nature genetics

A transcriptome atlas of rice cell types uncovers cellular, functional and developmental hierarchies

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The functions of the plant body rely on interactions among distinct and nonequivalent cell types. The comparison of transcriptomes from different cell types should expose the transcriptional networks that underlie cellular attributes and contributions. Using laser microdissection and microarray profiling, we have produced a cell type transcriptome atlas that includes 40 cell types from rice (*Oryza sativa*) shoot, root and germinating seed at several developmental stages, providing patterns of cell specificity for individual genes and gene classes. Cell type comparisons uncovered previously unrecognized properties, including cell-specific promoter motifs and coexpressed cognate binding factor candidates, interaction partner candidates and hormone response centers. We inferred developmental regulatory hierarchies of gene expression in specific cell types by comparison of several stages within root, shoot and embryo.

The systems perspective emerging in biology promises to explain many behaviors of cells and organisms based on modular networks of expression, interaction, regulation and metabolism[1]. A study of *Arabidopsis thaliana* root cell–specific transcriptomes recently demonstrated the value of rigorously comparable cellular data sets for identifying the genes and networks responsible for root cell–specific functions and interactions[2,3]. Several other plant studies have compared transcriptomes to resolve transcripts specific for stages or locations[4], but all are limited in scope to a few cell types or encompass zones or tissues combining multiple cell types.

We obtained transcriptome data for 40 rice cell types, comprising most types in seedling roots and shoots and in germinating seed. We acquired cell types by laser microdissection. Because laser microdissection does not require cell-specific tags or unique genetic lines, we were able to select cells entirely on the basis of their appearance and location in histological sections[5]. Among the 30,731 genes represented on the whole-genome long-oligonucleotide microarray platform, we detected the expression of 24,822 genes (80.8%) in at least one of the 40 cell types. A reference RNA pool provided a second probe against each cell type, allowing normalization of the entire database for quantitative comparisons between any or all cell types. We developed statistical procedures to address two atlas-related issues: (i) the transcript abundance for most expressed genes differed significantly between each experimental cell type and the control reference RNA to which it was compared, and (ii) each cell type–expressed gene set overlapped but was not identical to the common reference gene set. Cell types are described in **Supplementary Table 1** online (see also URLs section of Methods).

The atlas includes cell types from shoots, roots and germinated seeds, including three time points from seed imbibition (0 h, 12 h and 24 h), three root developmental zones (tip, elongation zone and maturation zone) and several leaf stages (leaf primordia P1, P2, P3 and P5 from 5- to 7-d-old seedlings). We validated the transcriptional profiles by RT-PCR, using an aliquot of the laser microdissection–isolated RNA to compare expression of representative genes (**Supplementary Table 2** online). The microarray data were consistent with published expression patterns of specific rice genes and of *A. thaliana* orthologs (for example, thioredoxin-h[6]).

Cell-specific transcriptomes showed qualitative and quantitative differences consistent with functional specialization. The transcriptome of each cell type was distinct, consisting of transcripts from 6,000–16,000 genes (26%–52% of all genes represented on the array, **Fig. 1a**). Many genes that were undetectable in the transcriptome of an entire organ were present at substantial amounts in one or more cell types within that organ. For example, we identified 879 genes, including several genes encoding transcription factors, that were expressed in one or more leaf cell types but that were below the

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Received 5 August 2008; accepted 17 October 2008; published online 4 January 2009; doi:10.1038/ng.282