# New class of transcription factors controls flagellar assembly by recruiting RNA polymerase II in *Chlamydomonas*

Lili Li[a,1], Guangmei Tian[a,1], Hai Peng[a,1], Dan Meng[b], Liang Wang[c], Xiao Hu[a], Cheng Tian[a], Miao He[a], Junfei Zhou[a], Lihong Chen[a], Cheng Fu[d], Weixiong Zhang[a,e,f,2], and Zhangfeng Hu[a,2]

[a]Institute for Systems Biology, Jianghan University, Wuhan, 430056 Hubei, China; [b]Tianjin Key Laboratory of Food and Biotechnology, School of Biotechnology and Food Science, Tianjin University of Commerce, 300134 Tianjin, China; [c]School of Life Sciences, Jiangsu Normal University, Xuzhou, 221116 Jiangsu, China; [d]Institute for Interdisciplinary Research, Jianghan University, Wuhan, 430056 Hubei, China; [e]Department of Computer Science and Engineering, Washington University in St. Louis, St. Louis, MO 63130; and [f]Department of Genetics, Washington University in St. Louis, St. Louis, MO 63130

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**Cells have developed regulatory mechanisms that underlie flagellar assembly and maintenance, including the transcriptional regulation of flagellar genes, an initial step for making flagella. Although transcriptional regulation of flagellar gene expression is required for flagellar assembly in *Chlamydomonas*, no transcription factor that regulates the transcription of flagellar genes has been identified. We report that X chromosome-associated protein 5 (XAP5) acts as a transcription factor to regulate flagellar assembly in *Chlamydomonas*. While XAP5 proteins are evolutionarily conserved across diverse organisms and play vital roles in diverse biological processes, nothing is known about the biochemical function of any member of this important protein family. Our data show that loss of XAP5 leads to defects in flagellar assembly. Post-translational modifications of XAP5 track flagellar length during flagellar assembly, suggesting that cells possess a feedback system that modulates modifications to XAP5. Notably, XAP5 regulates flagellar gene expression via directly binding to a motif containing a CTGGGGTG-core. Furthermore, recruitment of RNA polymerase II (Pol II) machinery for transcriptional activation depends on the activities of XAP5. Our data demonstrate that, through recruitment of Pol II, XAP5 defines a class of transcription factors for transcriptional regulation of ciliary genes. This work provides insights into the biochemical function of the XAP5 family and the fundamental biology of the flagellar assembly, which enhance our understanding of the signaling and functions of flagella.**

cilia | XAP5 | transcription factor | transcriptional regulation | *Chlamydomonas*

Cilia and flagella are subcellular organelles that protrude from the surface of cells (1). They have evolved to play crucial roles in cell motility, sensory reception, and signal transduction in various organisms (2). Cilia are highly conserved throughout evolution and are structurally dynamic, undergoing growth and resorption during the cell cycle (3). Ciliary dysfunction can cause a substantial number of human genetic disorders, including polycystic kidney disease, Joubert syndrome, and Bardet–Biedl syndrome, which are collectively referred to as "ciliopathies" (4).

Previous studies have shown that cilia are complex structures with hundreds of components involved in structure and function (5–7). These studies have also identified a number of human ciliopathy disease genes. They are involved in intraflagellar transport (IFT) and Bardet–Biedl syndrome (BBS) and are widely distributed and highly conserved among organisms with cilia and flagella (8, 9). IFT is a complex transport mechanism that regulates the trafficking of ciliary proteins along the axonemal doublet microtubules and is required for assembly and maintenance of cilia (8). The BBS subunits form a complex to transport ciliary proteins into the ciliary compartment (9). The expression of ciliary components is regulated at the transcriptional level, an important step during cilia formation (7, 10). Transcriptional regulation of ciliary gene expression was first recorded via studies of flagellar regeneration after experimentally inducing deflagellation in *Chlamydomonas* (11). Cells regenerate half-length flagella after flagellar amputation by using a preexisting pool of cytoplasmic proteins (12). Nevertheless, the synthesis of mRNA and proteins of the flagellar components must be switched on to complete reassembly of flagella (11, 12). Although the specific transcriptional regulation of ciliary genes' expression during cilia assembly has been widely studied in *Chlamydomonas*, the mechanisms by which cilia assembly is programmed at the transcriptional level remain elusive.

Emerging evidence suggests that transcription regulation may be critically important in the control of different functional and structural modules to generate ciliary diversity in various cell types (10, 13–15). The regulatory factor X (RFX) family proteins share a highly conserved winged-helix DNA-binding domain and are required for the transcriptional regulation of ciliary genes in primary cilia (13). FOXJ1 is a forkhead/winged-helix family transcription factor that is necessary for motile ciliogenesis in

## Significance

Transcriptional regulation of flagellar genes controls an initial step in flagellar assembly. In this study, we show that XAP5, a conserved protein of unknown function, defines a class of transcription factor for transcriptional regulation of genes involved in flagellar assembly. Phosphorylation of X chromosome-associated protein 5 (XAP5) during flagellar regeneration tracks flagellar length. Remarkably, recruitment of RNA polymerase II (Pol II) machinery for transcriptional activation depends on the activities of XAP5. Our data demonstrate that XAP5 functions as a transcription factor for transcriptional regulation of flagellar genes through recruitment of RNA Pol II. Our results enhance our understanding of the biochemical function of the XAP5 family and the transcriptional regulation of flagellar assembly.

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Data deposition: The *Chlamydomonas* XAP5 cDNA and protein sequences have been deposited in the National Center for Biotechnology Information (NCBI) GenBank data library (https://www.ncbi.nlm.nih.gov/nuccore/KU361202) (ID codes KU361202.1 and AMY57988.1). The RNA-sequencing data are available at the NCBI Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra/SRP131193) (accession no. SRP131193).

[1]L.L., G.T., and H.P. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: weixiong.zhang@wustl.edu or huzhangfenghzf@163.com.

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mammals, zebrafish, and *Xenopus* (14, 15). In sharp contrast to these results for metazoans, *Chlamydomonas* does not contain any of the orthologs of RFX, FOXJ1, or other transcription regulators that modulate the transcription of genes encoding ciliary proteins.

XAP5 proteins are evolutionarily conserved across diverse organisms. The *XAP5* gene was initially identified as a candidate disease gene in humans (16). *XAP5*-like (*X5L*) is highly expressed during spermatogenesis and is deregulated in testicular germ cell tumors (17). In addition, XAP5 proteins are also found in other nonciliated organisms (18, 19), including yeast (20). Accordingly, XAP5 domain-containing proteins have complex biological functions in diverse organisms. However, we currently know nothing about the biochemical function of any member of this protein family. In this context, the fundamental biological process by which ciliogenesis is programmed at the transcriptional level and the mode of action and biochemical function of the XAP5 family will provide insightful knowledge of flagellar assembly.

## Results

**Flagellar Assembly Is Defective in the *xap5* Mutant.** To search for mutants with defects in flagellar assembly, we performed a foreign DNA insertional mutagenesis screening of *Chlamydomonas reinhardtii* (3) and acquired a flagellar-assembly mutant, *af1-x*. The mutant cells formed aggregates, which were mostly palmelloid colonies, due to the failed release of daughter cells after cell division (Fig. 1 *A* and *B* and Movies S1–S3). Autolysin enzyme treatment caused the *af1-x* mutant cells to be released from the mother cell wall (Fig. 1*B*). This demonstrates that the cells were aflagellate or had very short flagella (<3 μm) and also showed that the flagella did not elongate over time (Fig. 1 *C* and *D*). The *af1-x* mutant was disrupted in the last intron of the *XAP5* gene (Fig. 1*E*). Further analysis showed that the *AphVIII* gene fragment was inserted into the gene, accompanied by a genomic addition of four nucleotides (Fig. 1*E* and Fig. S1*A*).

We first examined the conservation of XAP5 across diverse organisms (Fig. S1 *B* and *C*). It has an N-terminal coiled-coil region, an XAP5 domain at the C terminus, and a nuclear localization sequence predicted by the SMART (smart.embl-heidelberg.de/) and cNLS Mapper (nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi) websites (Fig. 1*E* and Fig. S1*C*). Transformation of *af1-x* with a cloned genomic fragment encoding untagged or HA-tagged XAP5 rescued the wild-type phenotypes (Fig. 1 *A* and *B* and Movies S1–S3), demonstrating that the mutant phenotypes of *af1-x* were caused by the disruption to the *XAP5* gene. An RT-PCR analysis showed that the transcripts of *XAP5* were missing in the *xap5* mutant (Fig. S1*D*). Thus, the foreign DNA fragment terminated *XAP5* gene transcription completely. A plausible explanation is that the fragment inserted in the intron of *XAP5* prevented proper transcription of *XAP5*, resulting in degradation of faulty and unstable transcripts. A polyclonal antibody generated against an XAP5 synthetic peptide detected a band corresponding to the predicted size of full-length XAP5 (44 kDa) in the wild-type and rescued cells. However, such a band was absent in *xap5* mutants, suggesting that the antibody is specific and that *xap5* is a null mutant (Fig. 1 and Fig. S1*E*).

XAP5 appeared to be normal in mutants defective in flagellar assembly (21, 22) (Fig. S1*F*), indicating that defective flagellar assembly does not affect the expression of XAP5 and that the absence of XAP5 in the *xap5*-mutant strain was unrelated to flagellar-assembly defects. The *xap5* cells had the same cell size as the wild-type and rescued cells (Fig. S1*G*), confirming that

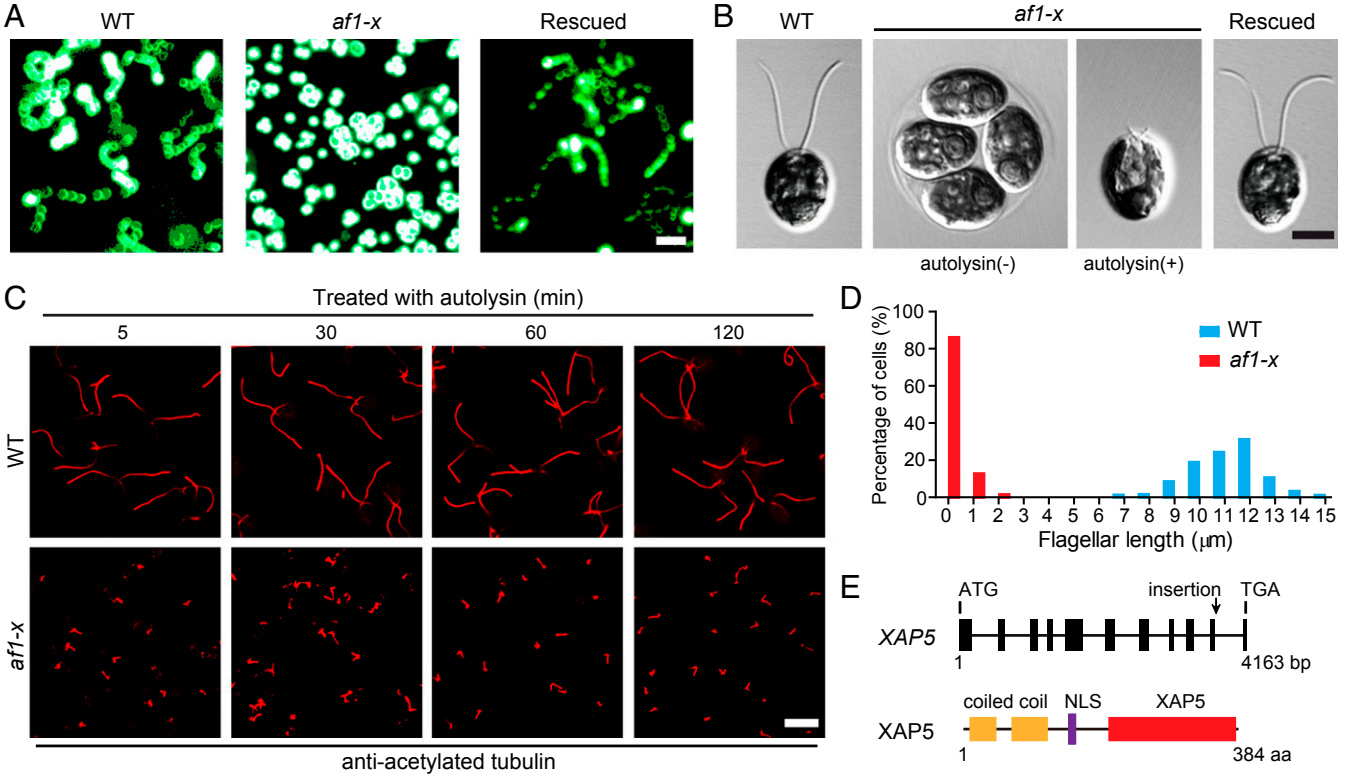


**Fig. 1.** Identification and characterization of a *xap5* mutant in *Chlamydomonas*. (*A*) Motile cells of the wild-type (WT) and rescued (*xap5::XAP5-HA*) strains were observed as long, winding tracks; conversely, palmelloid cells of *af1-x* were presented as bright focus. (Scale bar, 20 μm.) (*B*) Differential interference contrast (DIC) images of wild-type, *af1-x*, and rescued cells. Palmelloid *af1-x* cells were incubated with autolysin. (Scale bar, 5 μm.) (*C*) Immunofluorescence micrographs of wild-type and *af1-x* flagella labeled with acetylated tubulin antibodies after treatment with autolysin. (Scale bar, 10 μm.) (*D*) Flagellar length distribution of wild-type and *af1-x* cells after treatment with autolysin ($n = 500$). (*E*) Schematic view of the *XAP5* gene; the foreign DNA insertion site in *af1-x* and the domain structure of the XAP5 protein are indicated.

XAP5 is not required by processes essential for cell size or growth and supporting the idea that there is no necessary association between flagella phenotypes and cell size.

**Posttranslational Modification of XAP5 During Flagellar Assembly.** XAP5 proteins resided preferentially in the nucleus and seldom existed in the cytoplasm (Fig. S2 *A*–*C*). XAP5 contains a predicted nuclear-localization signal (NLS), KRKR, at amino acids 106–109 (Fig. S2*D*). In contrast to the wild-type gene, both *XAP5-ΔNLS-HA* and *XAP5-K106A-HA* failed to rescue the flagellar-assembly defects (Fig. S2 *E* and *F*). Deletion or mutation of the NLS KRKR disrupted the nuclear localization of XAP5 and led to a diffused cytoplasmic distribution (Fig. S2 *E* and *G*). Thus, the appropriate nuclear localization of XAP5 mediated by the NLS is required for flagellar assembly.

To understand the role that XAP5 plays in flagellar assembly, we examined its expression and subcellular localization. The expression and subcellular location of XAP5 had no significant change during flagellar regeneration (Fig. 2*A* and Fig. S2*H*). Unexpectedly, a posttranslational modification in XAP5 was reflected by a change in its electrophoretic mobility (Fig. 2*A* and Fig. S3 *A* and *B*). Importantly, XAP5 was modified immediately after deflagellation, and the modification of XAP5 disappeared as the flagella reached nearly their full length (Fig. 2*A* and Fig. S3 *A* and *B*). After lysates of *xap5::XAP5-HA* cells were incubated with a phosphatase, XAP5 was not modified (Fig. 2*B*), suggesting that XAP5 was phosphorylated during flagellar regeneration. To determine whether the phosphorylation of XAP5 occurred under the physiological conditions, we analyzed the status of XAP5 during the cell cycle. As expected, XAP5 was also phosphorylated during flagellar assembly after mitosis (Fig. S3*C*) (3). Therefore, XAP5 is an important regulator of flagellar assembly that occurs under physiological and nonphysiological conditions, and the phosphorylation of XAP5 may play a vital role in flagellar assembly. Nuclear XAP5, rather than cytoplasmic XAP5, underwent phosphorylation in response to pH shock-induced deflagellation (Fig. 2*C*). Correspondingly, the cytoplasmic localized XAP5-ΔNLS-HA and XAP5-K106A-HA mutants failed to undergo a mobility shift-associated phosphorylation after deflagellation (Fig. 2*D*). Thus, nuclear localization of XAP5 is necessary for its phosphorylation during flagellar regeneration.

Deflagellated cells regenerated to only half the original length after treatment with staurosporine (Fig. 2*E*), a protein kinase inhibitor which has been reported to block the phosphorylation of nuclear protein that may play a crucial role in transcriptional activation (23). Consistent with prior studies, blocking protein synthesis by cycloheximide resulted in regeneration of half-length flagella and a slow regeneration of flagella by rapamycin after deflagellation (Fig. 2*E*) (24), implying that cells have developed diverse regulatory mechanisms to ensure the formation of functional cilia. XAP5 did not undergo an obvious mobility shift in cells treated with staurosporine (Fig. S2 *D* and *E*). These data raise the possibility that staurosporine affects flagellar gene transcription, probably through the regulation of XAP5. Furthermore, in cells treated with actinomycin D and deflagellated by two successive pH shocks, flagellar assembly was completely inhibited after the second amputation (Fig. 2*F*), showing that inhibition of transcription impairs flagellar assembly.

To understand whether phosphorylation of XAP5 is required for flagellar assembly, we expressed in *xap5* HA-tagged XAP5 constructs with multipoint mutations or a single-point mutation at serine/threonine/tyrosine residue(s). We found that S119, a consensus phosphorylation site in the coiled-coil region of XAP5, is required for flagellar assembly (Fig. S4 *A*–*C*). The wild-type XAP5 and a phosphomimetic (*S119D*) mutant fully complemented the flagellar type, whereas phosphodefective (*S119A*) mutant cells were aflagellate (Fig. S4 *B* and *C*). The S119D and S119A mutant proteins did not undergo a mobility shift-associated phosphorylation after flagellar amputation (Fig. S4*D*), and these mutations did not affect the nuclear localization of XAP5 (Fig. S4*E*). Thus, S119 phosphorylation of XAP5 is essential for flagellar assembly. Furthermore, the phosphomimetic (*S119D*) mutant cells subjected to staurosporine treatment regrew approximately half-length flagella after amputation (Fig. S4 *F* and *G*), suggesting that staurosporine also suppresses other processes involved in flagellar assembly.

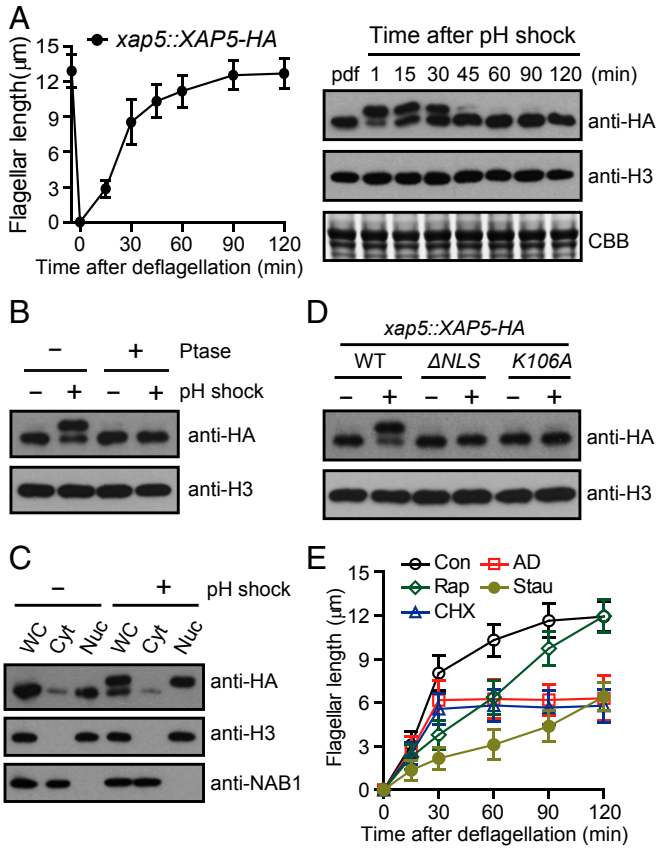


**Fig. 2.** XAP5 is phosphorylated in response to signals that induce flagellar assembly. (*A*) Immunoblot analysis of XAP5 during flagellar regeneration using an antibody against XAP5. Kinetics of flagellar regeneration upon shock-induced deflagellation is shown above the blot. Coomassie Brilliant Blue (CBB) showed an equal loading of all of the samples. H3, histone H3 antibody; pdf, cell sample before deflagellation. (*B*) Western blot analysis of whole-cell lysates from control and deflagellated *xap5::XAP5-HA* cells that were hatched with or without phosphatase (Ptase). (*C*) Control and deflagellated *xap5::XAP5-HA* cells were fractioned into whole-cell (WC), cytoplasm (Cyt), and nuclei (Nuc) fractions and were analyzed by immunoblotting. Antibodies against NAB1 and histone H3 were used to mark the cytoplasm and nuclei, respectively. (*D*) Immunoblot analysis of cell lysates from control and deflagellated *xap5* cells expressing the HA-tagged wild-type, ΔNLS, and K106A-XAP5 protein. (*E*) Inhibition of flagellar regeneration by staurosporine. Cells were triggered to regenerate flagella by pH shock in the presence of actinomycin D (AD) (100 μg/mL), cycloheximide (CHX) (10 μg/mL), rapamycin (Rap) (50 μM), or staurosporine (Stau) (1 μM). Con, control. Data in *A* and *E* represent mean ± SD. One flagellum from at least 50 cells was measured at each time point in three independent experiments.

**XAP5 Modulates the Transcription of Ciliary Genes.** To elucidate the mechanism by which XAP5 regulates flagellar assembly, we studied the possible function of XAP5 in modulating the expression of conserved genes necessary for ciliary assembly. We analyzed some of IFT- and BBS-related genes that broadly exist and are highly conserved in ciliated organisms. We found that *IFT144*, *IFT70*, and *BBS8* were down-regulated in *xap5* cells (Fig. 3*A*), whereas the expression levels of *IFT139*, *IFT20*, *BBS4*, and *α-tubulin* in *xap5* cells were not changed significantly (Fig. 3*A*). However, the expression of *IFT144*, *IFT70*, and *BBS8* appeared to be normal in other flagellaless mutants (Fig. 3*B*), suggesting

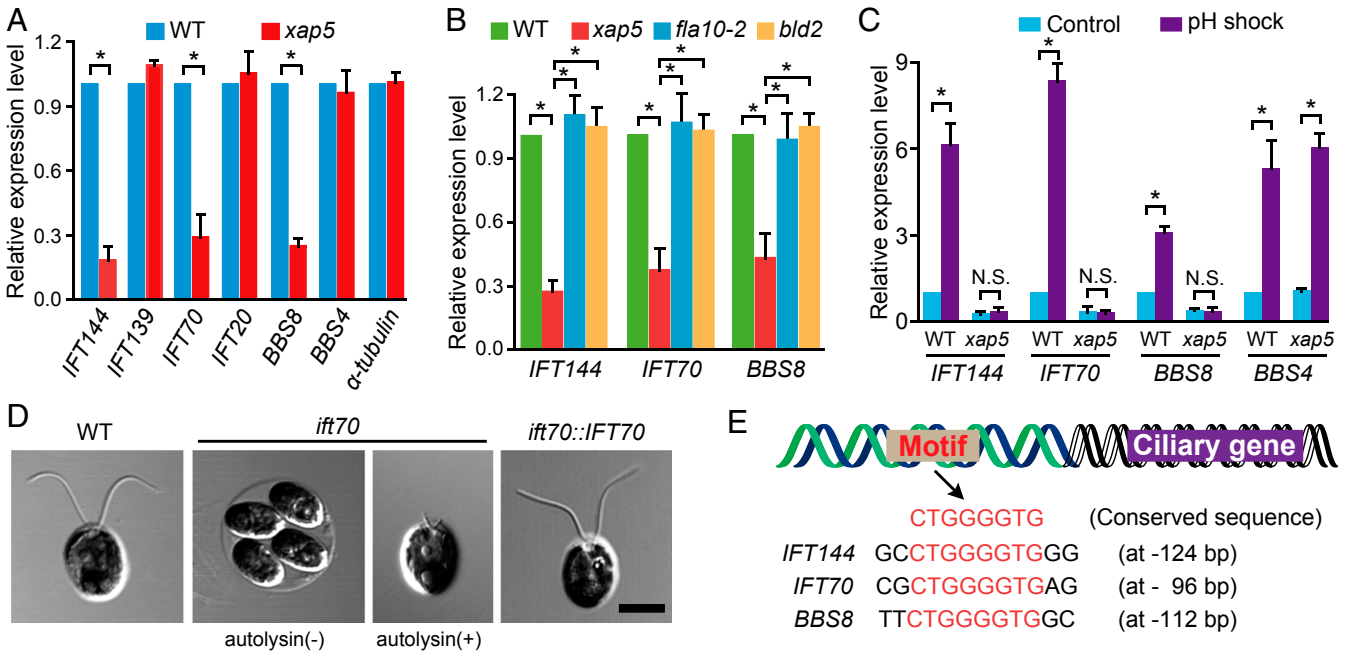


**Fig. 3.** XAP5 modulates the transcription of ciliary genes via directly binding to the CTGGGGTG motif. (*A*) Analysis of the transcript abundance of flagellar-associated genes in wild-type and *xap5* cells by real-time qPCR. (*B*) Quantification of the transcript abundance of ciliary genes in mutants defective in flagellar assembly. (*C*) Changes in the relative expression level of genes in wild-type and *xap5* cells after pH shock-induced deflagellation. (*D*) DIC images of wild-type, *ift70*, and rescued (*ift70::IFT70*) cells. Palmelloid *ift70* mutant cells were incubated with autolysin and released from the mother cell wall. (Scale bar, 5 μm.) (*E*) Schematic drawing representing the potential XAP5 target sites in the promoter regions of the ciliary genes. Data in *A*–*C* represent the mean ± SD of three independent experiments. $*P < 0.05$. N.S., not significant ($P > 0.05$).

that the lowered transcription levels of *IFT144*, *IFT70*, and *BBS8* in *xap5* cells were unrelated to defects in flagellar assembly.

*IFT144*, *IFT70*, and *BBS8* were up-regulated in wild-type cells during flagellar regeneration while their abundances in *xap5* cells were comparable (Fig. 3*C*). However, the BBS4 gene was up-regulated in *xap5* compared with wild-type cells after flagellar amputation (Fig. 3*C*). These data suggest that the transcriptional regulation of flagellar genes might be classified into two types: XAP5-dependent and XAP5-independent. In addition, the expression abundances of *IFT144*, *IFT70*, and *BBS8* were significantly reduced in *xap5::XAP5-ΔNLS-HA* and *xap5::XAP5-K106A-HA* cells compared with the wild-type cells (Fig. S5*A*), indicating that nuclear localization of XAP5 is essential for ciliary gene transcription. Moreover, IFT70 proteins in *xap5* cells exhibited distinctly decreased levels compared with the levels in wild-type cells and flagellaless mutants (Fig. S5 *B*–*G*). Consistent with the previous findings (25), the *IFT70*-null mutation impaired flagellar assembly (Fig. 3*D* and Fig. S5*G*). Collectively, these results reveal that XAP5 regulates flagellar assembly via modulating the transcription of XAP5-dependent flagellar genes.

**Sequence-Specific Binding of XAP5 in the Promoters of Ciliogenic Genes.** To assess whether XAP5 directly regulates ciliogenic gene expression, we first performed ChIP assays with an antibody against HA. Remarkably, the promoters of the candidate XAP5-target genes *IFT144*, *IFT70*, and *BBS8* were preferentially enriched in the *xap5::XAP5-HA* ChIP samples, especially near the predicted transcription start site (TSS) (Fig. S6*A*). Furthermore, XAP5 occupancy in the promoter regions was significantly elevated after flagellar detachment (Fig. S6*B*). However, when cells were treated with staurosporine, XAP5 occupancy exhibited no significant change during flagellar regeneration (Fig. S6*B*), implying that posttranslational modification directed the localization of XAP5 in chromatin regions. Therefore, XAP5 is presumably a potential factor affecting the activation of *IFT144*, *IFT70*, and *BBS8* promoters.

We observed that the 298-bp, 254-bp, and 224-bp regions upstream of the TSSs of *IFT144*, *IFT70*, and *BBS8*, respectively, were required for their normal promoter activities (Fig. S6*C*). Intriguingly, a BLAST analysis of these regions revealed a conserved CTGGGGTG sequence located immediately upstream of the TSS (Fig. 3*E*). An analysis by EMSA showed that XAP5 could bind directly to the motif (Fig. S6 *D* and *E*). Together, these results show that XAP5 regulates ciliary gene expression through direct binding to the CTGGGGTG core sequence motif in the ciliary gene promoters.

To investigate the potential scope of involvement of XAP5 in the transcription of flagellar and nonflagellar genes, we carried out genome-wide profiling of gene expression in *xap5* and in wild-type cells using deep sequencing (RNA-seq). The results showed that 2,126 genes, including 267 flagellar-associated genes, were down-regulated by at least twofold in *xap5* mutants (Fig. S7*A*). A gene ontology (GO) analysis revealed that these differentially expressed genes were enriched with functions for signal transduction-, cilium-dependent cell motility-, and cilium assembly-related biological processes (Fig. S7*B*), in motile cilium-, microtubule-, and intraciliary transport particle B (IFT B)-involved cellular components (Fig. S7*C*), as well as motor activity, structural constituent of cytoskeleton, and calcium ion binding in molecular functions (Fig. S7*D*).

We identified 111 known flagellar genes that were down-regulated in *xap5* cells (Fig. S7*E* and Table S1). Importantly, 106 of these genes contained the putative XAP5-binding motifs and thus may be directly regulated by XAP5 (Fig. S7 *F* and *G* and Table S1). The remaining five flagellar genes did not contain the putative binding motif (Fig. S7*G*). Their decreased expression might be due to changes in the abundance of other flagellar gene transcripts. We also examined the expression of three XAP5-dependent flagellar genes (*ODA13*, *FAP147*, and *FAP5*) and one XAP5-independent flagellar gene (*IFT20*) after amputation induced by pH shock in the wild-type and *xap5* cells. The expression of *IFT20* was elevated after pH shock in both wild-type and *xap5* cells. In contrast, the three XAP5-dependent

flagellar genes were up-regulated only in wild-type cells after flagellar amputation but were not significantly changed in *xap5* cells (Fig. S7*H*). Additionally, the abundance of XAP5-dependent flagellar genes was significantly decreased in the phosphodefective (*S119A*) mutant cells compared with the wild-type XAP5 and the phosphomimetic (*S119D*) mutant cells (Fig. S7*I*). Interestingly, the expression of the XAP5-dependent flagellar genes increased slightly in the *S119D* mutant compared with wild-type XAP5 cells (Fig. S7*I*). However, no change in the expression levels of two XAP5-independent flagellar genes (*IFT20* and *BBS4*) was observed in these cells (Fig. S7*I*). Thus, proper phosphorylation of XAP5 is required for normal transcriptional control of XAP5-dependent flagellar genes.

**XAP5 Facilitates Recruitment of the Polymerase II Apparatus to Promoters.** To directly demonstrate that XAP5 functions as a positive transcriptional activator of *IFT144*, *IFT70*, and *BBS8*, fragments of a putative promoter region containing the CTGGGGTG motif or a mutant form, AGTTTTGT, were assessed for their ability to activate the expression of a luciferase reporter gene. The result of luciferase activities showed that the CTGGGGTG motif in the promoter regions of *IFT144*, *IFT70*, and *BBS8* was critical for the expression of these genes (Fig. S8 *A* and *B*). Importantly, the *ift70* mutant phenotype was rescued by a transformation with the wild-type promoter driving *IFT70* expression. In addition, about 28% of transformants rescued the mutant flagellar phenotype (Fig. S8*C*). None of the transformants recovered the flagellar phenotype when *ift70* mutants were transformed with a mutated promoter driving *IFT70* expression (Fig. S8*C*). Therefore, XAP5 is a potent transactivator of *IFT144*, *IFT70*, and *BBS8* genes. Furthermore, we investigated the capacity of the promoters of wild-type ciliary genes to activate the expression of the luciferase (*Luc*) reporter gene in the wild type and *xap5* mutant (Fig. S8*D*). The *xap5* mutant showed a lower relative luminescence unit (RLU) average compared with the wild type (Fig. S8*D*). Additionally, the RLU value of the wild-type *IFT70* promoter directing the *Luc* gene was comparable in the wild type and flagellaless mutants (Fig. S8*E*), indicating that ciliary gene promoter activities are unrelated to ciliary defects.

A hallmark function of XAP5 is its direct regulation of ciliary gene expression, indicating the possibility that XAP5 could recruit the polymerase II (Pol II) machinery to the ciliary gene promoter for transcriptional activation. To further verify that loss of XAP5 reduces the levels of transcription of target genes, a ChIP analysis revealed that the occupancy of Pol II at the promoter regions of *IFT144*, *IFT70*, and *BBS8* genes with an antibody recognized the tandem heptapeptide repeats in the C-terminal domain (CTD) of Pol II's largest subunit (RPB1) (Fig. S8 *F* and *G*). An enrichment of Pol II at the target genes was observed in wild-type cells (Fig. 4*A*). In contrast, the occupancy of the Pol II apparatus at the *IFT144*, *IFT70*, and *BBS8* genes was reduced significantly in *xap5* cells (Fig. 4*A*).

We further reasoned that the ciliary genes were up-regulated during ciliogenesis as a result of the accumulation of Pol II at the genes. Strikingly, increased Pol II occupancy at ciliary genes was accompanied by a simultaneously increased presence of XAP5 at the proximal promoters after pH shock in *xap5::XAP5-HA* cells (Fig. 4*B*). In contrast, the occupancy of Pol II was not significantly changed in *xap5* mutants during flagellar regeneration (Fig. 4*B*). The component of the Pol II machinery was present in the immunoprecipitates prepared with an antibody against HA

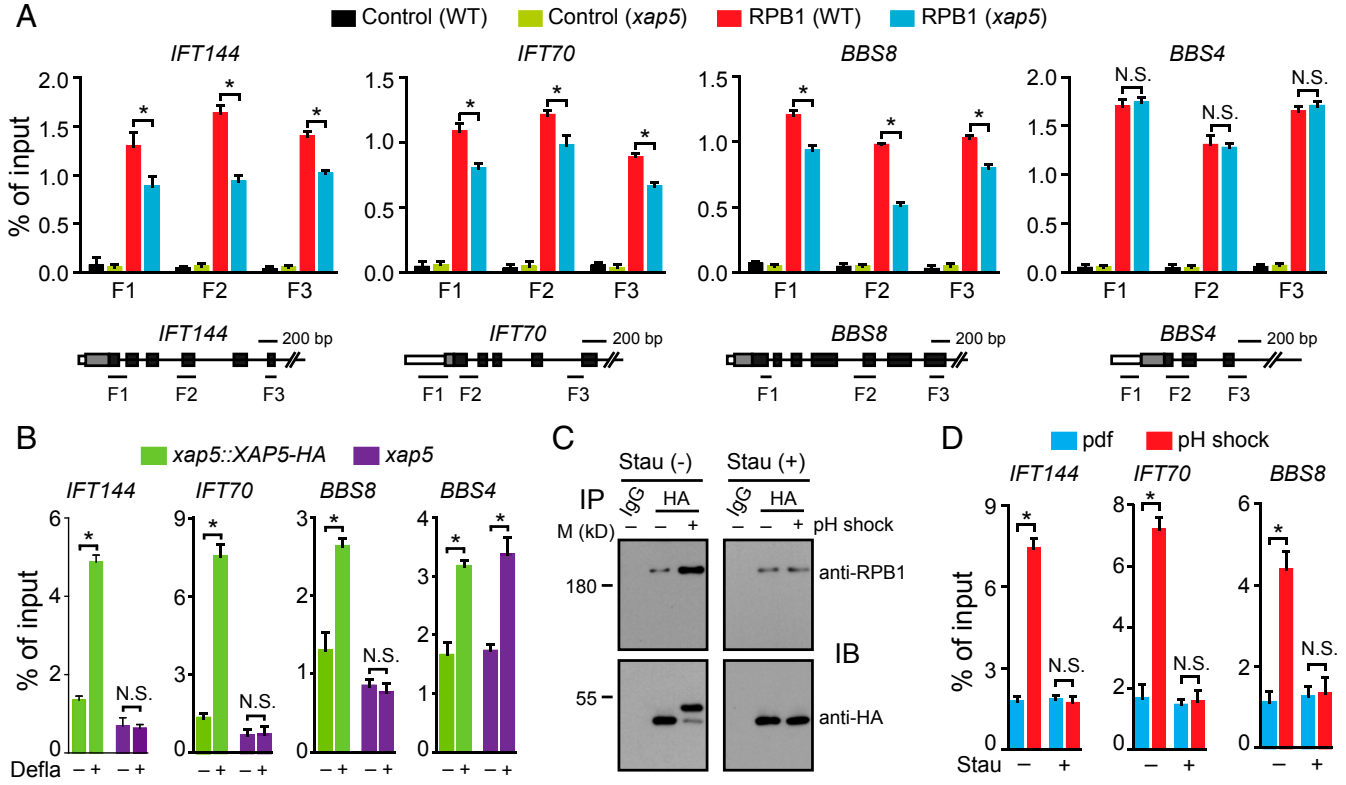


**Fig. 4.** XAP5-mediated recruitment of the Pol II machinery to ciliary gene promoters. (*A*) ChIP-qPCR analysis of the indicated genes using three independently prepared samples. ChIP was performed in wild-type and *xap5* cells with or without (Control) an antibody against RPB1. The regions amplified from chromatin immunoprecipitates by qPCR are indicated below each gene. (*B*) Detection of Pol II occupancy at ciliary genes in *xap5::XAP5-HA* and *xap5* cells after pH shock-induced deflagellation (Defla). (*C*) Coimmunoprecipitation of XAP5 and Pol II machinery. Isolated nuclei from cells in the presence or absence of staurosporine were immunoprecipitated with antibody against HA or with preimmune IgG, followed by Western blotting using antibodies against HA and RPB1, respectively. (*D*) Detection of Pol II occupancy at ciliary genes by ChIP-qPCR in control and deflagellated wild-type cells in the presence or absence of staurosporine. Data in *A*, *B*, and *D* represent the mean ± SD of three independent experiments. **P* < 0.05; N.S., not significant (*P* > 0.05).

and was absent in the control group (preimmune IgG) (Fig. 4*C*). Importantly, phosphorylated XAP5 had a stronger affinity to the Pol II machinery than did nonphosphorylated XAP5 (Fig. 4*C*). Furthermore, *xap5::XAP5-HA* cells treated with staurosporine failed to accumulate Pol II at target genes after deflagellation (Fig. 4 *C* and *D*). Combined, these results reveal the importance of phosphorylated XAP5-mediated recruitment of the Pol II machinery for transcriptional activation, suggesting that XAP5 acts as a transcription factor for transcriptional regulation of flagellar genes.

## Discussion

The specific transcriptional induction of ciliary genes is precisely controlled and coordinated during cilia formation, and inhibition of transcription impairs ciliogenesis (10). Although the significance of the transcriptional regulation of ciliary gene expression was initially documented in *Chlamydomonas* (11), the mechanisms underlying this regulation remain poorly understood. To our knowledge, the mechanisms and genes that regulate the transcription and expression of ciliary genes have not been reported in *Chlamydomonas* or other unicellular organisms.

It has been generally acknowledged that the last eukaryotic common ancestor is a flagellated unicellular organism (26). Despite flagellar genes being under direct transcriptional regulation in both unicellular and multicellular organisms, the evolution of the transcriptional program controlling flagellar assembly is largely unknown (26). Previous studies have shown that RFX transcription factors, FOXJ1 transcription factors, and ciliary genes all evolved independently (26–28). The transcriptional control of ciliary genes generally regulates the formation of one type of cilium in unicellular organisms. Nevertheless, in multicellular organisms, many ciliary genes are differentially regulated with cell type-specific patterns of expression to generate ciliary diversity (10). RFX and FOXJ1 are key regulators of ciliary gene expression in animals (10). However, both are absent from many unicellular organisms, including *Chlamydomonas*, indicating that the transcriptional control of the ciliogenesis is fundamentally different in unicellular and multicellular organisms.

Previous data suggest that XAP5 proteins play vital roles in many biological processes (17–20). However, little is known about the precise molecular function of the conserved nucleus-localized protein XAP5. In the current study, an in vitro binding analysis with predicted transcription regulatory elements revealed that XAP5 could specifically recognize and bind to a motif with a CTGGGGTG core in the promoter regions of ciliary genes (Fig. 3). Moreover, we demonstrated that XAP5 induced the promoter activities of targeted ciliary genes (Fig. 4). Therefore, our results show that XAP5 functions as a transcription factor to regulate flagellar assembly in *Chlamydomonas*. The transcriptional control of more than 100 flagellar genes was XAP5 dependent, whereas the expression of several flagellar genes was XAP5 independent, implying that the regulation of flagellar genes at the transcriptional level is a highly complex process and that XAP5-independent flagellar gene expression can be regulated by other, undiscovered transcriptional mechanisms. In addition, the promoter regions of almost all the XAP5-dependent flagellar genes contained the putative XAP5-binding motif. Thus, it is possible to use the XAP5-binding motif to predict XAP5 targets, particularly those among flagellar genes, which provides a way to identify genes potentially involved in ciliary assembly and function.

## Materials and Methods

The experimental materials and detailed experimental procedures can be found in *SI Materials and Methods*, which includes strains and cell cultures, transformation, screening for flagellar-assembly mutants, complementation of *xap5* and *ift70*, flagellar regeneration, flagellar length and cell size measurements, data availability, alignment and phylogenetic analysis, live-cell imaging and movies, real-time qPCR and RNA-seq analysis, ChIP, EMSA, luciferase activity assay, isolation of nuclei, and anti-IFT70 and anti-XAP5 antibodies.

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