Identification and Characterization of Two Novel Components of The Prp19p-associated Complex, Ntc30p and Ntc20p*

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The yeast *Saccharomyces cerevisiae* Prp19p protein is an essential splicing factor and a spliceosomal component. It is not tightly associated with small nuclear RNAs (snRNAs) but is associated with a protein complex consisting of at least eight proteins. We have identified two novel components of the Prp19p-associated complex, Ntc30p and Ntc20p. Like other identified components of the complex, both Ntc30p and Ntc20p are associated with the spliceosome in the same manner as Prp19p immediately after or concurrently with dissociation of U4, indicating that the entire complex may bind to the spliceosome as an intact form. Neither Ntc30p nor Ntc20p directly interacts with Prp19p, but both interact with another component of the complex, Ntc85p. Immunoprecipitation analysis revealed an ordered interaction of these components in formation of the Prp19p-associated complex. Although null mutants of NTC30 or NTC20 showed no obvious growth phenotype, deletion of both genes impaired yeast growth resulting in accumulation of precursor mRNA. Extracts prepared from such a strain were defective in pre-mRNA splicing *in vitro*, but the splicing activity could be restored upon addition of the purified Prp19p-associated complex. These results indicate that Ntc30p and Ntc20p are auxiliary splicing factors the functions of which may be modulating the function of the Prp19p-associated complex.

Splicing of pre-mRNA requires five small nuclear RNAs (snRNAs)1 and a large number of protein factors, which assemble into a large ribonucleoprotein complex called the spliceosome (for reviews, see Refs. 1–6). Spliceosome assembly is a multistep process that involves sequential binding of snRNAs to the pre-mRNA in an order of U1, U2, then U4/U6 and U5 as a preformed tri-snRNP particle. A subsequent conformational rearrangement results in dissociation of U1 and U4, accompanied by new base pair formation between U2 and U6 and between U6 and the 5′ splice site, leading to the formation of the active spliceosome on which the catalytic reactions take place.

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1 The abbreviations used are: snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein; ORF, open reading frame; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase pair(s).

Functional studies of snRNAs have revealed their important roles in recognition and alignment of splice sites mediated through base pair interactions between snRNAs and the intron sequences during spliceosome assembly. Although numerous protein splicing factors have been identified, their functional roles are not well understood. The DEx(D/H) box proteins are among the best characterized protein factors and have been shown RNA unwindase activity (6–10). It is generally believed that these proteins play essential roles in modulating structural change of the spliceosome during spliceosome assembly by either unwinding RNA base pairing or by hydrolyzing ATP to provide energy required for conformational rearrangement (6). In addition, a U5 protein with strong sequence similarity to ribosomal translocase EF-2 was demonstrated to have GTP binding activity and implicated in the structural rearrangement of RNA (11).

We have previously shown that the yeast *Saccharomyces cerevisiae* Prp19p protein is an essential splicing factor and is associated with a protein complex consisting of at least eight protein components (12). Prp19p is not tightly associated with snRNAs but is associated with the spliceosome immediately following or concurrent with dissociation of U4 from the spliceosome, suggesting a possible role in mediating conformational rearrangement or stabilizing the rearranged structure of the spliceosome during U4 dissociation (13). Two components of the Prp19p-associated complex have been identified. Ntc85p was identified by sequencing the components of the affinity purified complex (Ntc stands for PRP nineteenth) (14). It is essential for pre-mRNA splicing and may play a role in promoting binding of the Prp19p-associated complex to the spliceosome. Snt309p was identified by screening synthetic lethal mutants to the prp19 mutation (Snt stands for synthetic lethal to PRP nineteen) (15). Although the SNT309 gene is not required for yeast growth, Snt309p, through interaction with Prp19p, plays an important role in modulating interactions of Prp19p with other associated components to stabilize the Prp19p-associated complex (16). Both proteins are associated with the spliceosome immediately after or simultaneously with dissociation of U4. We report here the identification of genes complementing synthetic lethal mutants snt304 and snt384. We show that these two genes encode two other components of the Prp19p-associated complex, Ntc30p and Ntc20p, respectively. Both Ntc30p and Ntc20p are also associated with the spliceosome in the same manner as Prp19p, indicating that the entire complex may bind to the spliceosome as an intact unit.

EXPERIMENTAL PROCEDURES

Strains—The strains used were as follows: YSCC1, *M* at *a prc1 prb1 pep4 leu2 trp1 ura3 PRP19-HA; YSCC3, *Mata prc1 prb1 pep4 leu2 trp1 ura3 PRP4-HA PRP19-MYC; SEY6210.5, *Mata/Mata leu2/leu2 ura3/ura3 his3/his3 trp1/trp1 suc2/suc2 lys2/LYS2 ADE2/ade2; YD30, *Mata/Mata leu2/leu2 ura3/ura3 his3/his3 trp1/trp1 suc2/suc2 lys2/LYS2 ADE2/ade2 NTC30/NTC30::HIS3; YD20, *Mata/Mata leu2/leu2
Plasmids—The construction of plasmids was as follows. pCC30: the 4-kb Scal DNA fragment was inserted into the SmaI site of plasmid vectors RSH416.

pCC31: the 1-kb DNA fragment containing the NTC30 open reading frame (ORF) and a 300-bp downstream sequence retrieved from the yeast genome by PCR using primers 30-3 and 30-4 was digested with BamHI and inserted into the BamHI site of plasmid vector pGEM-1.

pCC311: the 1-kb BamHI fragment from pCC31 was inserted into plasmid vector pEG202.

pCC312: the 1-kb BamHI fragment from pCC31 was inserted into plasmid vector pACT2.

The construction of plasmids was as follows. pCC30: the PCR product with primers 30-1 and 30-2 using plasmid pCC31 as a template was digested with NruI and then self-ligated. pCC302: the 1.8-kb fragment containing the His3 gene was end-repaired and inserted into the NruI site of pCC301.

pW210: the 1.2-kb DNA fragment containing the NTC20 ORF and 400-bp upstream and 450-bp downstream sequences retrieved from the yeast genome by PCR on oligonucleotides 20-1 and 20-2 was ligated with the EcoRI- and BamHI-digested pRS416.

pW210: the 870-bp DNA fragment containing the NTC20 ORF and 450-bp downstream sequence retrieved from the yeast genome by PCR on oligonucleotides 20-2 and 20-3 was digested with BamHI and inserted into the BamHI site of plasmid vector pGEM-1.

pW210: the 170-bp SacI-Xhol fragment with the NTC20 gene was replaced with the 2-kb LEU2 fragment.

pW211: the 870-bp BamHI DNA fragment from pW210 was inserted into the BamHI site of plasmid vector pEG202.

pW212: the 870-bp BamHI DNA fragment from pW210 was inserted into the BamHI site of plasmid vector pACT2.

Cloning of the SNT304 and SNT384 Genes—The SNT304 and SNT384 genes were isolated by complementation of the synthetic lethal phenotype of the snt304 and snt384 mutants with a YCp50-based Sau3A genomic library obtained from M. Rose and P. Novick as described (15). The ends of the isolated DNA fragments were sequenced and compared with the yeast genome data base to identify the regions of the fragments in the genome. DNA fragments containing speculated ORFs were then subcloned for complementation analysis.

Construction of Gene Replacement—The ntc30::HIS3 allele was created by replacing the entire ORF of NTC30 with a 1.8-kb DNA fragment of the His3 gene. The ORF was deleted by reverse PCR with primers 30-1 and 30-2 using plasmid pCC30 as a template. The resulting plasmid pCC301 was digested with NruI and ligated with a blunt-ended 1.8-kb HIS3 fragment. This action yielded plasmid pCC302, which was digested with XbaI and Xhol to isolate the ntc30::HIS3 fragment for transformation into a diploid strain YER6210.5. Correct integration was confirmed by Southern blot analysis. The ntc20::LEU2 was created by replacing the SacI-Xhol fragment of plasmid pW210 with a 2-kb DNA fragment of the LEU2 gene. The resulting plasmid pW210 was digested with EcoRI and BamHI to isolate the ntc20::LEU2 fragment for transformation into YER6210.5. Haploid strains harboring disrupted NTC30 or NTC20 genes were isolated by sporulation of the heterozygous diploid strains followed by dissection of tetrads.

Splicing Reactions and Immunoprecipitation—Splicing assays were performed according to Lin et al. (17) using uncapped actin pre-mRNA as the substrate. Immunoprecipitation was carried out as described by Tarn et al. (18).

Northern Blot Analysis—Total yeast RNA was isolated as described by Vijayaraghavan et al. (19). RNA was electrophoresed in 5% polyacrylamide-4% urea gels and then electroblotted onto GeneScreen membranes in 25 mM NaPO4 (pH 6.5) at 4 °C overnight. Northern hybridization was performed according to Vijayaraghavan et al. (19) using the CRY1 gene as a probe.

Two-hybrid Assays—The NTC30, NTC20, NTC85, PRP19, and SNT309 genes were fused to the LexA-DNA binding domain in plasmid pEG202 and the GAL4-activation domain in plasmid pACT2, and each pair of plasmids was transformed into yeast strain EGY48 together with the β-galactosidase reporter plasmid pSH18-34. Two-hybrid assays were carried out according to procedures described in the manual for the Matchmaker system (CLONTECH).

RESULTS

Identification of NTC30 and NTC20—To identify components of the Prp19p-associated complex, we performed a screen for mutants that showed synergistic effects to a temperature-sensitive allele of prp19 using the ade2/ade3 sectoring system, isolating 15 such mutants (15). Cloning of the gene complementing the snt309 mutant phenotype identified Snt309p as Ntc25p of the Prp19p-associated complex (15). We further cloned genes conferring mutations snt304 and snt384 by complementation of their nonsectoring phenotype. SNT304 and SNT384 were found to correspond to ORFs YJR050w and YBR188c, respectively. YJR050w was recently reported by two-hybrid screening to be Isy1p as a protein interacting with Syf1p (20). It is a protein of 235 amino acid residues with a calculated molecular weight of 28,024. YBR188c is a previously unidentified gene containing 140 amino acid residues of molecular weight 15,968. Neither protein sequence contains any discernible motif.

To see whether SNT304 and SNT384 encode components of the Prp19p-associated complex, we raised antibodies against recombinant His-tagged Snt304p and Snt384p for immunoblot analysis. The Prp19p-associated complex was isolated by affinity chromatography (12), fractionated by SDS-polyacrylamide gel electrophoresis, and then subjected to Western blot analysis using anti-Snt304p and anti-Snt384p antibodies. Fig. 1 shows
that the anti-Snt304p antibody reacted with Ntc30p (lane 3), and the anti-Snt384p antibody reacted with Ntc20p (lane 6) of the Prp19p-associated complex. Preincubation of individual antibody with recombinant Snt304p or Snt384p abolished such reactions (lanes 4 and 7). Pre-immune sera from both rabbits also gave no reaction (lanes 2 and 5). These results indicate that Snt304p is Ntc30p and Snt384p is Ntc20p of the Prp19p-associated complex. In fact, Ntc20p was also identified to be ORF YBR188c by independent sequencing of the components of the affinity-purified Prp19p-associated complex.

Ntc30p and Ntc20p Are Spliceosomal Components and Are Associated with the Spliceosome in the Same Manner as Prp19p—We previously showed that Prp19p is not tightly associated with spliceosomal snRNPs, but is associated with the spliceosome during or after dissociation of U4 from the spliceosome (13, 18). Two other components of the Prp19p-associated complex, Snt304p and Ntc85p/Cef1p, also associate with the spliceosome in the same manner, suggesting that the Prp19p-associated complex may bind to the spliceosome as an integral complex (14, 15). To see whether Ntc30p and Ntc20p also associate with the spliceosome during the splicing reaction, anti-Ntc30p and anti-Ntc20p antibodies were used for immunoprecipitation of the spliceosome. Splicing reactions were carried out under normal conditions, and the reaction mixtures were precipitated with the anti-Ntc30p or anti-Ntc20p antibody conjugated to protein A-Sepharose. As shown in Fig. 2A, precursor RNA, splicing intermediates, and the intervening sequence, but only a small amount of the mature message, were precipitated by the anti-Ntc30p antibody (lane 4), indicating precipitation of the spliceosome. In the absence of the antibody (lane 2) or with the pre-immune serum (lane 3), no RNA was precipitated. Preincubation of the antibody with the recombinant Ntc30p protein also resulted in no precipitation of RNA (lane 5). Fig. 2B shows the same result when immunoprecipitation was carried out with the anti-Ntc20p antibody. These results indicate that both Ntc30p and Ntc20p are also spliceosomal components.

To see whether Ntc30p and Ntc20p also bind to the spliceosome at the same time as Prp19p, we performed the ATP titration experiment as before (14, 15). Fig. 3A shows the scheme of spliceosome assembly. We previously demonstrated that dissociation of U4 from the spliceosome is very sensitive to ATP concentration (13). Under normal conditions (1–2 mM ATP), U4 is dissociated from the spliceosome rapidly after binding of the tri-snRNPs. As a consequence, only very small amount of the U4 containing splicing complex A2-1 is detected. Dissociation of U4 is blocked at lower ATP concentrations with increasing amounts of A2-1 accumulated on decreasing ATP concentrations. It was demonstrated before that Prp19p is not associated with the spliceosome at low concentrations of ATP and is not present in A2-1 (13). We took advantage of this feature to analyze the steps of spliceosome assembly with which Ntc30p and Ntc20p are associated. In this experiment, we constructed a strain in which Prp19p is tagged with the c-Myc epitope, and Prp4p is tagged with HA. Prp4p was shown to bind to the 5' portion of U4 (21) and to be dissociated from the spliceosome with U4. Therefore, the anti-HA antibody could be used to follow U4 and anti-Myc antibody to follow Prp19p.

Splicing reactions were carried out in extracts prepared from such a strain at different ATP concentrations, and the reaction mixtures were subjected to immunoprecipitation with anti-Myc, anti-HA, anti-Ntc30p, and anti-Ntc20p antibodies. As shown in Fig. 3B, the extract showed high splicing activity at 1 mM ATP (lane 1) and 0.5 mM ATP (lane 7), low activity at 0.1 mM ATP (lane 13), but gave no spliced products or intermediates at 0.05 mM ATP (lane 19). The anti-Myc antibody precipitated the spliceosome efficiently from the reaction mixtures at 1 and 0.5 mM ATP (lanes 3 and 9). At 0.1 mM ATP, less splicing intermediates and products but more pre-mRNA were precipitated (lane 15). The pre-mRNA precipitated presumably reflects complex A1. At 0.05 mM ATP, no significant amount of the RNA was precipitated (lane 21). This finding is consistent with our previous results of precipitation using the anti-Prp19p antibody or the anti-HA antibody when Prp19p was tagged with HA (13, 14). In contrast, the anti-HA antibody, binding to Prp4p-HA, precipitated only residual amounts of pre-mRNA at 1 mM ATP (lane 4), more at 0.5 mM ATP (lane 10), and much larger amounts at 0.1 and 0.05 mM ATP (lanes 16 and 22). In all cases, no splicing intermediates or products were precipitated, reflecting the fact that U4 is dissociated prior to catalytic steps of the splicing reaction. Precipitation with anti-Ntc30p and anti-Ntc20p antibodies gave a similar pattern as with the anti-Myc antibody. Spliceosome-associated RNAs were precipitated with higher efficiency at high ATP concentrations (lanes 5, 6, 11, 12, 17, and 18), but negligible amounts were precipitated at 0.05 mM ATP (lanes 23 and 24). This indicates that like Prp19p, both Ntc30p and Ntc20p are associated with the spliceosome immediately after or concurrently with dissociation of U4.

Null ntc30/ntc20 Mutants Were Defective in Growth and Splicing—Among the two previously identified Prp19p-associated components, even though SNT309 was not required for cell viability (15), NTC855 was essential for yeast growth and for pre-mRNA splicing (14). To see whether NTC30 and NTC20

FIG. 2. Immunoprecipitation of the spliceosome with anti-Ntc30p and anti-Ntc20p antibodies. A, the splicing reaction mixtures (20 µl) were precipitated with the anti-Ntc30p antiserum (lane 4), or pre-immune serum (Pre, lane 3) or without serum (lane 2). Antiserum was also preincubated with recombinant Ntc30p prior to precipitation (lane 5). Lane 1 is 2 µl of the reaction mixture. B, the same as A, except the anti-Ntc20p antiserum and Ntc20p were used. RXN, reaction; PAS, protein A-Sepharose.
are required for cellular growth, null alleles of $NTC30$ ($\Delta NTC30$) and $NTC20$ ($\Delta NTC20$) were constructed. In these constructs, the entire ORF of $NTC30$ was replaced with a DNA fragment of the $HIS3$ gene, and a 170-bp fragment within the ORF of $NTC20$ was replaced with a DNA fragment of the $LEU2$ gene. Tetrad dissection of diploid strains containing one copy of $NTC30$ and one copy of $\Delta NTC30$ or $\Delta NTC20$ revealed that neither $NTC30$ nor $NTC20$ was essential for yeast growth, although $\Delta NTC30$ grew slightly less well than $NTC20$ or the wild-type cells (data not shown). However, deletion of both $NTC30$ and $NTC20$ resulted in severe growth defect as shown in Fig. 4A. Dissection of a diploid strain containing one copy of the wild-type and one copy of $\Delta NTC30$ or $\Delta NTC20$ yielded two types of spores. One type, accounting for approximately 70% of dissected spores, grew normally. The other type gave slow growth phenotype and yielded minute colonies, which were all leucine and histidine prototrophic on replica plating. This result suggests that cells from which both $NTC30$ and $NTC20$ genes were deleted ($\Delta NTC30/\Delta NTC20$), although still viable, were impaired in growth.

Because both Ntc30p and Ntc20p are spliceosomal components, growth defect of $\Delta NTC30/\Delta NTC20$ cells may be a consequence of splicing deficiency. To understand whether pre-mRNA splicing is defective in $\Delta NTC30/\Delta NTC20$ cells, RNA was isolated for Northern blot analysis using the $CRY1$ gene as a probe. Fig. 4B shows that as in the prp2 mutant (lanes 9 and 10), accumulation of pre-mRNA was seen in $\Delta NTC30/\Delta NTC20$ cells (lanes 7 and 8) and, to a much lesser extent, in $NTC30$ cells at 25 and 37 °C (lanes 3 and 4). Pre-mRNA accumulation was not seen in the wild type (lanes 1 and 2) and barely detected in $\Delta NTC20$ cells (lanes 5 and 6). This indicates that Ntc30p and Ntc20p, although not essential for pre-mRNA splicing, may play auxiliary roles in the splicing reaction.

As Ntc30p and Ntc20p are components of the Prp19p-associated complex, a splicing deficiency in $\Delta NTC30/\Delta NTC20$ cells may reflect a deficiency in the function of the Prp19p-associated complex. To test that assumption, extracts were prepared from $\Delta NTC30/\Delta NTC20$ cells and assayed for splicing. As shown in Fig. 4C, extracts prepared from $\Delta NTC30/\Delta NTC20$ cells gave very low splicing activity (lane 2). However, the splicing activity was restored to almost the wild-type level (lane 1) upon addition of the purified Prp19p-associated complex (lane 3). This indicates that the splicing deficiency of the $\Delta NTC30/\Delta NTC20$ extract was caused by a malfunction of the Prp19p-associated complex and that Ntc30p and Ntc20p may play roles in sustaining the full activity of the Prp19p-associated complex.

Sequential Interactions of Components in the Formation of the Prp19p-associated Complex—Two previously identified components of the Prp19p-associated complex, Snt309p and Ntc85p, were shown to interact directly with Prp19p (14, 15). To reveal the interactions between Ntc30p, Ntc20p, and other components of the Prp19p-associated complex, we performed two-hybrid assays. Prp19p, Snt309p, Ntc85p, Ntc30p, and Ntc20p were fused to both the LexA-DNA binding domain and the GAL4 activation domain, and interactions between each pair of proteins were analyzed by assaying the $\beta$-galactosidase activity. As shown in Fig. 5, neither Ntc30p nor Ntc20p interacted with Prp19p or Snt309p. Neither did they interact with each other. However, they both interacted with Ntc85p. The interaction of Ntc85p with Ntc20p was seen only when Ntc20p was fused to the DNA binding domain and Ntc85p was fused to the activation domain but was not detected when fusion was in

![Fig. 3 A](image)

**Fig. 3.** A, a scheme of the spliceosome assembly showing sequential binding of snRNAs and Prp19p. B, immunoprecipitation of the spliceosome using extracts in which Prp19p was tagged with the c-Myc epitope and Prp4p tagged with the HA epitope. The splicing reaction was carried out at 1 mM (lanes 1–6), 0.5 mM (lanes 7–12), 0.1 mM (lanes 13–18), and 0.05 mM (lanes 19–24) of ATP, and the reaction mixtures were subjected to immunoprecipitation with anti-Myc (lanes 2–6), anti-HA (lanes 4, 10, 16, and 22), anti-Ntc30p (lanes 5, 11, 17, and 23), and anti-Ntc20p (lanes 6, 12, 18, and 24) antibodies. RXN, reaction; PAS, protein A-Sepharose.
an opposite way. Ntc30p showed very strong self-interaction similar to Ntc85p and Prp19p (12, 14). No self-interaction of Ntc20p was detected.

We have previously demonstrated that Snt309p plays a role in modulating interaction of Prp19p with other associated components (16). In the absence of Snt309p, Prp19p becomes only loosely associated with other components. In addition, the association of Ntc85p with Ntc30p and Ntc20p was also weakened, but association between Ntc30p and Ntc20p was not affected (16). This indicates that Snt309p, although not interacting directly with Ntc85p, affects interactions between Ntc85p and Ntc30p and between Ntc85p and Ntc20p. A possible explanation for such a distant effect is that formation of the Prp19p-associated complex may involve sequential interactions of the Prp19p-associated components in an order of binding of Snt309p to Prp19p, followed by the binding of Ntc85p, and then Ntc30p and Ntc20p. In this case, depletion of Ntc30p or Ntc20p or both should have no effect on the association of Prp19p with Snt309p and Ntc85p.

To test this theory, we prepared splicing extracts from DNTC30, DNTC20, and DNTC30/DNTC20 strains. These extracts were subjected to immunoprecipitation with the anti-Ntc85p antibody followed by Western blotting to reveal components associated with Ntc85p. As shown in Fig. 6, the anti-Ntc85p antibody precipitated both Prp19p and Snt309p in all of these extracts regardless of the absence of Ntc30p and/or Ntc20p, indicating that association of Ntc85p with Prp19p and Snt309p was not affected by Ntc30p or Ntc20p in their combination. This result is in contrast to that of depleting Snt309p, which greatly destabilizes the association of Prp19p with all
other associated components. These results suggest that association of Prp19p with Snt309p and Ntc85p may be a prerequisite for the stable association of Ntc30p and Ntc20p with the Prp19p-associated complex.

**DISCUSSION**

By screening synthetic lethal mutants to the temperature-sensitive alleles of *PRP19*, we have identified three genes, *SNT309*, *NTC30*, and *NTC20*, that encode components of the Prp19p-associated complex. None of these genes is essential for cell viability. Yeast strains deleted of the *SNT309* gene gave a temperature-sensitive phenotype and accumulated pre-mRNA at the nonpermissive temperature (15). Deletion of the *NTC30* or *NTC20* gene alone did not show obvious growth phenotype. It is possible that Ntc30p or Ntc20p may play a less important role than Snt309p in the splicing reaction or in maintaining the integrity of the Prp19p-associated complex. Alternatively, *NTC30* and *NTC20* might be functionally redundant to other yeast genes. The genome of *S. cerevisiae* is thought to have undergone a duplication event some 100 million years ago, immediately followed by random deletion of individually duplicated genes from one or the other chromosome (22). Fifty-five duplicated regions were identified in the whole yeast genome as a consequence of such duplication and deletion events (22). Among them, the COR region of chromosome 10, on which *NTC30* resides, is ancestrally related to the ARC region of chromosome 5 (23). *NTC30*, originally named *UTR3* as an unidentified transcribed region, is related to *UTR5* in the ARC region (23). Although the protein sequences of these two genes do not share high homology, the possibility that they are functionally redundant cannot be ruled out. By tagging Utr5p with the HA epitope, we tested the possibility of whether Utr5p is a spliceosomal component or a component of the Prp19p-associated complex. Immunoprecipitation analysis revealed no association of Utr5p with Prp19p or with the spliceosome during the splicing reaction (data not shown). Thus, Utr5p is unlikely to be a functional homolog of Ntc30p.

On the other hand, *NTC30* and *NTC20* could be functionally redundant to each other based on the following observations. First, despite the fact that deletion of either the *NTC30* or *NTC20* gene showed a negligible growth phenotype, deletion of both genes severely impaired cellular growth. Furthermore, Ntc30p and Ntc20p showed similar patterns of interaction with other identified components of the Prp19p-associated complex. Both proteins interact with Ntc85p (Fig. 5) and Ntc40p, but neither interacts with Prp19p or Snt309p. Detailed biochemical analyses of these proteins are required for a direct proof.

We showed previously that Snt309p interacts strongly with Prp19p, and through this interaction, it modulates interactions of Prp19p with other associated components to form a stable complex. In the absence of Snt309p, the association of Prp19p or Ntc85p with other components is impaired, and the complex is dissociated into at least three parts, one containing Prp19p, one containing Ntc85p, and one containing Ntc30p and Ntc20p (16). Neither Ntc30p nor Ntc20p interacts directly with Prp19p or Snt309p, but both interact with Ntc85p. It is interesting that although Snt309p does not interact with Ntc85p, Ntc30p, or

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2 C.-H. Chen, W.-Y. Tsai, and S.-C. Cheng, unpublished results.
Ntc20p, it affects the interactions between these components and between Ntc85p and Prp19p. In contrast, neither Ntc30p nor Ntc20p affects association of Ntc85p with Prp19p and Snt309p. This finding suggests that formation of the Prp19p-associated complex may involve sequential interactions of the components in the following order: Snt309p binds to Prp19p first, followed by binding of Ntc85p, then Ntc30p and Ntc20p. The fact that Ntc30p and Ntc20p do not interact with each other but remain associated even after dissociation from Ntc85p (16) suggests that they may interact with another factor(s) to form a subcomplex regardless of the presence of Ntc85p. A scheme of interactions between the known components of the Prp19p-associated complex is shown in Fig. 7.

Like other components of the Prp19p-associated complex, both Ntc30p and Ntc20p are also associated with the spliceosome during the splicing reaction (14, 15). Furthermore, they also associate with the spliceosome in the same manner as Prp19p and other associated components. These results strongly suggest that the Prp19p-associated complex is added to the spliceosome as an integral complex and that the associated components may function in a coordinate fashion.

Ntc30p has recently been reported as Isy1p from the two-hybrid screening of Syf1p-interacting proteins (20). A sequence search identified Isy1p homologs in Schizosaccharomyces pombe, Caenorhabditis elegans, Drosophila melanogaster, and human (20), suggesting that the protein is conserved between the lower and higher eukaryotes. A similar search identified a protein with increased affinity with certain proteins. Fabrizio, P., Laggerbauer, B., Lauber, J., Lane, W. S., and Lührmann, R. (1999) EMBO J. 18, 2926–2937

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