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Redox signals in molecular adaptation

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Light stimulates translation of chloroplast *psbA* mRNA within 5 minutes of illumination probably by activating a protein complex associated with the 5' untranslated region of this message. The protein complex contains a regulatory redox-active site responsive to thioredoxin. The regulatory redox-active site is characterized as two vicinal (proximal) cysteines. We identified RB60, a protein disulfide isomerase-like member of the protein complex, as carrying the redox-active regulatory site, comprised of vicinal dithiol (VDS). From these experiments, we proposed that RB60 acts as sensor protein responsive to the 'light signal' which is transduced as a reductive signal by thioredoxin.

This suggests that for perception of the reductive signal *in vivo*, the regulatory VDS of RB60 has to be initially oxidized. We established, in parallel, the redox state *in organello* of the regulatory VDS and redox effects on D1 synthesis in intact chloroplasts. We have found that light activated specific oxidation of RB60 on one hand, and reduced RB60, probably via the ferredoxin-thioredoxin system, on the other. Higher light intensities increased the pool of reduced RB60 and the rate of *psbA* mRNA translation, suggesting that a counter-balanced action of reducing and oxidizing activities modulate translation of *psbA* mRNA in parallel with fluctuating light intensities. In the dark, chemical reduction of the vicinal dithiol site did not activate translation.

Hance, we proposed a mechanism by which light primes redox-regulated translation by a yet unknown mechanism, and then the rate of translation is determined by the reduction-oxidation of a sensor protein (RB60) located in a complex bound to the 5' untranslated region of the chloroplastic mRNA (Fig. 1). In the dark, *psbA* 5'PC rests in a translation-incompetent reduced form (1), potentially due to phosphorylation. The first light signal converts *psbA* 5'PC into a translation-competent form (2), potentially by dephosphorylation, and activates specific oxidation of RB60 (3), by a yet unknown factor. This light induced oxidation inactivates the translation-competent *psbA* 5'PC and confers it receptive to the reductive signal. This form of *psbA* 5'PC enters a cycle (4) of activation by reduction, mediated by the ferredoxin-thioredoxin system (5) in proportion to photosynthetic light intensity, and inactivation by oxidation by the RB60-specific activity (3). Higher light intensity increases the portion of reduced *psbA* 5'PC pool, and thereby translation of *psbA* mRNA (6). Whereas under

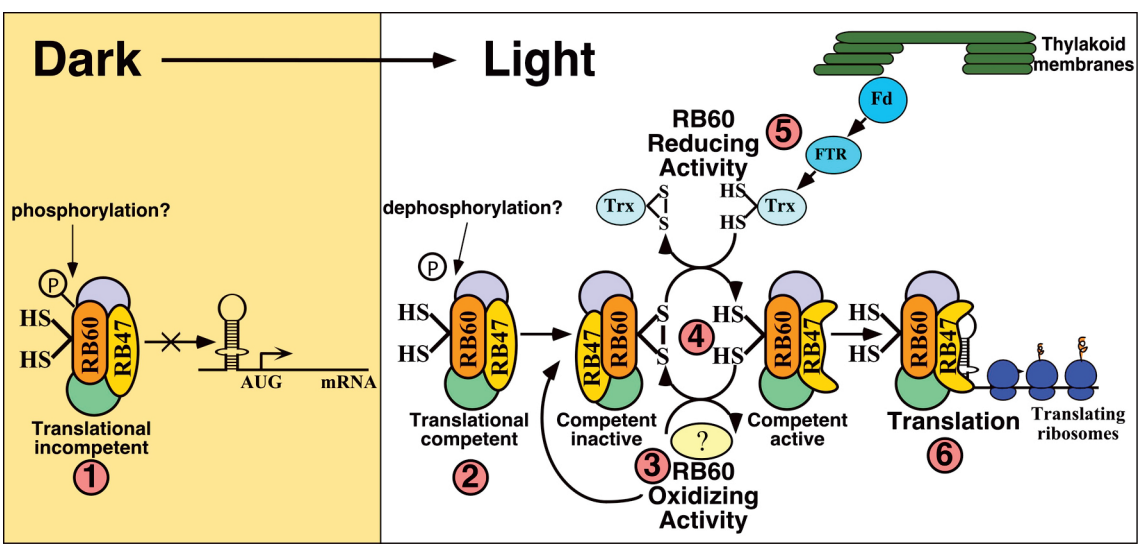

Fig. 1 *Model of redox signaling pathway of psbA mRNA translation.*

lower light intensity, oxidizing of RB60 increases the portion of inactive pool of *psbA* 5'PC and diminishes translation. Note: Our model is derived from our studies of regulation of *psbA* mRNA activity. It may be operative for additional chloroplast mRNAs.

VDS-containing proteins have been shown to participate in regulation of diverse and important biological activities, such as tyrosine kinases, receptors, enzymes of the reductive pentose phosphate cycle and translation. However it is yet unclear how the VDS-containing proteins mediate signal transduction and regulation by redox, how redox signals are transmitted in an otherwise redox-buffered milieu (under nonstress conditions), and how regulation specificity is attained. The relatively high abundance of the regulatory VDS-containing proteins in chloroplasts and fast kinetics of light signal transduction and activation of translation presents an excellent system for isolating and characterizing the function of these regulatory redox-active factors.

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