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Regulation of Ethylene Level and Ethylene Action in Climacteric Fruits; A Way of Reducing Post-harvest Losses

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Fruits are rich sources of vitamins, phytochemicals (e.g. flavonoids, phenolics etc.) and minerals like K, Ca, Mg etc. But most fruits are lost after harvesting due to over ripening or as a result of premature harvesting. The loss as mature or consumable fruits is the greatest loss of nutrients. Because mature fruits accumulate maximum nutrients as well as energy starting from initiation of flower buds to the maturity. Ethylene is the responsible hormonal factor for controlling fruit ripening process. We can promote ripening by application of exogenous ethylene or delay ripening by down regulating the endogenous ethylene production or by hampering the ethylene actions.

India is the second largest producer of fruits and vegetables in the world with a combined production of about 210 million metric tons per year (FAO). A study conducted by the Central Institute of Post-Harvest Engineering and Technology (CIPHET), Ludhiana, reported that about 18% of fruits and vegetables are lost in the post- harvest. So, to fulfill reduction in post-harvest losses, we must extend the shelf life of fruits and vegetables after harvesting there fulfilling the demand of the increasing consumers.

Ethylene

Ethylene is a gaseous plant hormone, which is the simplest known olefin (alkene) bearing molecular weight of 28 and synthesized by almost all parts of higher plants, gymnosperms and lower plants including ferns, mosses, liverworts, certain cyanobacteria, some fungi and bacteria. The rate of ethylene synthesis depends on type of tissue and the stage of development. In general meristematic and nodal regions are the most active in ethylene biosynthesis. However leaf abscission, flower senescence and fruit ripening also increases its production.

Structure and biosynthesis of ethylene

The chemical formula for ethylene is C_2H_4, which is synthesized from Yang Cycle. In the Yang cycle (given by Miyazaki Yang in 1987) , the S-adenosyl- methionine (AdoMet) is synthesized from L-methionine. The AdoMet comes out from the Yang cycle and is converted into 1- aminocyclopropane - 1- carboxylate (ACC) by the enzyme ACC synthase. The ACC is finally converted into Ethylene by the enzyme ACC oxidase.

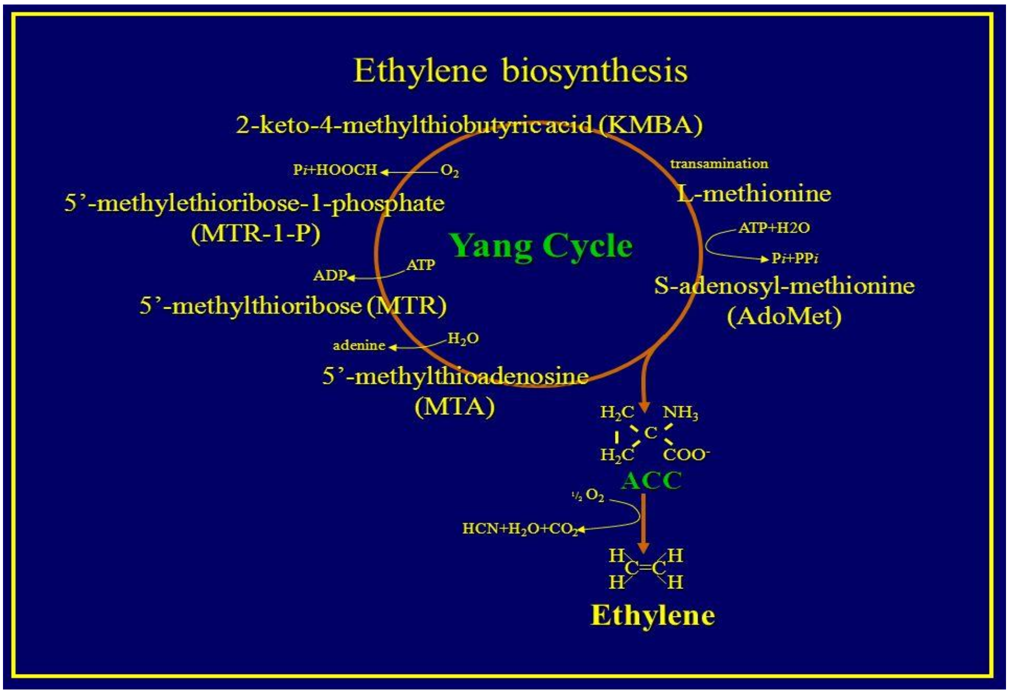

Fig.2. Ethylene biosynthesis

Commercial use of ethylene

Ethylene application promotes fruit ripening that makes this hormone commercially important. But our aim here is to delay ripening.

Ethylene concentration and duration of treatment

Physiological responses of plants to ethylene saturate at 100 ppm. Mature climacteric fruits generally initiate endogenous ethylene production within not more than 72 hours. De-greening should continue for not more than 72 hours or there is increased risk of peel senescence and decay.

Fruits

Fruits can be defined as the fleshy or dry ripened ovary of a flowering plant, enclosing the seed or seeds. Based on their respiratory peaks fruits can be classified into two types-

1. Climacteric fruits
2. Nonclimacteric fruits

Climacteric fruits are those, which can be harvested at physiological maturity and ripened off the plant. Example - apple, apricot, avocado, banana, guava, mango, papaya, pear, plum, tomato, sapota etc. Except avocado, banana and pear these fruits attain best flavour if ripened on the plant.
Fruits that are not capable of continuing their ripening process once removed from the plant are known to be nonclimacteric fruits. Example - berry, cherry, grapes, lemon, loquat, mandarin, muskmelon, pineapple, strawberry, water melon, pomegranate etc. Some muskmelon varieties are climacteric, but are best when harvested partially or fully ripe.

Fruit Growth and Ripening

Fruit Growth

Pollination induces fruit growth, because pollen is a rich source of auxin. Ovary produces hormones which sustain fruit growth: IAA or GA. Fruits grow initially by cell division later by cell enlargement.

Fruit ripening

Ripening can be defied as a process of physiological, biochemical and morphological changes of fruits leading to edible form from nonedible form. In order to accelerate ripening, fruit have to be exposed to naturally produced ethylene. Ethylene stimulates the synthesis of those enzymes which are involved in ripening process. Ethylene also stimulates its own synthesis (de-novo) via induction of ACC synthase transcription.

Changes in fruits on ripening

a. Functions of cell wall and middle lamella dissolution

↓

Change in texture from firm to soft

↓

Softening of fruit flesh

b. Loss of chlorophyll, synthesis of carotenoids and anthocyanins, chloroplasts get converted into other chromoplasts

↓

Loss of green colour and development of colours like red, yellow pink, red, purple

Compositional changes

- Conversion of starch to sugars (e.g.- glucose, fructose, sucrose)
- Production of aroma and flavor volatiles (alcohol esters)
- Polymerization of tannins (reduced astringency, e.g.- persimmons)
- Decrease in acidity

How can we delay ripening?

- By inhibiting ethylene biosynthesis with the help of some inhibitors like AOA, AVG, Co ion etc. which inhibit the action of ACC synthase or ACC oxidase.
- By using chemicals that absorb already synthesized ethylene e.g. $KMnO_4$
- Inhibiting ethylene action or hampering ethylene signal transduction.

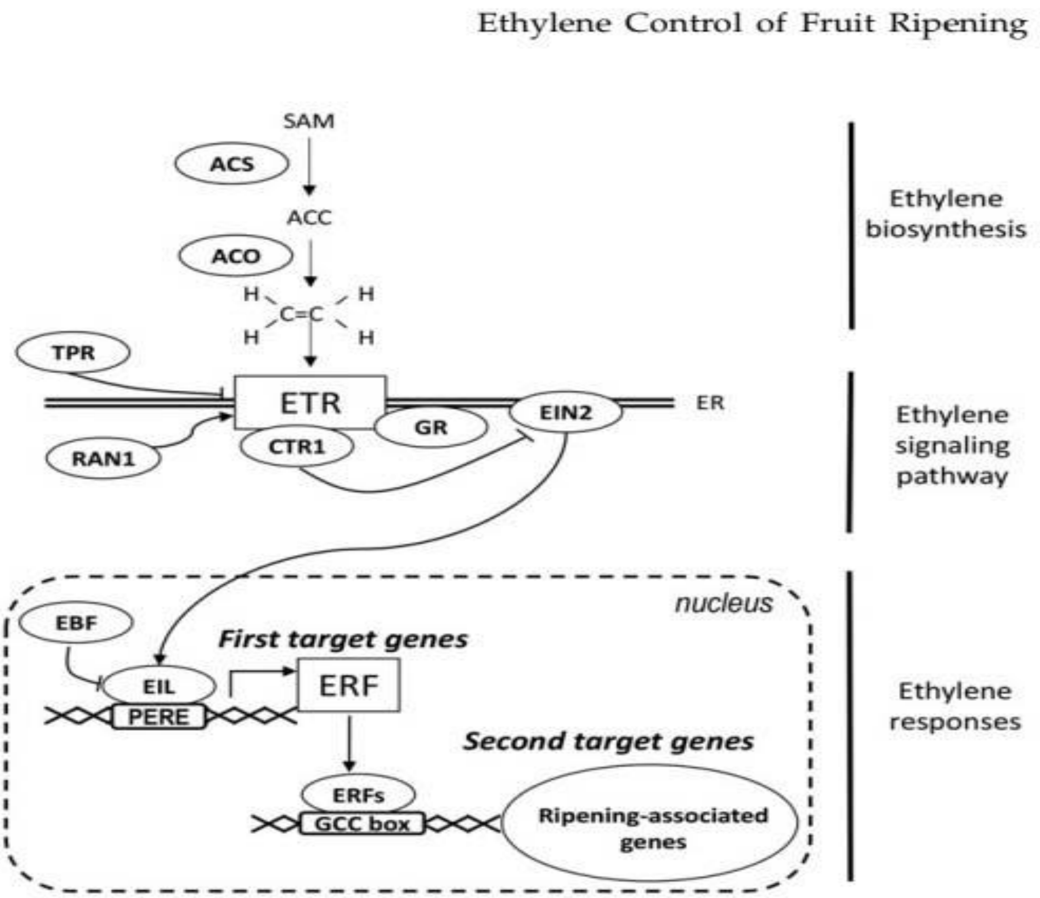

Fig. 3. ethylene perception and signal transduction

Regulation of ethylene action by disrupting ethylene receptors (inhibition of ethylene action)

The *ETR1* gene was the first plant hormone receptor gene cloned. The deduced protein has an amino-terminal ethylene binding domain and a carboxyl terminal domain homologous to the bacterial 'two- component' regulators which are receptors in various adaptation responses (Chang *et al*., 1993). When the mutant (ethylene resistant) form of the gene is inserted into tomato genome, ripening is delayed (Wilkenson *et al*., 1997).

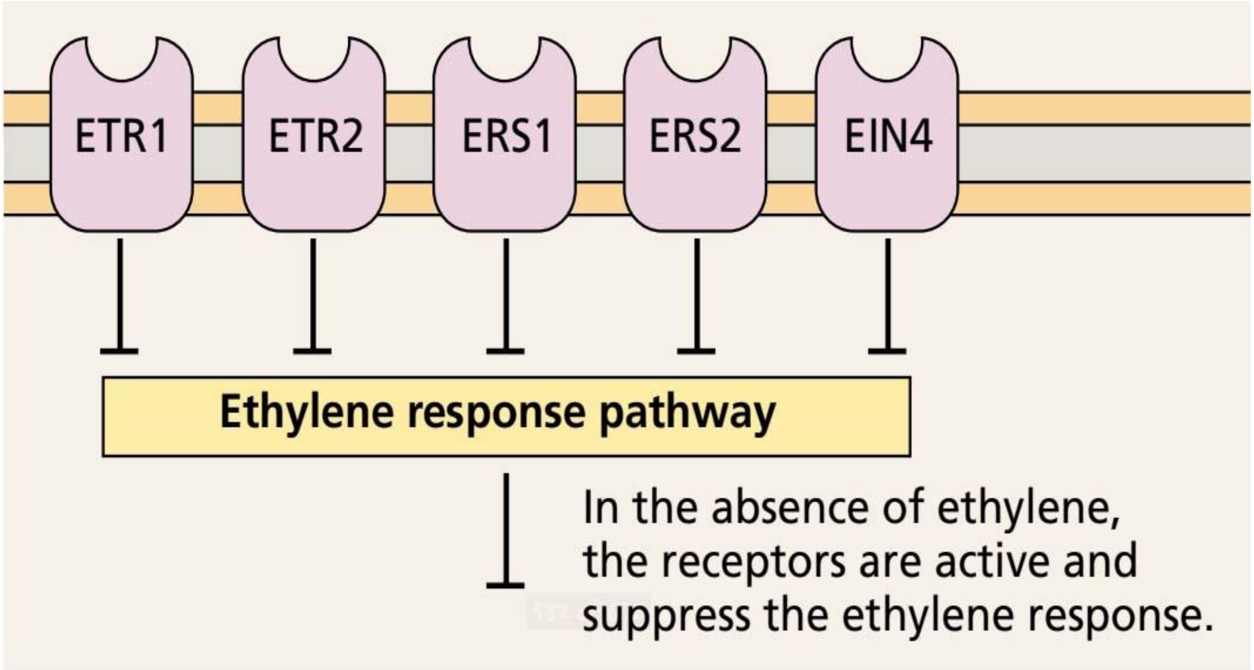

Fig.4.a. Active ethylene receptors in absence of ethylene

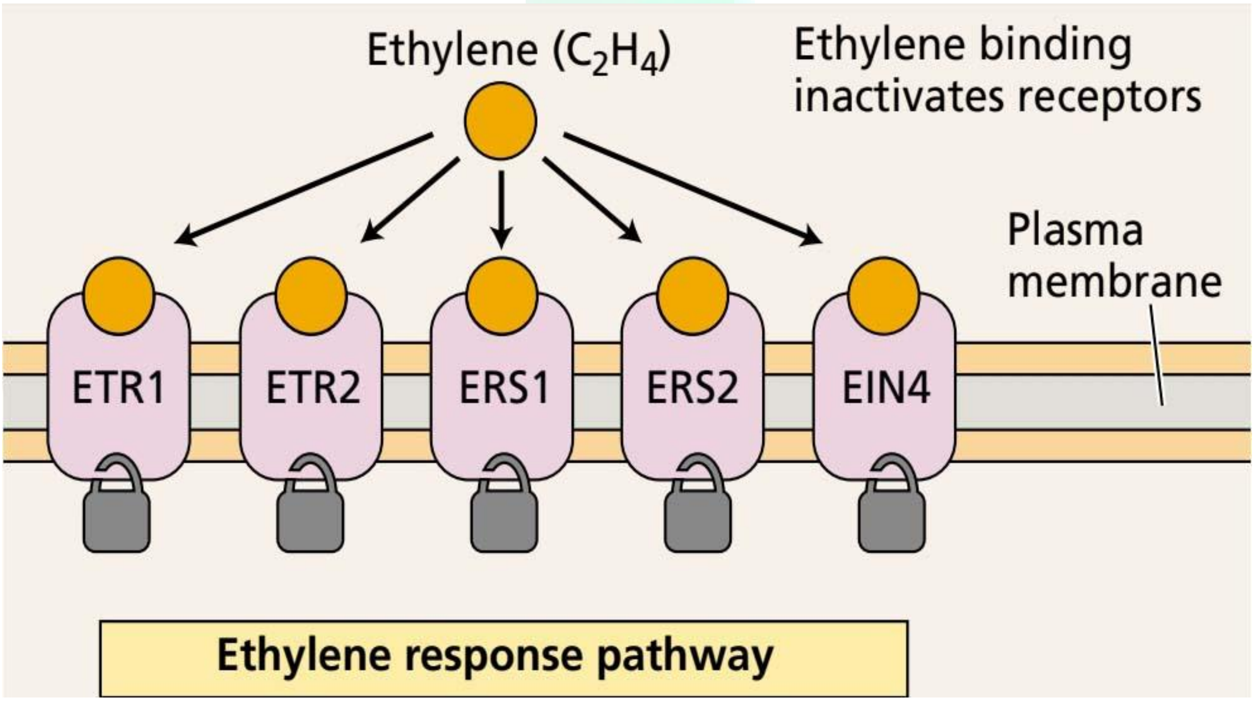

Fig.4.b. inactive ethylene receptors in presence of ethylene

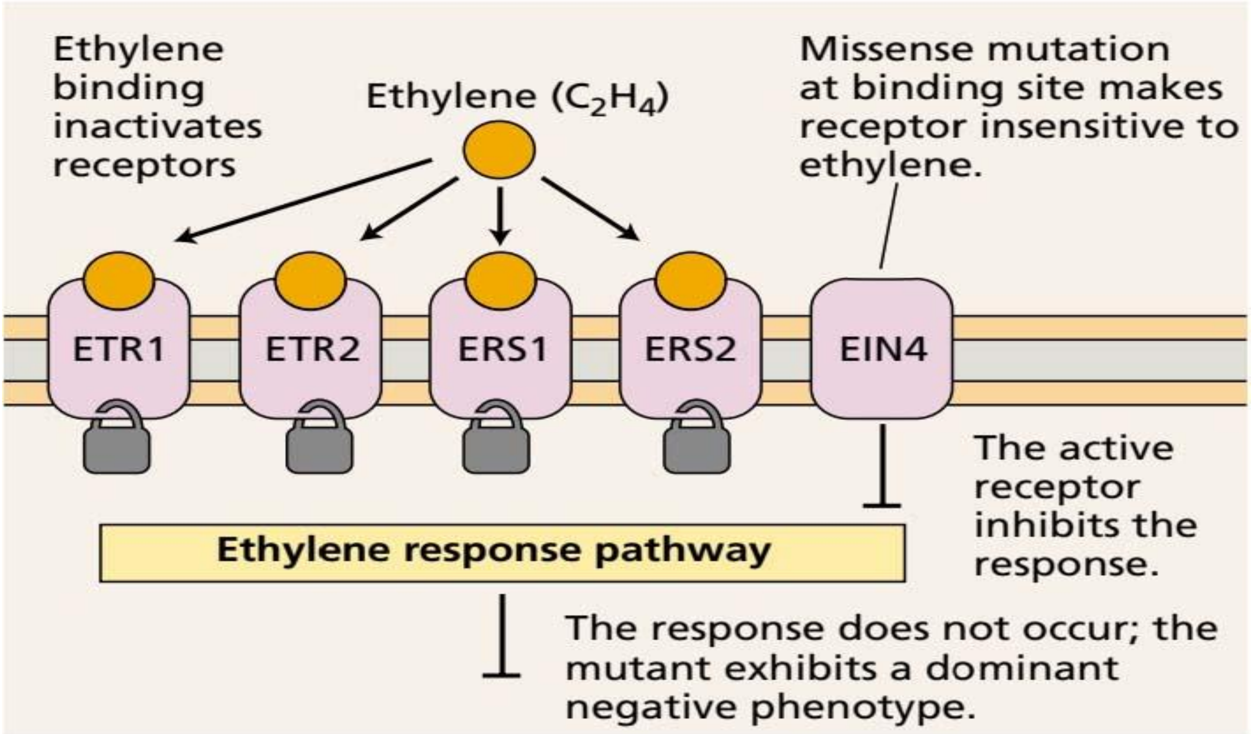

Fig.4.c. Mutation at binding site of ethylene receptor

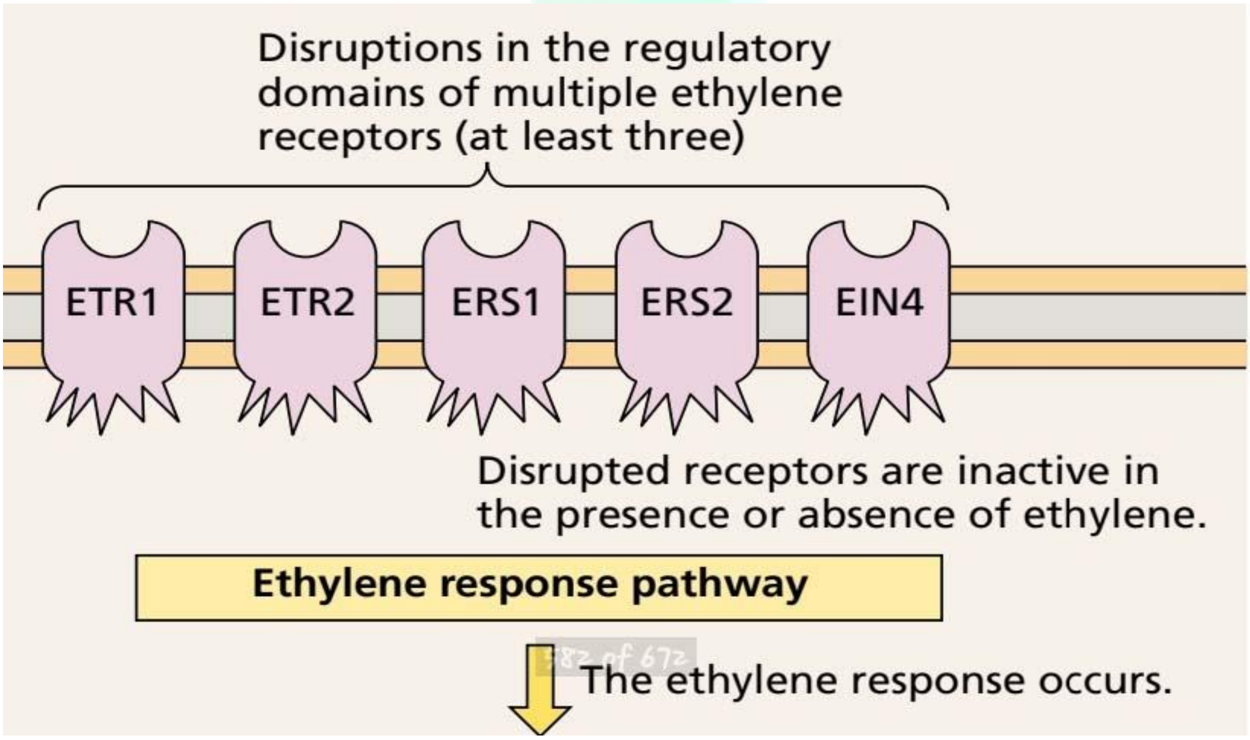

Fig.4.d. Disruption in the regulatory domains of ethylene receptors

So, to inhibit ethylene action or response, we have to mutate the binding site of the ethylene receptors and not the regulatory domains of the ethylene receptors

Some ripening mutants developed as per now

In *Arabidopsis*, a number of mutants defective in ethylene responses have been isolated such as *etr1*, *etr2*, *ein2*, *ein4*, ctr1 etc. There have been several mutants developed in tomato (*Lycopersicon esculentum*) also; Never ripe (Nr), ripening inhibitor (rin), nonripening (nor) and high pigment.

In tomato, the tomato *ripening-inhibitor* mutant is a single locus mutation which arrests the normal ripening response to endogenous ethylene. Tomatoes homozygous for this allele (rin) yield fruit which remain firm and green for weeks after their normal counterparts mature and senesce. *rin* mutant fruit fail to synthesize climacteric ethylene or accumulate lycopene (red carotenoid) in addition to being deficient in softening and remaining resistant to microbial infection.

The *Never-ripe* mutant of tomato is a semi-dominant, single gene mutation, originally characterized by delayed and incomplete fruit ripening. Recently, the *Nr* mutant has been shown to represent a block in ethylene signal transduction. The mutant only produces ~50% of the normal level of ethylene and ~20% of the normal level of lycopene (red carotenoid). The fruit are firmer than the fruit from the wild-type plants and are moderately pathogen resistant

The *high-pigment* mutant has higher pigmentation compared to normal tomato in all developmental stages. Red ripe *hp/hp* fruit are reported to contain 30 - 50% more beta-carotene and lycopene (the red pigment responsible for the characteristic red color of tomatoes) than their normal counterparts.

Conclusion

Fruit development and ripening are complex multilevel processes depending on the coordinated action of master regulators, including multiple hormonal signaling, microRNAs, epigenetic maintenance, and epigenetic modifying genes. The specific signal transduction pathway of ethylene can be regulated by altering the genes responsible for synthesis of the ethylene receptor molecules thereby delaying fruit ripening and ultimately increasing shelf life of perishable climacteric fruits.

Future prospects

More members in the putative ethylene receptor gene family are being discovered. Does each member have distinct roles in ethylene perception? How does ethylene regulate the activities of these receptors and how do these receptors regulate the downstream components? What are the downstream players in the pathway? These are the field of study for better understanding .

Thus it is necessary to widen and explore the scientific knowledge on how to utilize the immense application of this hormone for sustaining agricultural production in general and in reducing postharvest losses in particular.

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