Final Scientific Report

Cover Page

BARD Project Number: IS-4012-07

Date of Submission of the report: 30-1-2012

Project Title: Regulation of tomato fruit development by interacting MYB proteins

Investigators

Principal Investigator (PI): Rivka Barg

Co-Principal Investigator (Co-PI): Erich Grotewold

Collaborating Investigators: Yehiam Salts

Institutions

The Volcani Center, A.R.O.

Ohio State University, OH, USA

The Volcani Center, A.R.O.

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Differential cell expansion, fruit development, MYBI transcription factor, pericarp expansion, sub-cellular proteins localization, endoreduplication

Abbreviations commonly used in the report, in alphabetical order:

OEX- over expression, **RNAi-**RNA interference

Budget: IS: 138,000\$

US: 137,000\$

Total: 275,000\$

Signature

Principal Investigator

Signature

Authorizing Official, Principal Institution

Final Scientific Report

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1			
Submitted, in review, in preparation	1			
Invited review papers				
Book chapters				
Books				
Master theses			1 (Oxana Shaiman)	
Ph.D. theses		1 (Katja Machemer, in progress)		
Abstracts				
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.
None.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1 (for 10 days)	1 (for a month)		2
Longer Visits (Sabbaticals)				

Description Cooperation:

The project benefited from a tight and continuous cooperation, as evident in the paper published in Plant J. [Machemer K, Shaiman O, Salts Y, Shabtai S, Sobolev I, Belausov E, Grotewold E, Barg R. Interplay of MYB factors in differential cell expansion, and consequences for tomato fruit development. Plant J., 2011, 68(2):337-350], which summarizes a substantial portion of the project. The information included in this publication consolidates work performed in tight coordination between both labs: Many of the plasmids were constructed in OSU and others in the ARO, but all were used in both labs, according to the lab's expertise. All Y2H and co-IP experiments were done in the OSU lab, confocal analyses of co-agroinfiltrations experiments were done at both OSU and ARO, tomato transformation and histological analyses of transgenic tomato plants at ARO, and analysis of *Arabidopsis* orthologs was performed at OSU. Design of

Final Scientific Report

all experiments and their results were, and still are, regularly discussed by all partners involved.

Patent Summary (numbers)

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

Abstract

Background to the topic: Early tomato fruit development is executed via extensive cell divisions followed by cell expansion concomitantly with endoreduplication. The signals involved in activating the different modes of growth during fruit development are still inadequately understood. Addressing this developmental process, we identified *SIFSM1* as a gene expressed specifically during the cell-division dependent stages of fruit development. SIFSM1 is the founder of a class of small plant specific proteins containing a divergent SANT/MYB domain (Barg et al 2005). Before initiating this project, we found that low ectopic over-expression (OEX) of *SIFSM1* leads to a significant decrease in the final size of the cells in mature leaves and fruits, and the outer pericarp is substantially narrower, suggesting a role in determining cell size and shape. We also found the interacting partners of the Arabidopsis homologs of FSM1 (two, belonging to the same family), and cloned their tomato single homolog, which we named SIFSB1 (Fruit SANT/MYB-Binding1). SIFSB1 is a novel plant specific single MYB-like protein, which function was unknown. The present project aimed at elucidating the function and mode of action of these two single MYB proteins in regulating tomato fruit development. **The specific objectives were:** 1. Functional analysis of *SIFSM1* and its interacting protein SIFSB1 in relation to fruit development. 2. Identification of the SIFSM1 and/or SIFSB1 cellular targets. **The plan of work included:** 1) Detailed phenotypic, histological and cellular analyses of plants ectopically expressing FSM1, and plants either ectopically over-expressing or silenced for FSB1. 2) Extensive SELEX analysis, which did not reveal any specific DNA target of SIFSM1 binding, hence the originally offered ChIP analysis was omitted. 3) Genome-wide transcriptional impact of gain- and loss- of SIFSM1 and SIFSB1 function by Affymetrix microarray analyses. This part is still in progress and therefore results are not reported, 4) Search for additional candidate partners of SIFSB1 revealed SIMYBI to be an alternative partner of FSB1, and 5) Study of the physical basis of the interaction between SIFSM1 and SIFSB1 and between FSB1 and MYBI.

Major conclusions, solutions, achievements: We established that FSM1 negatively affects cell expansion, particularly of those cells with the highest potential to expand, such as the ones residing inner to the vascular bundles in the fruit pericarp. On the other hand, FSB1 which is expressed throughout fruit development acts as a positive regulator of cell expansion. It was also established that besides interacting with FSM1, FSB1 interacts also with the transcription factor MYBI, and that the formation of the FSB1-MYBI complex is competed by FSM1, which recognizes in FSB1 the same region as MYBI does. Based on these findings a model was developed explaining the role of this novel network of the three different MYB containing proteins FSM1/FSB1/MYBI in the control of tomato cell expansion, particularly during fruit development. In short, during early stages of fruit development (Phase II), the formation of the FSM1-FSB1 complex serves to restrict the expansion of the cells with the greatest expansion potential, those non-dividing cells residing in the inner mesocarp layers of the pericarp. Alternatively, during growth phase III, after transcription of FSM1 sharply declines, FSB1, possibly through complexing with the transcription factor MYBI serves as a positive regulator of the differential cell expansion which drives fruit enlargement during this phase. Additionally, a novel mechanism was revealed by which competing MYB-MYB interactions could participate in the control of gene expression.

Implications, both scientific and agricultural: The demonstrated role of the FSM1/FSB1/MYBI complex in controlling differential cell growth in the developing tomato fruit highlights potential exploitations of these genes for improving fruit quality characteristics. Modulation of expression of these genes or their paralogs in other organs could serve to modify leaf and canopy architecture in various crops. .

Achievements

1) **We identified *SlFSB1*** (*Solanum lycopersicum* Fruit SANT/MYB Binding protein1, *FSB1*), as a protein that physically interacts with FSM1. FSB1 is a founder member of a small plant specific gene family characterized by the presence of a single non-canonical MYB/SANT-like domain at the N-terminus and a conserved domain of unknown function (DUF3755) at the C-terminus. **We identified *SlMYBI*** (MYBI) as an additional FSB1-interacting partner, and established that the MYB-like domain of FSB1 participates in the interaction both with FSM1 and MYBI, while FSM1 and MYBI can not physically interact. It was also found that the **formation of the FSB1-MYBI complex is competed by FSM1**, thus establishing that these three MYB proteins can act in a common regulatory network.

2) **We established** the role of the small MYB like protein **FSM1 as a suppressor of differential cell expansion**. Following scrutinizing analysis of the histological effects of ectopic over-expression of FSM1, we could determine that it acts not merely as a suppressor of cell expansion but rather as an inhibitor of expansion of cell with the greatest longitudinal expansion potential. In the context of tomato fruit development post fertilization, the arrested ovary resumes growth, which initially involves a phase of cell division accompanied by cell expansion (designated growth phase II) continuing for 7-10 days. The phase II mitotic activity in the tomato fruit outer wall, the pericarp, takes place predominantly in the outer and inner epidermal layers and in the adjacent cell layers. In contrast, only sporadic cell divisions occur in the central (mesocarp) layers, which cells undergo appreciable expansion (Cheniclet *et al.*, 2005). Based on our findings, FSM1 can be defined as an important player in coordinating the very rapid development of the fruit as a whole during phase II (while increasing by ~ 500 fold within several days). And that by moderating/adjusting the rate of expansion of the inner mesocarp cells to meet the rate of growth due to cell division in the inner and outer cell layers. To our knowledge, this is the first report about a factor involved primarily in regulating differential cell expansion (Machemer et al 2011).

3) **FSB1 positively controls cell expansion**. Analysis of plants either over-expressing or silenced for FSB1 showed that opposite to FSM1, it acts as a positive regulator of cell expansion. The fact that unlike FSM1 it is expressed throughout fruit development (and in other developing organs as well), further supports its role as a positive regulator of cell

expansion (see Appendix). Whether it preferentially promotes the expansion of cells pre-programmed to become very large remains to be tested.

4) Although small MYB proteins have been previously described as inhibitory, particularly with regards to the regulation of trichome and root hair formation and anthocyanin accumulation (Kirik *et al.*, 2004a; Kirik *et al.*, 2004b; Dubos *et al.*, 2008; Tominaga *et al.*, 2008; Wester *et al.*, 2009), in all these cases, these proteins are believed to function by competing with the interaction of activatory R2R3-MYB factors with basic helix-loop-helix partners (Dubos *et al.*, 2010; Feller *et al.*, 2011). The possibility that **MYB domains serve as docking sites for other MYB domains**, as found for the FSM1/FSB1/MYBI interacting proteins, significantly expands the combinatorial space of the interactions in which this large group of plant regulatory proteins can function.

5). Based on the above described findings a model was developed explaining the role of this novel network of the three different MYB containing proteins in the control of tomato cell expansion, particularly during fruit development. In short, during early stages of fruit development (Phase II), the formation of the **FSM1-FSB1 complex** serves to **restrict the expansion of the cells with the greatest expansion potential**, those non-dividing cells residing in the inner mesocarp layers of the pericarp. Alternatively, during growth phase III, after transcription of FSM1 sharply declines, FSB1, possibly through complexing with the transcription factor MYBI serves as a positive regulator of the differential cell expansion which drives fruit enlargement during this phase.

6) Showing that the molecular mechanisms by which FSB1, MYBI and the RAD-like proteins FSM1 interact is conserved among their Arabidopsis homologs and even between the tomato and the Arabidopsis, we suggest that it is the developmental context in which they are expressed and the availability of binding partners that determines the phenotypic outcomes. According to the proposed model, the same complex could similarly participate in coordinating other, seemingly unrelated, developmental processes which are based on cell division and differential cell expansion. This implies that these genes or mutated version of them, are candidates for classic or transgenic breeding. The demonstrated role of the FSM1/FSB1/MYBI complex in controlling differential cell growth in the developing tomato fruit highlights potential exploitations of these genes for improving fruit quality characteristics. Modulating the expression of these genes or their homologs in other organs

could serve to modify leaf structure and/or canopy architecture in various crop species, especially in various vegetables where the leaves are the consumed organ.

Details of cooperation:

The project benefited from a tight and continuous cooperation, as evident in the paper published in Plant J. (Machemer et al Plant J., 2011, 68(2):337-350) which summarizes a substantial portion of the project. The information included in this publication and the data described in the Appendix consolidate work performed in tight coordination: Many of the plasmids were constructed in OSU and others in the ARO but all were used in both labs, according to the lab's proficiency. Based on the expertise of E.G. lab, all Y2H and co-IP experiments in the OSU lab, confocal analyses of co-agroinfiltrations experiments, both in OSU and ARO labs, and exploiting the experience of the Israeli partners, the tomato transformation and physiological and histological analyses of transgenic tomato plants were performed in the ARO, analysis of Arabidopsis orthologs was performed in the OSU lab. Design of all experiments and their results were, and still are, regularly discussed by all partners involved. Only by combining the different expertise of the cooperating partners, the research could both unravel the molecular basis underlying the interactions among the three studied MYB-like protein, and connect their mode of interactions to their functions in the control of the biologically interesting and agronomically important process of tomato fruit development. Tackling the research subject both at the biochemical and physiological levels enabled us to develop a general model explaining the relevance of this regulatory network to the control of different important developmental processes in plants which are executed via cell division and differential cell expansion.

Publications

Machemer K, Shaiman O, Salts Y, Shabtai S, Sobolev I, Belausov E, Grotewold E, Barg R. (2011) Interplay of MYB factors in differential cell expansion, and consequences for tomato fruit development. Plant J. 68(2):337-3350.