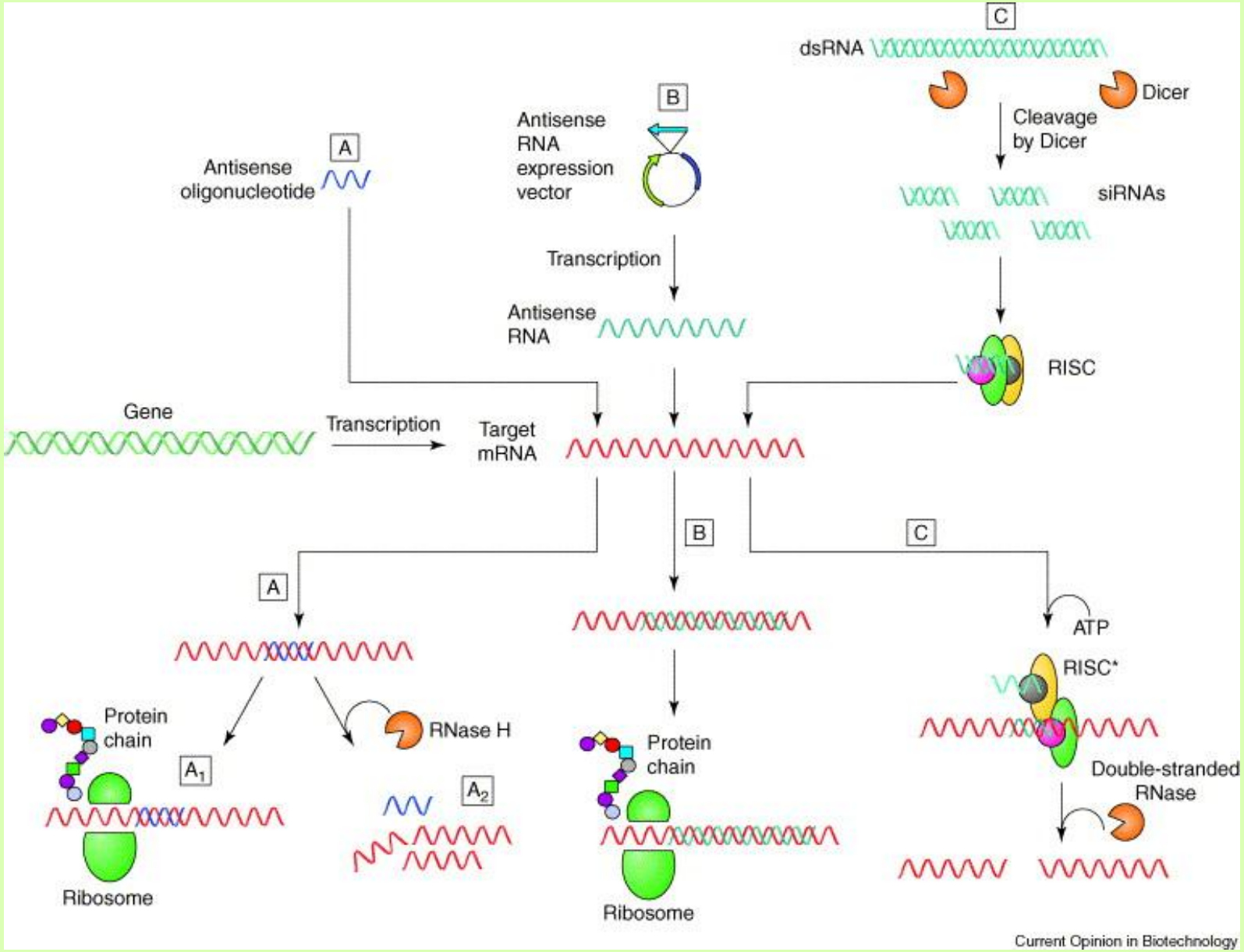
Antisense Technology

UNIT V

Post transcriptional Gene silencing



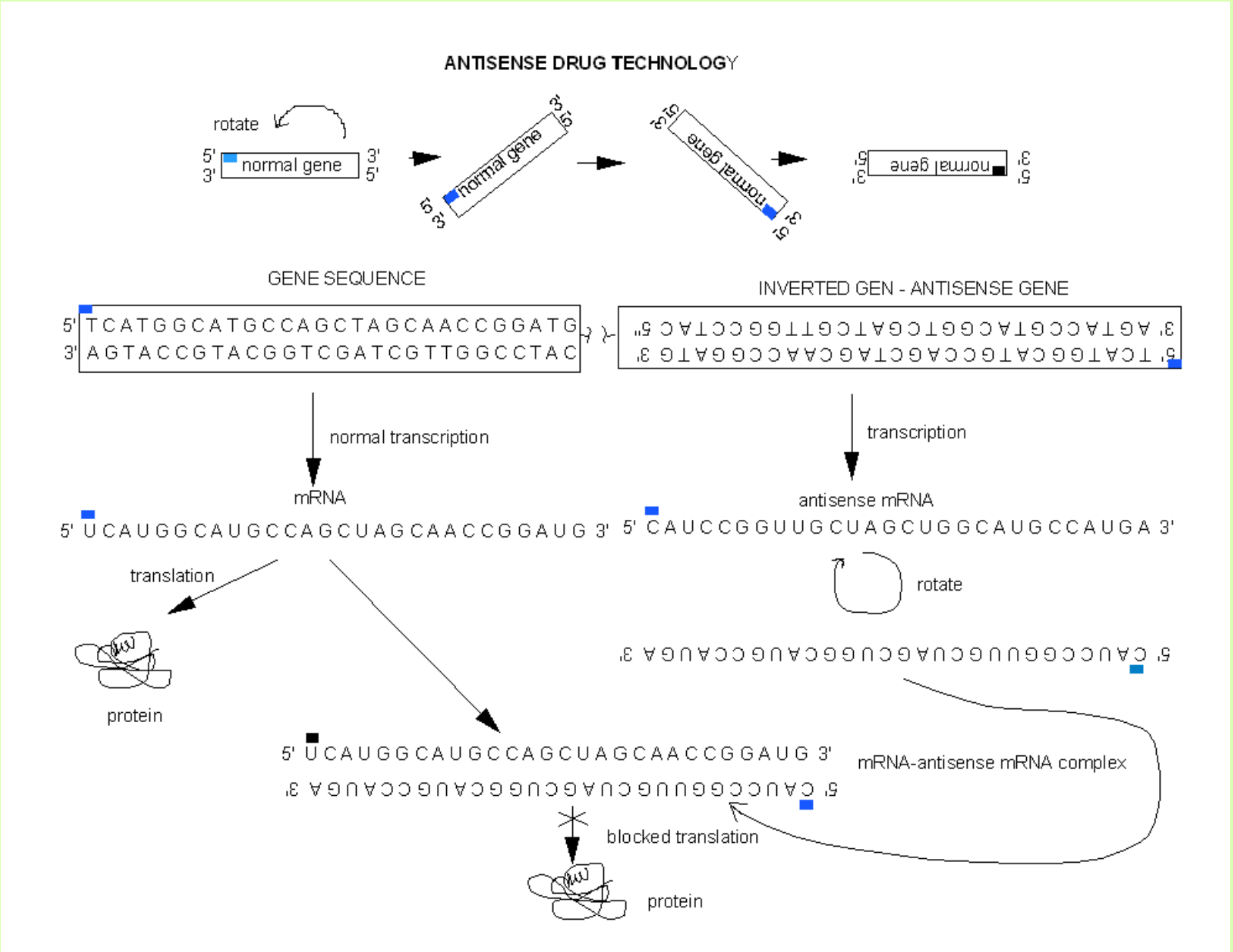
Introduction

- The tool that is used for the inhibition of gene expression is called Antisense technology. The antisense nucleic acid sequence base pairs with its complementary sense RNA strands and thus prevents it from being translated into a protein. The complementary nucleic acid sequence can be either a synthetic oligonucleotide, like oligodeoxyribonucleotides (ODN) having less than 30 mucleotides or longer antisense RNA (aRNA) sequences (Sczakiel, 1997). Example of sense and antisense RNA is: 5’ A C G U 3’ mRNA, and 3’ U G C A 5’ Antisense RNA.
- Dr. Hal Weintraub first developed this technology at Basic Science Division. Firstly, they showed that aRNA inhibits the gene expression in mouse cells by Berg, 2002. Dr. Meng-Chao Yao in 1996 showed that aRNA that was incorporated into non-conserved regions of ribosomal RNA (rRNA) disrupts translation and this was done by altering interaction of the mRNA, and the rRNA, mRNA chimera.

Introduction

- Sequence transcription of antisense DNA strand into the sense mRNA strand, which is then translated into polypeptide (Kimball, Nov 2002).
- The inhibition in which the theory works are as follows:
- When the RNA binds to the complementary mRNA, it forms a double stranded RNA (ds RNA) complex which is similar to double stranded DNA . The dsRNA complex do not allow translation to occur. This translation process was not known.
- Several theories include:
- dsRNA prevents ribosome from binding to the sense RNA and translating.
- dsRNA cannot be translated from nucleus to cytosol, where the translation occurs.
- dsRNA is susceptible to endoribonucleases that does not affect single stranded RNA, but degrade the dsRNA.

Antisense technology

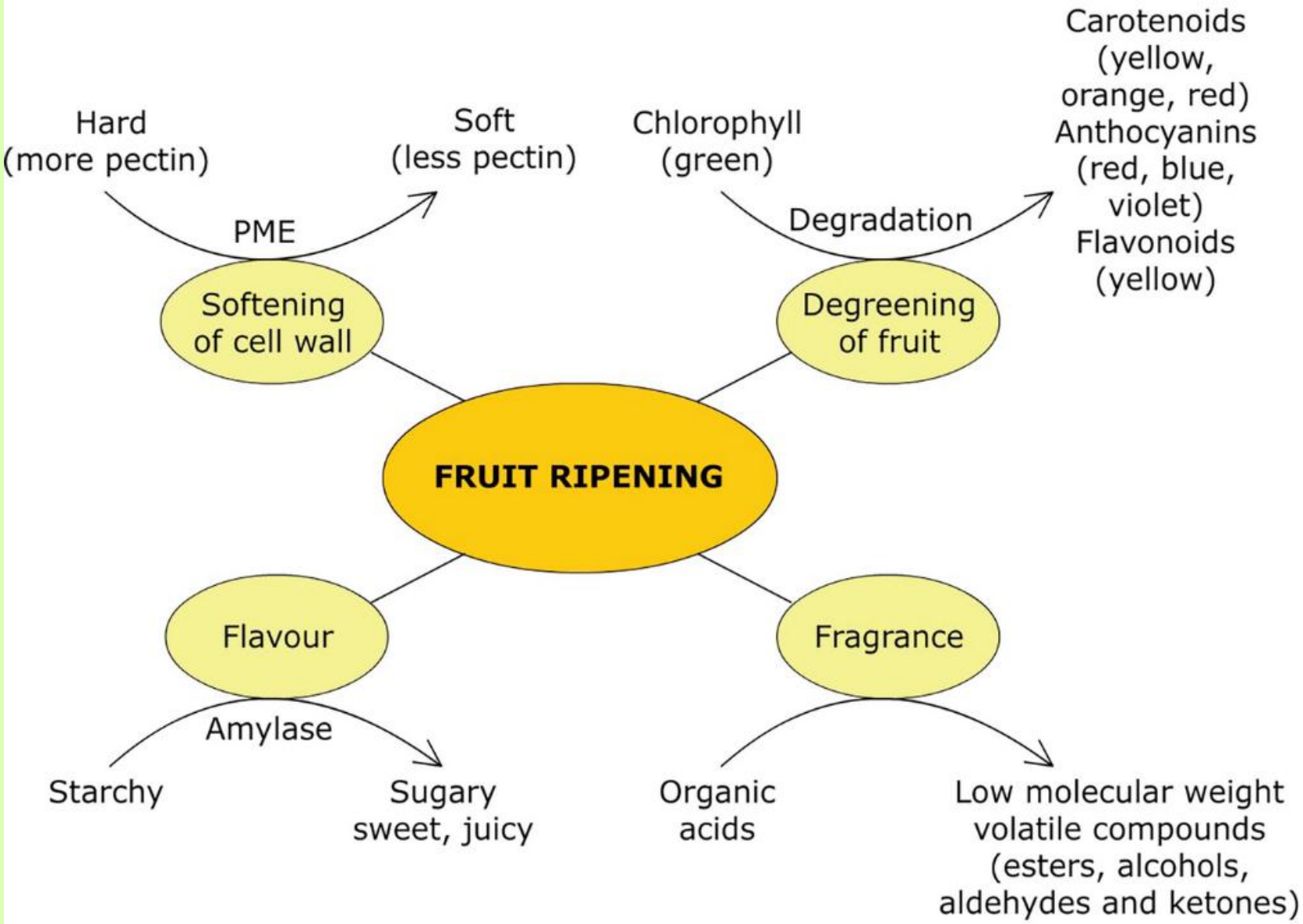


Flavr Savr Tomato

- **Flavr Savr** (also known as **CGN-89564-2**; pronounced "flavor saver"), a genetically modified tomato, was the first commercially grown genetically engineered food to be granted a license for human consumption.
- It was produced by the Californian company Calgene, and submitted to the U.S. Food and Drug Administration (FDA) in 1992. On May 18, 1994, the FDA completed its evaluation of the Flavr Savr tomato and the use of APH(3')II, concluding that the tomato "is as safe as tomatoes bred by conventional means" and "that the use of aminoglycoside 3'-phosphotransferase II is safe for use as a processing aid in the development of new varieties of tomato, rapeseed oil, and cotton intended for food use."
- It was first sold in 1994, and was only available for a few years before production ceased in 1997. Calgene made history, but mounting costs prevented the company from becoming profitable, and it was eventually acquired by Monsanto Company.

- Tomatoes have a short shelf-life in which they remain firm and ripe. This lifetime may be shorter than the time needed for them to reach market when shipped from winter growing areas to markets in the north, and the softening process can also lead to more of the fruit being damaged during transit.
- To address this, tomatoes intended for shipping are often picked while they are unripe, or "green", and then prompted to ripen just before delivery through the use of ethylene gas which acts as a plant hormone. The downside to this approach is that the tomato does not complete its natural growing process, and the final flavor suffers as a result.
- Through genetic engineering, Calgene hoped to slow down the ripening process of the tomato and thus prevent it from softening, while still allowing the tomato to retain its natural colour and flavour. This would allow it to fully ripen on the vine and still be shipped long distances without it going soft.
- The Flavr Savr was made more resistant to rotting by adding an antisense gene which interferes with the production of the enzyme polygalacturonase. The enzyme normally degrades pectin in the cell walls and results in the softening of fruit which makes them more susceptible to being damaged by fungal infections.
- Flavr Savr turned out to disappoint researchers in that respect, as the antisensed PG gene had a positive effect on shelf life, but not on the fruit's firmness, so the tomatoes still had to be harvested like any other unmodified vine-ripe tomatoes. An improved flavor, later achieved through traditional breeding of Flavr Savr and better tasting varieties, would also contribute to selling Flavr Savr at a premium price at the supermarket.
- The FDA stated that special labeling for these modified tomatoes was not necessary because they have the essential characteristics of non-modified tomatoes. Specifically, there was no evidence for health risks, and the nutritional content was unchanged.
- The failure of the Flavr Savr has been attributed to Calgene's inexperience in the business of growing and shipping tomatoe

Fruit Development and Ripening



Biochemical changes during tomato ripening

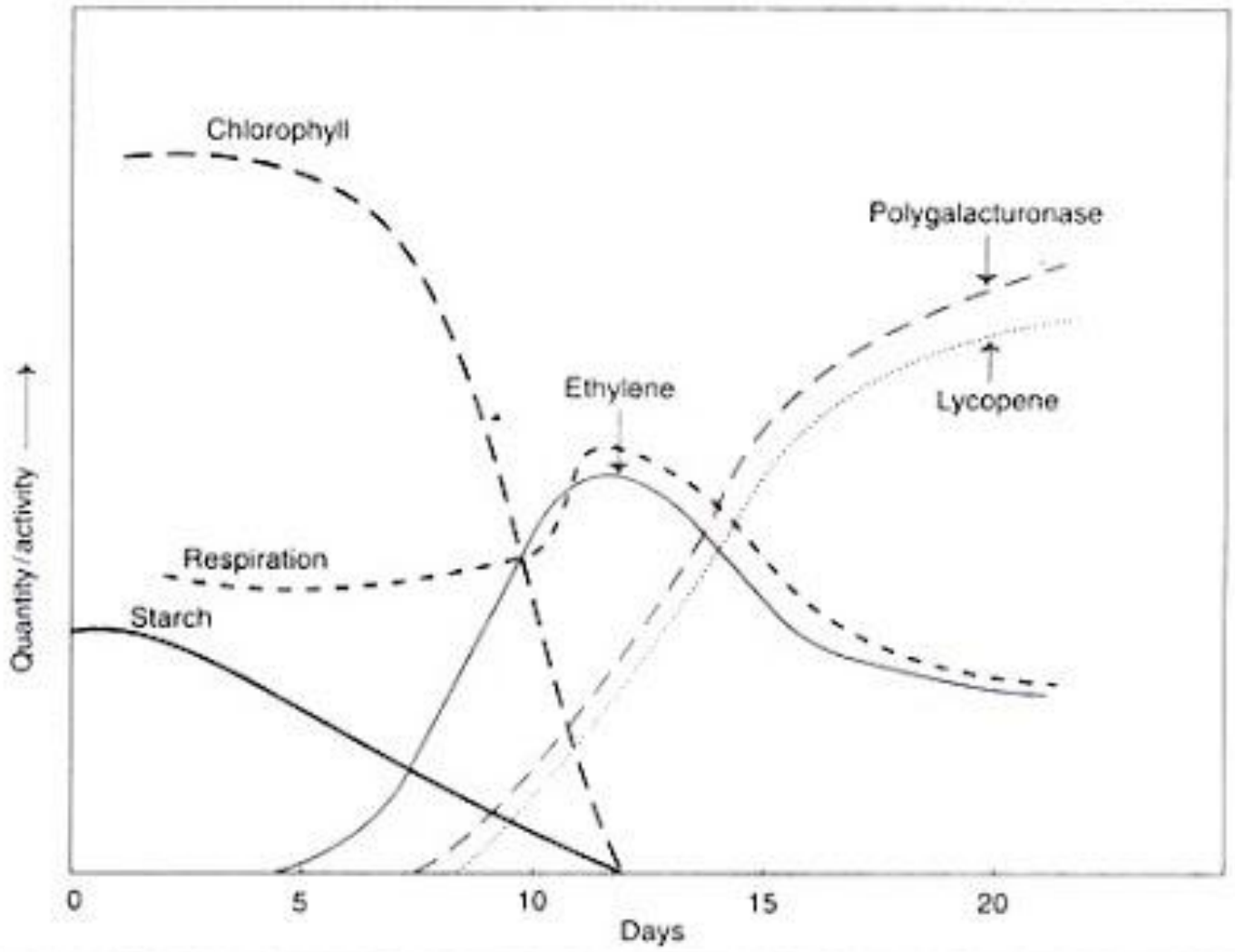
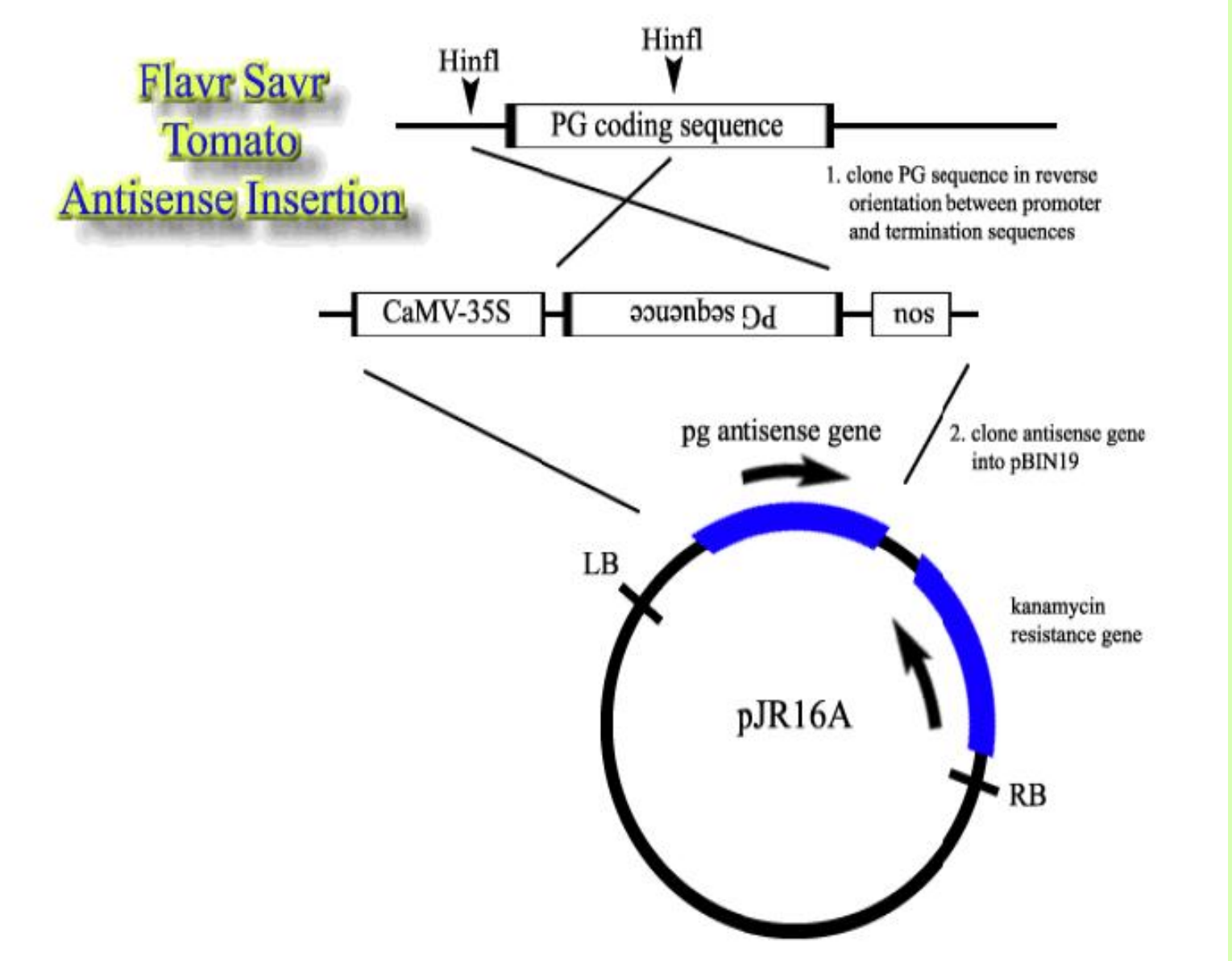


Fig. 50.8 : Biochemical changes during the process of tomato ripening.

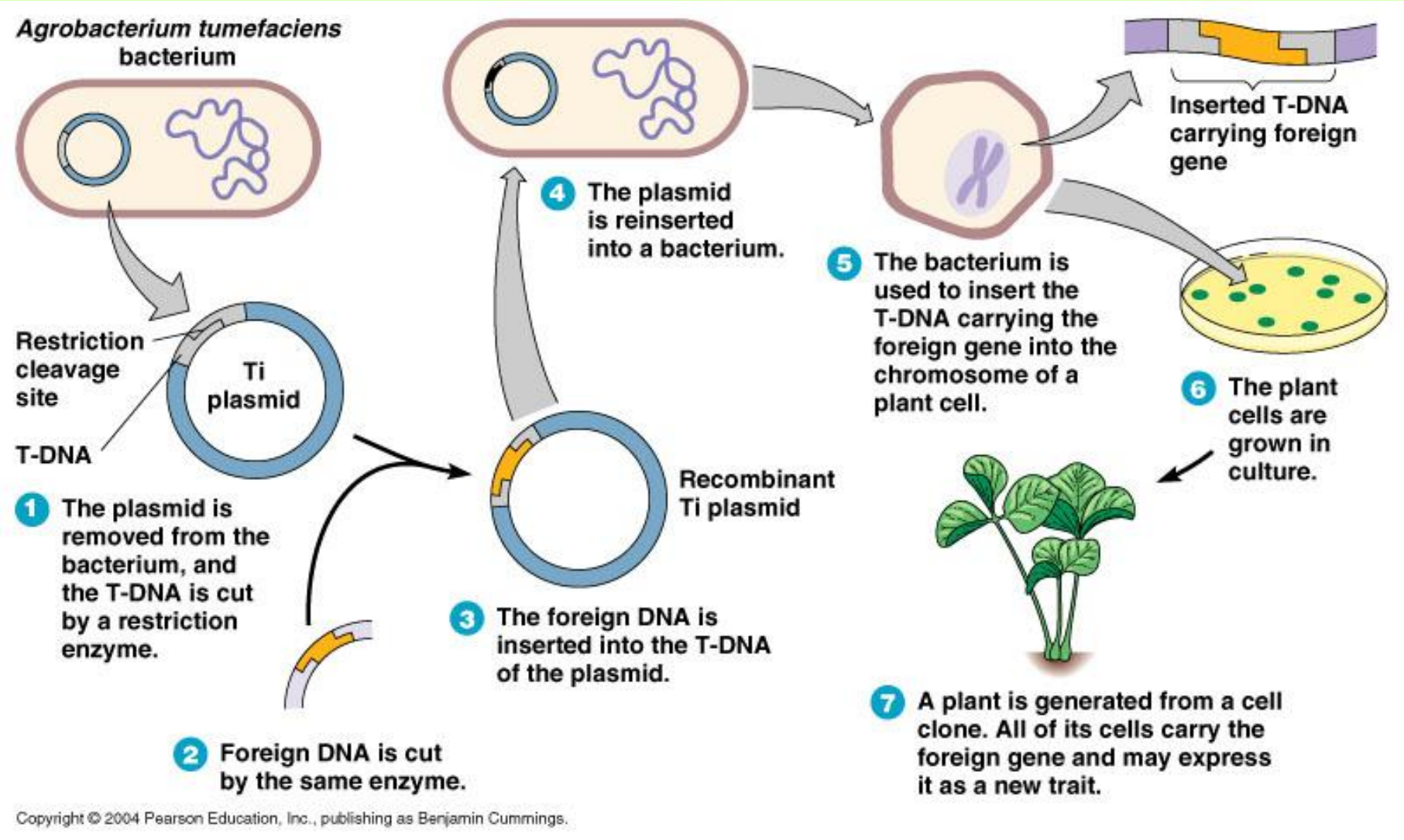
Ripening-related genes in tomato

Clone	Gene Product	Function	Role in ripening
pTOM5	Phytoene synthase	Lycopene synthesis	Red colouration
pTOM6	Polygalacturonase	Cell Wall degradation	Fruit softening
pTOM13	ACC oxidase	Ethylene ormation	Ripening trigger

Flavr Savr Tomato

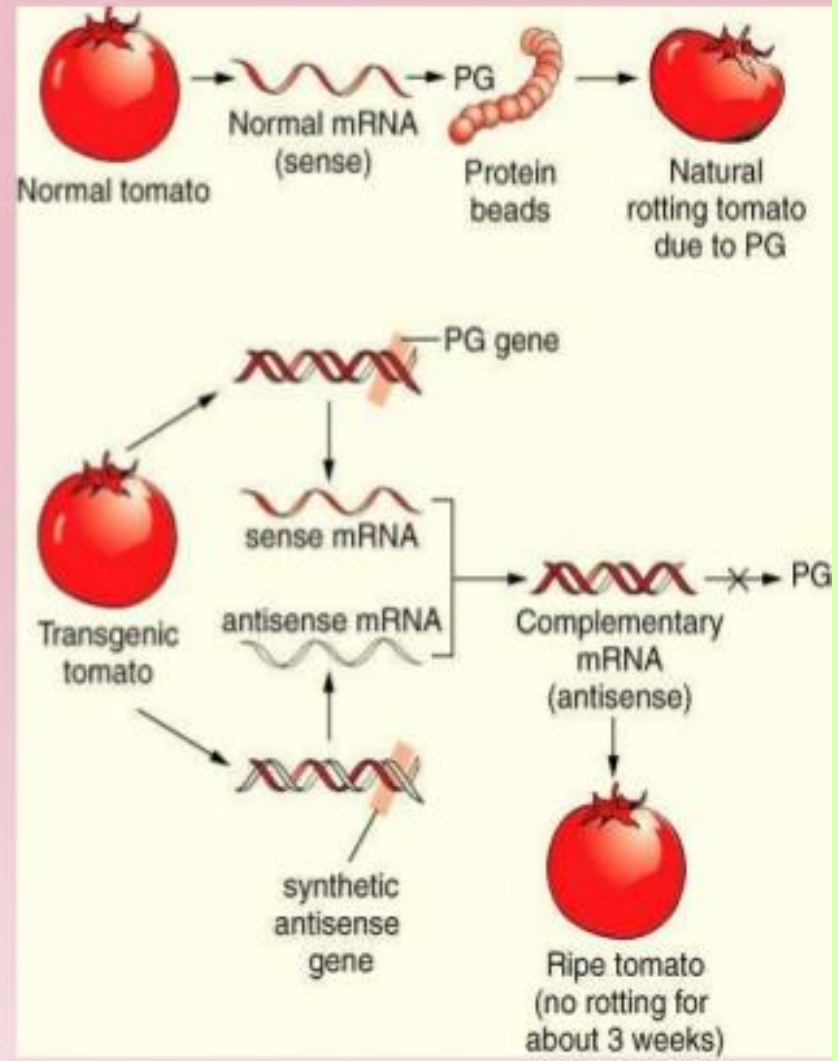


Genetic transformation of tomato



MAKING OF FLAVR SAVR

- Enzyme Polygalacturonase breaks down structural polysaccharide pectin in wall of a plant.
- This is part of the natural decay process in a plant
- Flavr savr tomatoes have been constructed that express an antisense mRNA complementary to mRNA for an enzyme involved in ethylene production
- These tomatoes make only 10% of normal amount of enzyme thus delaying ethylene production.



http://www.google.com/imgres?q=Flavr+Savr+Antisense technology

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Two tales of Annexin A2 knock-down: One of compensatory effects by antisense RNA and another of a highly active hairpin ribozyme

Elin Aareskjold[a], Ann Kari Grindheim[a], Hanne Hollås[a], Marianne Goris[a], Johan R. Lillehaug[b], Anni Vedeler[a,*]

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Application of RNA silencing to plant disease resistance

Cheng-Guo Duan[1,2], Chun-Han Wang[1] and Hui-Shan Guo[1*]

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The Flavr Savr Tomato, an Early Example of RNAi Technology

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