Utilization of transgenic maize plants for functional analysis of a histone promoter and host-geminivirus interactions

a Ph.D. Theses

by

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INTRODUCTION:

Plants, like all other higher eukaryotes go through a life cycle with a vegetative and a reproductive phase of growth. Plant organs develop throughout the vegetative growth period of a plant. Unlike other higher eukaryotes, most fully differentiated cells have the potential to dedifferentiate and to reenter the cell cycle. Due to the lack of cell migration, plant embryogenesis and development solely depend on cellular functions that regulate spatial and temporal control of cell division and expansion. To be able to build complex organs, plants must maintain strict control of cell division. Thus, in order to understand how plants regulate cell division and identify the nature of plant gene function during plant development number of tools have been developed and utilized. Genetic transformation, production and utilisation of transgenic plants for molecular and functional analysis of plant genes is a major achievement in plant science.

The transition from G1-S phase requires the synthesis of proteins that function directly on DNA metabolism; such as ribonucleotide kinases, proliferating cell nuclear antigen and histone proteins whose synthesis is closely related to DNA replication. Therefore, investigating the regulation of histone genes expression would reveal information on signal transduction mechanisms that link G1-S progression to S-phase specific histone gene expression in plant cells. To understand the effects of these phytohormones on cell division cycle, it is important to understand the basic regulatory switches controlling the transition from G_1-S, G_2-M phases of the cell cycle and how hormones affecting them. Cell cycle genes are thought to be candidates to play a role in mediating the effect of various growth regulators

STUDIES ON MAIZE STREAK VIRUS: VIRUS RESISTANCE AND HOST-VIRUS INTERACTIONS

Viruses provide useful tools and model systems, which can contribute to our understanding of cellular mechanisms, providing insights into processes that would otherwise be difficult to study.

Geminiviruses constitute a family of plant DNA viruses that cause seriuos damages to crop plants. At the same time, they are useful tools to study plant gene expression, DNA replication, regulation of plant gene expression during virus infection. Maize streak virus (MSV) is a member of cereal infecting geminiviruses and can be utilized for these molecular studies revealing host-virus interaction. However, these viruses also cause significant damage to crop plants, especially in tropical and sub-tropical regions.

Coat protein mediated protection against plant viruses

Replication, long-distance spread, and vector transmission of maize streak virus (MSV) depends on the presence of coat protein (CP). Expression of genes encoding nonfunctional CPs in transgenic plants can be tried in order to interfere with normal CP function. CP-mediated resistance describes the resistance that results from the expression of a virus CP gene in transgenic plants. There are few reports concerning the resistance to geminiviruses.

MSV and Maize Cell Cycle

Studies on the replication of geminiviruses during infection indicate the role of a viral protein (*Rep*) in creating a permissive environment for viral DNA replication (Oroczo, 1998). Current findings suggest that G1 regulation in plant cells is possibly effected by viral proteins in interaction with host factors, for maintaining a permissive environment for virus replication. It is therefore logical that geminiviruses, which also depend upon host DNA replication, would use a similar strategy to effect replication in non-dividing, differentiated tissues. All these increasing body of evidence supports a tight link between the cellular and geminiviral replication cycles and suggests that the cell cycle may be reset in a manner analogous to that used by tumor inducing animal DNA viruses.

AIMS:

- To develop efficient protocols for transient and stable gene transfer into maize plants. Evaluate the feasibilty of different gene transfer methodologies for maize.
- To demonstrate the basic science and applied aspects of genetic transformation through production and utilization of transgenic maize plants.
- To elucidate mechanisms how histone gene expression and G1-S phase progression regulated during plant development and in response to phytohormones.
- To produce transgenic maize plants resistant to MSV infection
- To utilise transgenic maize plants carrying MSV sequences for structural and functional analysis of MSV genome
- To understand how the host- cell division cycle and MSV replication related proteins interact, during virus infection.

MATERIALS AND METHODS:

For the production of transgenic maize plants a maize cell suspension HE/89 was utilized. Protoplast isolation and PEG mediated gene transfer was used for stable transformation. For transient assays, agro-inoculation and biolistic delivery techniques were used. Plant cell and tissue culture techiques were used for *in vitro* regeneration of transgenic maize plants.

Presence, integrity and expression of transgenes as well as endogenous host genes were monitored using Southern, northern, PCR, RT-PCR and western analysis.

Immunocytochemical detection tools and epi-fluorescent microscopy was utilized in sub-cellular localization of MSV-capsid protein. Cell cycle dynamics in synchronized cells was monitored by mitotic index determination and flow cytometry.

Insect transmission assays were performed for virus replication assays.

RESULTS AND DISCUSSION:

The term "genetic transformation" used in this thesis work, refers to revealing mechanisms of gene function in plants, complementing and supplementing traditional methods of crop improvement and have a profound impact of food production. Thus, the ability to create transgenic maize plants would be a tremendous advantage for those who are trying both to improve the commercial traits of maize and those using transgenics to explore more on the basic questions of "plant gene expression" in higher plants.

PEG mediated DNA uptake into protoplasts is an efficient and reliable way of gene transfer and subsequent analysis of the gene introduced. In this study, I have used two other gene delivery and genetic transformation protocols utilizing the physical (biolistic) and biological (agro-inoculation) means to drive DNA into plant cells and tissues. These methods have different characteristics and advantages over each other however, PEG mediated direct DNA uptake into protoplast stands to be the most versatile system for stable transformation into maize plants in my hands. Biolistics on the other hand, offered quick assessment of gene function. It has allowed me to test kinetics of reporter gene function when transiently expressed viral DNA in suspension cells and embryogenic calli. Moreover, it was possible to process statistically significant numbers of samples at a time. In agreement with other reports on protoplast derived transgenic cereal plants, the transformation efficiency, number of regenerable colonies of these experiment showed that PEG-mediated protoplast transformation is a feasible tools for genetic manipulations.

Analysis of the activity of the wheat histone H4 promoter in maize also exhibited expected expression patterns indicating its link to cell division dependent expression clustered in meristem tissues of the regenerated plants. The careful dissection of the patterns of mRNA accumulation originating from histone promoter-reporter gene cassettes also indicated that the replication-dependent activity started just before DNA replication at late G1 phase and is controlled independently of the DNA synthesis.(The activities of histone *H4*, histone *H2B*, *PCNA* genes exhibited late G1-S phase dependence, while the mRNA accumulation of B-type cyclin *CycB1;zm;1* suggested a role towards mitosis. Thus, in the present experiments, the expression of the analyzed maize genes showed characteristic cell cycle phase-specific patterns common to other members of the same gene families identified from other plant species.

Wheat histone H4 promoter activity in transgenic maize could be modified by exogenous application of phytohormones. These results support the potency of auxins triggering the G1/S phase progression while proving the negative regulatory effect of ABA as a stress related signal. As a potent cytokinin, zeatin did not significantly effect the histone promoter activity, probably due to its suggested role in late S-phase and M phases

Based on the results from the transgenic plants expressing MSV CP and data available on other virus resistance strategies via genetic modification clearly suggests the use of viral sequences other than the CP against MSV. The mechanism how the geminiviruses reach to the nucleus is not well understood. However, comparisons of amino acid sequences at the amino terminus of geminiviruses, including MSV revealed a potential nuclear localization sequence. I have also tested the hypothesis that NLS sequence in transgenic maize lines carrying MSV coat protein gene under the control of maize ubiquitin promoter directs the transgene product

into the nucleus of the plant cell. This results supports the findings on the possible role of CP to bind and transport ssDNA to the nucleus.

Studies on host cell cycle during MSV infection revealed that histone gene expression and transcripton of other S-phase associated genes were activated. This activation and virus replication could be blocked by a DNA polymerase inhibitor aphidicolin. Transient over-expression of Rep proteins also caused stimulation of host replication-dependent, S-phase specific histone gene expressio, indicating a role for Rep in host gene trans-activation. These results also suggests the usefulness of histone promoter::GUS fusions in monitoring changes in G1-S phase progression.

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Társszerzoi lemondó nyilatkozat

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