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**Control of Fertilization-Independent
Development by the *FIE1* Gene**

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	1	1		2
Submitted, in review, in preparation	1			1
Invited review papers				0
Book chapters				0
Books				0
Master theses			1	1
Ph.D. theses		1 (In progres)		0
Abstracts	9			9
Not refereed (proceedings, reports, etc.)				0

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				0
Issued (allowed)				0
Licensed				0

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Visits/Meetings		2	1	3
Sabbaticals				0
Postdoctorates		1		1

Cooperation, briefly explain whether synergistic, complementary or supportive.

The cooperation between the laboratories was synergistic, complementary and supportive. Each scientist provided his specific expertise. Dr. Ohad contributed his knowledge and expertise in plant anatomy, molecular biology and protein biochemistry, whereas Dr. Fischer furnished his experience and knowledge of genetics and plant. During the program there was free exchange of materials and information between the two laboratories. Our project has resulted in two publication (one joint publication Kiyosue et al., 1999). Dr. Ohad has visited Dr. Fischer laboratory twice and in addition Ms. Katz a graduate student from Dr. Ohad laboratory has spent nine weeks in the Berkeley laboratory conducting joint experiments.

Abstract

Title. Control of Fertilization-Independent Development by the *FIE1* gene.

A fundamental problem in biology is to understand how fertilization initiates reproductive development. During plant reproduction, one sperm cell fuses with the egg to form an embryo, whereas a second sperm cell fuses with the adjacent central cell nucleus to form the endosperm tissue that supports embryo and/or seedling development. To understand the mechanisms that initiate reproduction, we have isolated mutants of *Arabidopsis* that allow for replication of the central cell and subsequent endosperm development without fertilization. In this project we have cloned the *MEA* gene and showed that it encode a SET-domain polycomb protein. Such proteins are known to form chromatin-protein complexes that repress homeotic gene transcription and influence cell proliferation from *Drosophylla* to mammals. We propose a model whereby *MEA* and an additional polycomb protein we have cloned, *FIE*, function to suppress a critical aspect of early plant reproduction and endosperm development, until fertilization occurs. Using a molecular approach we were able to determine that *FIE* and *MEA* interact physically, suggesting that these proteins have been conserved also during the evolution of flowering plants. The analysis of *MEA* expression pattern revealed that it is an imprinted gene that displays parent-of-origin-dependent monoallelic expression specifically in the endosperm tissue. Silencing of the paternal *MEA* allele in the endosperm and the phenotype of mutant *mea* seeds support the parental conflict theory for the evolution of imprinting in plants and mammals.

These results contribute new information on the initiation of endosperm development and provide a unique entry point to study asexual reproduction and apomixis which is expected to improve crop production.

I. Introduction

Our long term objective is to understand how fertilization activates endosperm, embryo and seed development. In plants, two fertilization events are responsible for initiating reproduction. Within the ovule, the fertilized egg initiates embryogenesis, whereas the fertilized central cell forms the endosperm, a nutritive tissue that supports the growth and development of the accompanying embryo. Fertilization activates maternal developmental programs that transform the ovule integuments into a seed coat, and the carpels into a fruit. The mechanisms that initiate these distinct patterns of development are unknown. To address this issue, we have taken a genetic approach and have isolated a novel class of mutations in *Arabidopsis*, termed *FIE* (fertilization-independent endosperm (1) that form endosperm, seed coat and fruit in the absence of fertilization. Genetic analysis suggests that mutations in the *FIE1* genes exert their effect after meiosis in the female gametophyte, resulting in endosperm development when fertilization is prevented, and embryo abortion when fertilization occurs. These mutants provide a new approach to elucidate the very early steps that control plant reproduction.

II. Outline of original proposal and subsequent modifications

The primary goals of the original program were to understand at the molecular and cellular level how mutations in the *FIE1* gene activate aspects of reproductive development, and in particular, fertilization-independent endosperm development. To this end we specifically intended to:

1. Clone and sequence the *FIE1* gene allowing to predict its amino acid sequence and study its function.
2. Analyze the *fie1* mutant phenotype. We proposed to extend and complete the analysis of the *fie1* phenotype by studying the effect of the *fie1* mutation on egg cell and endosperm development, using light microscopy, in the presence and the absence of fertilization,

3. To unravel the mechanisms that regulates plant reproduction we proposed to study the temporal and spatial pattern of *FIE1* gene expression and gene product accumulation during plant development, and specifically in the central cell.

III. Comprehensive description of the research results

Gene Identification and cloning the *FIE1* Gene (Publication #1)

We have used a map-based strategy to clone the *FIE1* gene. Analysis of the *FIE1* sequence showed that it encodes a SET-domain polycomb protein. Subsequently, we found that *FIE1* was identical to *MEDEA*, a gene whose maternal-derived allele is required for proper embryo development (15). The identity between the *FIE1* gene we have cloned, and *MEA* was not predicted because of differences in the phenotypes of the two mutant alleles. Our results revealed new functions for the *MEA* gene, the control of central cell proliferation and endosperm development. The cloning of *FIE* gene, analysis of its phenotype and expression at the RNA level are presented in publication #1 (see: Kiyosue et al., 1999).

Imprinting of the *MEDEA* Polycomb Gene in *Arabidopsis* Endosperm (Publication #2).

Genetic analysis of *MEA* revealed that only the maternal wild type *MEA* allele is required for proper embryo and endosperm development. We have showed (See Publication#2; Kinoshita et al. 1999) that *MEA* is an imprinted gene that displays parent-of-origin-dependent monoallelic expression specifically in the endosperm. These results suggest that the embryo abortion observed in mutant *mea* seeds is due, at least in part, to a defect in endosperm function. Silencing of the paternal *MEA* allele in the endosperm and the phenotype of mutant *mea* seeds support the parental conflict theory for the evolution of imprinting in plants and mammals.

IV. Unpublished DATA

Localization of the MEA protein.

We have measure the level of *MEA* mRNA in deferent tissues and detected its presence in tissues for which there is no mutant phenotype. It is possible that translational or post-translational processes may be important in regulating *MEA* protein accumulation. To investigate this possibility, we have generate *MEA* antibodies, against the *MEA* protein fused to glutathione-S-transferase over-expressed in *E. coli*. (Fig.1A). The antibodies were purified on membrane containing over expressed *MEA* protein. Though our antibodies do recognize the expressed *MEA* protein (Fig.1B), so far we have not yet detected with our antibody the *MEA* protein in plant extracts. This work is now in progress.

Fig . 1. Expression and detection of GST-MEA fusion-protein expressed in *E. coli*

FIE and MEA Proteins Interact.

In mammals, insects and fungi, polycomb proteins have been shown to participate in the formation of complexes at specific sites within the genome (4). By influencing the degree of localized chromatin condensation, polycomb proteins play an important role in the suppression of homeotic gene transcription during insect and mammal embryo development (4). Polycomb proteins also influence patterns of cell proliferation (11). In *Drosophila*, (Fig. 2A) complex formation is thought to be initiated by the binding of a zinc finger transcription factor (i.e., Hunchback, Hb) to a "polycomb response element (pre)" DNA sequence (5). Hunchback then recruits a WD motif containing polycomb protein, Extra Sex Combs (Esc). Esc is thought to inhibit transcription of homeotic genes by two mechanisms (5). First, Esc may directly interfere with the basal transcription machinery, TFIID. Second, by virtue of its multiple WD domains, this class of polycomb group proteins has the capacity to bind simultaneously to various polypeptides, and thereby contribute to the assembly of multiprotein complexes (10, 11, 13). Thus, Esc recruits other polycomb group proteins that form stable complexes at specific sites within the genome and most likely package chromatin into a condensed form that prevents gene transcription. One class of polycomb proteins recruited by Esc is the SET-domain. These genes are the Suppressor of Position-Effect Variegation gene (Su(var)3), the Enhancer of Zeste Polycomb group gene (E(z)), and the Trithorax gene (trx-G) (6). This large class of diverse polypeptides function, in part, to maintain the silenced chromatin state until the next generation.

We (1, 2) and others (9) isolated *Arabidopsis* mutants, *fertilization-independent endosperm (fie)*, *fertilization-independent seed2 (fis2)* and *medea (mea)*, that act in the female gametophyte and allow for the central cell to replicate and form a diploid endosperm. The *FIE* (3), *MEA* (2, 14, 15) and *FIS2* (14) encode a WD-motif polycomb protein, SET-domain polycomb protein and zinc finger transcription factor, respectively. These proteins may participate in a complex similar to that found in other organisms. Recently, the *Drosophila* WD polycomb group protein has been shown to physically interact with a SET domain polycomb group protein, Enhancer of Zeste (E(z)) (6, 17). Similarly, the

mouse and human WD homologs have been shown to interact with their respective SET domain orthologs (8, 18).

Based on our results we proposed that FIE, MEA and FIS2, like insect and animal WD polycomb proteins (4), interact with each other and other polycomb proteins (Fig2.). The function of these complexes, at least in part, is to suppress transcription of endosperm genes. We hypothesize that when fertilization occurs, alteration of these complexes may allow initiation of endosperm gene transcription. According to this model, loss-of-function mutations in the *FIE*, *MEA* or *FIS2* genes prevents the formation of silencing complexes in the female gametophyte, resulting in fertilization-independent central cell nuclear replication and endosperm development.

To test our model we determined whether FIE and MEA interacted *in vivo* in the yeast two-hybrid system. As diagramed in Fig. 3, this system makes use of interacting pairs of proteins to bring GAL4 transcription activation and DNA binding domains together to activate transcription of β -galactosidase and *HIS3* reporter genes (19). To this end, we expressed a fusion protein consisting of the GAL4 DNA binding domain fused to a full-length FIE polypeptide, and the GAL4 activator fused to the full-length MEA polypeptide. Yeast containing either one of the individual constructs did not activate reporter gene transcription (Fig. 3). However, yeast containing both constructs displayed strong reporter gene transcription, indicating that FIE and MEA interact (Fig. 3). We (and others)

using *Drosophila* and animal homologs, have not been able to detect interactions between FIS2 and FIE or MEA, (data not shown) indicating that other proteins likely serve to establish the formation of the complex. Taken together, these results suggest that the mechanism for establishing and maintaining polycomb group complexes that silence gene transcription may

be conserved during the evolution of insects, mammals, and plants. Specifically, the molecular partnership of WD (i.e., FIE) and SET domain (i.e., MEA) polycomb proteins may be utilized in plants to suppress precocious endosperm development in the female gametophyte.

V. Conclusions

Seed, the end product of plant reproduction, represents one of the major primary nutrient source. Seeds are also the site of production of many important polymers such as oils and starches. The main goal of the proposed research was to elucidate the mechanisms by which reproductive development is controlled, namely endosperm and embryo development. The results obtained in this research program have provided tools permitting to better understand how polycomb proteins regulate reproduction, and in particular, the mechanism of fertilization-induced endosperm development. Such tools may lead in the future to development of novel strategies to generate plant varieties contributing to increase food production. Meanwhile, there are potentially beneficial to agriculture in Israel and the United States.

1. Seed Size. We have found that polycomb proteins (e.g., MEA) suppress endosperm development (2). As a result, reducing MEA activity may generate

seeds that overproduce endosperm (2). As described in our recently published manuscript (20), we believe that the MEA-mediated overproduction of endosperm is related to the very general phenomena of increased endosperm production and seed size caused by paternal genomic excess in *Arabidopsis* and many crop plants.

2. Apomixis. Apomixis is the formation of seed with a set of genes identical to that of the maternal parent. If the process of apomixis can be understood and harnessed, the benefits for agriculture are potentially enormous (21, 22). The fertilization-independent phenotype (i.e., endosperm, seed coat and fruit) associated with mutations in genes, such as *FIE*, *MEA* and *FIS2*, reveal that *Arabidopsis*, a sexually reproducing plant, has the genetic potential for aspects of fertilization-independent reproductive development (1, 2, 9). It is possible that alterations in the activity of polycomb proteins are responsible, at least in part, for the fertilization-independent reproductive development observed in apomictic plant species.

Aspects of the above program are currently under further research supported by a new BARD grant awarded to both laboratories.

VI. PUBLICATIONS THAT RESULTED FROM THE PROJECT.

Publication #1. Kiyosue, T., Ohad, N., Yadegari, R., Hannon, M., Dinneny, J., Wells, D., Katz, A., Margossian, L., Harada, J.J., Goldberg, R.B., Fischer, R.L.F. (1999) Control of Fertilization-Independent Endosperm Development by the *MEDEA* Polycomb Gene in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 96:4186-4191.

Publication #2. Kinoshita, T., Yadegari, R., Harada, J. J., Goldberg, R. B. & Fischer, R. L. (1999) Imprinting of the *MEDEA* Polycomb Gene in the *Arabidopsis* Endosperm. *Plant Cell* 11, 1945-1952.