Transcriptomes of the Anther Sporophyte: Availability and Uses

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An anther includes sporophytic tissues of three outer cell layers and an innermost layer, the tapetum, which encloses a locale where the gametophytic microspores mature to become pollen. The sporophytic tissues also comprise some vascular cells and specialized cells of the stomium aligning the long anther axis for anther dehiscence. Studies of the anther sporophytic cells, especially the tapetum, have recently expanded from the use of microscopy to molecular biology and transcriptomes. The available sequencing technologies, plus the use of laser microdissection and in silico subtraction, have produced high-quality anther sporophyte transcriptomes of rice, Arabidopsis and maize. These transcriptomes have been used for research discoveries and have potential for future discoveries in diverse areas, including developmental gene activity networking and changes in enzyme and metabolic domains, prediction of protein functions by quantity, secretion, antisense transcript regulation, small RNAs and promoters for generating male sterility. We anticipate that these studies with rice and other transcriptomes will expand to encompass other plants, whose genomes will be sequenced soon, with ever-advancing sequencing technologies. In comprehensive gene activity profiling of the anther sporophyte, studies involving transcriptomes will spearhead investigation of the downstream gene activity with proteomics and metabolomics.

Keywords: Anther • Anther development • Anther transcripts • Sporophyte transcripts • Tapetum • Transcriptomes.

Abbreviations: LCM, laser capture microdissection; LTP, lipid transfer protein; MPSS, massive parallel signature sequencing; PCD, programmed cell death; SBS, sequencing by synthesis

Introduction

An anther is the male organ in a flower. It consists of sporophytic tissues of three outer cell layers and an innermost layer, the tapetum. These sporophytic tissues enclose a locale in which the gametophytic microspores mature to become pollen (Goldberg et al. 1993, Hesse et al. 1993). The sporophytic tissues also include some vascular cells and specialized cells of the stomium aligning the long axis of the anther; these specialized cells will undergo programmed cell death (PCD) to split the anther and release the pollen (Goldberg et al. 1993). The cell composition of sporophytic tissues is substantially more complicated than that of gametophytic microspores and pollen. Pollen is uniform and, for some species such as maize, can be obtained in abundance and high purity for intensive studies at the microscopy and molecular levels. However, obtaining different sporophytic cells for detailed investigation has been difficult (Goldberg et al. 1993, Scott et al. 2004).

For decades, the anther sporophytic tissues have been studied mostly via microscopy (Goldberg et al. 1993, Hesse et al. 1993), and some studies involved use of mutants defective in anthers and pollen (Kaul 1998, Sanders et al. 1998). Among the sporophytic tissues, the tapetum has attracted the most attention. The tapetum cells are the only sporophytic cells that are not highly vacuolated, and their cytoplasm is densely packed, as seen with microscopy. These cells are important in nurturing the maturing microspores, and their ablation via genetic means leads to microspore death and thus male sterility (Sanders et al. 1998, Hesse et al. 1993). The cells are the site of action of genes involved in cytoplasmic male sterility and genic male sterility. A multiauthored book has described various aspects of the tapetum, mainly at the morphological level (Hesse et al. 1993). More recently, studies of the tapetum have expanded from the use of microscopy to that of molecular biology and other modern means, including the powerful tool of transcriptomes.

This mini review describes the recent use of gene activity profiling, mainly in the form of transcriptomes, to study anther sporophytic cells. It does not include similar studies of anther gametophytic microspores and detached pollen and tubes, except for comparison with sporophytic cells.
**Gene activity profiling: species, tissues and technologies**

Transcriptomes are produced with species whose genomes are well studied. The production is dictated largely by the available sequencing technologies that rely heavily on completely known genome sequences (Fig. 1). The species include rice, maize and Arabidopsis (Endo et al. 2004, Wang et al. 2005, Lu et al. 2006, Mandaokar et al. 2006, Ma et al. 2007, Wijeratne et al. 2007, Yang et al. 2007, Fujioka et al. 2008, Hobo et al. 2008, Ma et al. 2008, Endo et al. 2009, Huang et al. 2009, Johnson et al. 2009, Fujita et al. 2010, Deveshwar et al. 2011). Few other species have been used, with the exceptions in early studies of Brassica, an important crop and a close relative of Arabidopsis but with larger flowers (Scott et al. 1991, Amagai et al. 2003), and Lotus (Endo et al. 2002). Some of these studies involved use of male-sterile mutants defective in anthers, tapetum and/or pollen (Rubinelli et al. 1998, Mandaokar et al. 2006, Ma et al. 2007, Yang et al. 2007) or plants subjected to stress treatments (Endo et al. 2009). We believe that study of transcriptomes will expand from rice, maize and Arabidopsis to other crops and non-crop plants, whose genomes will be sequenced soon, with ever-advancing sequencing technologies.

A consideration is the floral organs and tissues used to produce anther transcriptomes (Fig. 1). Arabidopsis, because of its small flowers, has been studied with use of the whole inflorescence or flower, which includes both male and female parts as well as the sporophytic stalk, petal and sepal. Maize anthers can be obtained easily, because the tassels are large and have no female components. The anthers of rice flowers of progressive stages can be separated and obtained in sufficient amounts, albeit with intensive labor. Tapetum cells have been obtained (Hobo et al. 2008) with laser capture microdissection (LCM), which allows for knowing exactly what cell type is used. Nevertheless, the procedure probably produces contamination with other cell types (especially when relatively large amounts of cells are prepared), potential injury to the cells and thus low quality of the extracted RNA, and possible bias in the amplification of the RNA sample in minute amounts before sequencing.

In a totally different approach, in silico anther-minus-pollen subtraction of the transcriptomes can be used to obtain ‘sporophyte-specific’ transcriptomes (Huang et al. 2009). With this approach, a large amount of uninjured anther cells can be obtained for extraction of high-quality RNA. The resulting ‘sporophyte-specific’ transcriptome mainly represents transcripts of the tapetum (e.g. >95%) but also includes transcripts of other non-tapetum sporophytic and microspore-specific transcripts that are absent in pollen. The presence of these other transcripts in the anther-minus-pollen transcriptomes could be beneficial for future investigation of gene activities in non-tapetum cells. Regardless, investigators interested in a particular gene in a transcriptome will need to perform in situ hybridization or other analyses to confirm or negate the location of the transcript in a specific cell type (Endo et al. 2004, Lu et al. 2006, Wijeratne et al. 2007, Endo et al. 2009, Deveshwar et al. 2011).

Studies of transcriptomes of anthers and pollen began >20 years ago and have gained momentum in recent years with use of ever-advancing genomic technologies. Early studies involved differential screening of cDNA libraries of anthers and other organs, followed by sequencing of the cDNAs (Koltunow et al. 1990, Scott et al. 1991, Tsuchiya et al. 1994). These tedious procedures yielded only a few dozen transcripts, which usually represented the very abundant type.

Since then, researchers have used microarray technology (Fig. 1). Thousands of transcripts can be obtained from an anther sample (Endo et al. 2002, Endo et al. 2004, Wang et al. 2005, Lu et al. 2006, Mandaokar et al. 2006, Ma et al. 2007, Yang et al. 2007, Wijeratne et al. 2007, Ma et al. 2008, Hobo et al. 2008, Endo et al. 2009, Fujita et al. 2010, Deveshwar et al. 2011). The procedure is relatively inexpensive after the initial set-up, which is available at most research-oriented academic and research institutions. The limitations of the microarray technology are that the work is time-consuming, that transcript levels are not quantitative among different genes, that transcripts of closely related genes cannot be distinguished, and that the genome and/or expressed sequence tags (ESTs) of the species are needed, i.e. un-annotated genes may not be identified. We anticipate that the use of microarrays will diminish with time when more advanced sequencing technologies emerge.

More advanced technologies have included massive parallel signature sequencing (MPSS) and its related and superseding technologies.

**Fig. 1** Production and studies of anther sporophyte transcriptomes. Whole anthers (or flowers of Arabidopsis) or tapetum cells and microspores dissected via laser capture microdissection (LCM) from wild-type or mutant plants are used for transcriptome construction with microarray, massive parallel signature sequencing (MPSS), sequencing-by-synthesis (SBS) or RNA-Seq technologies. The transcriptomes are further analyzed directly or via in silico subtraction to identify sporophyte-specific genes and potential gene functions.
sequencing by synthesis (SBS), as well as the 454 sequencing technologies (Fig. 1). These technologies began to be available in 2005 and can produce transcriptomes of substantially better quality. More than 1 million transcripts could be identified from a single RNA sample from anthers, and the quantities of different transcripts can be directly compared (Huang et al. 2009). We anticipate that the transcriptomes produced by the latest next generation sequencing (NGS; RNA-Seq) (Wang et al. 2009) will produce transcriptomes of even better quality, small RNAs and from species whose genomes have not been sequenced.

Table 1 shows the available anther sporophyte transcriptomes listed by publication dates. The increasing quality of transcriptomes concomitant with advancing technologies is obvious.

One surprising absence in Table 1 is a set of high-quality transcriptomes of maize anthers. Maize is a major crop; its genome has been almost completely sequenced; and its male sterility genes are extensively used in manufacturing hybrid seed. Essentially all commercial maize grown in the USA is produced from hybrid seed manufactured by the seed industry, which has great incentive to make use of transcriptomes to aid its breeding and seed production. The industry had MPSS transcriptomes available early on. For example, Pioneer Hybrid Internationals, a major maize hybrid seed company in the USA, had MPSS sets of anther (tassel) samples long before the availability of publicly accessible transcriptomes (Brady et al. 2007). We anticipate that various maize anther transcriptomes of high quality will be available soon.

Another surprising absence in Table 1 is a set of high-quality transcriptomes of Arabidopsis anthers. The available transcriptomes are produced with microarray technology and whole flowers of different developmental stages. We also anticipate that various Arabidopsis anther transcriptomes of high quality will be available soon.

In gene activity profiling, we should expand from transcriptomes to proteomics and metabolomics. The current proteomics technology realistically cannot generate a complete spectrum of protein molecules from a complex sample such

Table 1 Available anther and anther sporophyte transcriptomes

| Species   | Methods                                      | Tissue/organ          | Progressive developmental stages | No. of genes tested | No. of anther-specific transcripts | References          |
|-----------|----------------------------------------------|-----------------------|----------------------------------|--------------------|-----------------------------------|---------------------|
| Tobacco   | Differential screening of cDNA library       | Anther                | 1                                | 58                 |                                   | Koltunow et al. (1990) |
| Brassica  | Differential screening of cDNA library       | Anther                | 2, mixed                         | 19                 |                                   | Scott et al. (1991)   |
| Rice      | Differential screening of cDNA library       | Anther                | 1                                | 2                  |                                   | Tsuchiya et al. (1994) |
| Arabidopsis | cDNA subtractive hybridization              | Flower buds from mutant | 1, mixed                        | 13                 |                                   | Rubini et al. (1998)  |
| Lotus     | Microarray                                   | Anther                | 2                                | 4,000              | 132                               | Endo et al. (2002)    |
| Brassica  | Microarray                                   | Anther                | 3                                | 3,000              | 52                                | Amagai et al. (2003)  |
| Rice      | Microarray                                   | Anther                | 3                                | 4,000              | 259                               | Endo et al. (2004)    |
| Rice      | Microarray                                   | Anther                | 1                                | 10,000             | 2,155                             | Wang et al. (2005)    |
| Rice      | Microarray                                   | Anther                | 4                                | 10,000             | 1,586                             | Lu et al. (2006)      |
| Arabidopsis | Microarray                                    | Anther, JA-treated mutant | 1                              | 22,000             | 1,296                             | Mandaokar et al. (2006) |
| Maize     | Microarray                                   | Anther from mutants   | 4                                | 22,000             | 1,192                             | Ma et al. (2007)      |
| Arabidopsis | Microarray                                    | Flower buds from mutants | 1, mixed                       | 22,000             | 1,954                             | Wijeratne et al. (2007) |
| Arabidopsis | Microarray                                    | Flower buds from mutant | 2, mixed                       | 22,000             | 260                               | Yang et al. (2007)    |
| Maize     | Microarray                                   | Anther                | 7                                | 44,000             | 1,952                             | Ma et al. (2008)      |
| Rice      | Microarray                                   | Tapetum and microspore | 5                                | 44,000             | 3,468                             | Hobo et al. (2008)    |
| Rice      | Microarray                                   | Anther, heat-treated  | 1, mixed                        | 22,000             | 1,439                             | Endo et al. (2009)    |
| Rice      | SBS                                          | Anther and pollen     | 6 (in silico subtraction)        | 52,000 (26,000)    | 2,359                             | Huang et al. (2009)   |
| Rice      | Microarray                                   | Anther and microspore | 8                               | 57,000             | 2,593                             | Fujita et al. (2010)  |
| Rice      | Microarray                                   | Anther                | 4                               | 57,000             | 1,000                             | Deveshwar et al. (2011) |

JA, jasmonic acid.

a In the microarray studies, the number of genes tested represents the number of different gene probes present in the microarrays. In the SBS study, which could provide more quantitative data, the number of genes tested (those whose GATC restriction sites were detectable) is shown; the number in parentheses represents the actual gene transcripts detected.

b Variations and conditions could be applied to the indicated numbers; readers should check the original papers.
as an anther extract. The only report of proteomics of rice anthers described 150 identified proteins (Kerim et al. 2003). No meaningful report of anther metabolomics is available. Such a study would be difficult and of insignificant meaning, because the metabolites detected could locate in one or many sites, such as the large vacuoles in the three outer anther sporophytic cells, small vacuoles in the tapetum cells, the locule and/or the vacuoles of the microspores.

**Information obtained or potentially obtainable from anther sporophyte transcriptomes**

The use of anther and anther–sporophyte transcriptomes of defined developmental stages has enhanced many aspects of delineating the functioning of the anther, especially the tapetum. These transcriptomes can be explored for direct discoveries or used to complement findings from traditional methods. The exploration can be further aided with specific mutants of anthers or pollen. A brief description of the discoveries or the potential for discoveries from the transcriptomes follows.

**Genes controlling anther development and the networking of their activities**

With help from traditional studies including the use of specific mutants, researchers can examine anther transcriptomes via analysis of their encoded proteins whose sequences possess putative DNA-binding domains (Zhao et al. 2002, Yang et al. 2003, Sorensen et al. 2003, Millar and Gubler 2005, Zhang et al. 2006, Yang et al. 2007). The sequential appearance of the transcripts during anther development allows for plotting the networking of these gene activities regulating anther development (Fig. 2). The network forms a working hypothesis, with which researchers can perform experiments to authenticate, fine-tune or negate the network.

Plant developmental processes are regulated by hormonal actions and interactions. Cytokinins, auxin, gibberellins and ethylene affect anther development (Hirano et al. 2008). Future studies can use anther transcriptomes to select all genes that have a recognized hormone-binding motif in the promoter regions and then analyze their expression patterns and their subanther locations during anther development. Such studies would have to be supplemented with delineation and differentiation of the subanther production and action sites of the hormones.

**Developmental changes of enzymes and metabolism domains**

Early morphological studies have revealed potential changes of metabolism in the anther, especially the tapetum, during development. The tapetum undergoes morphological changes observable via light and electron microscopy (Hesse et al. 1993). The cells gradually lose the cell wall facing the locule and change from uni- to di-nuclear, and then non-nuclear

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Fig. 2 A proposed gene regulation network of anther development in Arabidopsis. The diagram shows the transcription factors controlling tapetum cell formation, differentiation and programmed cell death. The tapetum cells in turn control the maturation of microspores. This figure is adopted from Wilson and Zhang (2009) and Murmu et al. (2010), which describe the names and potential actions of the transcription factors.
when PCD occurs. At an early stage of development, the cytoplasm possesses massive secretory machinery for exporting exine precursors to the microspore surface, and at a late stage generates temporary storage organelles before PCD (at least in Brassicaceae species) (Murgia et al. 1991, Platt et al. 1998). These and other changes can now be delineated with use of the transcriptomes. Transcripts producing enzymes that could catalyze these changes can be obtained from the transcriptomes and then analyzed (Wijeratne et al. 2007, Hobo et al. 2008, Huang et al. 2009), especially with the use of mutants (Grienenberger et al. 2010, Kim et al. 2010). During development, the greatest observed morphological changes could be linked to the peak levels of transcripts encoding specific enzymes, e.g. loss of cell wall linked to related wall hydrolases, exine appearance linked to enzymes in the biosynthetic pathways of fatty acids and phenylpropanoids (Fig. 3), and PCD linked to proteases (Yang et al. 2007, Huang et al. 2009).

### Secretomes

Early technology usually revealed only the abundant anther-specific or sporophyte-specific transcripts, such as those encoding lipid transfer proteins (LTPs) (Tsuchiya et al. 1994, Hihara et al. 1996, Rubinelli et al. 1998). Studies of high-quality transcriptomes reveal diverse quantities of individual transcripts. The most abundant transcripts encode LTPs, whose several genes produce >10% of the total sporophyte transcripts (Huang et al. 2009). The function of LTPs in transport of lipid (exine) precursors from the tapetum to the microspore surface has long been speculated on (Kader 1996). The enormous abundance of transcripts may indicate that LTPs also have structural and other roles. Less but still abundant are transcripts encoding oleosins in Arabidopsis; oleosins enclose alkane droplets inside the tapetosomes and move with the alkanes to the microspore surface to become major components of the pollen coat (Kim et al. 2002, Hsieh and Huang 2007). Nevertheless, the available Arabidopsis transcriptomes are from microarray technology (Wijeratne et al. 2007, Yang et al. 2007), which does not give quantitative data among different transcripts. The least abundant transcripts encode enzymes such as the endoplasmic reticulum P450 and transcription factors. Overall, the quantity of a transcript encoding a novel protein will provide hints regarding its function.

### Antisense transcripts and their possible roles

Abundant antisense transcripts are present in anther transcriptomes (Ma et al. 2006, Huang et al. 2009). Their levels are usually ~10% of, and increase and decrease before, during or after, their respective sense transcripts (Huang et al. 2009). Whether they play authentic functional roles in the regulation of their gene activities or are merely intermediates of RNA splicing remains to be elucidated.

### Small RNAs

Recent studies show that small RNAs regulate gene activities at the transcription and post-transcription levels. Small RNAs mediate gene activities that affect male and female reproduction (Millar and Gubler 2005, Wu et al. 2006). Obtaining transcriptomes of small RNAs requires new procedures not used in the traditional processing of RNAs for MPSS and SBS transcripts. The existing anther sporophyte transcriptomes do not include small RNAs. We anticipate that transcriptomes of small RNAs of sporophytic cells will be available soon.

### Tapetum-specific promoters for producing genetically modified male sterility

Hundreds of genes are expressed solely in the tapetum cells at specific developmental stages. The promoters of these genes can be used to drive the specific expression of a foreign gene for a deleterious effect (thus killing the pollen) (Mariani et al. 1992, Kriete et al. 1996) or to rescue a defective but essential native gene (thus restoring male fertility) (Li et al. 2007). The expression of such a deleterious or rescuing gene can be manipulated with the addition of an inducible motif to the promoter region to activate or inactivate the expression (Toppino et al. 2010). The selections for tapetum-specific promoters are enormous. Genes producing transcripts of high or low levels and expressed at early, mid or late stages of development, etc., can be selected. Nevertheless, researchers would have to check the authenticity of the selected promoters for the desired expression pattern (e.g. temporal or tissue specific) and their compatibility with the controlling genes.

### Discussion

Studies of transcriptomes per se can give an overall picture of gene activities and their regulation. In addition, transcriptomes of the anther sporophytic tissues can be goldmines for study of
the functioning of these tissues. Researchers need to comprehend the properties of different transcriptomes and design procedures for their mining. More and better anther sporophyte transcriptomes, including those of Arabidopsis and maize and of small RNAs, will be available soon. Progress in LCM, plus the ever-increasing capability to manufacture transcriptomes, will allow researchers to obtain specific single-cell transcriptomes. Gene activity profiling of sporophytic anthers with use of transcriptomes will spearhead the comprehension of downstream gene activities with use of proteomics and metabolomics. Technological advances in proteomics will allow researchers to obtain high-quality proteomics data for complementary studies with transcriptomes. Isolation of single cells in absolutely intact forms, plus the enhanced sensitivity of metabolomics technology, will allow for study of localized metabolomics in an anther. Together, transcriptomes, proteomics and metabolomics will complement one another to provide a comprehensive delineation of the functioning of different anther sporophytic tissues. Nevertheless, these libraries of mRNA, proteins and metabolites are simply tools, and we need to use these tools intelligently to accelerate the delineation of biological mechanisms.

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**Fig. 3** A model of exine precursor biosynthesis in the tapetum cells and transport of the precursors from the tapetum cell to the microspore surface. Fatty acids are synthesized in the plastid and transferred to the endoplasmic reticulum or cytosol for modifications. The resulting fatty components are covalently linked with phenylpropanoids to form exine precursors. The precursors are transported from the tapetum cell to the microspore surface via an ABC transporter or vesiculation, with the aid of lipid transfer proteins (LTPs). The model was drawn from results of Huang et al. (2009), Grienenberger et al. (2010) and Ariizumi and Toriyame (2011), which include the complete names of the abbreviated protein names.
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