

Addendum

From the Notch Signaling Pathway to Ribosome Biogenesis

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Addendum to:

Characterization of the Plant Notchless Homolog, a WD Repeat Protein Involved in Seed Development

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and

Underexpression of the Plant NOTCHLESS Gene, Encoding a WD Repeat Protein, Causes Pleiotropic Phenotype During Plant Development

S.-C. Chantha and D.P. Matton

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ABSTRACT

Nearly 240 WD repeat proteins have been identified from the *Arabidopsis* genome. Among these, some well characterized WDR proteins were shown to regulate various developmental processes in plants.¹ We have recently isolated in *Solanum chacoense* a homolog of the *Drosophila* NOTCHLESS gene. In *Drosophila*, NOTCHLESS regulates the activity of the Notch signaling pathway through a direct interaction with the intracellular domain of the Notch receptor. Although the Notch signaling pathway does not exist in yeast and plants, the NLE gene is conserved in animals, plants and yeast. Furthermore, functional conservation was suggested by expression of the plant NLE gene in *Drosophila*. In plants, underexpression of the plant NLE gene altered numerous developmental processes including seed development, and resulted in reduced aerial organ size and organ numbers, in delayed flowering, and in an increased stomatal index. Surprisingly, the link between these pleiotropic phenotypes is the recently discovered of the involvement of NLE in ribosome biogenesis, emphasizing its role in proper cellular growth and proliferation during plant development.

NOTCHLESS, A WD REPEAT PROTEIN INVOLVED IN A VARIETY OF DEVELOPMENTAL PATHWAYS IN PLANTS AND ANIMALS

Double-fertilization represents a pivotal event in the life cycle of angiosperms as it initiates the transformation of the ovule into a seed, a structure that carries the embryo of the next sporophytic generation. Following double-fertilization, a series of coordinated developmental processes are activated. One sperm cell fuses with the egg-cell to form the zygote and initiate embryogenesis, while another sperm cell unites with the central cell to initiate the development of an embryo-nourishing endosperm. Simultaneously, the integument(s) of the ovule differentiate into a protective seed coat while the ovary wall, within which the ovule is enclosed, differentiates into the fruit pericarp. Each of these developmental processes is linked to distinct genetic programs and, upon double-fertilization, their initiation involves major changes in gene expression.²⁻⁴

The ribosome is a key component in the machinery responsible for protein synthesis and, as such, plays a major role in controlling growth and development. The small 40S and large 60S subunits of the eukaryotic ribosome are made up of a total of four ribosomal RNAs (rRNAs) and about 80 ribosomal proteins (r-proteins). Unlike the prokaryotic ribosome, which is able to self-assemble, the biosynthesis of eukaryotic ribosomal subunits is highly coordinated in time and in space (from the nucleolus to the cytoplasm) and requires the participation of at least 170 nonribosomal factors.⁵ Nonribosomal factors and r-proteins are highly conserved in eukaryotes but, in contrast to other model organisms, limited information on their plant counterparts is available.^{6,7} Their importance during plant growth and development has however been shown by the expression of several plant r-protein genes being developmentally and environmentally regulated.⁸⁻¹² Plant r-protein types are likely all encoded by small gene families⁶ and members of a r-protein gene family can be differentially regulated.¹²⁻¹⁴ Disruption of r-protein gene expression was shown to cause general growth delay,¹⁵⁻¹⁷ hypersensitivity to genotoxic stress¹⁸ and early embryonic developmental arrest.¹⁹ Information on plant nonribosomal factors is scarce, although one was reported to be essential for female gametogenesis.²⁰

We recently reported that the NOTCHLESS (NLE) gene encodes a homolog of a yeast nonribosomal factor (YCR072, RSA4) and further provided evidences for an involvement of plant ScNLE protein in ribosome biogenesis by using yeast two-hybrid assays.^{21,22} In *Solanum chacoense*, transgenic plants with reduced levels of ScNLE transcripts displayed

pleiotropic phenotypes including a severe reduction in seed set.²³ Consistent with these phenotypes, we showed that *ScNLE* expression is associated to actively growing tissues of the shoot apex and is also strongly and transiently increased in ovules around fertilization time.^{21,23} In order to determine if the expression pattern of the *ScNLE* nonribosomal factor gene is shared by r-protein genes, we analyzed in this study the temporal expression profile of 288 ESTs corresponding to 65 r-proteins in ovules at different stages after fertilization by microarray analysis. DNA microarrays were performed from 7741 expressed sequence tags (ESTs) corresponding to 6374 ovule-expressed unigenes covering the zygotic to late torpedo embryonic developmental stages.² Because ESTs were only partially sequenced, we grouped the r-protein gene sequences on the basis of their amino acid sequence similarity to r-protein types (Table 1) and not to individual members of a r-protein type gene family.⁶ Thus from our EST set, more than 80% of all the cytoplasmic r-protein types were represented. Interestingly, our DNA microarray analysis revealed that gene expression from all the r-protein genes analyzed peaked two days after pollination (DAP), similarly to the *ScNLE* nonribosomal factor gene.²³ By 8 DAP, most had decreased to their basal level or below their level observed in unfertilized ovules (0 DAP). Self organization map (SOM) clustering revealed three distinct profiles (group 1 to 3). Most r-protein genes from group 1 showed a modest 1.5 to 2-fold increase in transcript levels 2 DAP and then decreased below the unfertilized ovule levels by 8 DAP. Most r-protein genes from group 2 showed a maximal 2-fold induction 2 DAP, and then returned to expression levels found in unfertilized ovules by 8 DAP. Most r-protein genes from group 3 showed greater than 2-fold increase in mRNA levels and a slower decrease back to levels found in unfertilized ovules.

Similarly to our observations, a significant increase in expression levels of several r-protein genes was observed in the zygote¹³ and two-celled proembryo.⁴ These data therefore suggest a rapid de novo transcription of r-protein genes upon fertilization as well as an increase in ribosome biogenesis and protein synthesis requirement at the onset of seed development. Our microarray analysis moreover showed a concerted upregulation of all the r-protein genes analyzed. Although members of a r-protein type family were shown to be differentially regulated, expression of most members seems to be upregulated when high supply of proteins is required, such as in actively growing and differentiating tissues.^{12,14} Since ribosome biogenesis depends on nonribosomal factors in eukaryotes,⁵ a similar transient upregulation profile in response to fertilization is expected for other plant nonribosomal factor encoding genes, as we previously demonstrated for the *ScNLE* gene.²³ Interestingly, in animals, the NLE homolog was shown to interact with the intracellular domain of the Notch transmembrane receptor and to be a modifier of Notch activity by an unknown mechanism.^{24,25} The Notch signaling pathway is however metazoan-specific and, with the exception of NLE, components and regulators of this pathway have

Table 1 **Ribosomal proteins clustering with the self organizing map (SOM) groups as determined by cDNA microarray analysis**

Group 1		Group 2		Group 3	
SSU	LSU	SSU	LSU	SSU	LSU
S3	L4	S8	L1	S16	L5
S4	L6	S9	L2	S17	L7a
S5	L11	S21	L8	S18	L9
S6	L21	S28	L10a	S23	L13
S10	L24		L12	S26	L13a
S11	L32		L34	S27	L14
S13	L36		L37	S30	L17
S14	L38		L37a		L18
S15	L41				L22
S19				RAP*	L23a
S20				P0	L26
S25				P1	L28
S29				P2	L29
S29a				P3	L30
				Sa (P40)	L31
					L33
					L35

*RAP, ribosomal associated proteins. Abbreviations: SSU, ribosome small subunit; LSU, ribosome large subunit.

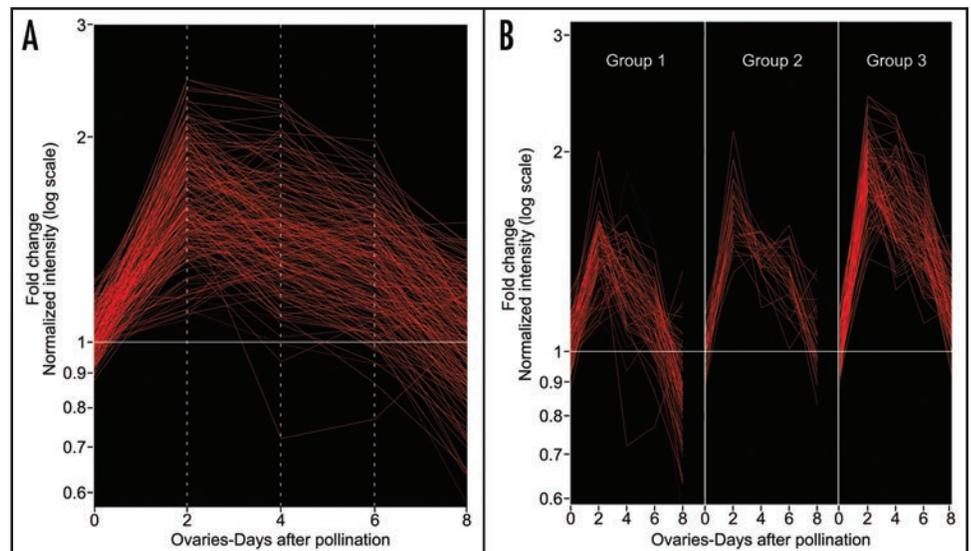


Figure 1. cDNA microarray analysis of ribosomal proteins expression following fertilization in *S. chacoense* ovules. (A) Gene expression changes observed using nonparametric Anova testing (Kruskal-Wallis t-test), along with a Benjamini and Hochberg multiple testing correction algorithm of all the ribosomal protein genes available in our 7K microarray. (B) Cluster analysis of the differentially expressed ESTs corresponding to the ribosomal proteins in Table 1. The clones were classified based on the similarity of their expression profiles using a Self organization map (SOM) clustering. Three distinct SOM profiles (Group 1 to 3) can be distinguished. RNA extraction, probe preparation, cDNA array hybridization and data analysis were performed as described previously.²⁷

no homologs in yeast and plant genomes.²⁶ Since the *NLE* gene and ribosome biogenesis as a whole are highly conserved in eukaryotes,⁷ this suggest that NLE is primarily involved in ribosome biogenesis and was likely later recruited as a regulator of the Notch pathway in the

animal lineage. This raises the possibility that the Notch pathway is somehow regulated by ribosome biogenesis through a direct interaction with a nonribosomal factor in animals. A possible link between ribosome biogenesis and a transmembrane signal receptor in plants has yet to be discovered.

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