Avihai Danon

Hadas Peled-Zehavi
Tal Alergand
Shira Avital-Lezer
Alexander Levitan
Inbal Dangoor
Sarit Rochkin

Redox Signals in Molecular Adaptation

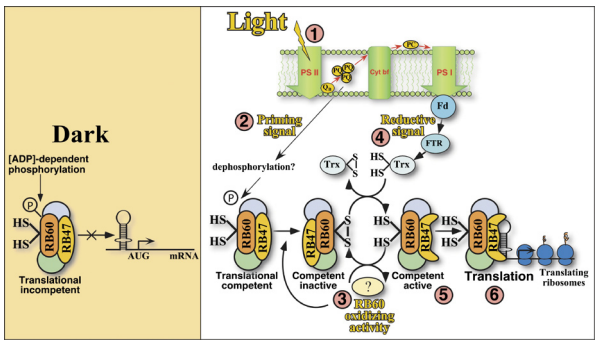
Department of Plant Sciences

Tel. 972 8 934 **2382** **Fax.** 972 8 934 **4181**
E-mail: Avihai.Danon@weizmann.ac.il
Web page: www.weizmann.ac.il/Plant_Sciences/idDanon.html

Signaling by redox-active proteins is ubiquitous in plants and animals, however, the molecular mechanisms by which these proteins regulate the expression of specific genes are not well understood. Conceivably, the redox state of these proteins must change uniquely according to their regulatory function, but the exact nature of these redox changes and the mechanism of their regulation is yet to be determined. Redox signaling of light-regulated translation in the chloroplast is a dynamic control that continuously adjusts the level of synthesis of several photosynthetic proteins to fluctuating light intensities. Hence, studying the properties of light-regulated redox signaling in the chloroplast should help clarify how redox signals are dynamically transmitted in an otherwise redox-buffered milieu (under nonstress conditions), and how regulation specificity is attained.

How does the fluctuating light-intensity control translation? Perception of the light-signal is mediated by the light-capturing reactions of photosynthesis that are localized in the thylakoid membranes. Light perception by the membranal photosynthetic reactions generates two signals (Fig. 1). The first light-signal (priming signal) turns on the translational regulatory pathways, that are inactive in the dark, and confers them receptive to the second light-signal. The second light-signal dynamically links light intensity with translational regulation, thereby modulating the rate of translation proportionally to the photosynthetically perceived light intensity. The second light-signal is transduced by the ferredoxin-thioredoxin system. In the light, electrons emanating from Photosystem I reduce thioredoxin through a series of oxidation-reduction reactions involving ferredoxin (Fd), ferredoxin-thioredoxin reductase (FTR), and thioredoxin (Trx). Reduced thioredoxin then drives the reduction of a regulatory disulfide in a protein complex (5'PC) implicated as translational regulator of psbA mRNA. Reduction of the regulatory disulfide stimulates the binding of 5'PC to the 5´-untranslated region of psbA mRNA. Binding of 5'PC to the psbA mRNA parallels the recruitment of psbA mRNAs onto polysomes and the subsequent synthesis of the D1 protein (encoded by psbA mRNA). This direct link between light and the synthesis of the D1 protein has the capacity to respond to fluctuating light levels and regulate the exchange of photooxidized reaction center proteins with de novo synthesized proteins at a rate proportional to the rate of photosynthesis. The properties of the regulatory disulfide play a crucial role in determining the specificity of the redox signal transduction. Once it is oxidized only Trx can reduce it to its dithiol form, and thereby its redox state is functionally coupled with that of Trx. We found that the regulatory disulfide site is carried by RB60, a member of 5'PC showing high similarity to protein disulfide isomerases. Characterization of the redox state of RB60 in chloroplasts showed that within minutes after illumination the pool of RB60 undergoes specific oxidation by a yet unknown mechanism. Under higher light intensities chloroplasts contained higher pool of reduced RB60 and exhibited stimulated rate of translation of psbA mRNA. Thus, the redox state of the pool of RB60 in the chloroplast appears to be controlled by a counter-balanced action of reductive and oxidizing activities that act specifically on RB60. The oxidation of RB60 renders it receptive to the reductive signal, and the properties of the regulatory disulfide of RB60 entrains it responsive specifically to reduction by thioredoxin carrying a reductive signal which is proportional to light intensity. The relatively high abundance of the regulatory disulfide-containing proteins in chloroplasts and fast kinetics of light signal transduction and activation of translation presents an excellent system for studying the mode

of function of these regulatory redox-active factors.



Model of redox signaling pathway of psbA mRNA translation. 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 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.

Selected Publications

Trebitsh, T., Levitan, A., Sofer, A., and Danon, A. 2000. Translation of chloroplast psbA mRNA is modulated in the light by counter-acting oxidizing and reducing activities. Mol. Cell. Biol. 20:1116–1123.

Trebitsh, T., Meiri, E., Ostersetzer, O., Adam, Z., and Danon, A., 2001. The protein disulfide isomerase-like RB60 is partitioned between stroma and thylakoids in Chlamydomonas reinhardtii chloroplasts. J. Biol. Chem. 276:4564-4569.

Trebitsh, T., Levitan, A., and Danon, A. 2001. Translation of chloroplast psbA mRNA is regulated by signals initiated by both photosystems II and I. Proc. Natl. Acad. Sci. USA, 98:12289-12295.

Shen, X., Danon, A., and Christopher, D.A. 2001. RNA binding-proteins interact specifically with the Arabidopsis chloroplast psbA mRNA 5' untranslated region in a redox-dependent manner. Plant & Cell Physiol. 42:1071-1078.

Katz, Y. and Danon, A. 2002. The 3' untranslated region of chloroplast psbA mRNA stabilizes binding of regulatory proteins to the leader of the message. J. Biol. Chem., 277:18665-18669.

Meiri, E., Levitan, A., Guo, F., Christopher, D.A., Schaefer, D.G., Zrÿd, J-P., and Danon, A. 2002. Characterization and knock out mutations of three PDI-like genes in Physcomitrella patens. Mol. Genet. Genomics 267:231-240.

Danon, A. 2002. Redox reactions of regulatory proteins: do kinetics promote specificity? Trends in Biochem. Sci.. 26:197-203.

Alergand, T., Trebitsh, T. and Danon, A. 2003. Redox signaling during light-regulated translation in chloroplasts. In: Cellular Implications of Redox Signaling, pp. 311-325. C. Gitler, A. Danon, eds. Imperial College Press, London, UK

Levitan, A., Danon, A. and Lisowsky, T. 2004. Unique features of plant mitochondrial sulfhydryl oxidase. J. Biol. Chem. 279: 20002-20008.

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