Ligand-induced transitions in the phosphorylation status of ethylene receptors in tomato fruit. Yusuke Kamiyoshihara ${ }^{1}$, Denise M. Tieman ${ }^{2}$, Donald J. Huber ${ }^{2}$, and Harry J. Klee ${ }^{2}$
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The plant hormone ethylene is perceived by a membrane-associated receptor family which is similar to the bacterial two-component histidine kinase receptors. Since ethylene receptors negatively regulate the signaling, the suppression is canceled upon ethylene binding, permitting responses including fruit ripening. Although receptors have autophosphorylation activity, the mechanism whereby signal transduction occurs has not been fully understood. Here we demonstrate that SIETR4, an important receptor for tomato (Solanum lycopersicum) fruit ripening, is multiply phosphorylated in vivo and the phosphorylation level is dependent on ripening stage and ethylene action. Although only phosphorylated isotypes were detected in immature and mature green fruits, the non-phosphorylated isotype appeared after ripening started. Furthermore, treatment of preclimacteric fruits with ethylene resulted in accumulation of SIETR4 with reduced phosphorylation while treatments of ripening fruits with ethylene antagonists, 1-methylcyclopropene and 2,5-norbornadiene, induced accumulation of the phosphorylated isotypes. A similar phosphorylation pattern was also found for Never ripe ( $\mathrm{Nr}=$ SIETR3), another ripening-related receptor. Alteration in the phosphorylation state of receptors is likely to be an initial response upon ethylene binding since treatments with ethylene and 1-methylcyclopropene rapidly influenced the phosphorylation state. The SIETR4 phosphorylation state was closely related to ripening progress, suggesting that the phosphorylation state of receptors is implicated in the ethylene signal output in the fruits. This phosphorylation may act as a regulator for the interaction with downstream components of signal transduction.

