FINAL REPORT

Project Title: Genetic regulation of apple fruit size

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OBJECTIVES AS STATED IN PROPOSAL

The overall research program is to understand the processes that control fruit size development in apple, and the molecular basis for its regulation. In this research we propose to:

1. Measure both the rate and duration of cell division in 5 apple cultivars differing in their fruit size potential to determine the contributions of receptacle size at flowering, rate of cell division, duration of cell division and cell expansion to final fruit size.
2. Identify and clone those genes associated with cell division from apple and relate the expression of these genes to the observed patterns of cell division.

Progress

We have made significant progress on the above objectives in the 10 months since this project received approval for funding. We have:

- Collected samples from 5 cultivars and collected images of transverse sections of fruit to allow for cell number and cell size measurements. We have also frozen tissue for RNA extraction and gene expression analyses.
- Almost completed cell number, cell size and FCM analysis of 3 cultivars, ‘Gala’, ‘Pixy Crunch’ and ‘Golden Delicious’.
- Cloned 4 cell cycle genes from apple tissue and completed gene expression work in the above 3 cultivars.

Some work, however, remains to be completed, and our goal is to have this completed during the next 3 months, at which time a final report will be submitted to the WTFRC.

SIGNIFICANT FINDINGS

- 'Golden Delicious', 'Gala' and 'Pixy Crunch' had a similar proportion of dividing cells during the early stages of cell division, but cultivars with higher fruit size potential sustained a higher proportion of dividing cells for longer than those cultivars with smaller fruit.
- 'Gala' and 'Pixy Crunch' had similar final fruit sizes but attained them in different ways. 'Gala' maintained a moderate cell production rate for a relatively long period of time whereas 'Pixy Crunch' had a high cell production rate, but for a short duration. 'Golden Delicious' had high cell production rates for an extended period resulting in larger fruit size.
- Fruit size was more closely related to cell number, rather than cell size.
- Two cell cycle genes (Cyc B2 and CDK B1) are expressed only during the period of cell division suggesting their expression could play a role in regulating cell division. One other cell cycle gene (CDK A) is expressed constitutively and so is unlikely to play a role whereas the role of another (Cyc D3) is uncertain.

RESULTS AND DISCUSSION

Cell size and number

Cell numbers are the product of the rate at which cells are dividing, and the proportion of cells undergoing active cell division. The sum of these functions is called the cell production rate (CPR). Expressed another way:

CPR = proportion of dividing cells X cell division rate

An apple fruit consists of an asynchronous population of cells, where the cells are not all doing the same thing at the same time. The proportion of dividing cells was estimated using flow cytometry. At bloom, about 20% of the cortical cells were dividing and this increased to 25-30% of cells dividing soon after the time we expect fertilization to have occurred (Figure 1). There was not a rapid cut-off in terms of the proportion of cells dividing, as is often implied in pomology text books, but rather a steady decline in fruit of all cultivars from the time of fertilization. For 15-20 days after fertilization, both 'Golden Delicious' and 'Gala' maintained a relatively high proportion of dividing cells. In 'Pixy Crunch' fruit, there was a rapid decrease in the proportion dividing cells starting about 9 days after full bloom. Both 'Golden Delicious' and 'Pixy Crunch' fruit had a second smaller peak in the proportion of cells dividing in late May, but this was absent in 'Gala' fruit.

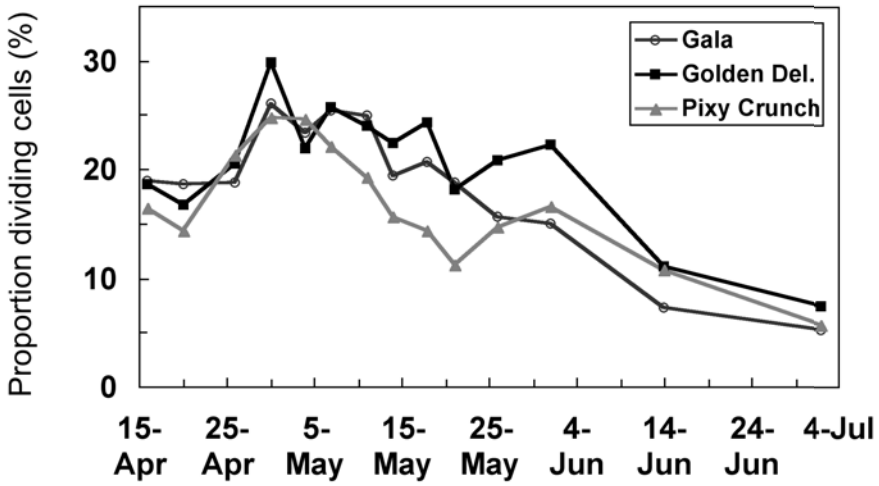

Figure 1. The proportion of actively dividing cells in apple cortical tissue of three apple cultivars as determined by flow cytometry analyses. Full bloom=April 22.

The cell number of fruit did not increase from 6 days prior to bloom until 4 days after bloom. In all likelihood, cell division did not start until fertilization, and this timing coincides with what would be expected (ie, approximately 4 days from pollination to fertilization). This suggests that fertilization acts as a trigger for cell division to start in the developing fruitlet. Cell number increased rapidly in 'Golden Delicious' and 'Pixy Crunch' after full bloom (FB) +4 days (Figure 2). The increase in cell number of 'Gala' fruit was slower. At FB+17 days (May 8) the increase in cell number of 'Pixy Crunch' fruit slowed, while that of the other two cultivars continued. The increase in cell number of 'Gala' fruit stopped at FB+26 days whereas 'Golden Delicious' continued until May 25 (FB+34). The relative cell production rate was calculated from cell numbers, to better show the rate of activity within developing fruit. In Figure 3a, it can be seen that 'Golden Delicious' and 'Gala' have similar patterns of relative CPR, but the early peak in activity (at FB+8 days) is much higher in 'Golden Delicious'. The divergence in cell numbers between these cultivars can also be clearly seen in Figure 2. While 'Golden Delicious' and 'Gala' had broadly similar patterns in relative CPR, 'Pixy Crunch' was clearly different. 'Pixy Crunch' fruit had a high early peak in relative CPR (again at FB+8 days), but this was short lived compared with both 'Gala' and 'Golden Delicious' (Figure 3b). These data clearly show that all three cultivars act differently with regard to their relative cell production rate and the cortical cell numbers in their fruit. These cultivars can be characterized as follows:

'Gala' – relatively long period of increase in cell number, but the increase is slow, resulting in small fruit size.

'Pixy Crunch' – rapid early increase in cell number but the duration is short, resulting in small fruit.

'Golden Delicious' – rapid early increase in cell number which persists for an extended duration. Fruit size is medium-large.

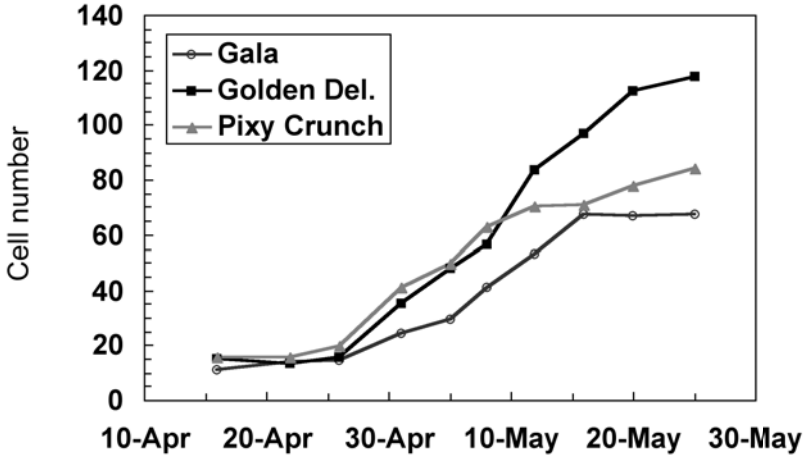

Figure 2. Number of cell layers in apple cortical tissue from the sepal vascular bundle to the epidermis in three apple cultivars. Full bloom=April 22.

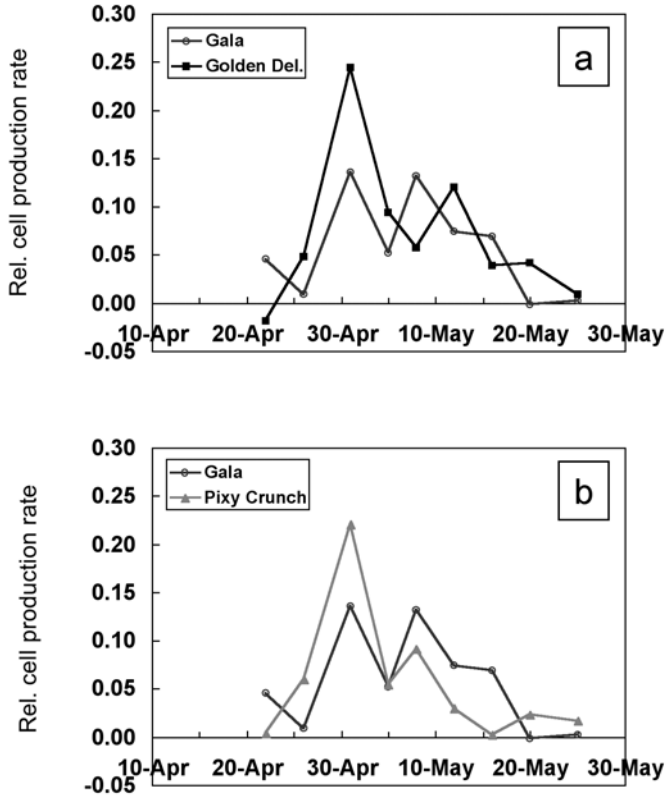

Figure 3. The relative cell production rate calculated from counts of the number of cell layers from the sepal vascular bundle to the epidermis of (a) 'Gala' and 'Golden Delicious', and (b) 'Gala' and 'Pixy Crunch' apple fruit. Full bloom=April 22.

Cell size increased at a very slow rate until May 5 (FB+14 days), at which point cells of 'Gala' and 'Pixy Crunch' enlarged faster than those in 'Golden Delicious' fruit (Figure

4). After 7-10 days, cells in all fruit had a similar rate of increase of fruit size, although 'Gala' and 'Pixy Crunch' cells were larger due to their brief growth spurt. It is interesting to note that for both 'Gala' and 'Pixy Crunch', their maximum rates of cell diameter increase occurred at the same time as their maximum increase in cell number (Figures 2, 4). This may be due simply to the available carbohydrate supply in the tree at that time, since this is about the time when shoots become net exporters of carbohydrates. In 'Golden Delicious' fruit however, it would appear that they sacrifice early cell enlargement in favor of continued rapid increase in the number of cells.

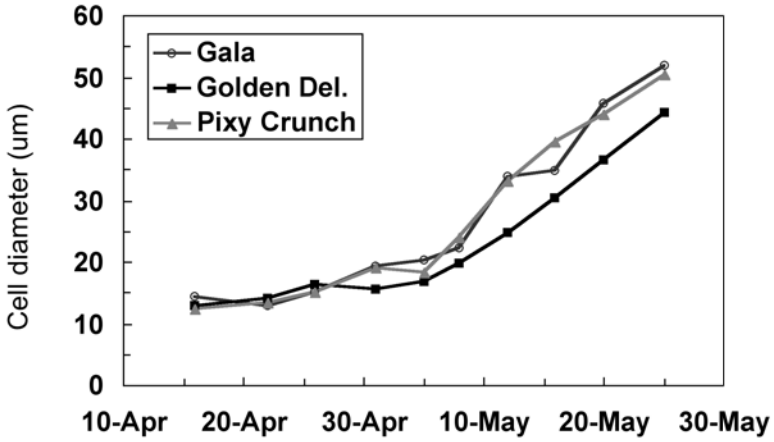

Figure 4. Diameter of fruit cortical cells in cell files from the sepal vascular bundle to the epidermis of three apple cultivars. Full bloom=April 22.

Gene expression

We successfully cloned four cell cycle genes from apple fruit. These genes consist of 2 cyclins (Cyc B2 and Cyc D3) and 2 cyclin dependant kinases (CDK A1 and CDK B1). There are probably many more cell cycle genes in apple, since even in *Arabidopsis* there are over 60. However we chose to study these 4 because they represent the more upstream genes in the cascade of events leading to their actions. The expression of these four genes in developing apple fruit were studied starting from full bloom until about three months later.

Two of the genes studied, Cyc B2 and CDK B1, were expressed strongly during the early period of fruit development in both 'Golden Delicious' and 'Gala' (Figures 5, 6 respectively), coinciding with the period of cell division. Not only were they expressed during the time that cells were increasing rapidly in number, but both these genes appeared to be more highly expressed in fruit with larger fruit size potential ('Golden Delicious') than those with smaller size potential ('Gala') (Figure 7). We are in the process of determining the expression of these genes in the other cultivars included in this study.

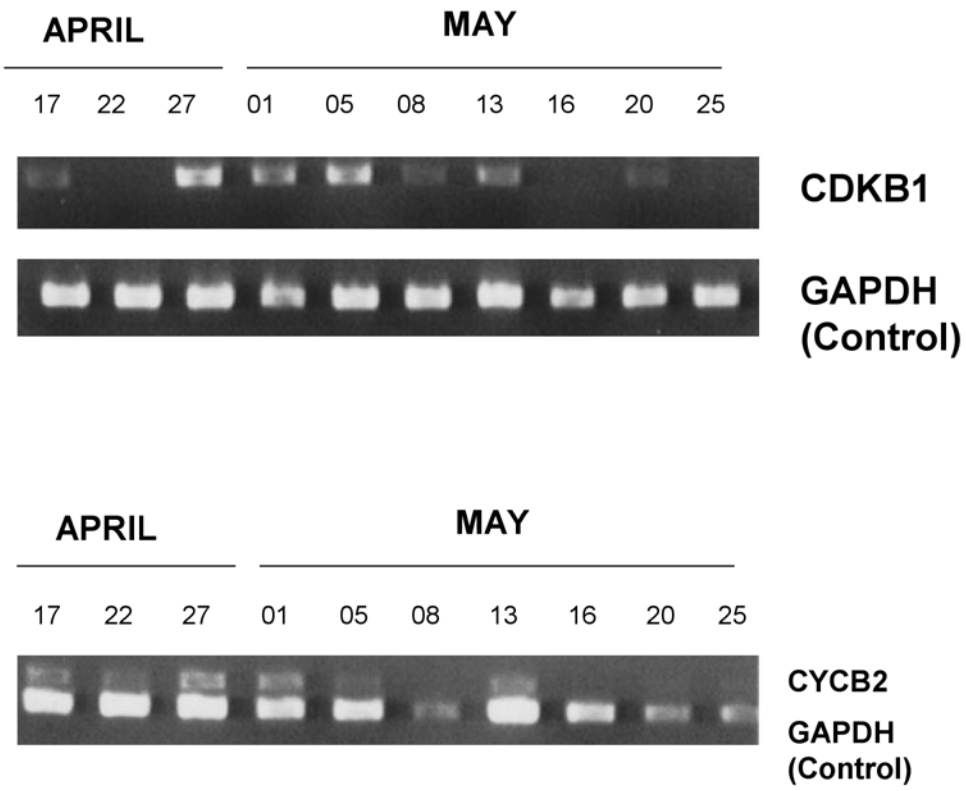

Figure 5. Expression of CDK B1 and Cyc B2 from 'Gala' apple fruit. Full bloom=April 22.

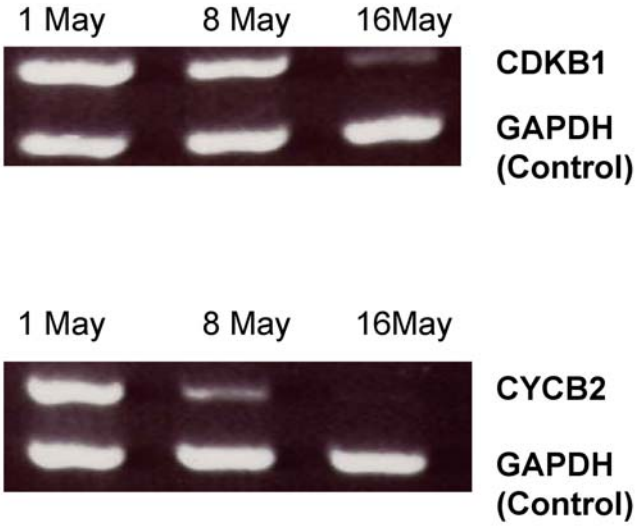

Figure 6. Expression of CDK B1 and Cyc B2 from 'Golden Delicious' apple fruit. Full bloom=April 22.

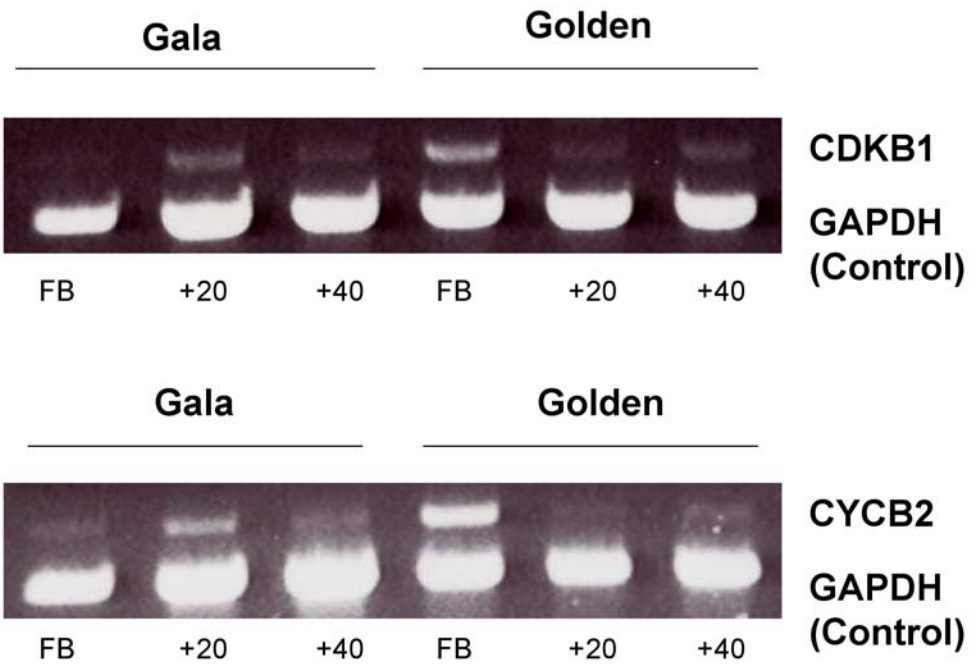

Figure 7. Expression of CDK B1 and Cyc B2 from 'Gala' and 'Golden Delicious' apple fruit. Full bloom=April 22.

Another of our candidate genes, CDK A1 appeared to be constitutively expressed (ie, expressed at all times) and therefore seems unlikely to play a central role in the regulation of cell division in these fruit (Figure 8). The expression of Cyc D3 was high until about 5-10 days after full bloom then it dropped to undetectable levels. It was expressed strongly again during July, well beyond the time that significant cell division occurs. It seems doubtful that this gene plays a role in cell division, but further analyses are being conducted to examine this conclusion.

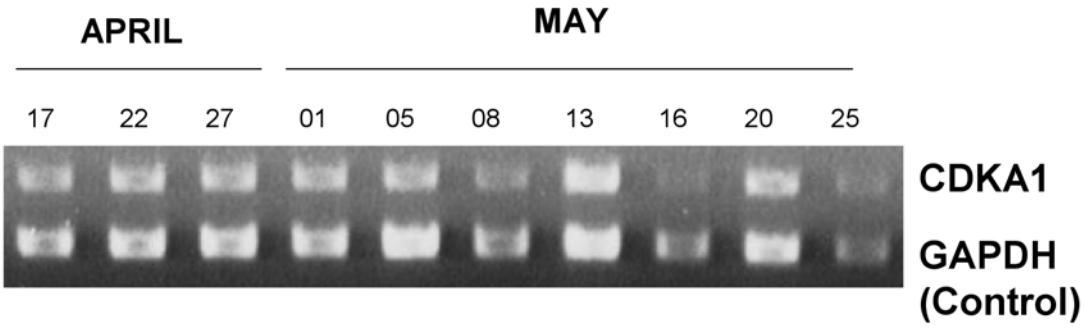

Figure 8. Expression of CDK A1 from 'Gala' apple fruit. Full bloom=April 22.

Significance to industry

We have made significant progress on further understanding the processes that lead to large sized fruit. We have also refuted 2 commonly held beliefs regarding fruit growth:

1. That cell division lasts for about 30 days and then stops. Our data show that there is a slow and gradual decline in the proportion of cells in a fruit that are actively dividing. At the time of fertilization, 25-30% of the cortical cells in an apple are dividing and this drops to about 5% at 70 days after bloom.
2. That fruit growth is first by cell division and then by cell expansion with no overlap. A considerable overlap in cell division and cell expansion is clearly shown in our data. Cells start a rapid enlargement beginning about 14 days after bloom but the number of cells keeps increasing until at least 25 days after full bloom.

We have identified cultivars that exhibit increased fruit cell numbers due to rapid but short CPR (‘Pixy Crunch’), lower CPR but over a longer period of time (‘Gala’) and rapid CPR over an extended duration (‘Golden Delicious’). This offers the potential to use these cultivars to understand what it is about ‘Golden Delicious’ that it is capable of both a rapid rate and long duration of CPR, and if these can be further increased.

We have identified two genes that may be involved in regulating cell division and cell production. Further analyses over the next several months will provide more conclusive data on the involvement of these genes in these processes. Once more definitive data on the function of these genes is gathered, methods to alter their expression to increase fruit size should be explored.

BUDGET

Project Title: Genetic regulation of apple fruit size

PI: Peter M. Hirst

Project duration: 2004

Current year: 2004

Project total: $20,000

Current year request: $20,000

Item	**2004**
Salaries	3740
Benefits	1283
Wages	4791
Benefits	484
Equipment	2000[1]
Supplies	7402
Travel	300
Total	**20,000**

1 Main equipment purchased was a Labnet Spectrafuge 24D microcentrifuge. The price was approximately $1300. Used during RNA extraction.

Funding from other sources.

NSF: Initiative for apple functional genomics. Collaborator on genetic control of apple fruit size. $300,000. Status – submitted.