological ecology and diseases, etc. Systems biology is used to sort and integrate these research results, furthermore explain the phenomenon of life at the protein level. This requires researchers of genetics, chemistry, biology, cell biology, engineering, mathematics, informatics and multi-disciplinary to make efforts collectively. In this study, researchers review the current status of proteomic technologies, discuss the research on proteomics in bovine and the development prospect of proteomics.

Key words: Proteomics, bovine, disease, genetic, bioinformatics

INTRODUCTION

In 1953, Watson and Crick's DNA double helix model was established which marks the arrival of the era of genes. After the Human Genomic Project (HGP) was completed (1990-2001) on February 15, 2001, an article was published in Nature "And now for the proteome from the Human Proteome" Organization (HUPO) (Abbott, 2001) and initiated the Human Proteome Project (HPP) which symbolized a mark of post-genomic era. Researchers have known there are three parts of the process from DNA, mRNA to protein: the transcription, translation and after translation, respectively. The expression of genes encoded in DNA begins with transcribing the gene into RNA then this transcript can be translated into protein, finally protein post-translational modification, showing the biological activity.

Protein is the executor of the physiological functions and directly manifeste of life phenomena. The study of protein structure and function will directly clarify the mechanisms of physiological or pathological changes in life. The form of the protein itself and the rule of the activity such as the modification after translation, protein conformation and interaction between proteins, etc., still rely on the direct study of protein to solve. Although, the variability and diversity of protein with special qualities cause the protein research technology to be much more complex and difficult than nucleic acid technology but it is these characteristics of protein participate and influence the whole process of life. The traditional approach to the study of a single protein has been unable to meet the requirements of the post genome era.

The concept of proteomics, first defined by Wasinger *et al*. (1995) referred to "the total proteins complement of a genome". Australian academic Wilkins (1997) was first used in the monograph and defined as proteins expressed by a genome or tissue. Now, proteomics can be understood as proteins of a biological, individual, organs, tissues, cells and body fluids expressed by the genomes (Kahn, 1995; Swinbanks, 1995; Marte, 2003).

Proteomics research including protein expression level, the translation, protein-protein interaction, etc. It should be pointed out that proteome are not a direct result of genome. He *et al*. (2002) in his book pointed out that the purpose of proteomics research is to find the pathogenetic mechanism of important physiological and pathological process. The amount of proteome is far more than the genome. Venter *et al*. (2001) speculated that the human genome's protein product categories, the results are shown in Table 1.

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Table 1: Speculated that the human genome's protein product categories

Protein species	Number	Percentage
Signal transduction proteins		
Signaling molecules	376	1.24
Receptors	1543	5.02
Kinase	868	2.83
Selective molecular switches	988	3.22
Nucleic acid binding protein		
Transcription factor	1850	6.02
Nuclease enzyme	2308	7.51
Transferase	610	1.99
Synthase, synthetase	313	1.02
REDOX enzyme	656	2.14
Lyase	117	0.38
Ligase	56	0.18
Isomerase	163	0.53
Hydrolytic enzymes	1227	3.99
Transfer/carrier protein	203	0.66
Viral protein	100	0.33
A mixture	1318	4.29
Cell adhesion	577	1.88
Molecular chaperone	159	0.52
Cytoskeleton binding protein	876	2.85
Extracellular matrix protein	437	1.42
Immunoglobulin	264	0.86
Ion channel proteins	406	1.32
Movement protein	376	1.22
Muscle binding protein	296	0.96
Light oncogene protein	902	2.94
Selective calcium binding protein	34	0.11
transport protein in the cell	350	1.14
Transport protein	533	1.74
Molecular functions of unknown protein	12809	41.70

A Protein-Protein Interaction (PPI) is a specific type of molecular function, controlling virtually every metabolic activity such as proliferation, differentiation, apoptosis and aging of cell, etc. The amount, type and timing of proteins expressed in the vital movement are different so body can react to the external environment stimulation (physics, chemistry, physiology, pathology, signal) to form gene buffer so as to adapt to environmental changes. Interactions between proteins are important for the majority of biological functions. It is an objective, complicated, interlaced and precision control response network. Therefore, it is a very difficult work to get a whole proteome in the cell. Humphery-Smith *et al.* (1997) defined the functional proteome as the proteins expressed by genome at a specific time, specific environment and under experimental conditions.

THE PROTEOMICS RESEARCH CONTENT

Proteomics research has penetrated into clinical medicine, preventive medicine and basic medicine, pharmacy, zoology, botany, plant pathology, nutrition and many other fields. Proteomics mainly divided into three directions: the mass of protein identification and fine feature analysis of processing modification after translation which is called constitutive proteomics, analysis of protein express with differential display method, defined as differential display proteomics also called comparative proteomics or expression proteomics and the application of mass spectrometry analysis or yeast two hybrid method to study the interaction between the proteins, drawing of Protein Interaction Networks (PINs) which is called interaction of proteomics. In 2000, it was a historic moment, the first network of large-scale protein-protein interactions was accomplished in the yeast *Saccharomyces cerevisiae* (Uetz *et al.*, 2000). The functional assignment of cell has progressed considerably by partnering proteins of similar functions.

Proteomics research generally divided into three steps: first, the proteome separation technology. Mainly with two-dimensional gel Electrophoresis (2DE) technology because of its advantages of high resolution is now being widely used but there also has short comings such as hard to detect low abundance proteins, extreme alkaline protein and hydrophobic proteins. The Nonequilibrium pH Gradient Electrophoresis (NEPHGE) is mainly used in alkaline protein separation. Blue Native-PAGE (BN-PAGE), it is mainly used for membrane protein complex and some big molecular weight proteins separate. In addition, researchers use some other technologies such as two-way high column chromatography technology and liquid column chromatography separation technology.

Second, the protein identified. In the late 80's the basis of the two technologies Matrix-Assisted Laser Desorption Ionization (MALDI) and Electro Spray Ionization (ESI) (Fenn *et al.*, 1989; Karas and Hillenkamp, 1988), developed Matrix-Assisted Laser Desorption Ionization (MALDI-TOF-MS) which is the most common analysis technology. Application of mass spectrometry can get protein Peptide Mass Fingerprinting (PMF) and then analysis to identify proteins. Now some separation and identification technology of combining the method is an effective method in practice such as 2DE and Western blotting technology of combining the method of Serum Proteomics Analysis (SERPA) has always lived, widely used in the screening of proteins associated with cancer. Protein chip and protein microarray is now used more protein chip combined with mass spectrometry of Surface-Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry, (SELDI-TOF-MS) technology. Biomolecular Interaction Analysis (BIA) combined with MALDI-TOF-MS form bio-sensor chip mass spectrum can be used in the functional properties of protein research and analysis of protein structure. Biochemistry type protein chip, combines known bioactive molecules to the surface of a chip to capture the target protein in the samples. Isotope label Relative Absolute Quantitative

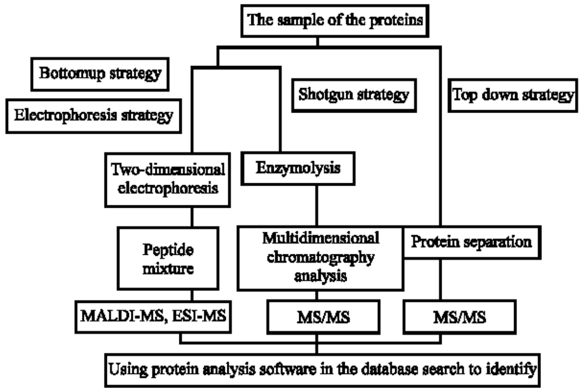

Fig. 1: The research of the bioinformatics and proteomics technology (Simpson, 2003); proteins and proteomics; a laboratory manual

(ITRAQ) technology to the relative quantitative MS/MS cans peptides, secondary mass spectrometry or tandem mass spectrum identification (Faulkner *et al*., 2012; Timothy *et al*., 2013). ITRAQ technology can detect the low abundance proteins, strong alkaline protein, <10 or >200 kD.

Third, the research of the bioinformatics and proteomics technology inculd set up proteomics bioinformatics database and appraisal and analysis the result with the application of bioinformatics. The specific process can be represented in Fig. 1.

THE PROTEOMICS IN QUALITY OF MILK

Milk has high nutritional value and is relatively expensive. In order to more accurately detect the adulteration in milk (Cosima *et al*., 2013) analyzed the trypsin digestion of protein in the fresh milk, milk and milk powder by using MALDI-TOF-MS. Rapid detection procedures of liquid milk established can detect the level up to 1% adulteration milk powder.

To understanding of the mechanism of estrogen to milk and milk protein, the proteome changes of Dairy Cow Mammary Epithelial Cells (DCMECs) were studied under the influence of estrogen (Huang *et al*., 2012). He selected including glycyl-tRNA synthetase belonging to the class-II aminoacyl-tRNA synthetase family, proteins involved in other cellular functions such as translation initiation factors, GTP-binding nuclear proteins, heat-shock proteins and proteins belonging to ubiquitin-proteasome system. Cow Milk Fat Globules (MFGs) have peculiar vesicle nature makes them an easily available source of biological material in monitoring the physiopathological state of the mammary gland. The research deals with the development of a suitable procedure for protein extraction from the cow Milk Fat Globules (MFGs) in order to qualitatively and quantitatively improve 2D electropherograms of the MFG (Bianchi *et al*., 2009) and the identifications were represented by proteins involved in lipid synthesis or in fat globule secretion. Based on the transcriptome and proteomics technology, probiotic *Lactobacillus casei* Zhang of growth mechanism in milk and soymilk was studied and milk of dairy colostrum and milk protein changes were studied (Wang *et al*., 2012; Yang *et al*., 2010a-e). Coscia *et al*. (2012) used proteomics technology to researched the milk protein and microconstituents. Zang *et al*. (2012) and then studied the milk protein changes under the milk heat treatment. Yang *et al*. (2013a) used the iTRAQ technology researched and analyzed cow, yak, water buffalo, goats and camels' milk whey protein, 211 proteins have been identified and 113 proteins have been categorized. The results showed that significant differences in proteomic patterns among five differentia milk and 177 differentially expressed proteins were submitted to advance hierarchical clustering. Li *et al*. (2013) used of proteomics strategies to studied the mechanism of rat intestinal lymphatic organization absorptive milk protein found that seven of the nine identified bovine-specific proteins are allergens in milk. He pointed out that most proteins identified in lymph were highly abundant proteins in the milk such as lactoglobulin and caseins.

THE PROTEOMICS IN THE CATTLE BREEDING

In the past few decades, researchers have observed that milk production and fertility exist negative correlation. At present, the most used method to measure the bull fertility is the Non-Return Rate (NRR) and Estimated Relative Conception Rate (ERCR). Researchers want to know whether use proteomics can find protein markers to speculate the bull fertility. Based on the different levels of field fertility expressed of 16 bull sperm proteomes were analysis (Soggiu *et al*., 2013) found that α-enolase was significantly down-regulated in the ERCR group while two other proteins, isocitrate dehydrogenase and triosephosphate isomerase were up-regulated in ERCR in comparison to ERCR+. It is possible that these protein markers associated with fertility. Frozen semen will lower the fertilization ability of sperm and accompanied by the change of protein decrease, expression, function, etc. Therefore, researchers committed to using proteomics techniques to clarify the mechanism of freezing injury and reveal the cryopreservation which caused the protein structure and function change.

Sooner or later, proteomics may open wider ideas and solutions and help researchers apply of frozen semen (Chen, 2008; Han *et al.*, 2009a, b; Li *et al.*, 2010). In order to solve the problem of sex of frozen semen's low breeding rate, the cow X, Y sperm comparative proteomics is good exploration (Han *et al.*, 2009a). In buffalo research, researchers have studied the water buffalo sperm proteome and identified the differentially expressed protein between the high rate of deformity and normal sperm (Wen *et al.*, 2012; Li *et al.*, 2012). Someone analyzed before and after maturation water buffalo oocytes and buffalo follicular fluid differential proteome (Liu *et al.*, 2012a, b; Fu *et al.*, 2012). In addition, Shamsi *et al.* (2011) find that differential protein expression existed in the tear among species, this research offered useful information for further study on tear proteins and the related ocular diseases.

THE PROTEOMICS IN CATTLE DISEASE

First of all researchers need to have a basic understanding normal cow proteome in different tissues and cells so as to continue to take further study the effect of disease in cattle proteome. Researchers studied the GSFs cell proteome; cow serum proteome by optimized of two-dimensional gel electrophoresis; the cow breast tissue and plasma proteome, different number of somatic cells in milk dairy protein, respectively (Wang *et al.*, 2006a; Lili *et al.*, 2009; Yang *et al.*, 2007a, b, 2010a, 2011a, b).

Wu *et al.* (2010) applied strong cation exchange capillary liquid chromatography-antiphase pressurized capillary electrochromatography two-dimensional system to analyze the cattle blood innocence proteolytic digestion. Li *et al.* (2011) has carried on the preliminary study to perinatal cow's serum proteomics. Lu *et al.* (2013) studied the dry milk issue related to the energy balance in cow's milk protein and metabolic changes. Liu *et al.* (2012a) used optimized two-dimensional electrophoresis method to research the cow mammary gland epithelial cells proteome and based on this study further research the Traditional Chinese Medicine (TCM) Cowherb seed how to influence of the cow mammary gland epithelial cells (Liu, 2012). The cow mammary gland epithelial cells and subcellular proteome were studied (Wang *et al.*, 2010a; Jin *et al.*, 2012; Huang *et al.*, 2011). The proteins selected in these experiments would be helpful to further studies on bovine mammary epithelial cells proliferation and lactation. D'Amato *et al.* (2009) studied combining the peptide ligand library whey proteome. Klein *et al.* (2013) studied the correlations between milk and plasma levels of amino and carboxylic acids in dairy cows.

Mastitis is the main diseases affecting dairy production. A lot of proteomics research have been done (He *et al.*, 2007; Yang *et al.*, 2007b; Zhang *et al.*, 2009; Yang *et al.*, 2010b). Researchers studied the *E. coli* mastitis (Yang *et al.*, 2010c) and *Staphylococcus aureus* mastitis by proteomic analysis (Yang *et al.*, 2011b; Reinhardt *et al.*, 2013). The sub-cellular proteomics play an important role in study of minor proteins which participating the intracellular metabolism. The clinical proteomics were used to study subclinical and clinical mastitis cows and screening the potential protein biomarkers from the different pathogens infection (Liu *et al.*, 2012a, b; Wang *et al.*, 2010b, 2012), suggested nuclei of mammary gland had experienced fundamental changes in structure and metabolism when contracted clinical mastitis. Proteomics provides a better research direction to understanding the pathogenesis of mastitis (Yang *et al.*, 2009, 2010e, 2012; Turk *et al.*, 2012).

Bacterium burgeri is a kind of gram-negative short coli. Br. Bovis can cause female infectious abortion. Brucella is one kind of comorbidities intracellular bacterial pathogens. Brucella virulence depends on its ability to transition to a host cell. Therefore, the pathogen must detect the intracellular localization and then to adjust gene expression within the host cell. Bioinformatics retrieval showed that the proteins were mainly associated with the energy metabolism, protein and amino acid syntheses, fatty acid metabolism as well as saccharide and coenzyme syntheses of brucella. Some high immunogenic membrane proteins were successfully screened from *Brucella melitensis* by immunoproteomics which provided a large quantity of candidate antigens for preparation of brucella subunit vaccine (Wu, 2008; Tang *et al.*, 2010; Yang *et al.*, 2010c; Tao *et al.*, 2012; Roset *et al.*, 2013).

Guo (2009) studied the cattle brucella weak poison vaccine strain s19 and build Δ s19 all mycoprotein by comparative proteomics. Zhao *et al.* (2012) analyzed 544 A macrophage proteomics of the infected cow. The cow's other clinical diseases were researched such as cow milk fever proteomics (Xia *et al.*, 2010); laminitis cows proteomics (Gao *et al.*, 2012; Dong *et al.*, 2012; Sun *et al.*, 2013); tuberculosis bacilli proteomics (Hu *et al.*, 2008). You *et al.* (2012) analyzed cows with vice TB plasma samples by MS/MS, screening six kinds of proteins which were raised more than two times including: transferrin, gelsolin isoforms and (Actin Binding Protein-ABP), complement subcomponent C1r, complement component C3, Amine Oxidase-copper Containing 3 (AOC3), coagulation factor II (thrombin) ($p<0.05$). Two lower expressions were Coagulation Factor XIII-B polypeptide

(COAFXIII) and fibrinogen. Prasad *et al.* (2013) analyzed the mycobacterium tuberculosis purified protein derivative of proteomics. Two-dimensional electrophoresis combined with Western blot were used to screen cattle mycoplasma immune related proteome (Chen *et al.*, 2012). Effect of Ure B subunit vaccine immunization on serum proteins in dairy cows was studied (Yuan *et al.*, 2012a; Li *et al.*, 2011) studied on changes of plasma proteome of dairy cow injecting lipopolysaccharide into external pudic artery. And 8 protein spots were identified to be 4 proteins including vitamin Dbinding protein precursor, serpin A3-6, alpha-1 antitrypsin and serpin A3-1 precursor. He suggested that vitamin D-binding protein precursor, serpin A3-6, alpha-1 antitrypsin and serpin A3-1 precursor played important roles in immune response, the identification of these proteins may be helpful to elucidate the molecular mechanism of host response to LPS challenge. A development of a Stress-Inducible Controlled Expression (SICE) System in *Lactococcus lactis* for the production and delivery of therapeutic molecules at mucosal surfaces were studied (Benbouziane *et al.*, 2013).

Ketosis is a common metabolic disease in cow. Researchers have known the ketosis diagnostic markers such as acetone and β-Hydroxy Butyric Acid (BHBA) but the disease prediction is still an unsolved challenge. Wang studied the pathogenic mechanism of ketosis and discovered proteins play a role such as energy metabolism, the degradation of carbohydrates and fatty acid metabolism, amino acid metabolism, antioxidant, cell structure, nucleotide metabolism and metabolism related protein. Klein *et al.* (2011) researched milk proteomics, founded that in the whole nursing, high milk Glycerophosphocholine (GPC) levels and high ratios of GPC to Phosphocholine (PC) allow for the reliable selection of healthy and metabolically stable cows for breeding purposes.

Pneumonia and diarrhea are the cow's common disease take place in the transportation. Cow serum proteome was studied before and after of transportation (Yuan *et al.*, 2012a, b). Senthilkumaran *et al.* (2013) collected 162 head calf after transport of Bronchoalveolar Lavage Fluid (BALF). The result showed that the low level of membrane protein A1 and A2 is the potential biomarkers of the weaned and transport fattening cattle pneumonia. Hand, foot and mouth disease is a highly contagious viral disease, infect wild and domestic cloven-hoofed animals. The complex relationship of FMDV and with the host cells leads to its replication and spread. BHK-21 cell line is an *in vitro* model for FMDV infection and is commonly used for viral seed preparation. In order to better understand the molecular basis of this relationship (Zhang *et al.*, 2010) made a proteomics study on baby hamster kidney cells infected with FMDV was performed. Mass spectrometry identified 30 altered protein spots (19 up-regulated, 9 down-regulated and 2 viral protein spots) which included metabolic processes proteins, cytoskeletal proteins, microfilament t-associated proteins, stress response proteins and FMD viral proteins. Western blot analysis further confirmed the differential expression of protein NME-2 in the proteomic profiles. Subcellular location demonstrated NME2 protein was distributed in BHK-21 cell cytoplasm and nucleolus.

Claw Horn Disruption (CHD) is a common underlying cause of lameness in dairy cattle which leads to compromised animal welfare and production losses. Tolboll *et al.* (2012) wanted to provide a relevant functional annotation of the proteins characterized in three different bovine claw tissues. A total of 388 different proteins were identified with 146 proteins available for identification in C, 279 proteins in D and 269 proteins in L. Three hundred and sixteen of the identified proteins could be subsequently grouped manually to one or more of five major functional groups related to metabolism, cell structure, immunity, apoptosis and angiogenesis.

THE PROTEOMICS IN CATTLE FOOD AND NUTRITION

With the development of proteomics, people want to further understand the effects of nutrition on proteome through this technology. Wang *et al.* (2012) studied the influence of proteomics on cow mammary gland epithelial cells by methionine. Aminophenylboronic acid interact with bovine serum albumin was studied (Wang *et al.*, 2006b). Low dose lipopolysaccharide perfusion was studied influence of cow serum proteome (Yuan *et al.*, 2012a). Yang *et al.* (2012b) analyzed the composition of the diet effects on rumen cow nipple protein expression. The results showed that acyl-CoA synthetase family member 2, hydroxymethylglutaryl-CoA synthase, peroxiredoxin-2 and voltage-dependent anion-selective channel protein 1 were up regulated in response to high concentrate diet while keratin 6A and Larva-specific Keratin (RLK) were up regulated in response to low concentrate diet. The identified proteins were mainly associated with functions related to stress, metabolism and signal transduction. Based on these findings, it was concluded that the changes of rumen papillae proteins affected by dietary composition that mediate rumen epithelial adaptation to dietary change. Zhang *et al.* (2010) founded that injecting V-AD3E solution into the periparturient dairy cows prior to calving up-regulated IgG and albumin levels in colostrum which besides transferring higher levels immune constituents to the offspring, fed the colostrum also promotes development and digestive tract protection in the neonate and defends the cows who are in immune suppression period against the invasion of pathogenic bacteria, fungi and virus. Kuhla *et al.* (2010) studied the hypothalamus-pituitary system to control the dynamic balance in the process of feed energy conservation and emissions reduction. Yang *et al.* (2013a, b) studied the duodenal infuse alpha linolenic acid effected on lactation cows plasma and milk proteome.

Researchers already know that high producing dairy cows in the early lactation can't get enough of feed to meet the nutritional needs. As a result, they are in negative energy balance and mobilizing body reserves including muscle protein, direct oxidation, glyconeogenesis. How to better understand and clarify the process of the change (Kuhla *et al.*, 2010) used 2DE and MALDI-TOF-MS analysis identified 43 differentially expressed muscle protein spots throughout the periparturient period. In early lactation, expression of cytoskeletal proteins and enzymes involved in glycogen synthesis and in the TCA cycle was decreased whereas proteins related to glycolysis, fatty acid degradation, lactate and ATP production were increased. And they proposed a model in which the muscle break down in early lactation provides substrates for milk production by a decoupled Cori cycle favoring hepatic gluconeogenesis and by interfering with feed intake signaling. Moyes *et al.* (2013) used iTRAQ identification of hepatic biomarkers for physiological imbalance of dairy cows in early and mid lactation. The result showed that pyruvate carboxylase and isocitrate dehydrogenase as potential hepatic biomarkers for Physiological Imbalance (PI) for cows during early lactation and alcohol dehydrogenase-4 and methylmalonate-semialdehyde dehydrogenase for cows in mid lactation. It provided a better understanding of the differences in coping strategies used for cows in PI.

PROSPECTS

Life science is an experimental science to prove the life science depends on the development of experimental technology. Although, MS-proteomics technology trend to mature and started by the instrument, sample preparation and calculation analysis of the combination of diversified development pattern but still need technical breakthrough to promote the development of proteomics (Altelaar *et al.*, 2013). Currently, there is no similarly a protein amplification technique like nucleic acid amplification of PCR, the detection of trace proteins is still a problem. Compared to the single subject on the other hand, it is difficult to interpret protein complex, dynamic, causal relationship. How to make this vast, abstraction and abound change problem analysis, Western classical process of scientific development is to divide the complicated things and to establish different models with different respective disciplines tools to study the details of a particular change, finally use the comparative method to analysis again. When accumulated to a certain extent of these studies and the concept of system causal hypotheses are put forward basis on certain theory. In the process of continuous screening repeated verification, put forward the hypothesis and to explain the changes of things. Use the classic theory of a subject (from another subject) in the study, tend to get a new awake. For example in the 19th century Hermann von Helmholtz was introduced first law of thermodynamics in the study of energy metabolism. It was laid a theoretical foundation for the research of biological energy balance in the body. And researcher can change the thought of the corresponding research train (DeLisi, 2004).

CONCLUSION

Therefore, it is not hard to see the new theory and the rational hypothesis are more than research technology innovation can lead the development of relevant disciplines. With the development of the discipline there is more and more discipline branch. Proteomics provides a new train of thought and through it researchers can directly and comprehensively applying interdisciplinary theory. Personal power is limited, scholars need more efforts to build the open, divergent and innovative research teams or organizations and carry out technical exchanges.

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