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Transcriptomics: A time-efficient tool with wide applications in crop and animal biotechnology

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Abstract

Transcriptomics is the study of RNA profile inside the cells at a given time point. Besides coding RNA, cells have the important regulatory non-coding RNA sequences also. It is not as simple as thought to study the transcriptome of a cell owing to its complexity. But, the recent advances in transcriptomic technologies have made it possible to characterize the transcriptome of a living cell and unravel molecular basis to help in the strategic improvement of both crop and animal production. Transcriptomics in recent years has extended to the vast range of agriculture and animal systems being studied thereby bridging the gap between the two sectors. There is always a scope to introduce newer methods to cover up the shortcoming of earlier technologies and allow for more detailed information of basic research questions.

Keywords: transcriptomics, crops, animals

Introduction

The concept of the central dogma of molecular biology which explains the basic idea of life and flow of genetic information within a biological system was given by Crick in 1970 (Crick, 1970) [5]. It essentially means that the genetic information encoded in DNA is transcribed to RNA, and RNA is translated to protein. Various forms of life including the biological activities of cells, tissues, and organisms are based on the central dogma of molecular biology where the key player required for mediating gene and protein expression is RNA itself. So, RNA plays a major role in deciphering the message encoded in DNA. There are two types of RNA inside the cell, noncoding RNA (ncRNA) and messenger RNA (mRNA or protein-coding RNA). Non-coding (ncRNAs) which were previously thought to be a part of junk genome play several key roles in gene regulation including transcriptional and posttranscriptional regulation (Peretea, 2012) [17]. This discovery was possible only due to the advent of transcriptomics which studies the whole transcriptome inside the cell (Bussotti and Leonardi, 2016) [3]. Regardless of the apparent similarities of RNA from animals and plants and their critical role in development, there is a slight difference in animal and plant transcriptome. The unique feature is that plants have shorter introns and they retain those introns during the alternative splicing events (Reddy, 2007) [20]. Genome-wide analysis of alternative splicing in model plants such as Arabidopsis (Marquez *et al.*, 2012) and in crop plants such as rice (Zhang *et al.*, 2010) [29] and soybean (Shen *et al.*, 2014) [22] has revealed that plant genes have one or more alternative transcript isoforms.

It is estimated that more than 90% of the genome inside cells is transcribed into RNA, where approximately 60% of all the transcribed RNA is the primary transcripts of the genes (Peretea, 2012) [17]. In this review, we describe the general application of transcriptomics in the field of crop and animal production improvement.

Before transcriptomics

Due to the complexity of the transcriptome of the living cells, it was challenging to study the genome behavior during earlier days. However, before the advent of transcriptomics to study the genes, several studies of individual transcripts were being performed. In, late 1970s cDNA libraries began to be formed with libraries of silk moth mRNAs collected and converted to complementary DNA (cDNA) for storage using reverse transcriptase (Sim *et al.*, 1979) [23] was first reported. In, the 1980s, low-throughput Sanger sequencing took over to sequence random individual transcripts from these libraries, called expressed sequence tags (ESTs) (Putney *et al.*, 1983) [18] which were employed to quickly categorized expressed genes and gene fragments. Scientists then started quantification of individual transcripts by northern blotting, nylon membrane arrays, and later reverse transcriptase quantitative PCR (RT-qPCR) began to be popular (Becker-Andre and Hahlbrock, 1989) [2]. However, these methods were difficult and captured only a small subsection of a transcriptome.

Development of high throughput techniques to study transcriptomics on a larger scale.

To make up for the demerits of the older technologies, tag-based methods took over such as serial analysis of gene expression, cap analysis of gene expression, and massively parallel signature sequencing. Nevertheless, these methods also failed to differentiate between genetic isoforms and were expensive to apply on a large scale. This led to the development of microarray (Grunstein and Hogness, 1975)^[7], which was used for genome-wide analysis and now has become the most widely used approach for transcriptomics. Both spotted oligonucleotide arrays and Affymetrix (Santa Clara, California) high-density arrays were the method of choice for transcriptional profiling until the late 2000s (Nelson, 2001). Although the technology was widely accepted, any discussion regarding microarray technology would be incomplete without a comprehensive examination of its various pitfalls and complexities innately presented. There are numerous sources of inconsistency in microarrays such as differences in arrays, dye labeling, efficiency in reverse transcription, and hybridization (Quackenbush 2001)^[19]. Recently, RNA sequencing (RNAseq) using next-generation sequencing technology has permitted the transcriptome to be characterized, and the number of studies using RNA-seq have gradually increased which ultimately covers the bias introduced by microarray (Yu and Lin, 2016)^[28]. Despite these minute shortfalls, the application of these tools has potentials to unravel the molecular basis of crop and animal production systems which will be instrumental for strategic improvement in both sectors. These tools allow researchers to simultaneously examine the expression of a large number of genes. Several studies have compared the accuracy of microarray and RNA-seq measurements (Su *et al* 2014)^[25] which is not the focus of the current review.

Transcriptomics: opportunities, applications, and research

Transcriptome study provides an essential platform to analyze the association between genotype and phenotype, which gives a better understanding of the underlying pathways and mechanisms controlling cell fate, development, and disease progression (Ruan *et al.*, 2004)^[21]. In, last few years with technological advances, researchers can use high throughput whole genome studies to an increasing range of species and extensively apply it in agricultural and biomedical research (Han *et al.*, 2015)^[8]. These technologies have alleviated the complexity of the methods used for characterization and quantification of genomes, epigenomes, and transcriptomes over the last few years. In effect this means that in the span of a relatively short period of time these techniques, once limited to a handful of species with completed genome sequencing, are now used for studying a diverse spectrum of biological systems. But to be both comprehensive and logical, we will limit the scope of this review to agricultural and animal systems only.

Transcriptomics: Bridging the gap between plant and animal science

From past a decade or more, number of agricultural researchers have made use of advances in next-generation sequencing techniques enabling them high- resolution linkage studies between gene variants and traits of interest (Imadi *et al.*, 2015)^[10] resulting in an upsurge in the scope of transcriptomic studies, not only in number but also in the range of agricultural systems that are being studied. The geo-climatic conditions have seen a dramatic shift in the recent

past which has led to an increase in environmental pressure. The result is an urgent need to accelerate breeding novel crops with more heat tolerance and higher disease and pest resistance to cope up with the challenges posed by the climate change. Advances in genomics offer the potential to speed up the process of developing crops with promising agricultural traits. Traditional or conventional plant breeding involves the exchange of genes between equally closely related species and is a difficult and laborious approach. To do away with these obstacles, molecular breeding through gene manipulation has widely been used to develop new high-yielding, stress tolerant crop varieties (Kissoudis *et al.*, 2014)^[12]. To date, the genome sequences for more than 55 plant species (not only model but non-model plants) have been produced (Wang *et al.*, 2017)^[26]. As a result, scientists are not totally reliant on genomic sequences of model plants to do research on non-model plants. Recent advances in transcriptomics have allowed researchers to describe the metabolites, transcription factors, and stress-inducible proteins involved in heavy metal tolerance in plants which may be useful in producing heavy metal-tolerant crops (Singh *et al.*, 2016)^[24]. With transcriptomics, it's possible to map the complex transcriptional changes that occur in the leaves of maize plants grown in acidic soil with phytotoxic levels, thus, indicating the importance of tolerance mechanisms working in the plants (Mattiello *et al.*, 2014)^[15]. Transcriptomics study vis-à-vis medicinal plants is gaining a strong foothold and has become the most active tool in medicinal plant genome research. It offers a complete understanding of gene expression and its regulation which has great implication in solving the questions of genetic evolution, genetic breeding, and ecology of medicinal plants (Wang *et al.*, 2015)^[27]. The biggest calamities which the world is facing right now are of food shortage and spread of diseases. To improve the nutritional quality of plant-based foods, the use of genetic manipulation and molecular breeding is an attractive and potentially useful contribution. This enables researchers to tackle these twin global health burdens of micronutrient deficiencies and diet-related non-communicable diseases (Martin *et al.*, 2011)^[14]. Transcriptomics enables us to study more about the genes and biochemical pathways that are essential for additional health benefits, moving beyond basic nutrition and into the development of functional foods, for example, seed oils that do not produce trans-fats but rather contain heart-healthy omega-3 fatty acids or cassava melons with increased protein content to help fight malnutrition. Improvements in protein quality and content for better human and animal nutrition increased vitamin and mineral levels to address nutrient deficiencies, and reduction of allergens and of anti-nutritional substances that diminish food quality can all be explored through omics (Ibanez *et al.*, 2013)^[9]. So, transcriptomics provides a stage of high-quality research on plants for health benefits of their dietary components for both animals and humans and in the process provides a vital link to relate agricultural and animal products use.

Domesticated animals have been selectively bred over thousands of years for different uses and environments, resulting in a wide variety of morphology and behavior (Zinsstag *et al.*, 2011)^[30]. In reality, for some traits, the variation observed is much greater than that found in laboratory or wild species. With the use of transcriptomics, many researchers have discovered candidate genes to be selectively bred for improving milk production (Canovas *et al.*, 2010)^[4], meat quality (Gorni *et al.*, 2011)^[6] in farm animals, egg production in chickens (Jonchere *et al.*, 2010)^[11]

and understanding the pathogenesis of complex diseases. With these technologies in hand, one system that has been well studied is a host-pathogen relationship in terms of immune response to various pathogens. We have recently shown how insecticide fipronil alters the transcriptome profile in lungs of mice using microarray approach (Pandit, 2018)^[1]. Transcriptomics research in domesticated animals has been used to study their acclimatization in different environments and stress conditions. It is likely that the outcomes of transcriptomic studies will influence how animal and plant productivity is managed, partly by producing new species and condition-specific plants as a diet for production animals.

Challenges and future viewpoints

Currently, microarray technology is a valuable biomedical tool and finds its use in a variety of diagnostic procedures. However, the very concept was not received well by many researchers during its beginning, and the technology had faced some challenges. Microarray data reliability and its clinical utility were criticized in the earlier days. Most of these challenges have been addressed now, and the credit goes to the advances in the original technologies as well as the incredible effort presented by the research community and commercial sellers. Currently, the microarray platform has achieved adequate calibration and method validation as well as well-organized probe printing, liquid handling, and signal visualization. But still, the uphill task with microarray technology is that is limited species coverage. It is available for model species only. This drawback has been covered by recent developments of high-throughput or next-generation sequencing (NGS) technologies which have greatly facilitated the study of transcriptomes, also in non-model species. Most of the transcriptome studies are done on tissue samples which have a different kind of cells and results in a mixture of transcripts which means that specific information on gene expression in certain types or subpopulations of cells is lost. This demands technology enabling gene expression profiling in single cells which could be a major advancement for biology. These technologies would allow for a more detailed information of basic research questions about different cell types, and their interaction with each other as well as throws more light on the selective effect of the different pathogen on certain cell types than the other.

References

- Pandit AA. Transcriptional profiling and quantification of genes associated with pulmonary immunomodulation n response to fipronil and endotoxin in interaction. Ph.D. (ABT) Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, 2018.
- Becker-Andre M, Hahlbrock K. Absolute mRNA quantification using the polymerase chain reaction (PCR). A novel approach by a PCR aided transcript titration assay (PATTY). *Nucleic Acids Res.* 1989; 17(22): 9437-46.
- Bussotti G, Leonardi T. Improved definition of the mouse transcriptome via targeted RNA sequencing. *Genome Research.* 2016; 26(5): 705-16.
- Canovas A, Rincon G, Islas-Trejo A, Wickramasinghe S, Medrano JF. SNP discovery in the bovine milk transcriptome using RNA-Seq technology. *Mamm Genome.* 2010; 21(11-12):592-8.
- Crick F. Central Dogma of Molecular Biology. *Nature.* 1970; 227:561.
- Gorni C, Garino C, IacuanIELlo S, Castiglioni B, Stella A, Restelli GL, Pagnacco G, Mariani P. Transcriptome analysis to identify differential gene expression affecting meat quality in heavy Italian pigs. *Anim Genet.* 2011; 42(2):161-71.
- Grunstein M, Hogness DS. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. *Proc Natl Acad Sci USA.* 1975; 72(10):3961-5.
- Han Y, Gao S, Muegge K, Zhang W, Zhou B. Advanced Applications of RNA Sequencing and Challenges. *Bioinformatics and Biology Insights.* 2015; 9(1): 29-46.
- Ibanez C, Simo C, Garcia-Canas V, Cifuentes A, Castro-Puyana M. Metabolomics, peptidomics and proteomics applications of capillary electrophoresis-mass spectrometry in Foodomics: a review. *Anal Chim Acta.* 2013; 802:1-13.
- Imadi SR, Kazi AG, Ahanger MA, Gucel S, Ahmad P. Plant transcriptomics and responses to environmental stress: an overview. *J Genet.* 2015; 94(3):525-37.
- Jonchere V, Rehault-Godbert S, Hennequet-Antier C, Cabau C, Sibut V, Cogburn L A, Nys Y, Gautron J. Gene expression profiling to identify eggshell proteins involved in physical defense of the chicken egg. *BMC Genomics.* 2010; 11:57.
- Kissoudis C, van de Wiel C, Visser RG, van der Linden G. Enhancing crop resilience to combined abiotic and biotic stress through the dissection of physiological and molecular crosstalk. *Front Plant Sci.* 2014; 5:207.
- Marquez Y, Brown JW, Simpson C, Barta A, Kalyna M. Transcriptome survey reveals increased complexity of the alternative splicing landscape in Arabidopsis. *Genome Res.* 2008; 22(6):1184-95.
- Martin C, Butelli E, Petroni K, Tonelli C. How can research on plants contribute to promoting human health? *Plant Cell.* 2011; 23(5):1685-99.
- Mattiello L, Begcy K, da Silva FR, Jorge RA, Menossi M. Transcriptome analysis highlights changes in the leaves of maize plants cultivated in acidic soil containing toxic levels of Al(3+). *Mol Biol Rep.* 2014; 41(12):8107-16.
- Nelson NJ. Microarrays have arrived: gene expression tool matures. *J Natl Cancer Inst.* 2011; 93(7):492-4.
- Pertea M. The Human Transcriptome: An Unfinished Story. *Genes.* 2012; 3(3):344-60.
- Putney SD, Herlihy WC, Schimmel P. A new troponin T and cDNA clones for 13 different muscle proteins, found by shotgun sequencing. *Nature.* 1989; 302(5910):718-21.
- Quackenbush J. Computational genetics: computational analysis of microarray data. *Nature reviews genetics.* 2001; 2(6):418.
- Reddy AS. Alternative splicing of pre-messenger RNAs in plants in the genomic era. *Annu Rev Plant Biol.* 2007; 58:267-94.
- Ruan Y, Le Ber P, Ng HH, Liu ET. Interrogating the transcriptome. *Trends Biotechnology.* 2004; 22(1):23-30.
- Shen Y, Zhou Z, Wang Z, Li W, Fang C, Wu M, Ma Y, Liu T, Kong LA, Peng DL, Tian Z. Global dissection of alternative splicing in paleopolyploid soybean. *Plant Cell.* 2014; 26(3):996-1008.
- Sim GK, Kafatos FC, Jones CW, Koehler MD, Efstratiadis A, Maniatis T. Use of a cDNA library for studies on evolution and developmental expression of the chorion multigene families. *Cell.* 1979; 18(4):1303-16.

24. Singh S, Parihar P, Singh R, Singh VP, Prasad SM. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. *Front Plant Sci.* 2016; 6:1143.
25. Su Z, Fang H, Hong H, Shi L, Zhang W, Zhang W, Zhang Y, Dong Z, Lancashire LJ, Bessarabova M, Yang X, Ning B, Gong B, Meehan J, Xu J, Ge W, Perkins R, Fischer M, Tong W. An investigation of biomarkers derived from legacy microarray data for their utility in the RNA-seq era. *Genome Biol.* 2014; 15(12):523.
26. Wang W, Cao X H, Miclăuș M, Xiong W and Xu J-H. The Promise of Agriculture Genomics. *International journal of genomics*, 2017.
27. Wang YL, Huang LQ, Yuan Y, Zha LP. Research advances on analysis of medicinal plants transcriptome. *Zhongguo Zhong Yao Za Zhi.* 2015; 40(11):2055-61.
28. Yu P, Lin W. Single-cell Transcriptome Study as Big Data. *Genomics, Proteomics & Bioinformatics.* 2016; 14(1):21-30.
29. Zhang G, Guo G, Hu X, Zhang Y, Li Q, Li R, Zhuang R, Lu Z, He Z, Fang X, Chen L, Tian W, Tao Y, Kristiansen K, Zhang X, Li S, Yang H, Wang J, Wang J. Deep RNA sequencing at single base-pair resolution reveals high complexity of the rice transcriptome. *Genome Res.* 2010; 20(5):646-54.
30. Zinsstag J, Schelling E, Waltner-Toews D, Tanner M. From one medicine to one health and systemic approaches to health and well-being. *Prev Vet Med.* 2011; 101(3-4):148-56.