5-2009

Rescuing Minute-like Phenotypes Due to RNAi-mediated Knockdown of Nopp140 by Deletion of p53 in Drosophila melanogaster

Renford Cindass Jr

Rescuing *Minute*-like Phenotypes Due to RNAi-mediated Knockdown of Nopp140 by Deletion of *p53* in *Drosophila melanogaster*

by

Renford Cindass, Jr.

Undergraduate honors thesis under the direction of

Dr. Patrick DiMario, PhD

Department of Biological Sciences

Submitted to the LSU Honors College in partial fulfillment of
the Upper Division Honors Program.

May 2009

Louisiana State University
& Agricultural and Mechanical College
Baton Rouge, Louisiana

Acknowledgements

I would like to thank the LSU Honors College for giving me the opportunity to conduct this thesis, and Dr. Patrick DiMario for guidance and allowing me to work in his laboratory. I would also like to thank Dr. David Donze and Dr. Jeffrey Tiger for agreeing to be on my thesis defense panel, Raphyel Rosby for the occasional advice, and Angie Serrano for much patience and support.

Table of Contents

List of Tables

Lists of Figures

Abstract

Nucleolar phosphoprotein 140 (Nopp140) and Treacle are two similar nucleolar proteins that play a role in the maturation of pre-rRNA. A deficiency in Treacle has been attributed to the occurrence of the human Treacher Collins Syndrome (TSC), which is an autosomal disorder causing abnormal craniofacial development. *Drosophila melanogaster* lacks Treacle, but it does express Nopp140, and it has been shown that a deficiency in Nopp140 causes developmental malformations similar to those observed in TCS. This research tests the hypothesis that the p53-dependent apoptosis pathway contributes to the abnormal phenotypes. Nopp140 was depleted by using the GAL4/UAS system to selectively express an RNAi construct in wings that targets mRNA encoding Nopp140. When incubated at 27° C, RNAi-expressing flies displayed obviously shriveled, vestigial-like wings. Similar RNAi-expressing flies were constructed that also had a deletion of the *p53* gene. The wings of these flies without p53 were curled only on the edges suggesting that the phenotype produced by the lack of Nopp140 was partially rescued by the loss of p53. The result supports the hypothesis that cellular stress ultimately caused by ribosome deficiency activates the p53-dependent apoptosis pathway, thus inducing cellular death.

Introduction

In all organisms, ribosomes have the essential role of protein synthesis within each cell. Translation of the genetic code in the form of mRNA occurs at ribosomes, which bind together individual amino acids to create proteins that can be used for cell structure, hormones, and ribosomal subunits among many other things. In eukaryotes, ribosome biogenesis is a highly complex process that occurs in the nucleolus. At least one hundred different small nucleolar ribonucleoproteins (snoRNPs) and 150 proteins are needed to build the ribosomal subunits, which are composed of four kinds of RNA (18S, 5.8S, 28S, and 5S) and eighty ribosomal proteins (Olsen 2004).

Ribosome biogenesis occurs in the nucleolus, which is divided into three regions: the fibrillar center (FC), the dense fibrillar components (DFCs), and the granular region (GR). Transcription of pre-ribosomal RNA (pre-rRNA) from rDNA occurs in the nucleolus, arguably on the border between the FC and the DFCs by RNA polymerase I (Olsen 2004). Proteins and snoRNAs associate with the nascent pre-rRNA to modify it by site-specific methylation and pseudouridylation, while at the same time cleaving it to form the mature 18S, 5.8S, and 28S rRNAs. These early processing steps occur within a 90S particle. As ribosomal proteins are added, the complex moves to the GR to continue assembly. The 5S rRNA is transcribed in the nucleus and transported into the nucleolus to be added to the new complex. The 90S complex is later split into two subunits (pre-60S and pre-40S). The maturation of the pre-60S subunit ends in the nucleus before it is transported into the cytoplasm, while the pre-40S subunit continues to be modified even after export into the cytoplasm (Fromont-Racine, Senger et al. 2003).

The complexity of ribosome biogenesis increases the chance for error. One such error causes the Treacher Collins Syndrome (TCS), which is an autosomal dominant disorder typically characterized by abnormal craniofacial development that occurs with an incidence of 1 in 50,000 live births (Gorlin, Cohen et al. 1990). There have been 51 mutations indentified in the human *TCOF1* gene located at 5q32-q33.1 causing TCS: 32 are deletions, 10 are insertions, and 7 are missense/nonsense mutations (Marszalek, Wojcicki et al. 2002). These changes in the genetic code lead to a loss-of-function mutation in *TCOF1* resulting in a deficiency of the nucleolar phosphoprotein Treacle, which has only been identified in vertebrates thus far (Cui and DiMario 2007). Normally, Treacle is highly expressed in neural crest cells that migrate to populate the first and second brachial arches during embryogenesis. Without Treacle, patients with TCS present with midface hypoplasia (underdevelopment), micrognathia (undersized jaw), microtia (underdeveloped ears), conductive hearing loss, and/or cleft palate with variable expressivity and penetrance (Marszalek, Wojcicki et al. 2002).

The precise cellular function of Treacle remains uncertain, but evidence suggests that Treacle serves two possible functions in ribosome biogenesis. It has been demonstrated that Treacle interacts with Upstream Binding Factor (UBF), which is a RNA polymerase I transcription factor (Valdez, Henning et al. 2004). It also interacts with Nop56, which is a component of a ribonucleoprotein complex that catalyzes 2'-O-methylation of 18S pre-rRNA (Gonzales, Henning et al. 2005). Haplo-insufficiency of *TCOF1* has been suggested to describe the pathogenesis of TCS, since less of the protein product is produced. If Treacle is not produced in sufficient amounts due to one nonfunctional gene, there will be less ribosomes available for protein synthesis, and the

proper proliferation and/or differentiation of specific neural crest cells will not occur (Mogass, York et al. 2004).

Treacle is structurally and functionally related to Nucleolar Phosphoprotein 140 (Nopp140). Although both proteins contain unique N and C termini, ten acidic serine clusters alternating with alanine-, lysine-, and proline-rich stretches populate the large central repeat domains of both proteins (Isaac, Marsh et al. 2000). Both proteins co-localize in the DFC component of the nucleolus, and due to the similar large central repeat domain, they both interact with casein kinase 2 (CK2) leading to a high degree of phosphorylation; however, Nopp140 localizes in nuclear Cajal bodies while Treacle does not. Aside from that difference, Nopp140 and Treacle are very similar functionally. Nopp140 associates with box C/D and box H/ACA snoRNPs that guide site-specific 2'-O-methylation and pseudouridylation of pre-rRNA, respectively (Yang, Isaac et al. 2000). Nopp140 regulates rRNA transcription within mammalian nucleoli by interacting with RNA polymerase I (Chen, Pai et al. 1999). Other functions of Nopp140 include potentially acting as an assembly factor for snoRNPs or as a chaperone to transport snoRNPs between Cajal bodies and the nucleoli (Isaac, Yang et al. 1998), and shuttling rapidly between the nucleus and the cytoplasm to facilitate the import of nucleolar ribosome assembly factors or the export of nucleolar products (Meier and Blobel 1990).

As shown by Cui and DiMario (2007), morphological defects resulting from a deficiency in Nopp140 in *Drosophila* are reminiscent of the human craniofacial deformities caused by TCS. BLAST searches have shown that the closest homologue to human Treacle in *Drosophila* is Nopp140, since BLAST searches are not able to identify a *TCOF1* gene homologue. The defects caused by the lack of Nopp140 result in *Minute*-

like phenotypes. These traits are likely due to insufficient ribosome functions in larval imaginal discs, pupal histoblasts, and adult germ cells (Cui and DiMario 2007). This leads to the classic *Minute* phenotypes, which include shortened and thin thoracic bristles, rough eyes, missing or deformed antennae, abnormal wings, defective abdominal cuticle segmentation, reduced viability and fertility, prolonged development, and recessive lethality (Schultz 1929).

In *Drosophila melanogaster*, two isoforms of Nopp140 exist. The first 583 residues of the two proteins are identical, and alternative splicing creates different carboxy termini. The Nopp140-True isoform contains 686 residues, and its unique carboxy terminus is 65% identical to that in human Nopp140 (Waggener and DiMario 2002). BLAST searches of the *Drosophila* genome further show that Nopp140-True is the closest homologue to human Treacle (Cui and DiMario 2007). The Nopp140-RGG isoform contains 720 residues, and contains a long RGG (arginine and glycine-rich) carboxy terminus. Similar RGG domains are found in many RNA-binding proteins.

Regulating gene expression is essential to conducting genetic research with *Drosophila*. Three methods have been established to control gene expression. The heat shock method drives gene expression from the heat shock promoter, and the gene is turned on by increasing the temperature to a level that activates the promoter. This method allows inducible expression; however, ectopic expression is ubiquitous, basal levels of expression are observed from heat shock promoters, and heat shock itself can produce unwanted phenocopies (Brand and Perrimon 1993). Another method is the use of tissue-specific promoters that allows transcription to be limited to certain cells. Selective expression is one of the advantages, but this method is restricted by the availability of

cloned and characterized promoters that can direct expression in the desired pattern. If the gene product is toxic, transgenic lines would not be able to be created (Brand and Perrimon 1993). The GAL4/UAS system developed by Brand and Perrimon (1993) is the third tool that has proven useful in *Drosophila* research. This method has several advantages over the previous two. It allows for the rapid creation of individual strains that have gene expression targeted to specific cells, the target gene is separated from its transcriptional activator, and this method is designed to generate lines that express a transcriptional activator rather than an individual target gene in various patterns (Brand and Perrimon 1993). There are two parts to the GAL4/UAS system: the responder and driver. The target gene is termed the responder, and it is controlled by the presence of Upstream Activating Sequences (UAS) that act as a promoter region. GAL4, the driver, is the transcription factor, and it expresses the gene in a particular pattern. The responder and the driver are kept separate in two distinct transgenic lines. Without GAL4, the responder lines remain transcriptionally silent, but once the two lines are bred together, their progeny will express the target gene in the particular tissue-specific GAL4 pattern.

GAL4 activity is controlled by the temperature at which *Drosophila* is incubated. Minimal expression activity is witnessed at 16° C, and at 29° C, a balance between maximum GAL4 activity and effects on fertility and viability due to growth at high temperatures is seen (Duffy 2002). Routine uses of the GAL4/UAS system include (1) identification of genes involved in the process of interest via enhancer- or gene-trapping, (2) analysis of cellular autonomy of a gene product through targeted mosaics, (3) cellular marking to aid in screens for mutations affecting the process of interest, (4) analysis of loss-of-function phenotypes through targeted expression of RNAi and dominant-negative

constructs, and (5) genomic approaches to the identification of genes whose misexpression affects the process of interest (Duffy 2002).

RNA interference (RNAi) mediated knockdown of mRNA has proved to be an effective tool to simulate the effects of protein deficiency (Meister and Tuschl 2004). The endonuclease Dicer cleaves double-stranded RNA molecules that are then incorporated into the RNA-induced silencing complex (RISC). RISC selectively binds and cleaves mRNA that microRNA (miRNA) or small interfering RNA (siRNA) complement to prevent translation of that mRNA. RNAi effectively silences the expression of particular genes.

Cui and DiMario (2007) showed that RNAi-mediated knockdown of Nopp140 causes *Minute*-like mutations in *Drosophila melanogaster*. Three types of RNAi targeting different regions of the mRNA for Nopp140 were created. RNAi that targeted the unique 3' ends of the two isoforms were extremely weak to non-existent. The most effective RNAi targeted the common 5' end of mRNA for both isoforms of Nopp140 causing the *Minute*-like mutations. The lack of functional ribosomes could explain the formation of this phenotype, and it has been suggested that this disruption of ribosome biogenesis induces the p53-dependent nucleolar stress pathway (Prieto and McStay 2007).

p53 is a transcription factor that plays key roles in the destruction of stressed or abnormal cells preventing cancerous growth. Normally, p53 is highly ubiquitinated preventing high concentrations of the transcription factor from accumulating, but when a cell is stressed or DNA is damaged, p53 is stabilized by phosphorylation which blocks the ubiquitination. In mice, once p53 stabilizes, it binds to promoter regions on DNA allowing for the transcription of apoptotic genes and cell-cycle arrest genes. As proposed

by Jones *et al*, 2008, p53 induces cell-cycle arrest due to a deficient amount of mature ribosome. It is proposed that in mice p53 represses RNA polymerase I activity by preventing the interaction between UBF and promoter selectivity factor (SL1), and thereby directly interfering with transcriptional initiation at the promoter of rRNA (Jones, Lynn et al. 2008). However, in *Drosophila*, although p53 is required for apoptosis to occur, it is not necessary for cell-cycle arrest (Ollmann, Young et al. 2000). In *Drosophila*, p53 is stabilized by MNK/Chk2 phosphorylation (Brodsky, Weinert et al. 2004). The similarity between Treacle and Nopp140, and the related p53-dependent apoptosis pathways in humans and *Drosophila* suggest that ribosome deficiencies cause cell stress leading to p53-dependent apoptosis in cells critical for the normal development of the organism. The following research tests the hypothesis that p53-dependent apoptosis contributes to the abnormal phenotypes observed by the loss of Nopp140 in *Drosophila melanogaster*.

Methods and Materials

This project builds upon the experiment conducted by Cui and DiMario, 2007. The *w⁻/w⁻; TCom.C4/TCom.C4* strain was used from that experiment. This strain is homozygous for the RNAi construct knocking down Nopp140 mRNA levels by 60%, and when crossed to *Da-GAL4* (stock #5460) to induce ubiquitous expression, pupal lethality is witnessed. The first chromosome balancer, *FM4*, and the third chromosome balancer, *TM3,* were used to follow genes when performing crosses. Unless stated otherwise, all fly stocks were obtained from the Bloomington *Drosophila* Stock Center at Indiana University. Strains included in this study include the X chromosome GAL4 driver line, *A9-GAL4* (stock #8761) and a third chromosome stock $p53^{5A\text{-}1\text{-}4}$ (stock #6815). The *A9-GAL4* driver causes expression of the RNAi insert in the wings. The $p53^{5A\text{-}1\text{-}4}$ strain of *Drosophila* has a deletion of the *p53* gene (*Δp53*) and does not exhibit p53-mediated apoptosis. Additional details on the various strains, chromosomes, and marker genes are available at FlyBase (Grumbling, Strelets et al. 2006).

The various strains of *Drosophila* were crossed to completely remove both copies of the *p53* gene establishing the experimental driver line *A9-GAL4/FM4; + /+; Δp53/Δp53* (see **Figure 2**). FM4 was maintained in the stock due to a problem with female fertility when the genotype was homozygous for both *A9-GAL4* and *Δp53*. The experimental responder line *w⁻/w⁻; TCom.C4/TCom.C4; Δp53/Δp53* was also created (see **Figure 1**). To create a control strain to compare the progeny of the experimental driver and experimental responder *Drosophila* strains, *w⁻/w⁻; TCom.C4/TCom.C4* flies were crossed with *A9-GAL4/A9-GAL4; +/+* flies. These flies were incubated at 22° C and 27° C.

The creation of the experimental driver and responder stocks was conducted by following certain phenotypes produced by the balancers, the driver, and the RNAi construct. *TM3* produced serrated wings and causes the thoracic bristles to become short like stubble. *FM4* produced the bar eye phenotype. When either heterozygous or homozygous for the *A9-GAL4* driver, dark red colored eyes were produced. The RNAi construct (*TCom.C4*) produced light shades of orange eyes when heterozygous, but when homozygous for *TCom.C4*, dark orange eyes were seen.

Stocks of the experimental driver and responder lines were stabilized and maintained prior to crossing the two lines together. When the experimental driver males and experimental responder females were crossed together, female progeny were heterozygous for the GAL4 driver on the X-chromosome and the RNAi construct on the second chromosome, while homozygous for *Δp53* on the third chromosome (see **Figure 2**). Male progeny did not inherit the driver; therefore, males did not express the RNAi construct. Therefore, males served as a baseline for the examination of females. All crosses were conducted at 22°C (room temperature) until the experimental driver and responder lines were crossed. All crosses (experimental driver crossed to experimental responder and control driver crossed to control responder) were conducted simultaneously at 22° C and 27° C to take advantage of the temperature sensitivity of the GAL4/UAS system.

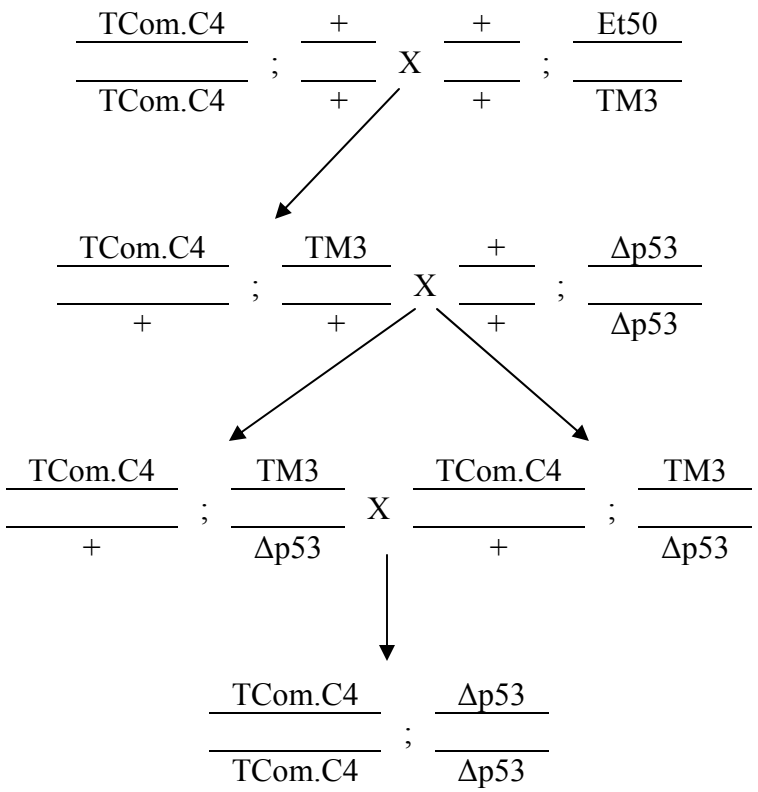

Figure 1. Creation of the experimental responder *Drosophila* strain homozygous for *TCom.C4* and *Δp53*. Starting with the homozygous *TCom.C4* stock, *p53* was removed by using the balancer *TM3* to mark the 3rd chromosome and then crossing with the strain homozygous for *Δp53*. Light orange eye color was followed to ensure progeny had *TCom.C4*. The final progeny homozygous for *TCom.C4* and *Δp53* had dark orange eyes without serrated wings or stubbled bristles. The stock was continuously crossed to maintain the existence of the stock. Only the 2nd and 3rd chromosomes are shown. The X-chromosomes in all cases were w^-.

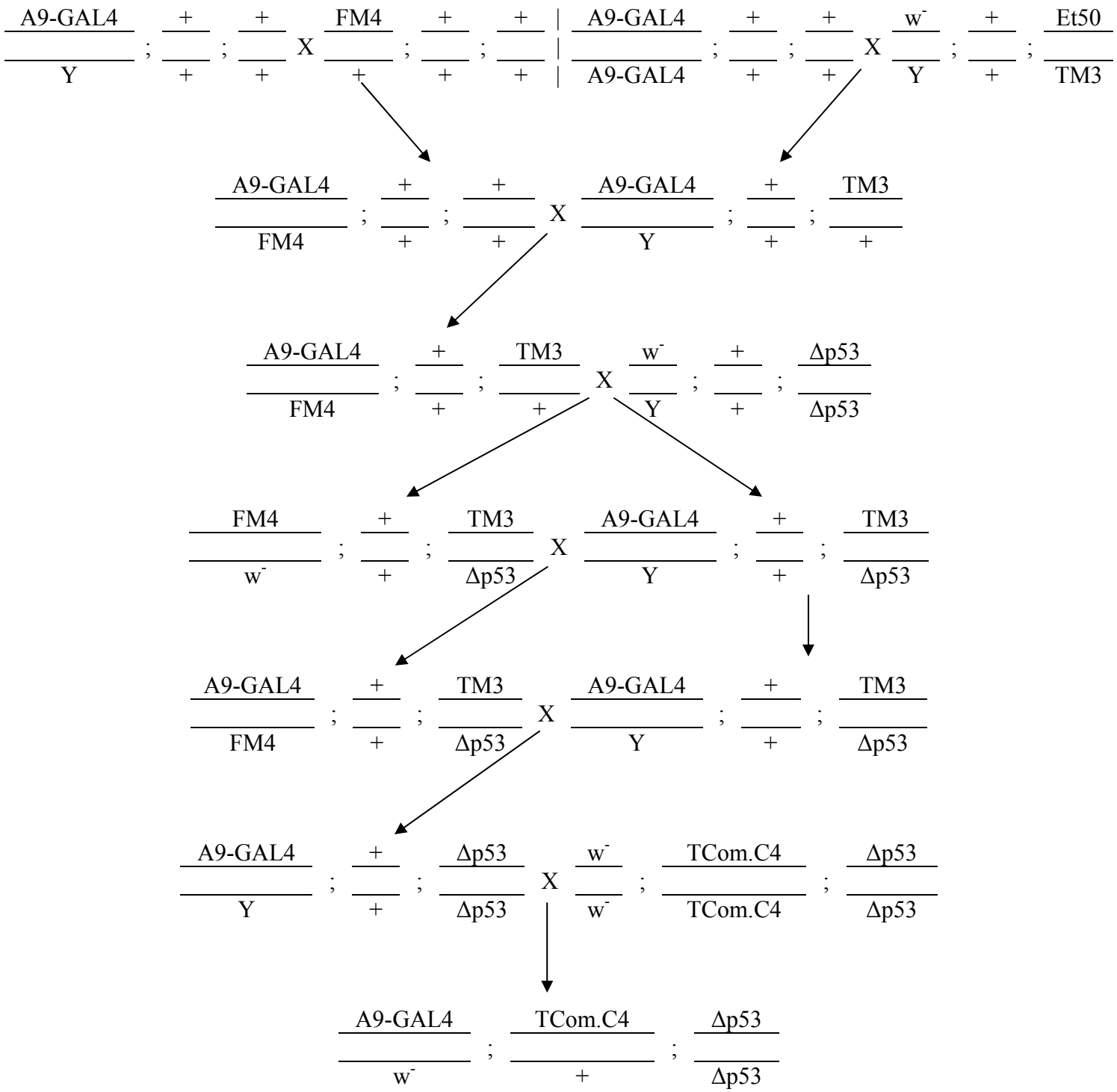

Figure 2. Creation of the experimental driver *Drosophila* strain homozygous for *A9-GAL4* and *Δp53*, and creation of experimental progeny. The *FM4* and *TM3* balancers were added to place a marker on the 1st (X) and 3rd chromosomes, respectively. Dark red eyes were followed to ensure that progeny had *A9-GAL4*. Male *A9-GAL4/Y; +/+; Δp53/Δp53* were crossed with the experimental responder strain *w-/w-; TCom.C4/TCom.C4; Δp53/Δp53*. Chromosomes 1, 2, and 3 are shown.

Results

The *A9-GAL4* driver directed RNAi expression only in the wing discs of *Drosophila* larvae. Normally, both wings are straight and rounded at the ends. When RNAi is expressed to deplete Nopp140 in wing discs with p53 present (depending on the temperature of incubation), the wings would deform, bending upward on the edges creating a slight bowl shape (incubated at 22°C) (see **Figure 3**) to completely shriveled taking on a vestigial-like appearance (incubated at 27°C) (see **Figure 6**).

All crosses were designed to establish an internal control for the comparison of gene expression. All control males had the genotype *w^-/ Y; TCom.C4/+* on the first and second chromosome. Since males did not inherit the *A9-GAL4* driver, the RNAi construct could not be expressed. Control male flies, regardless of whether or not p53 was present, had normal wings, which were straight with rounded ends (see **Figure 3 and Figure 4**).

Control females inherited the genotype *A9-GAL4/ w^-; TCom.C4/ +*. When incubated at 22° C, wings curved upward on the sides and on the ends creating a bowl shape (see **Figure 3 and Figure 5**). Female flies had a penetrance of 74±23%. The ratio between female to male ratio was .91±0.1. When incubated at 27° C, wings completely shriveled with 100% penetrance (see **Figure 6 and Figure 7**). The female to male ratio was found to be 0.28±0.12.

For the experimental *Drosophila* strains, as with the control strains, only female flies inherited the *A9-GAL4* driver, and therefore only females expressed the RNAi construct. Both male and female flies were homozygous for the *Δp53* gene. The male progeny of the cross between the experimental driver and experimental responder produced flies with the genotype *w^-/ Y; TCom.C4/ +; Δp53/Δp53*. These male flies were

indistinguishable from male control flies with the genotype *w⁻/ Y; TCom.C4/+*. Males had straight wings with round ends regardless of the temperature that they were incubated (see **Figure 6, Figure 8, and Figure 10**).

Female progeny from the experimental cross had a genotype *A9-GAL4/w⁻; TCom.C4/ +; Δp53/Δp53*. After incubation at 22° C, wings curved upward with a penetrance of 47±6% (see **Figure 4 and Figure 5**). The female to male ratio was 1.81±0.46. When incubated at 27° C, wings were not completely shriveled but highly curved on the sides and completely extended (see **Figure 8**). There was a 100% penetrance of females with this phenotype (see **Figure 9**), and the female to male ratio was 1.16±0.43.

	22° C		27° C	
	Penetrance	♀:♂ ratio	Penetrance	♀:♂ ratio
♂(p53)	0%	.91±.1	0%	.28±.12
♀(p53)	74±23%		100%	
♂(Δp53)	0%	1.81±.46	0%	1.16±0.43
♀(Δp53)	47±6%		100%	

Table 1. Incubation temperature, the penetrance of the abnormal wing phenotype, and the female to male ratio.

a.

b.

Figure 3. Incubated at 22° C, comparison of (a) a male with genotype *w⁻/Y; TCom.C4/ +; +/ +* and (b) a female with genotype *A9-GAL4/w⁻; TCom.C4/ +; +/ +*. Both have the *p53* gene. The male shows a wing with the typical wild type phenotype, which is a straight wing with a rounded end. The female shows how RNAi expressed in the wings cause a curling on the edges.

Figure 4. Incubated at 22° C, comparison of (a) a male with genotype *w⁻/Y; TCom.C4/ +; Δp53/Δp53* and (b) a female with genotype *A9-GAL4/w⁻; TCom.C4/ +; Δp53/Δp53*. Neither have the *p53* gene. The male with the *p53* gene deleted still shows a wing with the typical wild type phenotype, which is a straight wing with a rounded end. The female shows that even though *p53* is deleted, the wing is still slightly curled.

a. **b.** **c.** **d.**

Figure 5. Comparison of the wings of (a) a male with the *p53* gene (b) a female with the *p53* gene (c) a male without the *p53* gene and (d) a female without the p53 gene. Stocks were incubated at 22° C. Male wings expressed a normal phenotype. Both female wings show slight curling on the edges of the wings.

a.

b.

Figure 6. Comparison of (a) a male with genotype *w⁻/Y; TCom.C4/ +*; *+/ +* and (b) a female with genotype *A9-GAL4/w⁻; TCom.C4/ +; +/+*. Both have the *p53* gene, and incubation occurred at 27° C. The male shows a wing with the typical wild type phenotype, which is a straight wing with a rounded end. The female shows how RNAi expressed in the wings shrivels the wings almost completely. This is a vestigial-like phenotype.

Figure 7. Females with the genotype *A9-GAL4/w⁻; TCom.C4/ +; +/+*. All are seen with shriveled, vestigial-like wings showing 100% penetrance.

a.

b.

Figure 8. Comparison of (a) a male with genotype *w⁻/Y; TCom.C4/ +; Δp53/Δp53* and (b) a female with genotype *A9-GAL4/w⁻; TCom.C4/+; Δp53/Δp53*. Neither have the *p53* gene, and incubation occurred at 27° C. The male with the *p53* gene deleted still shows a wing with the typical wild type phenotype, which is a straight wing with a rounded end. The female shows how when the *p53* gene is deleted, the wings extend and are no longer completely shriveled.

Figure 9. Females with the genotype *A9-GAL4/w⁻; TCom.C4/ +; Δp53/Δp53*. All wings are extended with curling on the edges similar to stocks incubated at 22° C. There was a 100% penetrance for partial rescue.

a. **b.** **c.** **d.**

Figure 10. Comparison of the wings of (a) a male with the *p53* gene (b) a female with the p53 gene (c) a male without the p53 gene and (d) a female without the p53 gene. Stocks were incubated at 27° C. Male wings expressed a normal phenotype. The female wing with *p53* expresses a shriveled vestigial-like phenotype, while the female wing with without *p53* is only curled on the edges, which is a less severe phenotype.

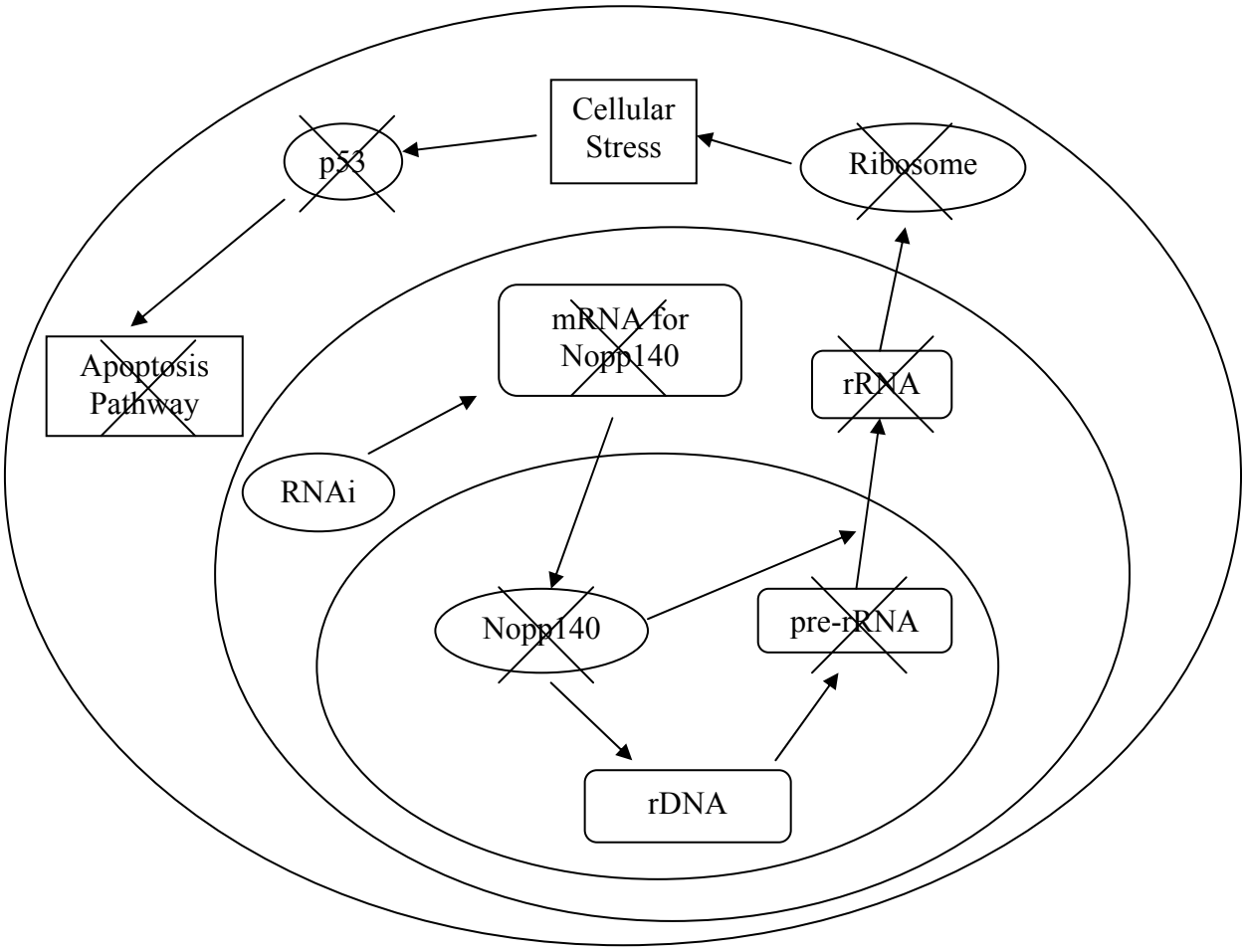

Figure 11. A visual depiction of the cellular processes involved in this experiment. RNAi knocks down the mRNA for Nopp140. This in turn causes Nopp140 levels to be reduced; thus, interfering with rDNA transcription and pre-rRNA modification. Mature rRNA is not created and cannot be incorporated to form a functional ribosome. This causes cellular stress, which normally would activate the apoptosis pathway; however, when p53 is deleted, the p53-dependent apoptosis pathway will not be activated.

Discussion

This experiment has demonstrated that the p53-dependent apoptosis pathway is involved in the morphological deformities caused by a depletion of Nopp140 in *Drosophila melanogaster*. Due to the temperature dependence of the GAL4/UAS system, expression varied between 22° C and 27° C. Less of the RNAi construct for Nopp140 was expressed at 22° C than at 27° C. This explains why the phenotypes of stocks incubated at 22° C were not as severe as the stocks incubated at 27° C (Duffy 2002).

The lack of Nopp140 by RNAi expression presumably resulted in a deficiency of functional ribosomes causing cellular stress. This stress activated the p53-dependent apoptosis pathway in the larval wing discs suggesting that there are fewer cells present, and this lead to the morphological malformations. This explains why the wings had a slight curl at 22°C and were completely shriveled at 27° C. However, when strains were homozygous for the deletion of *p53* (*Δp53*), a decrease in penetrance of the slight curl occurred (22° C), and wings tended to be elongated curving upward only at the edges (27° C). Since the *p53* gene was not present, p53 could not be transcribed to activate the apoptosis pathway. Although complete rescue of the phenotype was not achieved, deletion of the *p53* gene did prevent the wings from completely shriveling at 27° C demonstrating that the cellular stress produced by a lack of Nopp140 does cause programmed cellular death.

Jones *et al.* (2008) have demonstrated how deletion of *p53* can rescue the effects of a deficiency of Treacle in mice. When the production of Treacle is decreased, severe facial deformities resulted in mice with the *p53* gene; however, when the function of p53 was completely lost, the facial deformities were completely rescued as opposed to the

partial rescue of this experiment. They explained that in addition to the activation of the p53 apoptosis pathway, cyclin G1-mediated cell-cycle arrest occurs. In *Drosophila,* p53 does not influence cell-cycle arrest. If the lack of Nopp140 also influences the cell-cycle arrest pathway in *Drosophila*, then inhibition of this pathway along with the p53 apoptosis pathway may completely rescue the deformity. The complete rescue can also be due to the fact that the knockdown of Treacle was only 30%. As stated previously, the mRNA levels for Nopp140 was knocked down by 60% (Cui and DiMario 2007). The increased knockdown levels could be another reason for achieving only a partial rescue.

Nopp140 is the closest analogue to Treacle in *Drosophila*; therefore, the results of this experiment have good indication that deletion of *p53* can rescue the phenotype produced by Treacher Collins Syndrome. However, *p53* serves more functions in humans than in *Drosophila*, and although the deletion of *p53* may seem like an attractive route to cure TCS, p53 has a key role in preventing the development of cancer in humans. Currently TCS can be corrected by invasive surgery, and if patients were presented with this option, it would seem that surgery would be the better option over the chance of developing cancer. An alternate cure rather than complete p53 inhibition would be preferred.

Cancer seems to be the reverse of TCS. Where TCS can be explained by a decrease in ribosome biogenesis, cancer is an increase. It has been suggested by Jones *et al*. (2008) that the control of Treacle may serve as a potential modifier of tumor progression. Although Nopp140 and Treacle are similar, it has not been directly shown in humans that a deficiency in Nopp140 causes a decrease in cellular proliferation as does a deficiency in Treacle. Nopp140 does regulate rRNA transcription within mammalian

nucleoli by interacting with RNA polymerase I. This suggests that a deficiency in Nopp140 can cause cellular stress. If it is shown that a lack of Nopp140 can induce cell death in humans, its regulation in human cancer cells may serve to be an attractive field of study to control tumor growth.

Drosophila has proven to be a good model for genetic research and is proving itself as a useful model organism for research towards TCS. Although the biochemistry that occurs within *Drosophila* is different from humans, it can still provide effective avenues of insight into this disease.

References

Brand, A. and N. Perrimon (1993). "Targeted Gene Expression as a Means of Altering Cell Fates and Generating Dominant Phenotypes." Development **118**(2): 401-415.

Brodsky, M. H., B. T. Weinert, et al. (2004). "*Drosophila melanogaster* MNK/Chk2 and p53 Regulate Multiple DNA Repair and Apoptotic Pathways following DNA Damage." Mol. Cell. Biol. **24**(3): 1219-1231.

Chen, H., C. Pai, et al. (1999). "Human Nopp140, Which Interacts with RNA Polymerase I: Implications for rRNA Gene Transcription and Nucleolar Structural Organization." Mol. Cell. Biol. **19**(12): 8536-8546.

Cui, Z. and P. J. DiMario (2007). "RNAi Knockdown of Nopp140 Induces *Minute*-like Phenotypes in *Drosophila*." Molecular Biology of the Cell **18**: 2179-2191.

Duffy, J. B. (2002). "GAL4 System in *Drosophila*: A Fly Geneticist's Swiss Army Knife." genesis **34**(1-2): 1-15.

Fromont-Racine, M., B. Senger, et al. (2003). "Ribosome Assembly in Eukaryotes." Gene **313**: 17-42.

Gonzales, B., D. Henning, et al. (2005). "The Treacher Collins Syndrome (TCOF1) Gene Product is Involved in pre-rRNA Methylation." Hum. Mol. Genet. **14**(14): 2035-2043.

Gorlin, R. J., M. M. Cohen, et al. (1990). Syndromes of the Head and Neck. Oxford, Oxford University Press.

Grumbling, G., V. Strelets, et al. (2006). "FlyBase: Anatomical Data, Images and Queries." Nucl. Acids Res. **34**(suppl_1): D484-488.

Isaac, C., K. L. Marsh, et al. (2000). "Characterization of the Nucleolar Gene Product, Treacle, in Treacher Collins Syndrome." Mol. Biol. Cell **11**(9): 3061-3071.

Isaac, C., Y. Yang, et al. (1998). "Nopp140 Functions as a Molecular Link Between the Nucleolus and the Coiled Bodies." J. Cell Biol. **142**(2): 319-329.

Jones, N. C., M. L. Lynn, et al. (2008). "Prevention of the Neurocristopathy Treacher Collins Syndrome through Inhibition of p53 Function." Nat Med **14**(2): 125-133.

Marszalek, B., P. Wojcicki, et al. (2002). "Clinical Features, Treatment and Genetic Background of Treacher Collins Syndrome." J. Appl. Genet. **43**(2): 223-233.

Meier, U. and G. Blobel (1990). "A Nuclear Localization Signal Binding Protein in the Nucleolus." J. Cell Biol. **111**(6): 2235-2245.

Meister, G. and T. Tuschl (2004). "Mechanisms of Gene Silencing by Double-Stranded RNA." Nature **431**(7006): 343-349.

Mogass, M., T. P. York, et al. (2004). "Genomewide Analysis of Gene Expression Associated with TCOF1 in Mouse Neuroblastoma." Biochemical and Biophysical Research Communications **325**(1): 124-132.

Ollmann, M., L. M. Young, et al. (2000). "*Drosophila p53* is a Structural and Functional Homolog of the Tumor Suppressor *p53*." Cell **101**(91-101).

Olsen, M. O. J. (2004). The Nucleolus. New York, Kluwer Academic/ Plenum Publishers.

Prieto, J.-L. and B. McStay (2007). "Recruitment of Factors Linking Transcription and Processing of pre-rRNA to NOR Chromatin is UBF-dependent and Occurs Independent of Transcription in Human Cells." Genes & Development **21**: 2041-2054.

Schultz, J. (1929). "The Minute Reaction in the Development of *Drosophila melanogaster*." Genetics **14**(4): 366-419.

Valdez, B. C., D. Henning, et al. (2004). "The Treacher Collins Syndrome (TCOF1) Gene Product is Involved in Ribosomal DNA Gene Transcription by Interacting with Upstream Binding Factor." Proceedings of the National Academy of Sciences of the United States of America **101**(29): 10709-10714.

Waggener, J. M. and P. J. DiMario (2002). "Two Splice Variants of Nopp140 in *Drosophila melanogaster*." Mol. Biol. Cell **13**(1): 362-381.

Yang, Y., C. Isaac, et al. (2000). "Conserved Composition of Mammalian Box H/ACA and Box C/D Small Nucleolar Ribonucleoprotein Particles and Their Interaction with the Common Factor Nopp140." Mol. Biol. Cell **11**(2): 567-577.