ABSTRACT

The hypusination is a spermidine dependent post-translational modification required to activate the translation factor eIF5A and it is essential in all eukaryotes. Recent experimental evidences suggest an important role for eIF5A in the processes of senescence and response to environmental stress in plants, in the establishment of cell polarity in yeast and it has been also involved in human diseases such as diabetes, HIV-1 and cancer. In order to characterize at the molecular level the biological activity of eIF5A in plants, we have established a methodology based on biochemical and immunological techniques to determine the hypusination pattern of eIF5A in *A. thaliana*. The implementation of this methodology allowed us to demonstrate that treatment with abscisic acid inhibits activation by hypusination of the eIF5A1 isoform. In addition, with the aim to understand the functions of eIF5A during the development of *A. thaliana*, we have carried out functional studies based on the characterization of transgenic plants for genetic inactivation of the eIF5A pathway by means of RNA interference conditional to dexamethasone application. Conditional genetic inactivation of the hypusination enzyme deoxyhypusine synthase, produced alterations during development and in response to adverse growing conditions, such as early flowering, root growth inhibition, alterations in root hairs, exacerbated stem branching, completely lignified tracheal elements in hypocotyls, reduced levels of nitric oxide and hypersensitivity to glucose, salt, and abscisic acid. Recently it has been shown that eIF5A is necessary for the translation of proteins with more than 3 successive prolines in their sequence. The ontology analysis revealed an enrichment of proteins with poly-prolines between those involved in the organization of the actin cytoskeleton. Alteration of the eIF5A activity caused defects in the structure of the actin filaments in *A. thaliana, S. cerevisiae* and *H. sapiens*. The study of temperature-sensitive mutants of yeast showed eIF5A requirement during the process of sexual mating through the translation of the formin Bni1. Translational regulation experiments in HeLa cells showed that

silencing via RNA interference of eIF5A1 caused a defect in the translation rate of the formin FMNL1 and protein ezrin. These results confirm that the essential eIF5A ribosome activity seems to be conserved in eukaryotes, and affects essentially to proteins with poly-prolines involved in the organization of the actin cytoskeleton.