

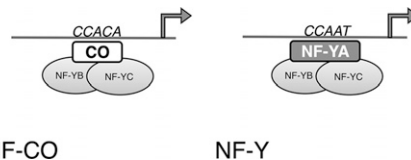
IN BRIEF

CONSTANS Companion: CO Binds the NF-YB/NF-YC Dimer and Confers Sequence-Specific DNA Binding ^{OPEN}

One of the central players in the complex regulation of flowering, CONSTANS (CO), functions as a center for integration of the various signals that determine the timing of flowering. As output, CO regulates the expression of *FLOWERING LOCUS T* (*FT*) and other genes (reviewed in Shim et al., 2017). CO contains a CCT (CONSTANS, CONSTANS-LIKE, TOC1) domain similar to the conserved domain in NF-YA, a subunit of the Nuclear Factor-Y (NF-Y) transcription factor complex. NF-Y binds to a CCAAT box in the *FT* promoter and CO binds to a CO-responsive element (CORE) in the *FT* promoter.

NF-Y contains three subunits; two of these subunits, NF-YB and NF-YC, contain histone fold domains and interact through these domains to form a dimer, analogous to the dimer formed by histones H2B and H2A. The third subunit, NF-YA, binds this dimer and provides sequence specificity for binding to the CCAAT box. Animal genomes generally encode only one or two versions of each subunit and plant genomes have about a dozen genes for each subunit. Many of these genes have diverged in expression pattern but show strong conservation of the histone fold domain and a stretch in NF-YA required for sequence-specific binding of DNA and binding the histone fold domain.

Work in *Arabidopsis thaliana* showed that NF-YB and NF-YC physically interact with CO. To examine whether CO forms a trimeric complex analogous to NF-Y, Gnesutta et al. (2017) conducted electrophoretic mobility shift assays (EMSAs) with the purified CO CCT domain, *Arabidopsis* NF-YB2 and NF-YC3, and a probe with the *FT* CORE2 sequence. Indeed, the EMSAs showed that CO plus NF-YB2/NF-YC3 bound the CORE2 specifically and did not bind the CCAAT box and that CO alone showed little or no binding to CORE2. The authors named the CO/



Model for the NF-CO and NF-Y complexes. Association of NF-YB and NF-YC subunits with either CO or NF-YA gives sequence-specific DNA binding. (Reprinted and modified from Gnesutta et al. [2017], Figure 8.)

NF-YB2/NF-YC3 complex the NF-CO complex.

The authors next examined the preferred binding sequences of NF-CO. To this end, they examined the ability of mutant probes to compete for binding to the NF-CO complex in EMSAs and found that this complex prefers to bind the CORE element CCACA, with specific flanking sequences. The authors used RNA-sequencing analysis of *co-sail*, *nf-yb*, and *nf-yc* mutants to identify the genes potentially regulated by NF-CO, which included *FT* (as expected) and circadian regulation genes such as *GIGANTEA*; this analysis also refined the definition of the matrix of sequences NF-CO can potentially bind. Intriguingly, some genes that showed alterations in *nf-yb* and *nf-yc* mutants did not show alterations in the *co-sail* mutants, indicating that other proteins with CCT domains might interact with these NF-CO subunits. The *Arabidopsis thaliana* genome encodes 40 proteins with predicted CCT domains, indicating many potential binding partners for NF-YB/NF-YC. Coimmunoprecipitation assays in the various mutants also showed that CO did not bind to the *FT* promoter in the absence of NF-YB2 and NF-YC3.

In the NF-Y complex, NF-YA interacts with a region formed by specific α -helices

of NF-YB and NF-YC. Analysis of NF-YB2 and NF-YC3 mutations in amino acids that are important for heterotrimer formation in NF-Y showed that these mutations affected formation of NF-CO, indicating that CO and NF-YA may bind to NF-YB/NF-YC in a similar way. Likewise, mutation of a conserved amino acid in CO abolished binding to CORE2. The similarities of binding indicate that CO and NF-YA might compete for binding, providing a possible explanation for the observation that overexpression of some NF-YAs can affect flowering, and yet another cautionary tale for interpretation of experiments involving protein overexpression.

The large number of different NF-Y subunits, binding sites, and CCT domain-containing proteins presents a staggering number of potential complexes. Untangling their combinatorial activities and the multitude of processes where these complexes act is an exciting possibility for future research.

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