

Multiple roles and interaction factors of an E-box element in *Chlamydomonas reinhardtii*.

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Projects

Functional characterization of molecular components of the circadian clock of the green alga *Chlamydomonas reinhardtii*

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Abstract

The two subunits of the circadian RNA-binding protein CHLAMY1 from *Chlamydomonas reinhardtii* are involved in maintaining period (C1 subunit) and phase (C3 subunit) of the circadian clock. C1 coregulates the level of C3. Overexpression of C1 causes a parallel increase in C3. Both subunits can also integrate temperature information, resulting in hyperphosphorylation of C1 and up-regulation of C3 at low temperature. Temperature-dependent up-regulation of C3 is mediated predominantly by an E-box element and only partially by two DREB1A-boxes that are situated within the C3 promoter. The E-box element is also involved in circadian C3 expression. Here, we show that the C3 promoter region drives C3 coregulation by C1. We also found that replacement of the E-box prevents the coregulation of C3 in strains overexpressing C1. In contrast, replacement of any of the two DREB1A-boxes does not influence either the coregulation of C3 by increased levels of C1 or circadian C3 expression. Thus, the E-box has multiple key roles, including temperature-dependent up-regulation of C3, its circadian expression, and its coregulation by C1. Using mobility shift assays and DNA-affinity chromatography along with mass spectrometry, we characterized proteins binding specifically to the E-box region and identified five of them. By immunoblotting, we could further show that C3 that was detected in nuclear extracts can be found in the E-box-binding protein complex. Our data indicate a complex transcriptional mechanism of C3 up-regulation and a positive feedback of C3 on its own promoter region.

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