

EDITORIAL: REFLECTIONS ON PLANT CELL CLASSICS

Reflections on the Issue of Regulation in Molecular and Cellular Biology

In reflecting on important papers published in *The Plant Cell*, we consider a letter from Eran Pichersky in 2005, which posed the question ‘Is the concept of regulation overused in molecular and cellular biology?’ (Pichersky, 2005). Pichersky noted that authors often use the term loosely and erroneously. For example, if a gene regulates the production of the protein it encodes, then all genes are regulatory and the term becomes meaningless. Similarly, showing that a product is no longer made when an enzyme is knocked out does not mean that the enzyme is ‘regulatory’, simply that it is necessary. The misuse of the term ‘regulatory’ persists, and there remains a need to define and use this term more precisely.

REGULATORY FUNCTION VERSUS REGULATORY ACTIVITY

Part of the difficulty in defining ‘regulatory’ is that we have come to use the term in two different ways. Certainly some components of biological processes have functions that are exclusively regulatory. Transcription factors (TFs) are obvious examples. The essence of their function is that they regulate the transcription of other genes, by binding DNA specifically and usually by modulating the rate of initiation of transcription by the basal transcriptional machinery. Use of the term ‘regulatory’ cannot be challenged in such cases – it is what these proteins do. However, it is equally true that any one transcription factor may not be ‘determining’ the rate of expression of its target genes all, or even most, of the time. This is where we use ‘regulatory’ in the context of systems, and it is the application or misapplication of the term in this sense that Pichersky’s letter sought to address.

LESSONS FROM MYBs AND miRNAs

Although transcription factors may all have regulatory activities, not all have regulatory functions. An example of this can be seen in the control of anthocyanin biosynthesis. Analysis of loss-of-function mutants affecting the activity of the MYB and bHLH regulatory components of the MYB/bHLH/WDR (MBW) complex revealed that their activity is necessary for the transcription of the genes in the anthocyanin biosynthetic pathway, (Paz-Ares et al., 1987, Ludwig et al., 1989, Walker et al, 1999; Ramsay and Glover 2005). MYBs and bHLHs both have regulatory functions, the WDR probably acts to stabilize the complex, and there is no evidence to support the idea that its activity limits anthocyanin biosynthesis except in mutants.

The creation of over-expression alleles, although often criticized by reviewers, provides a means to identify processes in metabolic pathways that may truly be defined as having ‘regulatory functions’ under any one set of conditions. For example, the overexpression of the MYB factor component of the MBW complex usually results in significant increases in anthocyanin accumulation all over the plant (Borevitz et al, 2000) whereas overexpression of the bHLH factor component does not,

in most tissues in *Arabidopsis* (Zhang et al, 2003). These data suggest that although both MYB and bHLH factors have necessary regulatory activity in the anthocyanin biosynthetic pathway, only the MYB factor exercises significant regulatory functionality (control) in determining the amount of anthocyanin that is produced in particular tissues.

Such distinctions between regulatory functions and regulatory activities are important, for they identify factors that will be effective, if modified, in engineering flux significantly. This point is borne out by examination of the factors underpinning natural variation in pigment production in flowers; in *Antirrhinum*, apple, grape, *Petunia*, and others, this trait is due to variation in the activity of the MYB factors that have regulatory functions in this pathway (De Jong et al, 2003, Quattrocchio et al, 1999, Schwinn et al, 2006, Tacos et al, 2006, Walker et al, 2007) although the balance of regulatory function may vary between different species (for example overexpression of the bHLH factor in tomato results in significant increases in anthocyanin production in leaves; Mooney et al, 1995) and between different cells and environmental conditions (Shang et al., 2011; Taylor and Briggs, 1990).

Another example of the need to separate regulatory function in systems from the regulatory activity of components is illustrated by the activity of many miRNAs. Clearly the activity of miRNAs is regulatory: they limit the transcript levels or translation of their targets, wherever they are expressed. However, is it true to claim that miRNAs control or regulate specific processes?

In many cases, such as in the regulation of HD-ZIPIII transcript levels by miR165 and miR166 in leaf polarity determination (Kidner and Martienssen, 2004) the answer to this question is probably no—usually changing miRNA levels is not the process that determines induction of target transcript levels in particular cells; their function more often is to prevent or buffer against inappropriate expression. In other words, they may be required for regulatory circuits to operate but are not directly involved in the nonlinear modulation of such regulatory processes. Indeed, the expression of miR165 and miR166 has been shown to be regulated by the HD-ZIPIII transcription factors REV, PHAB, and PHAV in combination with HD-ZIPII TFs (that are also targets of PHAB, PHAV and REV), emphasizing that miR165 and miR166 have regulatory activities rather than having primary regulatory functions (Merelo et al., 2016).

UNIFIED DEFINITIONS OF REGULATORY FUNCTION?

We find arguments in favor of developing clear definitions of ‘regulatory function’ and ‘regulatory activity’ to be very strong. However, there are also equally strong arguments in favor of expanding our concepts of regulation. Metabolic flux control theory, first proposed by Kacser and Burns (1973), has revolutionized thinking on how control works in metabolic pathways, showing that regulation may be a property of many steps in a pathway and that regulatory function may change under

changing conditions. It would be a welcome development to see the ideas underpinning this theory applied to plant developmental processes as well. Application of flux control theory could make important contributions to understanding the control of development—that regulatory functions can change depending on the particular conditions and that the control of the end point may be assigned to many steps in the pathway. Although many developmental processes may not fit easily into the models for flux control analysis (for example, because of the need to measure gene/protein activity), the general principles that have come from this theory and its broadening of our understanding of how regulatory functions may be allocated and regulation exercised could ultimately be as useful to developmental research as it has become to research in metabolism.

We still endorse fully Pichersky's call for clearer definitions of regulation, exemplified here by numerous references to seminal papers published in *The Plant Cell*, while adding our own plea for discussion to broaden our concepts of regulatory function in plants, as a whole.

Cathie Martin
Department of Metabolic Biology,
John Innes Centre,
Norwich NR4 7UH, UK
cathie.martin@jic.ac.uk
ORCID: 0000-0002-3640-5080

Richard A. Jorgensen
University of Arizona
Department of Plant Sciences
Tucson AZ 85721-0036 USA
raj@ag.arizona.edu
ORCID: 0000-0002-0382-2371

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*References highlighted for the 30th anniversary of *The Plant Cell*.

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Cathie Martin and Richard A. Jorgensen

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