TOMATO BIOTECHNOLOGY

Tomato Biotechnology…..

Why tomato was targeted as one of the first crops to be altered by Biotechnology?

How fruit ripening Tomato is regulated?

Approaches to altering fruit ripening by modifying ethylene biosynthesis

Tomato Biotechnology…

Tomato was an early target for genetic modification and “improvement” for a number of reasons

- Tomato is a member of Solanaceae family
  - same family as tobacco
  - many members of this family, including tomato, are easy to transform
- high value crop

Tomato Biotechnology…

More reasons for tomato biotechnology

- Several characteristics that could be improved
- fresh tomatoes for market
  - quality, taste, shelf life, seasonal availability
- Tomato processing
- product yield, reduced processing costs, improved quality

Improvement of Tomato

Tomato has been a model system for many studies in plant genetics

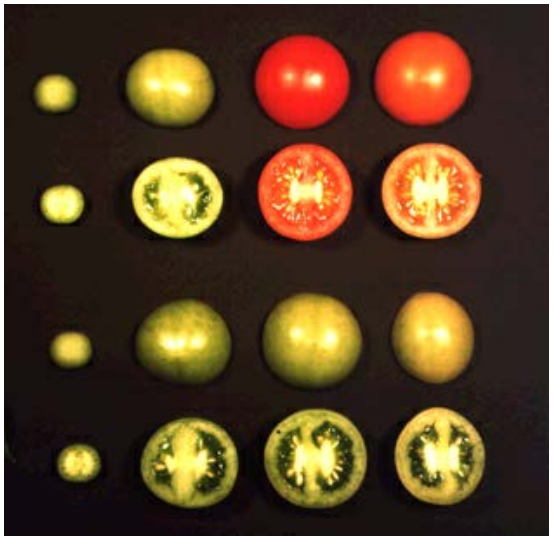
Lot of interesting mutants

Among these are mutations that affect ripening of fruit

***rin* - ripening inhibited**

Nr - never ripen

***nor* - non-ripening**



Modification of fruit ripening

Fruit ripening has been one of the primary targets for modification because

- Ripening impacts handling, shelf life, quality of fresh tomatoes
- Ripening affects processing properties
- Tomato fruit ripening is a useful model for ripening of some other fruits
- Methods developed to alter tomato ripening may be applied to other fruits

Ripening and fruit development

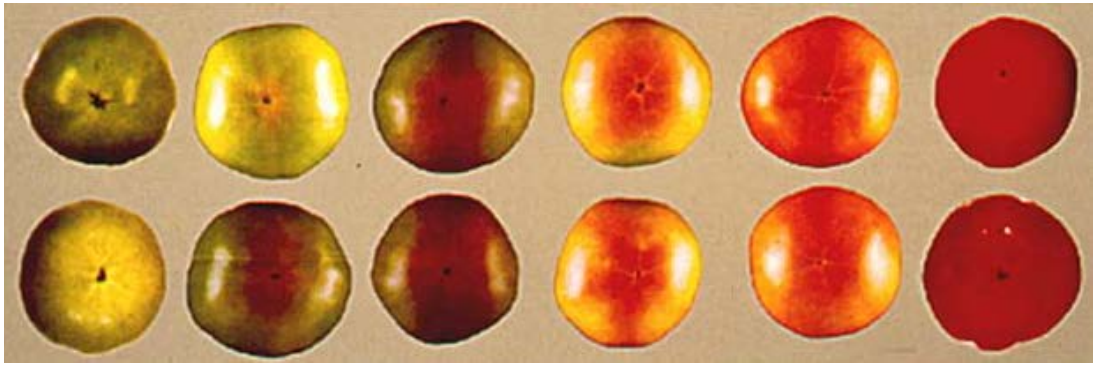
The penultimate stage in fruit development

- Flowering
- Fertilization
- Fruit development and growth
- Fruit ripening
- Senescence

Fruit development

- Fertilization is followed by cell division and cell expansion
- It takes 40 to 50 days for the fruit to reach its maximum mature green size
- It switches from growth to ripening
- Several dramatic changes occur that are collectively referred to as ripening

Tomato fruit ripening



The following occurs:

- Gaseous hormone ethylene is produced
- Increased respiration
- Synthesis of red pigments (lycopene)
- Softening of the fruit
- Conversion of starches to sugars
- Development of flavors

Fruit ripening

- All of these processes are highly regulated; ripening is not just a random deterioration of the fruit
- Ethylene plays a central role in ripening of tomato fruits
  - and in ripening of many other fruit, e.g. banana, peach, melon
  - and in other developmental processes, e.g. flower senescence

Ethylene

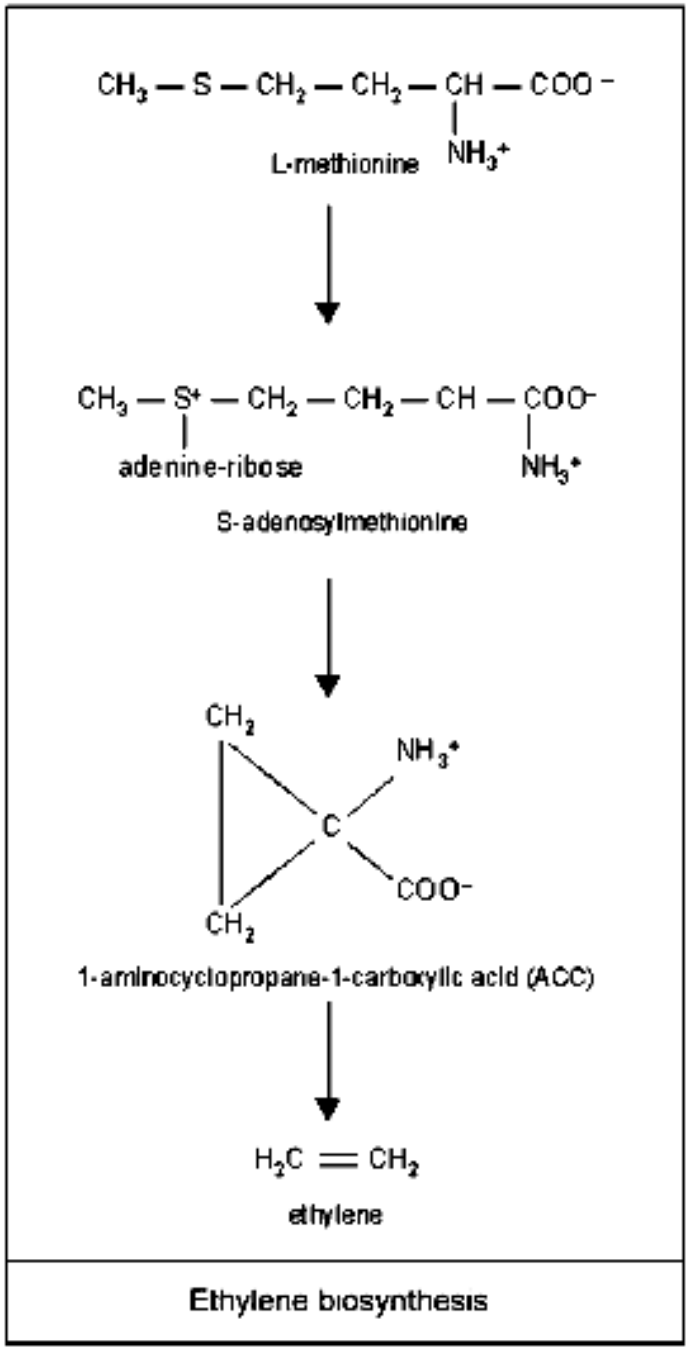
Synthesis of ethylene

starts with the amino acid methionine

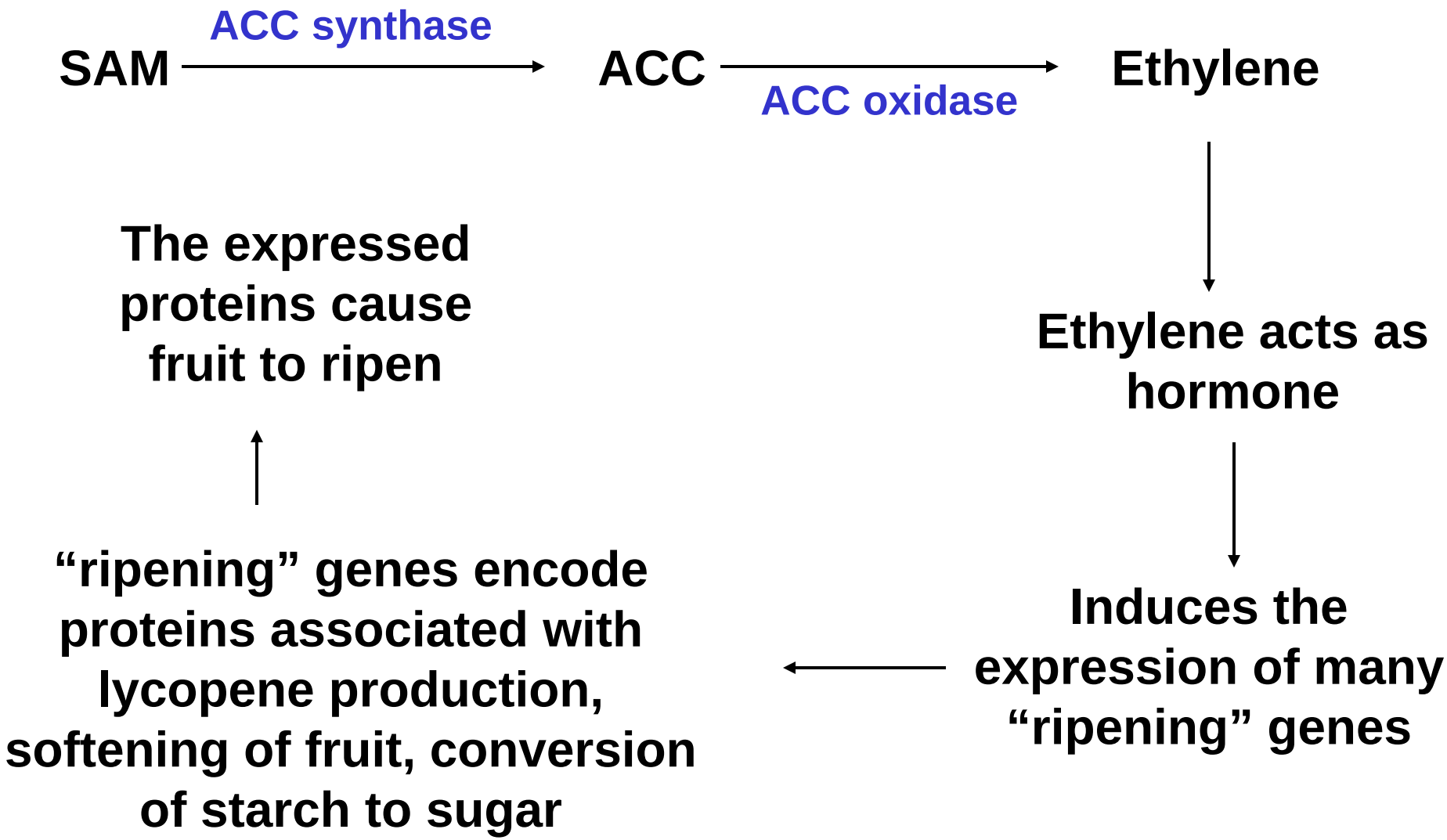
methionine to S-adenosyl methionine (SAM)

SAM to aminocyclopropan 1-carboxylic acid (ACC)

Finally ACC is converted to ethylene



Ethylene and Fruit ripening



Strategies to modify ripening

How can the over-worked and under-appreciated biotechnologist use this information to change fruit ripening?

Two approaches

- Reducing the synthesis of ethylene in tomato fruits
- Reducing or altering the effects of ethylene

Reducing ethylene synthesis....

Three methods

Metabolizing SAM to something other than ACC

Metabolism of ACC so it cannot be converted to ethylene

Inactivating the genes for ethylene biosynthesis

Reducing ethylene's effects….

Ethylene → **Fruit ripening**

Three methods

- Identifying mutants with reduced responses to ethylene (conventional)
- Inactivating genes that cause specific ripening processes (e.g. softening) to occur
- Making plants or specific tissues (e.g. fruits) unresponsive to ethylene

Reducing ethylene synthesis….

SAM decarboxylase metabolizes SAM and prevents the formation of ACC

Without ACC, no ethylene will be produced, ripening will be altered

Reducing ethylene synthesis….

Gene for SAM decarboxylase cloned from a virus that infects bacteria, T3 bacteriophage

Open reading frame of this gene was used to produce a chimeric gene

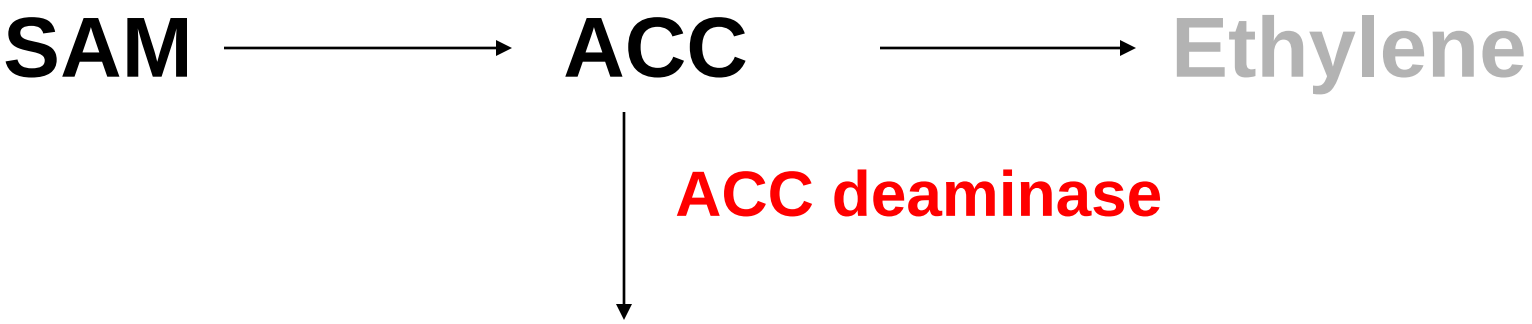
What sort of promoter should be used to drive the expression of this gene?

- should be expressed in fruit when they are just starting to ripen (late mature green stage)
- or a promoter from a gene that is activated in response to ethylene

Reducing ethylene synthesis

- Chimeric SAM decarboxylase gene transferred into tomato by *Agrobacterium*-mediated transformation
- Reduced or eliminated expression of ethylene in tomato fruits
- Developed by Agritope (now part of Exelixis, a CA biotech company)
- At one time, they were also applying this technique to raspberry and melon

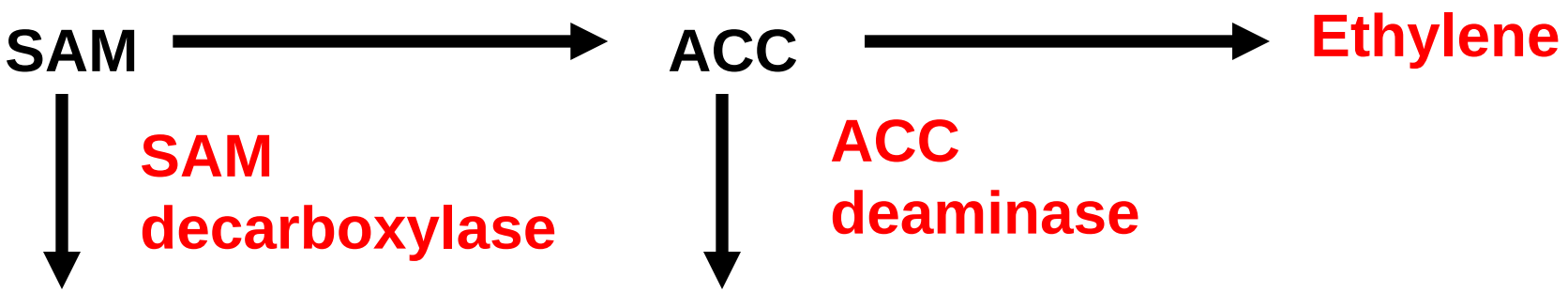
Reducing ethylene synthesis….



Approach used by Monsanto

- ACC deaminase gene identified in a bacterium, *Pseudomonas chlororaphis*
- Chimeric gene transferred into plants
- Reduced ethylene synthesis and delayed fruit ripening

Reducing ethylene synthesis by metabolic interference



Both methods prevent ethylene from being synthesized by interfering with the metabolic pathway

Diverting substrates away from ethylene