Functional genomics and physiology of plants an overview

Dénes Dudits

Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

Creation of knowledge in science is largely dependent on the success in the inventions of new methodologies. The synergetic interaction between original ideas and instrumentation has been a significant driving force in research also with higher plants. Introduction of recombinant DNA technology, the progress in molecular and cellular studies have essentially restructured the plant biology research that could discover new dimensions in understanding of basic physiological functions in plants. We can hardly find any field of plant physiology related to plant growth, development, photosynthesis, environmental adaptation, crop improvement in which the gene isolation, production of recombinant protein, antibodies, or generation of transgenic plants would not be used as basic experimental tools. As the consequence of developments in high capacity DNA sequencers and bioinformatics, the genomic area provided the complete nucleotide sequence of plants such as Arabidopsis and rice (Goff et al. 2002; The Arabidopsis Genome Initiative 2000). The available databases are logarithmically increasing in size, and the genomic sequences are completed with large sets of EST (expressed sequence tag) data derived from random sequencing of cDNA clones. The cDNA libraries represent the set of active genes from different cell types, organs or physiological conditions (Crookshanks et al. 2001; Ewing et al. 2000). The various EST programs are essential components of the genomic approaches in which the discovery of gene function is in the focus.

The functional genomic projects are extensively based on the use of different mutants collections. Transposons or T-DNA insertions can destroy the subsequent function of genes and phenotypic, biochemical characterization of the loss of function mutants provides original insight to the role of genes (Agrawal et al. 2001; Greco et al. 2001; McElver et al. 2001 Young et al. 2001). The insertion mutants can be directly used for the cloning of the corresponding gene. The establishment of a link between the nucleotide sequence of a defined gene, the predicted protein structure and the mutant phenotype became a routine procedure in plant biology research as well. Special type of mutants can be produced by random transformation of an active promoter or enhancer DNA sequence into the plant genome. If these regulatory elements can cause an overexpression of the neighbouring genes, the resulted gain of function, dominant phenotype gives information about the possible function of a gene (van der Fits et al. 2001).

In general, we can conclude that production and analysis

of transformants is the most efficient way in the discovery of the gene function. Transgenic plants carrying the gene of interest under the control of a new promoter (constitutive, tissue or cell type specific, inducible) can be an ideal experimental materials for molecular, biochemical or physiological studies. Frequently, the overexpression of gene product by a strong, constitutive promoter alters variety of characters in the analyzed plants. This approach was successfully used to change morphology or growth of plants (Postma-Haarsma et al. 2002; Wyrzykowska et al. 2002; Wang et al. 2000). Transgenic plants can show improved environmental adaptation or increased resistance against pathogenic infections (Polidoros et al. 2001; Oberschall et al. 2000; Verberne et al. 2000; Deák et al. 1999; Holmberg and Bülow 1998).

The transformation technology can also allow the silencing of a gene or gene families (De Buck et al. 2001; Vaucheret et al. 2001). Recently gene silencing and epigenetic control mechanisms gained a special attention as key players in the control of plant development. Several examples support a conclusion that the major switches during plant development (fertilization, organ initiation, vernalization, flowering) are based on chromatin remodelling that activates previously silenced genes. Development of endosperm without fertilization in FIE mutants, the lack of vegetative growth in embryogenic flowering mutants (EMF2) or embryo formation from roots in the PICKLE mutants demonstrate the primary role of chromatin remodelling mechanisms during the completion of developmental programs in plants (see reviews: Grossniklaus et al. 2001; Habu et al. 2001).

Our present understanding of signalling pathways involved in stress or hormonal responses was largely extended by studies with transgenic plants. We can consider several physiological processes as series of phosphorylation/ dephosphorylation events. So the control of plant cell division cycle is based on set of kinases and phospahatases that can interact with regulatory proteins of stress or hormonal signalling (Zwerger and Hirt 2001; Ayaydin et al. 2000; Dudits et al. 1998). Targeted protein degradation is also a basic molecular mechanism in hormonal signalling (Leyser 2001). The molecular elements of signal transduction cascades establish a bridge between sensing of external factors such as light, temperature, nutritions, hormones and the reprogramming of the gene expression patterns. Synthesis of new sets of proteins contribute to new cellular functions. The functional genomic approaches aim to provide an overall information about the activity of large number (several thousands) of genes. The DNA-chip technologies can very efficiently outline the transcriptional profile of plant cells, organs under different environmental conditions or developmental stages. The co-ordination between activity of genes can be reflected by the characteristic changes in expression patterns of defined set of genes. Other alternative methods such as the differential display or cDNA-AFLP (amplified fragment length polymorphism) can also be used for transcript profiling (Breyne and Zabeau 2001; Lievens et al. 2001; Qin et al. 2001). The actual physiological status of plant cells can be characterized by the comparison of 2Dprotein patterns. The high through put micro sequencing of proteins with mass-spectrometry (MALDI-TOF) is an important method in proteome analysis (Mathesius et al. 2001).

Considering the leading publications in the field of plant biology and the increasing role of high technology based methodologies, we have to recognize a new research era that dissolve the boundaries between disciplines of plant sciences. There is a need to deal with the complexity of cellular, developmental events that requires the simultaneous application of structural, anatomical, metabolic, genetic, physiological, biochemical, bioinformatical, recombinant DNA, immunological methods and approaches. It is a special task for plant biologists to integrate the knowledge from studies on fundamental biological processes in plants with those generated by research with other higher eukaryotes.

References

- Agrawal GK, Yamazaki M, Kobayashi M, Hirochika R, Miyao A, Hirochika H (2001) Screening of the rice viviparous mutants generated by endogenous retrotransposon Tos17 insertion. Tagging of a zeaxantin epoxidase gene and a novel ostatc gene. Plant Physiol 125:1248-1257.
- Ayaydin F, Vissi E, Mészáros T, Miskolczi P, Kovács I, Fehér A, Dombrádi V, Erdődi F, Gergely P, Dudits D (2000) Inhibition of serine/threoninespecific protein phosphatases causes premature activation of cdc2MsF kinase at G2/M transition and early mitotic microtubule organization in alfalfa. The Plant J 23:85-96.
- Breyne P, Zabeau M (2001) Genome-wild expression analysis of plant cell cycle modulated genes. Curr Opin Plant Biol 4:136-142.
- Crookshanks M, Emmersen J, Welinder KG, Nielsen KL (2001) The potato tuber transcriptome: analysis of 6077 expressed sequence tags. FEBS Lett 506:123-126.
- De Buck S, Van Montagu M, Depicker A (2001) Transgene silencing of invertedly repeated transgenes is released upon deletion of one of the transgenes involved. Plant Mol Biol 46:433-445.
- Deák M, Horváth GV, Davletova S, Török K, Sass L, Vass I, Barna B, Király Z, Dudits D (1999) Plants expressing the iron-binding protein, ferritin ectopically are tolerant to oxidative damage and pathogens. Nature Biotech 17:192-196.
- Dudits D, Magyar Z, Deák M, Mészáros T, Miskolczi P, Fehér A, Brown S, Kondorosi É, Athanasiadis A, Pongor S, Bakó L, Koncz Cs, Györgyey J (1998) Cyclin-dependent and calcium-dependent kinase families: response of cell division cycle to hormone and stress signals. In Plant Cell Division: Molecular and Developmental Control, eds., Francis D, Dudits D, Inzé D, Portland Press, London 21-46.
- Ewing R, Poirot O, Claverie JM (2000) Comparative analysis of the *Arabidopsis* and rice expressed sequence tag (EST) sets. In Silico Biol 1:197-213.

Goff SA et al. (2002) A draft sequence of the rice genome (Oryza sativa

L. ssp. japonica). Science 296:92-100.

- Greco R, Ouwerkerk PB, Taal AJ, Favalli C, Beguiristain T, Puigdomenech P, Colombo L, Hoge JH, Pereira A (2001) Early and multiple Ac transpositions in rice suitable for efficient insertional mutagenesis. Plant Mol Biol 46:215-227.
- Grossniklaus U, Spillane C, Page RD, Köhler C (2001) Genomic imprinting and seed development: endosperm formation with and without sex. Curr Opin Plant Biol 4:21-27.
- Habu Y, Kakutani T, Paszkowski J (2001) Epigenetic developmental mechanisms in plants: molecules and targets of plant epigenetic regulation. Curr Opin Genet Dev 11:215-220.
- Holmberg N, Bülow L (1998) Improving stress tolerance in plants by gene transfer. Elsevier Science Ltd. review 3:61
- Leyser O (2001) Auxin signalling: the beginning, the middle and the end. Curr Opin Plant Biol 4:382-386.
- Lievens S, Goormachtig S, Holsters M (2001) A critical evaluation of differential display as a tool to identify genes involved in legume nodulation: looking back and looking forward. Nucl Acids Res 29:3459-3468.
- Mathesius U, Keizers G, Natera SH, Weinman JJ, Djordjevic MA, Rolfe BG (2001) Establishment of a root proteome reference map for the model legume *Medicago truncatula* using the expressed sequence tag database for peptide mass fingerprinting. Proteomics 1:1424-1440.
- McElver J, Tzafrir I, Aux G, Rogers R, Ashby C, Smith K, Thomas C, Chetter A, Zhou Q, Cushman MA, Tossberg J, Nickle T, Levin JZ, Law M, Meinke D, Patton D (2001) Insertional mutagenesis of genes required for seed development in *Arabidopsis thaliana*. Genetic 159:1751-1763.
- Oberschall A, Deák M, Török K, Sass L, Vass I, Kovács I, Fehér A, Dudits D and Horváth VG (2000) A novel aldose/aldehyde reductase protects transgenic plants against lipid peroxidation under chemical and drought stresses. The Plant J 24:437-446.
- Polidoros AN, Mylona PV, Scandalios JG (2001) Transgenic tobacco plants expressing the maize Cat2 genes have altered catalase levels that affect plant-pathogen interactions and resistance to oxidative stress. Transgenic Res 10:555-569.
- Postma-Haarsma AD, Rueb S, Scarpella E, den Besten W, Hoge JH, Meyer AH (2002) Developmental regulation and downstream effects of the knox class homeobox genes Oskn2 and Oskn3 from rice. Plant Mol Biol 48:423-441.
- Qin L, Prins P, Jones JT, Popeijus H, Smant G, Bakker J, Helder J (2001) GenEST, a powerful bi-directional link between cDNA sequence data and gene expression profiles generated by cDNA AFLP. Nucleis Acids Res 29:1616-1622.
- The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796-815.
- van der Fits L, Hilliou F, Memelink J (2001) T-DNA activation tagging as a tool to isolate regulators of a metabolic pathway from a genetically non-tractable plant species. Transgenic Res 10:513-521.
- Vaucheret H, Beclin C, Fagard M (2001) Post-transcriptional gene silencing in plants. J Cell Sci 114:3083-3091.
- Verberne MC, Verpoorte R, Bol GF, Mercado-Blanco J, Linthorst HJM (2000) Overproduction of salicylic acid in plants by bacterial transgenes enhances pathogen resistance. Nature Biotech 18:779-783.
- Wang H, Zhou Y, Gilmer S, Whiwill S, Fowke LC (2000) Expression of the plant cyclin-dependent kinase inhibitor ICK1 affects cell division, plant growth and morphology. The Plant J 24:613-623.
- Wyrzykowska J, Pien S, Shen WH, Fleming AJ (2002) Manipulation of leaf shape by modulation of cell division. Development 129:957-964.
- Young JC, Krysan PJ, Sussman MR, (2001) Efficient screening of Arabidopsis t-DNA insertion lines using degenerate primers. Plant Physiol 125:513-518.
- Zwerger K, Hirt H (2001) Recent advances in plant MAP kinase signalling. Biol Chem 382:1123-1131.