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Review

Ribosomal composition and control of leaf development

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ABSTRACT

Protein synthesis in plants occurs at three sub-cellular locations that have their own specific ribosomal compositions: the cytoplasm, mitochondria and plastids. An increased demand for functional and efficient translational machinery is required during development of leaves. The role of specific ribosomal protein (RP-) encoding genes in the regulation of development has been underestimated as housekeeping. However, in *Arabidopsis thaliana* several RP loss-of-function mutations have been identified that affect cell division or cell expansion and consequently result in deformed leaf size and shape, indicating cell- or development-specific roles of RPs during leaf growth. This view is strengthened by the observation that the expression of many RP genes follows distinct patterns during leaf development. Moreover, translatomics data demonstrate that ribosomal composition is dynamic and organized in a spatio-temporal manner. The regulation of RP gene transcription via different promoter-localized *cis*-elements allows additional control relevant for leaf growth. We conclude that RPs have a more distinct role in regulating specific processes in leaf development than previously anticipated, and envisage fascinating novel insights in the near future.

Contents

1. Introduction

Protein synthesis involves the binding and translation of mRNA by ribosomal complexes. There are three major protein synthesis locations in the plant cell, each with a distinct composition of its

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ribosomal complex. rRNA molecules form the core of the translational complex while the ribosomal proteins (RPs) are mainly found at the surface [1]. The discovery that the catalytic activity of the ribosome comes from the rRNA and not the RPs led to the idea that the ribosome has evolved as an entire RNA-catalysing enzyme [2]. Later on, apparently more specialized functions evolved that are dependent on the RPs that conjugate with the rRNA molecules. Since RPs are mainly present at the surface of the particle they are in an excellent position for mediating the many interactions of the ribosome with other components, including regulatory proteins of the cell [3].

One typical aspect of multicellular organisms is the demand for the co-ordination of cell proliferation and expansion during development. Leaf size and shape largely vary between plants; such differences arise from genetic and environmental factors. The rapid advances within genome biology have helped to identify dozens of genes that affect leaf size or shape. The regulatory mechanisms that underlie these processes are still insufficiently understood. Leaf development involves a series of characteristic processes such as the initiation of periclinal cell divisions at the shoot apical meristem (SAM), resulting in cellular outgrowth. This group of cells gradually differentiates into a determinate organ with a symmetric architecture, a network of veins, and a regulated distribution of specialized cell types, such as guard cells and trichomes in the epidermal cell layers.

The size of leaves is determined by only two physiological processes: cell division leading to an increase in cell number, and cell expansion resulting in enlargement of cells [4]. The basic metabolic and energetic costs of protein synthesis and turnover directly affect the capacity of cellular growth [5]. It was already demonstrated three decades ago [6] that the amount and composition of polysomes change dramatically in growing bean leaves during cell division and the first phases of leaf expansion. The amount of polysomes increases, suggesting an increased demand for protein synthesis. The number of cytoplasmic polysomes drops rapidly after cessation of cell division. However, the number of organelle-specific complexes still increases until the leaf has reached its final size. At leaf maturity the total abundance of polysomes drops and a low level is then maintained. Taken together, these data demonstrate that the level of ribosomes during leaf growth substantially fluctuates. Duplicated RP-encoding genes in *Brassica napus* have undergone functional divergence into highly specialized paralogs and coexpression networks [7]. Translation in plants is most likely also regulated by altering the proteins in the ribosome. This is nicely exemplified by the mere fact that 251 genes in *Arabidopsis* encode the 81 possible proteins present in the cytoplasmic ribosome [8]. The presence of small multigene families encoding for the various RPs allows for a highly dynamic composition of the ribosome.

Most RP mutants are characterized by a decrease in cell number [9]. It can be stated that the total number of cells that arise through cell division contributes to the final size of a leaf. However, in several cases a lower number of cells is compensated by an increase in cell size [10], demonstrating that cell division and expansion are not regulated at the single-cell level alone, but that growth is regulated at the whole-organ level reaching a set final size [11]. The mechanisms behind this compensation effect have been subject of recent studies as discussed below.

Ribosomes are generally perceived as housekeeping components within the cell, with a non-selective role in polypeptide synthesis. However, the specific phenotypes of several RP mutations suggest that ribosomal composition may play a fundamental role during development. The big questions here are whether ribosomal composition changes mRNA preference (and thus defines which transcripts are translated) and whether a specific ribosomal composition exists that stimulates leaf growth.

1.1. Ribosomal protein mutants and developmental defects

1.1.1. Cytoplasmic RPs

The cytoplasmic ribosome contains 81 RPs encoded by small gene families encompassing a total of 251 genes in *Arabidopsis* [8]. The presence of multiple copies of individual RPs suggests that functional specialization might have occurred among family members. Still several copies might only act as pseudogenes without any biological function. If they are all housekeeping genes, as it is sometimes taught, it is clear that a mutation in a single RP might cause dysfunction of the whole translational machinery, as with the *EMBRYO-DEFECTIVE* (*EMB*) mutants [12]. RPS6B, RPS11A, RPL8A, RPL19A, RPL23C, RPL40B have been identified as *EMB* mutants. They all belong to families with two or three members, suggesting that there is low functional redundancy or that some of them are weakly active paralogous. Loss of *RPS6B* is lethal, while partial down-regulation results in an altered leaf pattern [13]. Of the two-gene *RPS5* family, *RPS5A* is mainly expressed in dividing cells, whereas *RPS5B* is preferentially expressed in differentiating and elongating cells [14], fitting with specification of different family members. Knock-out of *RPS5A* is embryo lethal, whereas loss of a single allele causes delayed development and altered leaf vascular patterning. A T-DNA insertion in the coding region of *RPL23aB* has no reported effect on plant morphology [15], whereas suppression of *RPL23aA* transcript level leads to reduced cell division, retarded growth, morphological abnormalities and altered vascular patterning [16]. The phenotypes of RP mutants show a striking overlap with those of auxin-related mutants [17–19]. The phytohormone auxin is of major importance in plant development, suggesting that translation and auxin signaling might share a common role in regulating leaf growth. The first described RP affecting leaf growth is caused by a T-DNA insertion in the *RPS18A* gene resulting in the characteristic *pointed first leaf* (*pfl*) phenotype [20]. Although the *RPS18* family has three members, the other copies cannot compensate for the loss, indicating functional divergence between the three proteins (or slight but important differences in expression patterns). Activation insertion mutants of *RPS13B* have a similar phenotype and are therefore called *pfl2* [21]. A *pfl1 pfl2* double mutation does not further enhance the mutant phenotype, demonstrating that both proteins might be needed for the same process. A knock-out of *RPL24B* leads to a gynoecium development defect in addition to a pointed leaf phenotype [22]. The observed reduction in leaf width of the *pfl* mutants can either be the result of a lower cell number or a reduction in cell size. In the case of the *rps13b* mutant fewer but larger cells were present in leaves when compared to wild-type [21]. Conversely, the *rpl24b* mutant has no change in cell length, which might indicate a defect in cell division. Thus, the change in leaf size is caused both by a defect in cell expansion or cell division depending on the RP gene affected. Two recent studies demonstrate that RPs specifically modulate cell expansion, in line with this conclusion. First, the pointed leaf phenotype of the *angusta3* (*ang3*) mutant is caused by an amino acid change in RPL5B [23]. Interestingly, the total cell number is not affected but leaf epidermal cell size is significantly reduced. Microarray analysis of total RNA of the *ang3* mutant revealed no differential expression of genes known to control leaf development suggesting a putative post-transcriptional regulation of cell expansion [23]. The loss of function of individual RP genes often results in detrimental growth defects; however, knock-out of *RPS15aE*, a gene of a six-member family, results in larger leaves, longer roots, and increased subepidermal palisade cell size [24]. The authors suggested that RPS15aE is a negative growth regulator; however, further in-depth studies are needed to pinpoint the exact mechanism behind the regulation of cell expansion. Taken together, the reported leaf phenotypes for RP mutants may imply that modulation of ribosome composition is a mechanism that can stimulate or repress growth. With this