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NUCLEAR FACTOR-Y: still complex after all these years?

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The NUCLEAR FACTOR-Y (NF-Y) families of transcription factors are important regulators of plant development and physiology. Though NF-Y regulatory roles have recently been suggested for numerous aspects of plant biology, their roles in flowering time, early seedling development, stress responses, hormone signaling, and nodulation are the best characterized. The past few years have also seen significant advances in our understanding of the mechanistic function of the NF-Y, and as such, increasingly complex and interesting questions are now more approachable. This review will primarily focus on these developmental, physiological, and mechanistic roles of the NF-Y in recent research.

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Introduction

Due in large part to their sessile nature, plants have developed sophisticated signaling mechanisms to perceive and respond to the environment, allowing them to fine-tune their growth, development, and stress responses. Alterations in gene regulation underlie this plasticity, and, for a number of reasons described below, NUCLEAR FACTOR-Y (NF-Y) transcription factors are particularly interesting research candidates to better understand how plants integrate endogenous and environmental signals so effectively.

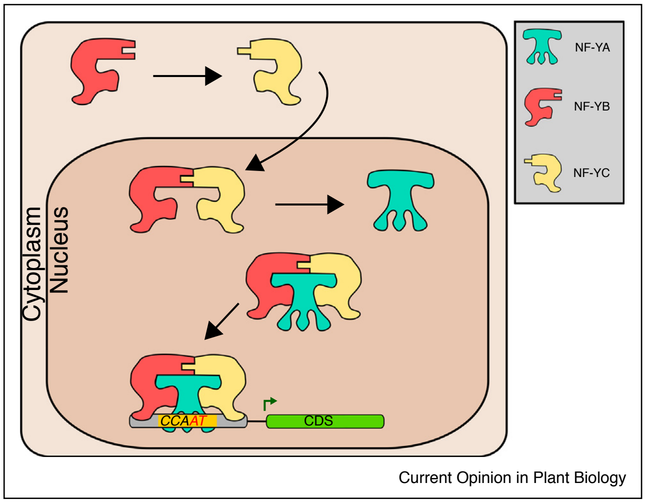
NF-Y transcription factors consist of protein subunits from three distinct families (NF-YA, NF-YB, and NF-YC) that are traditionally understood to function as a heterotrimeric transcription factor complex. The functional NF-Y complex recognizes and binds the consensus sequence *CCAAT*; however, not all *CCAAT* pentamers are bound by NF-Y complexes, suggesting that additional influences of flanking nucleotides, local chromatin structure, and interactions with other trans-acting factors also contribute to NF-Y binding. NF-YB and NF-YC subunits contain histone-fold domains that facilitate their dimerization and subsequent translocation from the cytosol to the nucleus, where NF-YA is then recruited to form a complete NF-Y complex that can bind DNA and affect transcription (Figure 1). Protein crystallography studies have shown that NF-YA subunits make all of the direct physical contacts with *CCAAT*, while NF-YB and NF-YC form a binding platform for NF-YA and make stabilizing contacts with the DNA backbone [1]. Individual NF-Y subunits are not generally found to independently bind DNA with high affinity or regulate transcriptional activity, though recent discoveries have suggested that functional, non-canonical trimeric complexes are also a natural fate for histone-fold containing NF-YB/NF-YC subunits [2••].

While NF-Y are found in all eukaryotes, the individual NF-Y families have significantly expanded across the plant lineage — for example, the *Arabidopsis thaliana* (Arabidopsis) genome has ~10 genes encoding each subunit type [3]. The implications of this expansion are likely quite significant when considering that the NF-Y function as a heterotrimeric complex, with as many as 1000 theoretical NF-Y complexes being able to form in Arabidopsis [4]. Unsurprisingly, the NF-Y have been implicated in many developmental and physiological processes and have recently become a target of translational approaches to create more resilient and nutritious crops [5–7]. This review focuses on the most impactful developmental, physiological, and mechanistic NF-Y research of the last few years, and we encourage interested readers to look through any of a number of high-quality NF-Y reviews to obtain a more complete picture [8–11].

Roles of the NF-Y in development and physiology

Arguably the best understood role of the NF-Y in plants is their regulation of the principle florigen in Arabidopsis, *FLOWERING LOCUS T* (*FT*), during photoperiodic flowering. While this line of research can be traced back over a decade [12–15], a more precise understanding of how the NF-Y regulate *FT* has only recently emerged. Prior to 2016, a series of observations indicated that NF-Y regulation of *FT* was mediated through CONSTANS (CO), a

Figure?1



Stepwise formation of the NF-Y complex. Studies in animal systems have shown that assembly of the complete NF-Y complex occurs in a stepwise manner, where NF-YB and NF-YC dimerize in the cytoplasm, move into the nucleus, recruit an NF-YA component, and subsequently bind DNA and affect transcription.

transcription factor previously suspected to directly regulate *FT* [16,17]. Two schools of thought developed to describe the relationship between NF-Y and CO — the 'recruitment model,' positing that a complete NF-Y trimeric complex recruited CO to the *FT* promoter, and the 'replacement model,' suggesting that CO replaced NF-YA to form a CO/NF-YB/NF-YC trimer (NF-CO) that could bind to and regulate the *FT* promoter. This debate has largely been settled, and the standing model supports aspects of both ideas.

The prevailing hypothesis is that NF-Y regulates *FT* through the action of a pair of protein complexes (NF-Y and NF-CO) that are brought into close proximity of each other while also binding the *FT* promoter at two distinct binding sites separated by over 5 kb [2••,18,19••,20–23]. The interaction of these two complexes is facilitated by the formation of chromatin loops, with a canonical NF-Y complex binding a distal *CCAAT* box ~5.3 kb upstream of the *FT* start and a non-canonical NF-CO complex binding at a proximal CORE (CO Responsive Element) site immediately upstream of the *FT* start [2••,18]. The key insight, which addressed the original confusion, is that both NF-YA and CO appear to be separately utilizing NF-YB/NF-YC dimers to facilitate DNA binding. In addition to advancing our specific, mechanistic understanding of NF-Y/CO regulation of *FT* during flowering, these observations are likely to be broadly paradigmatic for describing the actions of the larger CCT (CO, CO-LIKE, and TOC1) family of proteins, of which CO is just one member (further discussed below).

Most research into NF-Y regulation of photoperiodic flowering has focused on the NF-Y/CO relationship, though a few reports have emerged suggesting that this is not the only relevant NF-Y role. One alternative role is as a regulator of histone H3K27me3 de-methylation in the *SUPPRESSION OF OVEREXPRESSION OF CO 1 (SOC1)* promoter through interaction with RELATIVE OF EARLY FLOWERING 6 (REF6 [24]). This regulation appears to also integrate gibberellic acid (GA) signaling, as binding of NF-Y to the *SOC1* promoter is enhanced in response to application of exogenous GA. As only NF-YA specifically bound the SOC1 promoter fragment and the putative binding site did not contain a canonical *CCAAT* box, the exact mechanism of this regulation requires additional examination. In rice, NF-Y actually inhibit flowering in response to long days [25], though several rice NF-YB and NF-YC orthologs are able to complement Arabidopsis late-flowering mutants [26].

A recent report identified and characterized NF-Y regulation of miR156, a central component of the floral induction aging pathway in chrysanthemum [27]. This is the first published instance of an NF-Y modulating miRNA biogenesis, though previous research demonstrated that several Arabidopsis NF-YA proteins were themselves targeted by miR169, including a recently identified relationship between miR169 and several NF-YA subunits in regulating lateral root development [28,29]. The relationship between NF-YA and miR169 also extends to nodule formation during legume-rhizobia symbiotic interactions, where NF-YA is an important factor regulating the function and persistence of nodule meristems [30]. More recent work has shown that NF-YA proteins are also necessary for the earliest stages of nodule formation, and that a conserved suite of NF-YB and NF-YC proteins are also important in these developmental processes [31,32]. A more precise understanding of this process is beginning to emerge, where it appears that NF-Y mediated regulation begins following the first cortical cell divisions preceding nodule formation [33].

While some aspects of NF-Y research have matured to the point of understanding mechanistic function, others remain less concrete. Of particular note, several groups have recently reported roles for the NF-Y during light-mediated and dark-mediated seedling development. In particular, NF-YB9, also known as LEAFY COTYLEDON 1 (LEC1), has been implicated as a positive regulator of dark-mediated development [34], while NF-YC1, 3, 4, and 9 appear to be positive regulators of light-mediated development [35•,36•]. In both cases some uncertainty remains regarding the mechanistic function of the relevant NF-Y subunit(s). NF-YB9 regulation of dark-mediated development appears to be mediated through interaction with PHYTOCHROME INTERACTING FACTOR 4 (PIF4, [34]); however, because first, hypocotyl elongation was used to measure dark-