Review Article
The animal nuclear factor Y: an enigmatic and important heterotrimeric transcription factor

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Abstract: Nuclear factor Y (NF-Y) is a heterotrimeric transcription factor with the ability to bind to CCAAT boxes in nearly all eukaryotes and has long been a topic of interest since it is first identified. In plants, due to each subunit of NF-Y is encoded by multiple gene families, there are a wide variety NF-Y complex combinations that fulfill many pivotal functions. However, the animal NF-Y complex usually has only one type of combination, as each subunit is generally encoded by a single gene. Even though, mounting evidence points to that the animal NF-Y complex is also essential for numerous biological processes involved in proliferation and apoptosis, cancer and tumor, stress responses, growth and development. Therefore, a relatively comprehensive functional dissection of animal NF-Y will enable a deeper comprehension of how lesser combinations of the NF-Y complex regulate diverse aspects of biology processes in animal. Here, we focus mainly on reviewing recent advances related to NF-Y in the animal field, including subunit structural characteristics, expression regulation models and biological functions, and we also discuss future directions.

Keywords: Nuclear factor Y, expression regulation model, proliferation and apoptosis, cancer and tumor, growth and development, stress response

Introduction

Transcription factors (TFs) are known as proteins that recognize specific DNA sequences in the control regions of target genes and thus negatively or positively regulate their expression, and different TFs can form various groups to combinatorially and synergistically play context-dependent regulatory roles [1, 2]. Nuclear factor Y (NF-Y) is a TF that exists in almost all organisms, from prokaryotes to eukaryotes [3-7]. It is a heterotrimeric complex that consists of three different subunits, NF-YA, NF-YB and NF-YC, all of which are essential for the function of NF-Y. Over the past few years, NF-Y has attracted considerable interest, and multiple lines of evidence reveals that NF-Y plays important roles in many biological processes both in plants and animals.

We recently review the characteristics of plant NF-Y; each subunit of the complex is encoded by multiple gene members, which further form different subfamilies to drive diverse functions during stress responses, growth and development in plants [8]. In contrast to the observations in plants, in yeast and mammals, each subunit of NF-Y is usually encoded by a single gene, but the genes have multiple splicing forms and undergo various posttranslational modifications. In addition, each animal NF-Y subunit can interact with multiple proteins to regulate the transcription of target genes (Table 1), indicating the potentially diverse functions of the NF-Y complex in animals [9-11]. Consistent with these observations, recent studies find that animal NF-Y plays essential roles in cell proliferation and differentiation, various diseases, stress responses, growth and development. Therefore, it will be very helpful to fully review the functions of NF-Y, address some important questions related to the past and future study of NF-Y and discuss future investigations of animal NF-Y. In this review, we mainly synthesize and summarize the research progress related to the subunit structural charac-
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Functional dissection of nuclear factor Y in animal

teristics, expression regulation characteristics and biological functions of NF-Y in animals. We also provide future perspectives on this topic.

Discovery of NF-Y

NF-Y is first discovered and named in mice in 1987, at which time nuclear proteins that specifically bind to two conserved transcriptional control elements, the X box and the Y box, in the major histocompatibility complex (MHC) class II promoter, are referred to as X box-binding protein (NF-X) and Y box-binding protein (NF-Y), respectively [12]. Subsequent studies prove that NF-Y consists of three varying subunits, which are named NF-YA, NF-YB and NF-YC [9, 13, 14]. In the 1990s, the sequences of NF-YA, NF-YB and NF-YC are cloned in mice by Huijsduijnen et al. (1990) and Sinha et al. (1996). Through sequence analyses, it is found that NF-YA, NF-YB and NF-YC show high sequence homology to the transcriptional activators heme activator protein 2 (HAP2), HAP3 and HAP5 in yeast, respectively, and they can

Figure 1. Phylogenetic relationship analysis among NF-Y genes from different species. Phylogenetic relationships of NF-YA, NF-YB and NF-YC from various animal species are presented in the red shadow, orange shadow and green shadow, respectively.
Form a heterotrimer as HAP2/HAP3/HAP5 to coregulate the expression of target genes [9, 13, 14].

A recent report demonstrates that NF-Y actually binds to a CCAAT box that is harbored in the Y box element (consists of 14 bases) in the reverse orientation [6], and the three different subunits of NF-Y are all necessary for binding to the CCAAT box [11, 13]. Therefore, NF-Y is also known as CCAAT-binding factor (CBF), and the protein that recognizes the full-length Y box sequence is usually referred to as Y box-binding protein 1 (YB-1) [15-17]. By using Chromatin immunoprecipitation-sequencing (ChIP-seq) and systematic evolution of ligands by exponential enrichment (SELEX), it is revealed that when binding to target genes, NF-Y prefers DNA sequences, while YB-1 prefers single-stranded DNA and RNA sequences [16, 18-22]. NF-Y can also bind to RNA under certain circumstances. For example, the solitary long terminal repeat element of antisense RNA from endogenous retroviruses includes a variable number of CCAAT binding site that functions as traps or decoy targets for physical binding by NF-Y, resulting in the inhibition of human cancer cell growth [23]. More than that, recent ChIP-seq analyses suggest that NF-Y is capable of binding the CCAAT motif not only in the promoter region but also in nonpromoter regions, such as in 5′ untranslated regions (UTRs), 3′UTRs, exons, introns and intergenic regions [24, 25].

Structural characteristics of NF-Y subunits

Each animal NF-Y subunit is usually encoded by a single gene accompanied by different splicing isoforms, generating one type of NF-Y complex [8-11]. However, recent studies reveal that there are two NF-YA genes and two NF-YB genes in the planarian Schmidtea mediterranea [26], and two NF-YB genes in Caenorhabditis elegans [27]. The difference in the number of NF-Y genes between the planarian Schmidtea mediterranea and Caenorhabditis elegans and other animals is likely a result of evolution. Of course, the specific underlying cause remains elusive and requires further investigation. The results from phylogenetic tree analysis in different species demonstrated that NF-Y has a closer genetic relationship to mammals, such as humans, mice and cattle, with close to 100% similarity overall, than to other animals (Figure 1). However, only the conserved domain presents high homology between mammals and insects, between prokaryotes and eukaryotes, and among different insects (Figure 2A-C). These findings suggest that the conserved domain of NF-Y may be necessary for its function.

The structure of NF-Y has been illuminated by using various biological and chemical methods, such as X-ray crystallography, hydroxyl radical footprinting, mutational analysis and electrophoretic mobility shift assay (EMSA) experiments [28, 29]. Through structural analysis, NF-YA is found to have two domains (a glutamine-rich (Q-rich) domain and a core domain), with the core domain containing two α helices (A1 and A2) (Figure 2A). The A1 helix (mostly positively charged) is responsible for the recognition of the NF-YB/NF-YC dimer by binding to a composite crevice (mainly positively charged) that is centered on the αC helix of NF-YC. The A2 helices can be divided into an α-helical N-terminal region and a coiled C-terminal region, and these helices can identify specific DNA sequences by inserting into the DNA minor groove with the help of a conservative GxGGRF motif that is present in the subsequent loop [30, 31]. The A1A2 linker between the A1 and A2 helices offers the conformational flexibility that is necessary to direct the A2 helix toward specific DNA sequences and maintains the stable connection of the A1 helix to the NF-YB/NF-YC interface [28, 32, 33]. The Q-rich domain is also known as the transactivation domain and possesses transcriptional activation characteristics [28, 32, 33]. Although recent studies find that the Q-rich domain influences the bend angle and transcriptional activation of target genes [28, 34], the role of the Q-rich domain must be further clarified.

Both NF-YB and NF-YC have a histone fold domain (HFD) that contains four α helices (α1, α2, α3 and αC), which function in protein-DNA and protein-protein interactions, and NF-YC also includes a Q-rich domain in its N-domain (Figure 2B, 2C) [28, 33]. Experimental mutational analysis indicates that the α1 helix of NF-YB affects the DNA recognition ability of the NF-YA A2 helix, possibly by influencing the α-helical N-terminal region and positioning it in the correct orientation. Structurally, the α2 helix of NF-YB and the α1 and αC helices of...
Figure 2. Conserved domain analysis of three animal NF-Y subunits and modulation model of the NF-Y heterotrimer. A-C. Multiple amino acid alignment of conserved domains in NF-YA, NF-YB and NF-YC from Caenorhabditis elegans, Drosophila melanogaster, Homo sapiens, Mus musculus, Schmidtea mediterranea and Xenopus tropicalis. The black shadow represents identical amino acids. The actual amino acid numbers within each protein correspond to the numbers between the brackets. D. Molecular mechanism by which the NF-Y trimer modulates target genes. The heterodimer of NF-YB/NF-YC is assembled in the cytoplasm and translocates into the nucleus in an importin 13-dependent manner, where it binds to NF-YA that enters the nucleus by an importin β-dependent pathway to form the NF-Y trimer. The NF-Y trimer can recruit other TFs or proteins that may be activators or repressors via its three subunits to positively regulate or negatively control target gene expression through the CCAAT box.
NF-YC play pivotal roles in binding to NF-YA, as they provide a negatively charged and wide surface groove. Furthermore, the NF-YC αC helix is also a target for vital regulatory proteins, such as p53 and c-Myc [14, 28, 33, 35-37]. All of these structural features of the NF-Y subunit are important for the formation of the trimeric complex, the recognition and binding of the CCAAT regulatory element and the function of NF-Y.

NF-Y subcellular localization and its regulatory mechanism for target genes

In plants, NF-YA usually localizes to the nucleus, and NF-YC can accumulate in both the nucleus and cytoplasm. The subcellular localization of plant NF-YB is variable, and the nuclear transport of NF-YB is mediated by its interaction with NF-YC [3, 8]. For example, although Arabidopsis NF-YB is located predominantly in the cytoplasm [38], HAP3b, a putative CCAAT-binding TF, has been proven to be a nucleoprotein in the root tip and leaf epidermal cells in Arabidopsis [39]. Similar to the observations in plants, NF-YA has been shown to localize to the nucleus due to its evolutionarily conserved nonclassical C-terminal nuclear localization signal in human HeLa cells and mouse NIH 3T3 cells [40, 41]. Moreover, a previous study proves that NF-YA levels increase in the nucleus during the oocyte maturation process and decrease in maturing spermatozoites in Schistosoma mansoni [42], indicating that NF-YA may participate in the regulation of Schistosoma mansoni reproductive cell growth and development. The locations of NF-YB and NF-YC differ between animals and plants. NF-YB localizes in the nucleus in HeLa cells and NIH 3T3 cells [40]. NF-YC localizes in both the nucleus and cytoplasm in NIH 3T3 cells, and its location can change during the regulation of the cell cycle [41]. For example, NF-YC is detected mainly in the NIH 3T3 cell cytoplasm but accumulates in the nucleus during the cell cycle at S phase onset [41]. There is usually only one form of the NF-Y complex in animals, and various forms of the NF-Y complex in plants [3, 43, 44]. The subcellular localization differences between NF-YB and NF-YC and the changes in NF-YC localization in various cells and growing environments may be a remedial measure of a single form of animal NF-Y heterotrimer during the course of evolution, and conducive to NF-Y timer plays multiple roles under different conditions in animal.

Animal NF-Y regulates target gene expression through a specific mechanism (Figure 2D). NF-YA is imported into the nucleus in an importin β-dependent manner, and the NF-YB/NF-YC heterodimer is first tightly assembled in the cytoplasm and then translocated into the nucleus for NF-YA binding by an importin 13-mediated pathway in HeLa cells [40, 41]. After the formation of the NF-Y complex, the A2 helix of NF-YA deeply inserts into the DNA minor groove, the HFD of NF-YB/NF-YC binds to the DNA sugar-phosphate backbone, and finally finish the NF-Y trimer binding to target genes via the CCAAT motif [32, 33]. It is worth mentioning that the NF-Y heterotrimer binds to a CCAAT motif that contains 25 bp [32], which may mean that two CCAAT boxes that are less than 25 bp apart cannot be bound at the same time. Mutation of the NF-Y subunit results in the loss of NF-Y function. For example, a dominant-negative NF-YA subunit still interacts with the NF-YB/NF-YC dimer. However, this NF-Y trimer is inactive with respect to DNA binding [25, 45-47]. Although all three subunits of NF-Y are necessary for DNA binding to target genes, a previous study suggests that Mes4 can replace NF-YC to form a complex with NF-YA and NF-YB to activate mesoderm-specific gene expression in the Drosophila mesoderm [48]. NF-YC is rapidly lost in Drosophila early embryos, but Mes4 is first detected in the presumptive mesoderm [48], suggesting that Mes4 may be a tissue-specific substitution for NF-YC during Drosophila embryogenesis. Further analysis is essential for clarifying this possibility. Future studies are likely to identify additional proteins that can take the place of the NF-Y subunit in the NF-Y trimer.

Furthermore, the regulatory role of NF-Y in targeting genes can be positive, negative, both positive and negative or bidirectional, and this effect depends mainly on the binding site of NF-Y in the target gene regulatory region. For example, NF-Y can positively regulate the transcription of the xanthine oxidoreductase gene by binding to its promoter region (-135 to -107) [49], and can negatively regulate the promoter activity of tbx-2 through the binding site at -203 bp [50]. By using supershift gel mobility assays, DNA methylation interference analysis and gel
mobility competition assays in human HEK 293 and HeLa cells, it is found that when bind to the CCAAT box at position -18 to -14, NF-Y is an activator of von Willebrand factor, but when bind to the downstream CCG element (+226 CCGNNNCCC +234) in the first exon, NF-Y is a repressor of von Willebrand factor, indicating that NF-Y can both positively and negatively regulate the expression of von Willebrand factor [51]. NF-Y functions in the inhibition of oncoprotein 18 transcription by binding the repressive site at -599 and activates oncoprotein 18 expression by binding the stimulatory site at -65 in the mouse NIH 3T3 cell line and the human HEK 293 embryonic kidney cell line [52, 53]. NF-Y can both positively regulate and negatively regulate the expression of TRBP through the CCAAT-390 site and CCAAT-347 site, respectively, in human astrocytes [54]. The bidirectional regulatory role of NF-Y in target genes is also revealed in recent years. A recent study proves that NF-Y can bind to a CCAAT box in the bidirectional promoter of GHR and CCDC152 to induce their transcription. A high protein level of CCDC152 represses both this bidirectional promoter activity and cell migration and proliferation. In contrast, a low protein level of CCDC152 increases the activity of this bidirectional promoter and triggers cell migration and proliferation [55]. The same bidirectional transcription role of NF-Y is also uncovered in the head-to-head gene pair PRR11-SKA2 [56].

Due to genome differences among various animals, the regulatory mechanism of NF-Y may vary. Mammalian NF-Y interacts with p53 through the NF-YC αC helix and a region similar to the tetramerization domain in the C-terminus of p53 [57-59]. However, this tetramerization domain is not conserved in Drosophila. Instead, the promoter activity of the Drosophila p53 gene relies on NF-Y, as its 5'-flanking region contains NF-Y binding sites (CCAAT box) [60-62]. Moreover, NF-Y can bind to the CCAAT motif of HSP70 and HSP40 in humans and Xenopus [63, 64]. However, the HSP70 promoter activity of Drosophila may not be regulated by NF-Y because its promoter region does not contain a CCAAT cis-acting element [65]. The multiple regulation characteristic of NF-Y may be involved in its functional diversity. This effect may be one reason why each NF-Y subunit is usually encoded by a sin-

Figure 3. Function of NF-Y in cell apoptosis and cell proliferation in mammals. A. NF-Y can interact with wild-type (wt) p53 and mutant (mut) p53 to regulate cell apoptosis and cell proliferation. B. The regulatory characteristics of NF-Y, wt-p53, mut-p53 and mut-NF-Y during cell apoptosis and cell proliferation caused by DNA damage after stress signals.
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gene (in contrast to plants, where each subunit of NF-Y is encoded by multiple members), but NF-Y complex plays one of the most important roles in animal survival.

Roles of NF-Y during various biological process

By using various bioinformatics approaches, which are based mainly on searching for cis-regulatory elements, coupled with different genome-wide molecular biology tools, including cDNA microarrays, RNA-seq, ChiP-seq and ChiP on ChiP, many studies have revealed that animal NF-Y plays essential roles in many biological processes, such as the cell cycle, disease, growth and development, and stress responses. In addition, the specific biological roles of NF-Y in various animals have similarities and differences related to whole genome sequences and inherited traits [11, 43, 44, 66, 67].

Roles of NF-Y in disease

Growing evidence in patient samples and cells supports the concept that NF-Y, as a common TF, plays critical roles in many human diseases, such as various types of cancer, tumors, neurological diseases, and metabolic diseases [16, 43, 44, 68-70]. In these processes, NF-Y mainly recruits many coactivators or corepressors to regulate disease-related genes (Figure 2D), then to better drive a role in disease [43, 44, 71].

It is well known that the normal operation of the cell cycle is crucial for biological survival. Accumulating evidence suggests that NF-Y affects apoptosis and proliferation by influencing the cell cycle, and this function of NF-Y is often related to p53 (Figure 3), which is a tumor inhibitor that plays an essential role in cell apoptosis and results in different consequences, depending on a number of variables, such as genetic background, cell type and cellular environment [44, 72-75]. When cells are subjected to stress, NF-Y can interact with wild-type p53 (wtp53) and subsequently recruit histone-deacetylases (HDACs) to represses many key genes (such as CDK1, CDC25C, cyclin A, and cyclin B1) that are involved in the G1/S and G2/M cell cycle transitions through binding to CCAAT boxes in their promoters. This process corrects the cell cycle checkpoint and results in normal cell apoptosis [44, 57, 58, 72]. However, mutated p53 (mutp53) recruits acetyltransferases (HATs), such as p300, resulting in the acetylation of mutp53 and NF-YA, and subsequently triggers the transcription of CDK1, CDC25C, cyclin A, cyclin B1 and CDK1-related kinase and the deregulation of the cell cycle, which may ultimately cause tumor progression (Figure 3B). Except that, it is found that when NF-Y is mutated, p53 cannot interact with NF-Y, and the target genes involved in cell proliferation cannot be activated by NF-Y. Thus, cell apoptosis can occur (Figure 3B).

Aberrations in cell cycle, cell proliferation and cell apoptosis regulation constitute a common platform for the establishment of neoplasms [76]. The function of NF-Y in the cell cycle, cell proliferation and cell apoptosis implies the possible involvement of NF-Y in tumors. Indeed, recent studies demonstrate that NF-Y is connected to tumors, mainly by regulating some key genes and signaling pathways related to the generation of tumors [44, 77, 78]. For example, a recent study proves that ectopic expression of NF-YA accelerates osteosarcoma cell malignant phenotypes, partly by activating the fatty acid synthase signaling pathway, which is a possible promising target for osteosarcoma management [79]. The NF-Y complex can be recruited by c-Jun and E1A to bind to the CCAAT element and regulate the basal transcription of the bone sialoprotein gene in osteosarcoma, and the recruitment mechanisms of c-Jun and E1A are similar but independent [80]. In addition, the expression of NF-yc progressively increases with the grade of glioma, as indicated by immunohistochemical assessments in glioma samples from human patients [77]. Further study finds that NF-yc is involved in human glioma cell cycle, growth and proliferation progression by suppressing the cyclin-dependent kinase inhibitor p21, and the knockdown of NF-yc is capable of decreasing tumor size in the brain in vivo [77]. All these findings indicate that NF-yc is likely to be an independent and novel predictor of glioma patient survival. NF-ya also plays an important role in the regulation of tumors, especially during angiogenesis. The main mechanism lies in the ability of NF-ya to regulate VEGF expression and STAT3 activity by inducing EZH2 expression in melanoma [81].

Cancer is also a disease of cell cycle, cell proliferation and cell apoptosis deregulation, which
can evolve from malignant tumors. Recent studies show that NF-Y can induce various types of cancer, such as colorectal cancer, pancreatic cancer, lung cancer and hepatocellular carcinoma, by regulating the expression of cancer-related genes (Figure 4). During the process of colon cancer regulation, NF-Y can interact with SOX9 through NF-YA and the C-terminus of SOX9 to upregulate the expression of cell-cycle-related genes, such as TOP2A, CDK1, CCNB1 and CCNB2 [71]. On the other hand, by using ChIP-seq and an anti-NF-YA antibody in human colon adenocarcinoma SW480 cells, Falcone et al. first reveal that NF-Y also functions in colon cancer by upregulating the transcription of miRNA-17, -21, -130b, -301b, -181a and -181b [25]. In addition, many detailed analyses have revealed that NF-Y plays a vital role in the regulation of pancreatic cancer. The clear mechanism from current research is that NF-Y can interact with p73 through NF-YB and repress the transcriptional activation of PDGFRb, resulting in noninvasive cells in p53-null pancreatic cancer cells. However, when p53 is mutated, mutp53 interacts with p73, which interferes with or disrupts the formation of the p73/NF-Y complex and thereby abrogates the ability of the p73/NF-Y complex to inhibit the expression of PDGFRb, triggering an increase in its transcription and promoting pan-

![Figure 4](image-url)
Functional dissection of nuclear factor Y in animal

Figure 5. Function of NF-Y in Drosophila growth and development. NF-Y plays various roles during the growth and development of Drosophila through the regulation of multiple signaling pathways. The roles of the NF-Y heterotrimer, NF-YA, NF-YB and NF-YC are indicated in the green oval, orange oval, red oval and olive oval, respectively.

creatic cancer cell invasiveness [82]. Although mutp53 can interact with NF-Y upon DNA damage (Figure 3) [57], no physical interaction between NF-Y and mutant p53 is found in the pancreatic cancer cells used by Weissmueller et al. (2014). This discrepancy may be caused by the use of different biological settings and extraction techniques. NF-Y also participates in the regulation of lung cancer. The results from coimmunoprecipitation assays and luciferase reporter assays reveal that p53 can negatively regulate the bidirectional promoter activation of PRR11-SKA by interacting with the NF-YB subunit of the NF-Y trimer. The loss of p53 relieves its inhibitory effect on PRR11-SKA2 bidirectional transcription, resulting in inducible expression of PRR11-SKA2, which accelerates the motility and proliferation of lung cancer cells [59]. Hepatocellular carcinoma is a type of inflammation-related cancer. During hepatocarcinogenesis, the activated IL-1β inflammasome can bind to IL-1RI, which recruits IRAK1. Phospho-IRAK1 interacts with TRAF6 to further phosphorylate and activate JNK, which causes the phosphorylation of p300, the recruitment of the NF-Y complex to p300 and CBP, and the acceleration of NF-YA acetylation, triggering Gankyrin gene transactivation. The upregulation of Gankyrin is closely associated with hepatocarcinogenesis [83].

In addition to tumors and cancer, recent studies have also revealed the role of NF-Y in some other human diseases. For example, related research has found that human globin genes have a CCAAT motif in their promoters that is bound by NF-Y. Mutation of the γ-globin CCAAT motif triggers the hereditary persistence of fetal hemoglobin, while mutation of the β-globin CCAAT box results in β-thalassemia [84-86]. NF-Y is also involved in some neurological diseases. The inactivation of NF-Y results in typical neurodegeneration, which is accompanied by endoplasmic reticulum disorganization and p62 and ubiquitin accumulation [87]. Inducing the expression of NF-YC promotes neuronal apoptosis via the pro-apoptotic protein Bim in the hippocampus of mice following middle cerebra artery occlusion [88]. These results indicate that NF-Y may play essential role in human diseases.

Roles of NF-Y during growth and development

NF-Y is crucial for animal embryo growth and development. The deletion of NF-YA alleles causes early embryo lethality in mice due to the loss of cell survival and differentiation ability [89]. Similarly, the knockdown or overexpression NF-YA with different GAL4 also drives lethality in the Drosophila pharate adult stage, possibly by influencing disc specification [43, 90]. Despite these findings, further studies are needed to explore the detailed molecular mechanism of NF-Y in embryo growth and development. With the application of RNA-seq and ChIP-seq, more growth- and development-related molecular mechanisms of NF-Y will be identified in the future.
NF-Y also plays multiple roles during the growth and development of the *Drosophila* eye, R7 photoreceptor and thorax via the regulation of different signaling pathways (Figure 5). The lethality phenomenon that accompanies the headless phenotype in pharate *Drosophila* adults is caused by the expression of NF-YA with eyeless-GAL4, and decreasing the dose of eyeless genes enhances this phenotype, indicating that NF-YA can hamper *Drosophila* eye disc specification [90]. The knockdown of NF-YA in *Drosophila* eye-antennal discs with UAS-NF-YAIR and GMR-GAL4 triggers R7 photoreceptor signal loss and a rough eye phenotype, which can be rescued by the expression of an ERK pathway gene (sev), whose promoter contains two CCAAT boxes that are bound by NF-Y. These results suggest that NF-Y is essential for the development of R7 photoreceptor cells because it regulates the expression of sev [91, 92]. Similarly, the knockdown of *Drosophila* NF-YB in eye imaginal discs can also induce an adult rough eye phenotype due to the upregulation of caspase-dependent apoptotic functions related to apoptosis-induced proliferation [43]. Although the knockdown of *Drosophila* NF-YB in eye imaginal discs also represses cell differentiation in the R7 photoreceptor, this process does not rely on the apoptotic function of NF-YB [43], and further studies are required to uncover the underlying mechanism. Furthermore, NF-YC has an important regulation role in R7 photoreceptor neurons. Pros and R7-specific rhodopsins are essential in *Drosophila* R7 neuron development, and Sens and R8-specific rhodopsins play important roles in the development of *Drosophila* R8 neurons [93, 94]. In the R7 neuron of *Drosophila*, the R8 targeting program is partially repressed by NF-YC, as NF-YC can suppress the signaling pathway involved in Sens. Mutant NF-YC in R7 neurons causes the expression of Sens and R8 rhodopsins but not Pros and R7 rhodopsins, and when NF-YC is absent, the axons of R7 end in the same layer as the axons of R8 [7]. Although these findings elucidate the function NF-YC in the regulation of the R7 targeting program, further analysis is needed to clarify the specific inhibitory mechanism of NF-YC for Sens. In addition to the eye and R7 photoreceptor, NF-Y plays key roles in the development of the *Drosophila* thorax. Recent studies find that the knockdown of NF-YA in the *Drosophila* wing discs notum compartment causes a thorax disclosed phenotype. Both mutation of the p53 gene and reduction of the dose of the JNK bsk gene can enhance the phenotype induced by NF-YA knockdown. This effect is caused by the positive regulatory role of NF-Y in the transcription of p53 and bsk [60, 95].

The function of NF-Y in growth and development has also been revealed in mammals. In human embryonal carcinoma cells, NF-Y can positively regulate the transcription of the ID gene family and the FGF-4 gene, both of which are essential for the coordinated regulation of development, cell growth and differentiation by a sophisticated living system [96-100]. In addition, recent studies demonstrate that NF-Y functions in the switch of muscle from proliferation to differentiation. Forced expression of NF-YA triggers impaired downregulation of many cell cycle genes, such as cyclin A, cyclin B1, CDK1, cdc25B and cdc25C, and induces myogenin, p21 and creatine kinase activity, resulting in delayed myogenin induction in mice [101-103]. Alternatively, splicing of exon 3 (encoding 28 amino acids in the Q-rich activation domain) of NF-YA causes two splicing isoforms, NF-YAs (short) and NF-YAl (long) [10]. Although the two isoforms present similar activation potential in transcriptional activation experiments, a recent study shows that NF-YAs boost cell proliferation, while NF-YA1 enhances cell differentiation by directly activating Cdkn1c and Mef2D and indirectly activating MRF in C2C12 myoblast cells [74]. Moreover, NF-Y can connect with other proteins or TFs to influence myoblast proliferation and differentiation. MEIS1 and CREB seem to rely on the presence of NF-Y to balance the activity of myostatin P/E, maintaining basal levels of myostatin transcription during myoblast proliferation in mice [104]. Another research reveals that NF-Y can coordinate with Rev-Erα through NF-YB to coregulate myogenesis gene expression in differentiated myotubes and differentiating and proliferating myoblasts in mouse models [4].

In addition to mammals and insects, NF-Y also functions in *Caenorhabditis elegans* and *Schistosoma mansoni* growth and development. Unlike the mammalian gene, *Caenorhabditis elegans* NF-Y is differentially regulated during development rather than ubiquitously expressed, and it can regulate the expression of
tbp-2 and egl-5, both of which play important roles in the development of all multicellular animals [27, 50, 105, 106]. The Schmidtea mediterranea NF-YA and NF-YC play significant roles in neural lineages and epidermal early differentiation, and NF-YB of Schmidtea mediterranea is essential for spermatogonial stem cell proliferation and selfrenewal [26, 107]. Though these findings, further studies are needed to fully clarify the role of NF-Y in Caenorhabditis elegans and Schistosoma mansoni growth and development.

Role of NF-Y in stress responses

The functions of animal NF-Y in stress responses have been revealed in recent years. The expression of NF-YA and NF-YB is induced and reaches its highest levels 48 h after cisplatin and radiation treatment, while the transcript levels of NF-YC do not show significant changes in nasopharyngeal cancer cells under cisplatin and radiation stress [108]. These results suggest that although NF-YA, NF-YB and NF-YC function as a complex, the transcription of these genes is regulated at different levels after cisplatin and radiation exposure. This study is the first report that involved the stress response of NF-Y itself. However, the expression profiles of NF-Y under other environmental stresses have not been extensively researched since then. Future studies should focus on this topic to fully understand the changes in NF-Y mRNA and protein levels that occur in response to environmental stresses.

In addition, the regulatory roles of NF-Y in target stress response genes have also been uncovered. Heat shock proteins (HSPs) are known to play important roles in heat shock stress. The promoters of HSPs in many species contain a CCAAT cis-acting element that can be bound by NF-Y. For example, NF-Y is not only acetylated by p300 but also interacts with p300 to regulate the expression of Hsp70 after heat shock treatment in Xenopus [63]. The coordination of NF-Y with HSF-1, CREB and NF-κB plays crucial roles in the transcriptional regulation of Hsp70.3 under ischemia-like or heat shock conditions in mice [64]. HSP-CBF interacts with the B subunit of NF-Y to coactivate the transcription of Hsp40 and Hsp70 in Xenopus [65]. These findings suggest that NF-Y is likely to participate in the heat stress response, partially through HSPs. Moreover, the NF-Y trimer associates with the HDAC1-RFP dimer complex through NF-YC and the RFP domain of the RFP protein to form a heteromeric pentamer, which is a negative regulatory factor in the transcription of TBP-2 during oxidative stress in human cancer cells [109]. A functional NF-Y heterotrimer complex and a high-affinity CCAAT box-binding site are needed for optimal stimulation by ATF6 after thapsigargin treatment in mouse NIH 3T3 cells [110]. Along with Sp1, NF-Y is a double-stranded CCAAT motif binding protein, which mediates MDR1 transcriptional activation in response to ultraviolet irradiation in human KB-3-1 epidermoid carcinoma cells [46]. Despite these reported results, more work is needed to obtain a detailed understanding of the regulatory mechanism by which NF-Y affects stress response genes.

Other roles of NF-Y

In addition to the above mentioned functions, NF-Y also plays roles in other processes, such as preadipocyte and adipocyte metabolism, hematopoietic stem cell (HSC) survival, and the circadian clock. NF-Y is important for preadipocytes and adipocytes. In preadipocytes, a knockout experiment demonstrates that NF-Y exacerbates adipocyte depletion by disrupting preadipocyte pool maintenance and/or generation, resulting in age-progressive fat loss in NFY-KO mice. In adipocytes, mouse NF-Y plays a key role in maintaining the basal expression of adiponectin during adipocyte differentiation and the nutrient response [111]. Furthermore, NF-Y play a key role in the regulation of fatty acid synthase during the refeeding/fasting transition in adipocytes [112]. NF-Y plays a role in adipocyte development by regulating the transcription of leptin. Abnormal control of these genes by NF-Y may trigger lipodystrophy in mice [113].

A recent study suggests that NF-Y is an excellent candidate for the genetic regulation of HSC survival, proliferation and selfrenewal in mice [114]. NF-YA is preferentially expressed in bone marrow subpopulations, where HSCs are enriched, and the transcriptional level of NF-YA rapidly decreases following HSC differentiation [115]. In primitive mouse HSCs, the overexpression of NF-YA increases the mRNA levels of LEF-1, Notch-1, telomerase RNA and multiple...
HOX4 paralogs [115]. The deletion of NF-YA is accompanied by the dysregulation of Bcl-2, Notch1, Bmi-1, HoxB4, p21 and cyclin in mice [114]. These genes play crucial roles in HSCs and are involved in selfrenewal, apoptosis and cell cycle control [114, 116-119], suggesting an important role of NF-Y in HSCs. The circadian clock allows organisms to adapt to daily changes in the environment and synchronize various behavioral, physiological, molecular and biochemical processes accordingly. NF-Y has been identified as an additional candidate transcriptional regulator of the circadian clock by using DNA-array meta-analysis data and large-scale CCG promoter analysis in rodent tissues [120].

A recent study also prove that NF-Y cooperates with Rev-Erb and Sp1 to positively regulate the transcription of brain-muscle Arnt-like protein 1, which is a core circadian clock gene in mouse embryonic fibroblast NIH 3T3 cells [121]. Furthermore, NF-Y functions in cardiovascular homeostasis, ischemia-reperfusion injury and immune responses by regulating the transcription of the human type A natriuretic peptide receptor gene, the xanthine oxidoreductase gene and MHC genes, respectively [49, 67, 122]. These findings highlight the functional diversity of NF-Y, and with additional studies, more detailed molecular mechanisms will be uncovered in the near future.

Concluding remarks and outstanding questions

Whether in plants or animals, due to NF-Y plays vital roles in many biological processes, it has attracted extensive attention in recent years. However, according to the current research on NF-Y, there are some obvious differences in the regulatory mechanisms of NF-Y between plants and animals. In particular, in plants, each subunit of NF-Y is encoded by multiple gene members that further form different subfamilies to drive diverse functions [8-11]. In contrast, in yeast and mammals, each subunit of NF-Y is encoded by a single gene. Even so, this observation does not mean that NF-Y is less important in animals than in plants. In recent years, it has been uncovered that NF-Y plays significant roles in animal science and will remain to do so. Because animal NF-Y is a key regulator of many biological processes, such as development, cancer, tumors, and stress responses, and target genes activated by NF-Y are prominent candidates for maintaining the normal status of organisms under different environmental conditions [11, 43, 44, 66, 67]. In this case, it can say that NF-Y is a multifunctional TF.

Despite the reported observations related to animal NF-Y to date, many outstanding questions still need to be addressed in the future. First, it is well known that the NF-Y complex functions in the nucleus. However, few studies have revealed the precise subcellular localization of NF-Y subunits in various species and the differences among animals. Second, the expression levels of NF-Y during different biological processes are not fully understood. Recent studies focused mainly on the transcriptional regulation role of NF-Y in target genes. It is also important to explore the mRNA and protein expression profiles of NF-Y during these processes to better reveal its functional mechanisms. Third, though recent studies investigate the transcriptional regulation of NF-Y (for example, the U7 snRNA plays a role as a transcriptional inhibitor to control NF-Y [123], and Musashi-1, an RNA-binding protein, binds to the mRNA of NF-YA, represses its translation and sequesters it in glioma-initiating cells and breast cells via a posttranslational mechanism [124]), the transcriptional regulation of NF-Y is not fully understood. A detailed map of NF-Y transcriptional regulation and posttranslational modifications must be explored, which may contribute to functional understanding of NF-Y. Fourth, some studies have shown that the NF-YC subunit of the NF-Y complex can be replaced by Mes4 in Drosophila [48]. It is worth investigating whether other proteins can replace NF-YA or NF-YB to form a heterotrimer with NF-YB/NF-YC or NF-YA/NF-YC. Finally, noncoding RNAs, including long noncoding RNA and small noncoding RNA, perform a remarkable variety of biological functions, including the regulation of gene expression at the transcriptional, translational and RNA processing levels [125-128]. It has been proved that the long noncoding RNA PANDA sequesters the NF-Y trimer from target gene promoters by interacting with NF-YA in human diploid fibroblast strains, neonatal foreskin tissue and fetal lung tissue [129]. Recent research also suggests that NF-Y regulates the transcription of some miRNAs in human colon cancer [25]. In the near future, more studies that reveal the connections between NF-Y and noncoding
RNAs will enrich our understanding of the functional mechanisms of NF-Y. Addressing these questions may uncover novel mechanistic principles related to animal NF-Y.

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Disclosure of conflict of interest

None.

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Functional dissection of nuclear factor Y in animal


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