The Pennsylvania State University

The Graduate School

Department of Neural & Behavioral Sciences

INVESTIGATING STAT3 ISOFORM EXPRESSION AND GENE TARGETS IN THE RETINA

A Thesis in

Biomedical Sciences

by

Patrick Thomas Brown

Submitted in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science

May 2015

The thesis of Patrick Thomas Brown was reviewed and approved* by the following:

Colin J. Barnstable
Professor and Chair of Neural & Behavioral Sciences
Thesis Advisor

Ralph L. Keil
Chair of Biomedical Sciences Graduate Program

Gregory Yochum
Assistant Professor of Biochemistry and Molecular Biology

Samuel S. Zhang
Assistant Professor of Neural & Behavioral Sciences

*Signatures are on file in the Graduate School

ABSTRACT

Signal transducer and activator of transcription 3 (STAT3) regulates cell fate decisions in the developing retina through transcriptional control of its target genes. Properly timed inactivation of STAT3 is required for rhodopsin expression and rod photoreceptor development. Exogenous application of the STAT3 activator CNTF blocks rod development and increases the number of bipolar, Müller glia, and amacrine cells. A microarray with probes for 12, 282 genes was used to determine expression changes in embryonic retina cultured with or without CNTF. Only 2% of the genes exhibited a change in expression. The majority of those changed were rod photoreceptor genes with the rest being bipolar, amacrine, or Müller glial cells. This evidence supports the conclusion that the phenotypic changes caused by CNTF are through cell-type specific gene changes. In the adult retina, STAT3 is activated after injury to mount an immunologic response and induces cell proliferation in reaction to cell death. Expression of the two STAT3 isoforms, α and β, was examined during retinal development and adulthood to determine if the different roles are a result of differential isoform expression. RT-PCR revealed that the mRNA messages for both isoforms are present in the perinatal and mature retinal tissue, but *STAT3α* is expressed in much greater abundance compared to *STAT3β*. Western blot detection confirmed that only STAT3α was made into protein. Therefore, it is the α isoform of STAT3 that is responsible for the different functions of STAT3 in the retina.

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Dr. Colin Barnstable, for his leadership and insightful comments on various topics throughout this academic process. He has the unique ability to see the broad scope of projects and the precision to understand the details required to make that project come to fruition. Special thanks are also due to Drs. Samuel Zhang and Evgenya Popova who frequently let me call upon their scientific knowledge and technical experience. I must also acknowledge the rest of my thesis committee for their guidance during my Master's research. Lastly, the utmost gratitude is bestowed upon my friends and family. Their tireless love and support has given me strength to continue down the difficult path that is scientific research.

Chapter 1

Introduction

1.1 STAT proteins

1.1.1 STAT family

As their name suggests, the signal transducer and activator of transcription (STAT) family of proteins function to propagate extracellular signals through the cytoplasm and into the nucleus where they activate transcription of target genes. When a STAT-activating ligand binds its receptor, intracellular kinases phosphorylate STAT monomers. This causes STAT dimerization and subsequent translocation to the nucleus where STAT dimers bind to DNA and induce transcription of target genes.

There are seven members of the STAT family (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) and each responds to specific cytokines and growth factors (Akira, 1999). Research on the STAT pathway began in the 1990s when the first STATs, STAT1 and STAT2, were discovered. They were identified as part of a three-protein complex, called interferon-stimulated gene factor 3 (ISGF3). The third protein belongs to the interferon-regulatory factor family of transcription factors. ISGF3 was purified during a screen looking for transcription factors that bound promoters of genes up-regulated after interferon stimulation (Levy et al., 1988; Fu et al., 1990; Schindler et al., 1992). Although researchers interested in viruses and the cell's response to interferon originally identified the STAT family, STATs play roles in many tissues during development and in the adult.

Currently it is known that STAT1 and STAT2 form heterodimers that are activated after interferon-α, β, or γ stimulation and induce transcription of the genes interferon regulatory factor 1, monocyte chemotactic protein 1, and others, which mobilize the immune response (Ramana et al., 2002). However, STAT2 may also function in heterodimers with STAT6 or with IRF9 when stimulated by

interferon-β and TNFα. In this manner STAT2 can promote expression of the *Duox2* gene, whose protein product generates H_2O_2 as part of a defense mechanism against microbes (Fink and Grandvaux, 2013; Geiszt et al., 2003). In mature animals, STAT3 promotes expansion of $CD4^+CD25^+/Foxp3^+/Lag\text{-}3^+$ Tregs, is required for the differentiation of Th-17 $CD4^+$ T cells, and suppresses the immune response in melanoma cells (Kortylewski and Yu, 2008). While many of its functions are restricted to the immune system, it has become clear that it has a much broader role. Phosphorylation of STAT4 is stimulated by IL-12. After cytokine activation, STAT4 promotes transcription of genes involved in lymphocyte proliferation and Th1 helper cell development (Wurster et al., 2000). Although different genes encode STAT5a and STAT5b, they are both phosphorylated in response to an IL-2 signal. STAT5a induces splenocyte proliferation, whereas STAT5b induces both splenocyte and thymocyte mitosis. Additionally, both are required for natural killer cell differentiation (Lin and Leonard, 2000). The primary activators of STAT6 are IL-4 and IL-13, which lead to transcription of MHC Class II, CD23, and allows B lymphocytes to switch between IgG1 and IgE (Wurster et al., 2000).

The gene targets of STAT3 are vitally important for life of an organism, especially during early development. STAT3 is the only member that when knocked out creates nonviable embryos (Stephanou et al., 2009). Crossing STAT3 +/- mice yielded STAT3 -/- embryos that were not viable past embryonic day 6 (E6) (Takeda et al., 1997). To determine more precisely the role of STAT3 in early embryo development, STAT3 was knocked out in both sperm and oocytes prior to fertilization. STAT3 embryos at E3.5 are normal; however, morphological changes are seen at E4.5. Using lineage markers, cell loss was identified in the inner cell mass but not in the trophectoderm (Do et al., 2013). The importance of STAT3 is also marked by the presence of homologues in other vertebrates namely *Danio rerio* and *Xenopus*, although it is not the only STAT member with homologues (Nishinakamura et al., 1999; Oates et al., 1999).

1.1.2 STAT3 protein and pathway

STAT3 has two isoforms – STAT3α and STAT3β. STAT3α is 770 amino acids in length, while STAT3β contains only 722 amino acids. The truncation is due to a splice site acceptor within exon 23 that removes 50 bases from exon 23 and inserts a stop codon. Both isoforms contain several regions of interest, which can be visualized in Figure 1 (Lim and Cao, 2006).

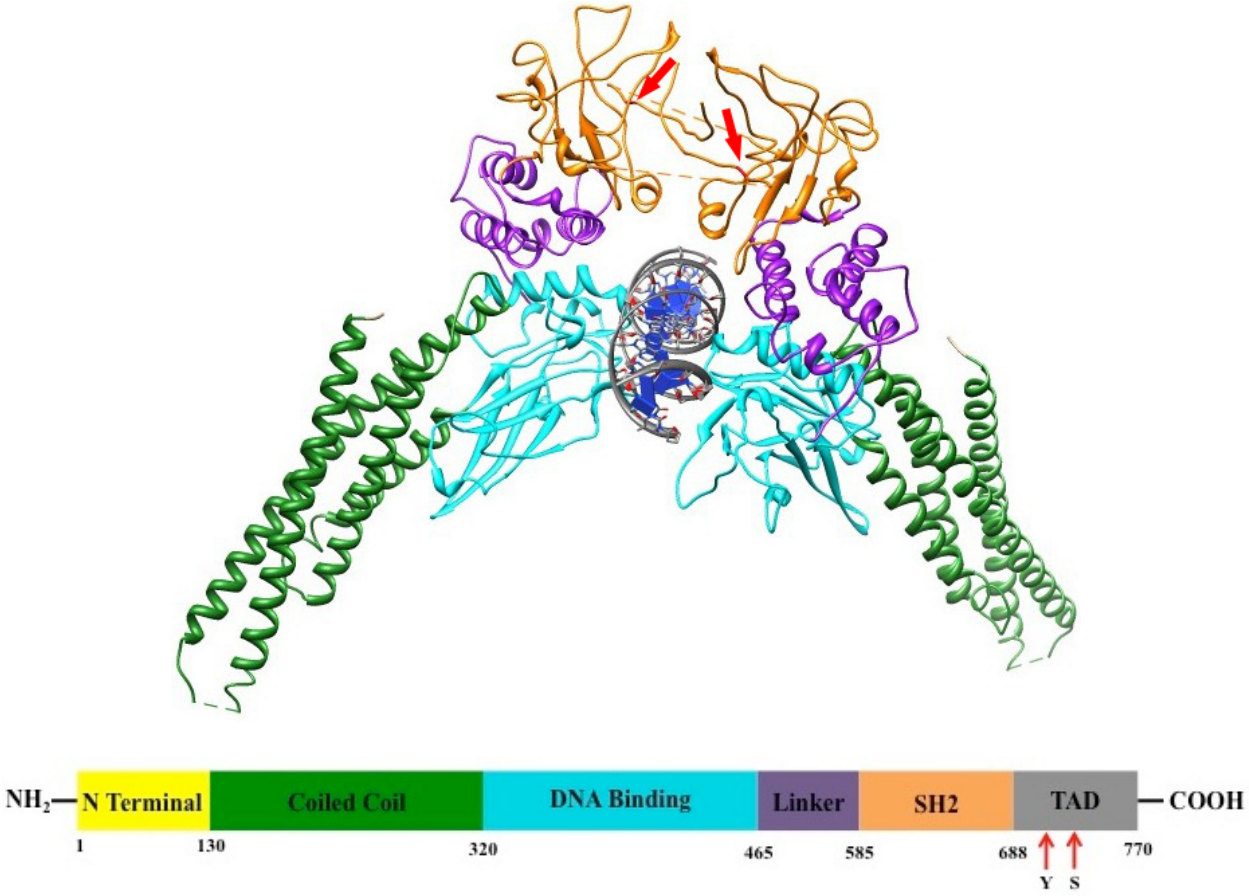

Figure 1: STAT3 protein domains. Top: STAT3β homodimers bound to DNA; PDB file 1BG1. Red: Tyrosine 705. Orange: SH2 domain. Purple: linker domain. Blue: DNA-binding domain. Green: Coil-coil domain. Not shown is the transcriptional activating domain that is present in the STAT3α isoform, which would be located beyond the SH2 domain. Red arrows point to tyrosine 705 residues. Bottom: A schematic drawing of the STAT3 protein domains. The two red arrows point to the sites of phosphorylation, Y705 and S727. TAD; transcriptional activating domain.

The coiled-coil domain is located at the N-terminal side of the protein and facilitates protein-protein interactions either in the cytoplasm or in the nucleus. The DNA-binding domain lies in the middle of the protein and upon STAT3 dimerization the two DNA-binding domains interact with DNA on opposing sides. A linker domain bridges the gap between the DNA-binding domain and a Src Homology 2 (SH2) domain. It is at the SH2 domain that two tyrosine-phosphorylated STAT3 monomers homodimerize or a phosphorylated STAT3 monomer and pSTAT1 monomer can heterodimerize. The bond is formed between the tyrosine-phosphorylated residue of one monomer and the SH2 domain of the other monomer. Following the SH2 domain is the transcriptional activating domain, within which lie two phosphorylation sites – tyrosine 705 and serine 727 (Horvath, 2000). Because STAT3β is truncated, it lacks most of the transcriptional activating domain including serine 727. However, it maintains tyrosine 705, some transcriptional activating properties, and expression of STAT3β can overcome the embryonic lethality of a STAT3 knockout (Yoo et al., 2002; Maritano et al., 2004).

It is widely accepted that Y705 phosphorylation is required for dimerization and transcriptional activity; however, unphosphorylated STAT3 in cancer can still induce transcription through interaction with NF-κB (Wen et al., 1995; Yang et al., 2005, 2007). Unphosphorylated STAT3 binds to unphosphorylated NF-κB and competes away IκB. Then, using STAT3's nuclear localization signal, the complex enter the nucleus where it enhances transcription of NF-κB target genes (Yang et al., 2007).

Compared to tyrosine phosphorylation, the role of S727 phosphorylation is more ambiguous. Some groups claim that phosphorylation at both residues is required for full transcriptional activation but not through regulation of DNA binding; other labs claim that S727 phosphorylation coordinates dephosphorylation of Y705; another group of researchers contend that S727 has its own function separate of Y705 (Chung et al., 1997b; Huang et al., 2013a; Miyakoshi et al., 2014; Wakahara et al., 2012; Wen and Darnell, 1997; Wen et al., 1995). It is likely that the role of S727 phosphorylation differs depending on cell type and cellular response. Besides phosphorylation, STAT3 can be modified with other post-translational marks including lysine 685 acetylation, lysine 140 methylation, lysine 180 methylation, and S-glutathionylation on cysteines 328 and 542 (Butturini et al., 2013, 2014; Kim et al., 2013; Yang et al., 2010; Yuan et al., 2005). Gene transcription of STAT3 targets is up-regulated following STAT3 lysine 685 acetylation and lysine 180 methylation, but down-regulated by cysteine S-glutathionylation and lysine 140 methylation.

STAT3 can be activated by a number of extracellular signals such as cytokines and growth factors (Appendix A). The most common of these extracellular activators is the IL-6 family of ligands. This family contains 11 members, but the relevant retina molecules are leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), and oncostatin M (OSM). They belong to the same family because they bind to the gp130 receptor, although each ligand requires a unique subunit that confers specificity (Figure 2). CNTF and LIF are similar in that their receptors have one gp130 and one LIFRβ subunit (Rhee et al., 2004). Additionally, the CNTF receptor contains a CNTFRα subunit that makes it selective for CNTF. The OSM receptor contains a gp130 subunit and an OSMR subunit; however, OSM can also bind the LIF receptor, which explains its effect on retinal cells (Xia et al., 2011). STAT3 can be activated by other non-IL-6 family ligands and their cognate receptors, such as TNFα and Wnt3a. A comprehensive list of STAT3 activators in many cell types can be found in Appendix A.

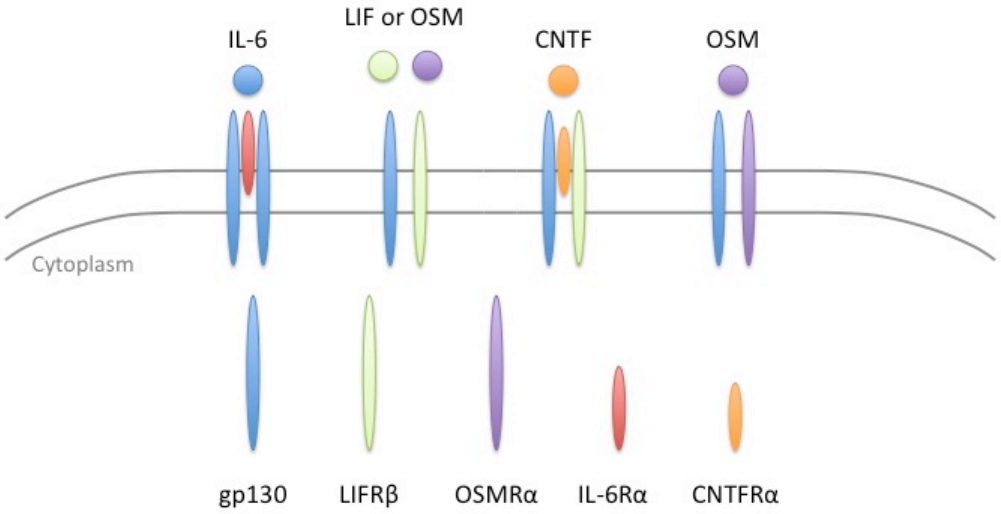

Figure 2: Common IL-6 family ligands and their corresponding receptors.

Once a ligand binds its receptor, an intracellular kinase is activated that tyrosine phosphorylates STAT3. It is Jak2, a member of the Janus kinase family, that is canonically associated with tyrosine phosphorylation of STAT3. Upon binding of CNTF or LIF to their receptor, Jak2 bound to the intracellular portion of the receptor becomes phosphorylated and the extracellular signal is propagated into the cytoplasm. Jak2 phosphorylates Y705 allowing STAT3 monomers to dimerize. Once a pSTAT3 dimer is formed, it transits into the nucleus via importin-α, -β, and Ran where it binds to DNA and activates transcription of its gene targets (Figure 3) (Darnell et al., 1994; Ihle, 1995; O'Shea, 1997; Schindler and Darnell, 1995; Cimica et al., 2011; Ma and Cao, 2006; Ushijima et al., 2005). STAT3 is not the only target of Jak2; it can also tyrosine phosphorylate other proteins including Grb2 (Chauhan et al., 1995; Giorgetti-Peraldi et al., 1995) and PI3 kinase regulatory subunit α (Fuhrer and Yang, 1996).

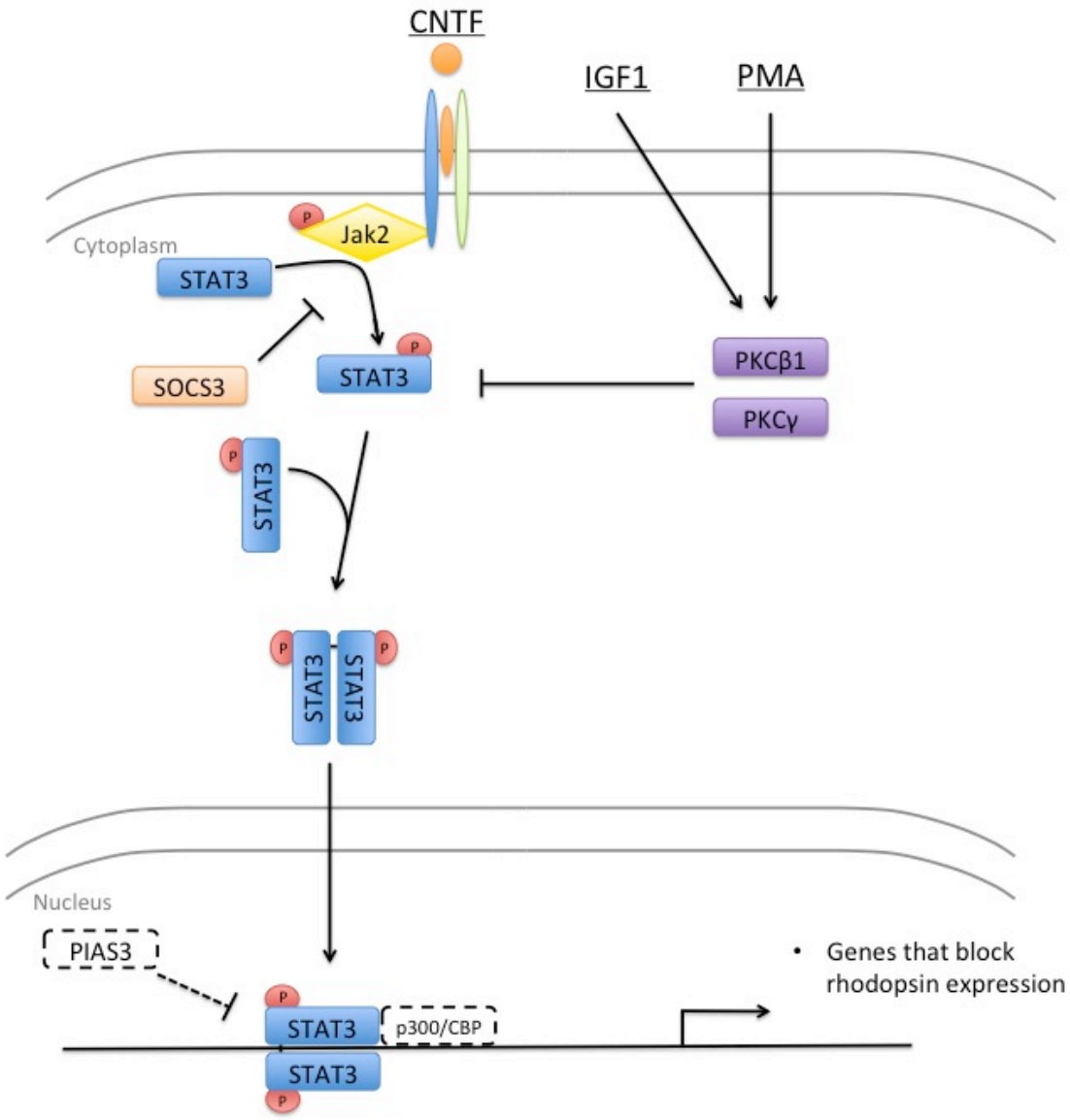

Figure 3: The canonical signaling pathway of STAT3. Dashed lines represent molecules/pathways that are hypothesized in the retina due to their interaction in other tissues and their known role in the retina.

In addition to cytokine stimulation, it has been observed that STAT3 can be activated by the downstream Notch effector Hes1. Hes1 is a basic helix-loop-helix transcription factor that restricts gene

transcription; however, several labs have shown that pSTAT3 levels can be modulated by Hes1. Overexpression of an active form of Notch or Hes1 protein increased pSTAT3 and transfection with Hes1-targeted siRNA diminished the ability of EGF to stimulate pSTAT3 in COS-1 cells. The direct Hes1-STAT3 and Hes1-Jak2 interactions were confirmed by co-immunoprecipitation (Kamakura et al., 2004). The current model is that Hes1 acts as a scaffold protein enabling Jak2 to phosphorylate STAT3 independent of a cytokine receptor. This interaction has recently been linked to many physiologic events including delaying differentiation of pluripotent cells, prevention of hepatic insulin resistance, and the IL-22 mediated antimicrobial response (Murano et al., 2014; Zhou et al., 2013).

Although phosphorylation of STAT3 is required for transcriptional activation, it is likely that STAT3 must interact with other proteins in the nucleus to coordinate gene transcription. P300/CBP is a transcriptional co-activator that promotes gene transcription by altering chromatin conformation. When bound to a transcription factor, p300/CBP transfers acetyl groups onto histone proteins and opens chromatin, allowing transcriptional machinery to bind. STAT3 has been shown to activate transcription of its target genes through recruitment of p300/CBP in multiple tissues (Ray et al., 2002, 2005; Yuan et al., 2005). Knockout studies showed that lack of p300/CBP results in malformed cone and rod photoreceptors and impaired vision in mice. Gene analysis identified many photoreceptor genes whose expression levels were decreased in p300/CBP knockout retina, one of which was cone-rod homeobox (Crx) (Hennig et al., 2013). Furthermore, studies revealed that Crx requires p300/CBP, and subsequent histone acetylation, in order to drive opsin expression (Peng and Chen, 2007; Yanagi et al., 2000). While it has not been shown that STAT3 recruits p300/CBP to its target genes in the retina, it is a plausible mechanism for retinal transcriptional activation.

After cytokine stimulation of STAT3 in the cytoplasm, pSTAT3 dimers translocate into the nucleus. Several labs showed that this process was dependent on importin-α, importin-β, and Ran (Cimica et al., 2011; Ma and Cao, 2006; Ushijima et al., 2005). It has been shown that even in the absence of tyrosine phosphorylation, STAT3 can transit between the nucleus and cytoplasm (Liu et al., 2005).

Within STAT3's coiled-coil domain lies a nuclear localization signal, as mutating either of two arginine residues in this region abrogates importin-α binding and STAT3 nuclear localization (Ma and Cao, 2006; Ma et al., 2003). After stimulation, STAT3 must exit the nucleus and return to the cytoplasm. Studies have shown that STAT3 contains three nuclear export signals (NES) that act in a Crm1-mediated mechanism. Treatment of cells with leptomycin B showed that two of the NESs are required to export basal levels of nuclear STAT3 to the cytoplasm, whereas the third NES is used to export stimulated nuclear STAT3 (Bhattacharya and Schindler, 2003).

Several intracellular proteins have been found to abrogate the STAT3 signal. Suppressors of cytokine signaling 3 (SOCS3) and protein inhibitor of activated STAT, 3 (PIAS3) are perhaps the most well known, but several others exist. A yeast two-hybrid assay revealed that SOCS3 binds to Jak2 and other kinases. When bound to Jak2, SOCS3 blocks the interaction between Jak2 and STAT3, thus inhibiting STAT3 phosphorylation. As a result, STAT3 cannot promote transcription of its target genes (Masuhara et al., 1997; Minamoto et al., 1997).

While SOCS3 functions in the cytoplasm, PIAS3 regulates pSTAT3 from inside the nucleus. It is only found interacting with STAT3 after cytokine stimulation of pSTAT3 and works to decrease the ability of pSTAT3 to activate transcription. Electrophoretic mobility shift assay (EMSA) indicated that PIAS3 blocked STAT3 from binding DNA. Besides homodimerization, STAT3 can heterodimerize with STAT1, yet EMSA revealed that PIAS3 did not block STAT1 binding, indicating selectivity for STAT3. Both SOCS3 and PIAS3 play important roles in the retina (Chung et al., 1997a).

Additionally in the nucleus, the protein Tip60 can act by itself as a histone acetyltransferase; however, it can complex with transcription factors to repress transcription. Tip60 forms a complex with HDAC7 and STAT3 in HEK293 cells and decreases transcription of STAT3 target genes (Xiao et al., 2003). It was first discovered in hepatocytes that cyclin D1 binds to the transactivating domain of STAT3 and prevents it from activating transcription. It does so by exporting pSTAT3 from the nucleus to the cytoplasm via an unknown mechanism (Bienvenu et al., 2001). In transfection studies carried out in COS-

1, it was shown that the MAP kinase protein ERK2 can negatively regulate STAT3. Activated pERK2 phosphorylates serines, including S727 in STAT3. It also decreases Y705 phosphorylation and inhibits DNA binding of STAT3 through direct association of ERK2 and STAT3 in the nucleus (Jain et al., 1998). Although to our knowledge there is no literature regarding these molecules and their interaction with STAT3 in the retina, they are present and may regulate the pathway like they do in other systems.

Just as STAT3 controls transcription of target genes, so too is the transcription of *STAT3* message controlled by transcription factors, one of which is STAT3 itself. Using luciferase assays it was determined that IL-6 treatment increased *STAT3* mRNA through a region of the STAT3 promoter that contained a STAT3 binding element. A STAT3 dominant negative protein confirmed that after IL-6 treatment, STAT3 protein binds the *STAT3* gene to promote transcription. Further analysis revealed that STAT3 and a cAMP-response element binding-protein were both required for the regulation of the *STAT3* gene by the STAT3 protein (Ichiba et al., 1998). Besides the STAT3 protein, β-catenin/TCF4 and estrogen receptor α also bind to the *STAT3* gene promoter to increase its transcription (Yan et al., 2008; Gao et al., 2006).

STAT3 is also regulated at the translation level, specifically through micro RNAs. Micro RNAs are small RNA oligos approximately 22 nucleotides long and, due to their sequence, bind to the 3'-untranslated region of a gene's mRNA. When bound they prevent translation of that gene from beginning. Researchers have identified miR-124 and miR-125b as negative post-transcriptional regulators of STAT3. Their expression is suppressed in ulcerative colitis and cancer respectively, which allows for increased STAT3 expression (Koukos et al., 2013; Liu et al., 2011). Micro RNAs also play a role in regulating STAT3 during development where miR-17 family members miR-17, miR-20a, and miR-106b diminish STAT3 levels to allow proper develop of lung epithelial cells (Carraro et al., 2009).

STAT3's most prominent function is as a transcription factor. Examination of mouse embryonic stem cells found that STAT3 induces expression of genes required for maintenance of an undifferentiated phenotype like Klf4 and Rgs16, genes that are active during embryogenesis, which perhaps sheds light

onto the reason STAT3 deletion is embryonic lethal (Bourillot et al., 2009; Ohnishi et al., 2000; Villasenor et al., 2010). STAT3 protein also induces expression of its own gene as well as a negative regulator of STAT3, SOCS3 (Bourillot et al., 2009). A review of STAT3 in hematological tumors describes how STAT3 drives gene expression of *cyclin D1*, *Bcl-2*, and other genes that promoted cell growth and survival (Al Zaid Siddiquee and Turkson, 2008). In addition to cell survival, STAT3 also targets genes responsible for muscle differentiation and wound healing (Dauer et al., 2005; Snyder et al., 2008). Immune system genes such as the B-cell differentiation factor *Rag1* or *toll-like receptor 6* are also targets (Snyder et al., 2008). Several publications have compiled lists of genes bound and regulated by STAT3, a few of which are referenced here (Alvarez et al., 2005; Carpenter and Lo, 2014; Kidder et al., 2008). What these genes have in common is that they have a STAT3 consensus sequence in their promoter. STAT3 was originally named the acute phase response factor and it's consensus binding site is the acute phase response element (CTGGGA) (Wegenka et al., 1993).

Although STAT3 was originally identified as an activator of transcription, it regulates cellular respiration in the mitochondria independent of its transcription activating properties. STAT3 was observed in mitochondrial fractions of heart and liver tissue, primarily in the mitochondrial matrix (Boengler et al., 2010). Further investigation revealed that STAT3 co-immunoprecipitated with complexes I and II of the electron transport chain. To test the function of complex I and II, oxidation rates were tested in the presence of glucose and succinate respectively. Pro-B cells lacking STAT3 had decreased oxidation rates compared to controls. These assays were duplicated in pro-B cells transfected with various STAT3 constructs to test the requirements of tyrosine and serine phosphorylation in cellular respiration and it was found that normal oxidation rates could be recapitulated with a S727 phosphomimetic, but not a Y705 phosphomimetic. Additionally, the DNA binding domain of STAT3 was found to be unimportant for proper complex I and II function. A STAT3 construct was made that cannot form dimers, yet showed normal oxidation function. This indicates that dimerization of STAT3 is not required for complex I and II function. These biochemical results were confirmed in mice lacking cardiac

STAT3 as heart mitochondria tested for complex I and II function were found to be deficient, similar to the pro-B cells lacking STAT3 (Wegrzyn et al., 2009). It is also through this mechanism that STAT3 promotes Ras-dependent tumorigenesis (Gough et al., 2009). Translocation of STAT3 into the mitochondria is hypothesized to involve Tom20 and cyclophilin D. Tom20 is part of a complex that shuttles cytosolic proteins into the mitochondria and cyclophilin D regulates mitochondrial pore opening (Ramage et al., 1993; Basso et al., 2005). When STAT3 was immunoprecipitated from cardiac tissue, both proteins bound to STAT3 (Boengler et al., 2010).

In the cytoplasm STAT3 also stabilizes microtubules. The protein stathmin breaks down microtubules through depolymerization, but STAT3 binds to stathmin to inhibit microtubule degradation. Deletion of STAT3 resulted in microtubule destabilization and subsequent siRNA blockade of stathmin expression countered the effect of STAT3 deletion and gave the microtubules a normal phenotype (Ng et al., 2006). Stathmin is present in adult retinal ganglion cells, and thus may explain STAT3s presence in healthy adult RGCs (Liedtke et al., 2002).

1.2 STAT3 in the retina

While the family of STAT proteins was first identified by their activation by interferon, it has been discovered that they play important roles outside of the immune system such as in the retina. The mature retina contains seven cell types (rod and cone photoreceptors, bipolar, amacrine, horizontal, Müller glia, and retinal ganglion cells) that are located in five distinct layers (outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, and ganglion cell layer) in addition to the retinal pigment epithelial cells. At birth, retinal cell differentiation is not yet complete and only two distinct layers are visible. Investigating STAT expression in the retina showed that all members are present. STATs 1, 2, 4, 5a, 5b, and 6 are expressed in the embryo and adult retina. They are found in the RPE as well as various neural retina cell types. However, the STAT3 expression pattern was the most interesting. Prior to birth, STAT3 was expressed and Y705 phosphorylated in the inner retinal layer, cells

that would later become Müller, bipolar, amacrine, and horizontal cells. However, by postnatal day 1 (PN1) expression switched to the outer retinal layer, which contains rod and cone photoreceptor precursors (Figure 4). STAT3 expression waned one week after birth, but was present again by eight weeks postnatal (Zhang et al., 2003).

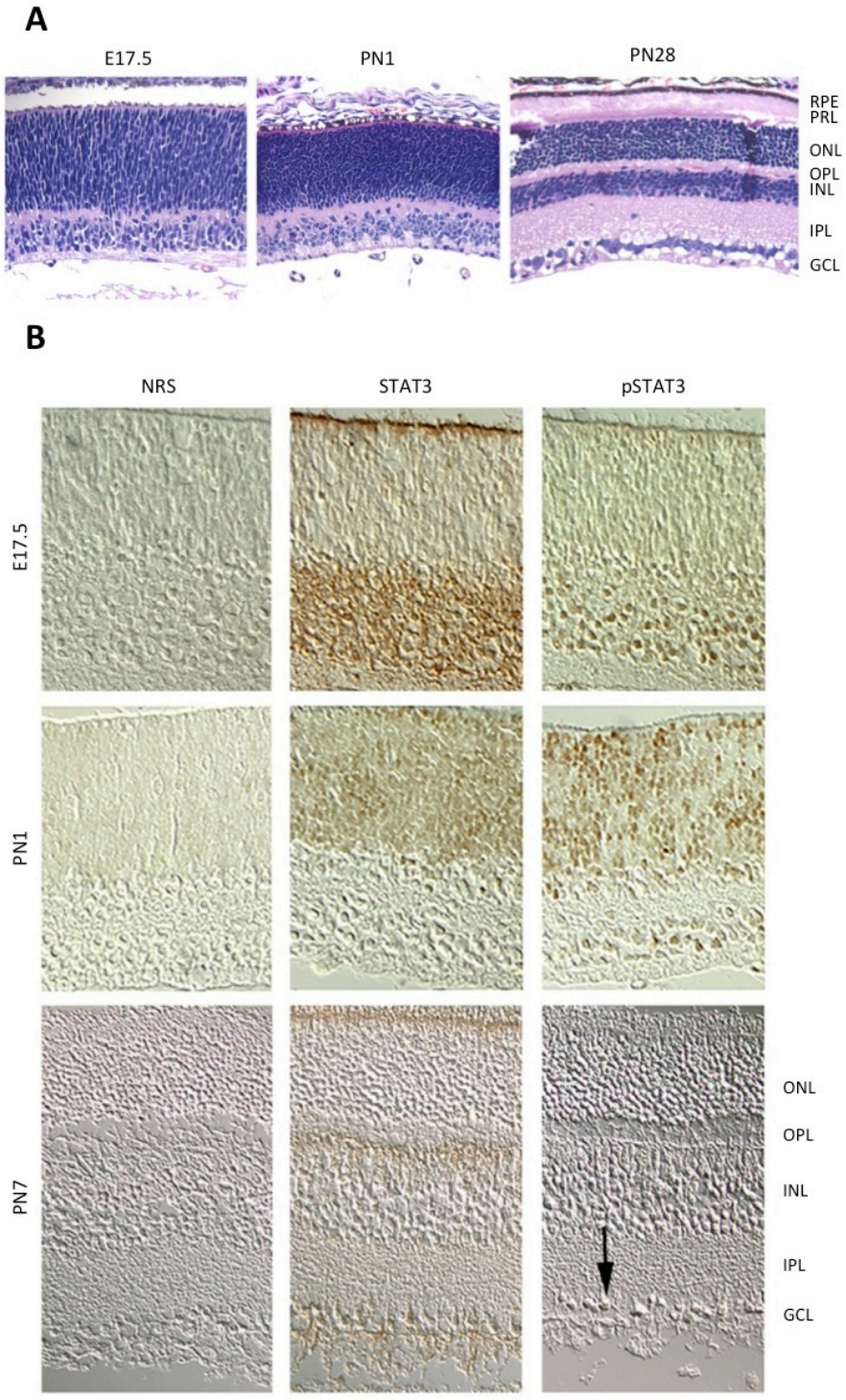
A
E17.5
PN1
PN28
RPE
PRL
ONL
OPL
INL
IPL
GCL
B
NRS
STAT3
pSTAT3
E17.5
PN1
PN7
ONL
OPL
INL
IPL
GCL

Figure 4: H&E stain of retinal layers and STAT3 expression in the retina. A) H&E stain of retinal layers from E17.5, PN1, and PN28 mice. Figure is unpublished work from Samuel S. Zhang. B) Immunohistochemistry of E17.5, PN1, and PN7 retina stained with normal rabbit serum (NRS), STAT3, or pSTAT3. Arrow indicates singular ganglion cell expressing pSTAT3 (Zhang et al., 2003). RPE; retinal pigment epithelium, PRL; photoreceptor layer, ONL; outer nuclear layer, OPL; outer plexiform layer, INL; inner nuclear layer, IPL; inner plexiform layer, GCL; ganglion cell layer.

1.2.1 Development

In the late 1990s retinal cell culture systems were used to determine the growth factors mediating rodent rod photoreceptor development. Some factors such as retinioc acid, basic fibroblast growth factor and S-laminin induced rod development, while others including transforming growth factor-α (TGF-α), CNTF, and LIF blocked this process (Anchan et al., 1991; Elliott et al., 2006; Ezzeddine et al., 1997; Hicks and Courtois, 1992; Kelley et al., 1994; Kirsch et al., 1996, 1998; Lillien and Cepko, 1992). To more accurately model *in vivo* conditions, the Raff lab used a mouse retina explant culture system to examine the effects of LIF and CNTF on rod differentiation. When retinas were removed at PN1 and grown in culture, the number of rhodopsin+ cells increased up to 8 days as visualized using immunostaining. After 7 days in media supplemented with either LIF or CNTF there was a decrease in the number of rhodopsin+ cells in a concentration dependent manner (Neophytou et al., 1997). A similar study cultured rat retina with IL-6, which is in the same ligand family as LIF and CNTF, for 10 days and found that the number of cells expressing opsin was unchanged compared to control (Ezzeddine et al., 1997). More extensive research on CNTF found that not only does it retard photoreceptor differentiation, but also increases the percentage of bipolar cells. At birth many retinal cells have not undergone their terminal division. Label experiments have identified that 80% of these progenitor cells will become rods; however, treating PN1 retina with CNTF for 10 days resulted in only 4% of cells becoming rods. In

response to this, the percentages of other cell types were increased: bipolar cells were increased 13-fold, amacrine were increased 2-fold, and Müller glia cells were increased 5-fold. The effect of CNTF was solely on cell identity as CNTF treatment did not alter proliferation or cell death (Ezzeddine et al., 1997). This suggests that CNTF has an effect on the process of cell fate determination. Other treatments can also affect rhodopsin expression in a mechanism similar to CNTF and LIF. For example, addition of fetal calf serum (FCS) or TGF-α to media was sufficient to decrease the number of rhodopsin-expressing cells through activation of Müller glia. Media from conditioned Müller cells (MCM) was just as effective at blocking rods as FCS, TGF-α, CNTF, or LIF. Inhibition of the CNTF and LIF receptors revealed that the mechanism of MCM's action was through secretion of a CNTF- or LIF-like substance (Neophytou et al., 1997). These experiments showed that the presence of either exogenous or endogenous cytokines could block rod differentiation in a retinal explant culture model.

Knockout mice and inhibitors were used to confirm that the CNTFRα and LIFRβ regulate rod differentiation. Retina from mice lacking either CNTFRα or LIFRβ were removed at PN1 and cultured for 10-11 days. Compared to homozygous wild-type retina, the number of opsin+ cells was greater, while the number of bipolar, amacrine, and Müller glia cells were unchanged. Additionally, when CNTF was added to retinal cultures of CNTFRα knockout retina there was no change in the number of opsin+ cells compared to untreated retina lacking CNTFRα (Ezzeddine et al., 1997). The importance of LIFRβ in rod differentiation was confirmed with a modified version of human LIF, hLIF05, that antagonizes the receptor. Mouse retinas were cultured starting at PN1 with CNTF, MCM, or FCS and treated with hLIF05 every 3 days for a duration of 7 days. While stimulation with cytokines alone decreased the number of rhodopsin+ cells, addition of the LIFRβ antagonist returned the number of rhodopsin+ cells back to control levels. High concentrations of LIF were able to compete away hLIF05 and reduce the number of rhodopsin+ cells, verifying the role of LIFRβ in rhodopsin expression (Neophytou et al., 1997). There was a time difference between CNTFRα and LIFRβ expression. CNTFRα was highly expressed at PN1, but steadily decreased until PN12 where it stayed through adulthood. LIFRβ expression, on the other

hand, was moderate at PN1 and increased until PN6 where it stayed into adulthood (Hertle et al., 2008). These results show that the specific CNTF and LIF receptors are necessary for ligands to decrease rod differentiation in the developing retina.

There was interest in determining what cellular signals in the retina were activated by CNTF or LIF. Instead of using CNTF, a modified, more potent form of CNTF called Axokine was used. The change in efficacy was due to a C-terminal truncation of 15 amino acids and two amino acid substitutions. Axokine was injected intravitreally into rat retina and retina were harvested between 5 minutes and 7 days post-injection. Phosphorylated STAT3 was first apparent at 15 minutes post-injection and steadily increased until 2 days. Total STAT3 protein increased from 16 hours to 7 days post-injection. In contrast, pSTAT1 was only evident after 16 hours and only lasted until 2 days after the Axokine injection. Similar to STAT3, STAT1 total protein increased 15 hours to 4 days post-injection. The MAP kinase pathway was also investigated and it was found that Axokine only increased pMAPK from 1 – 4 hours after injection. These pieces of evidence suggest that the CNTF/LIF pathway selectively stimulated the STAT3 signaling cascade over a long period of time in retinal tissue (Peterson et al., 2000).

Several labs used cytokines to show that STAT3 regulates rod photoreceptor development. Early postmitotic photoreceptor precursors express the protein Crx, but lack the progenitor marker PCNA and the late postmitotic photoreceptor marker rhodopsin. In immunofluorescence experiments, PN1, PN3, and PN5 retina treated with CNTF for 15 minutes were stained for proliferating cell nuclear antigen (PCNA), Crx, pSTAT3, and rhodopsin. In each experiment the PCNA+ and Crx+ cells were mutually exclusive, while pSTAT3 colocalized with those cells expressing Crx. Cells expressing rhodopsin did not express pSTAT3. Newly postmitotic photoreceptor precursors expressing Crx were sensitive to CNTF stimulation of pSTAT3, but this sensitivity was lost in further differentiated rhodopsin+ cells. Thus, STAT3 is sensitive to CNTF activation as early as PN1 and as late as PN5, but only in cells that have yet to fully differentiate into rod photoreceptors (Rhee et al., 2004). Work in two labs went on to show that rod photoreceptor development required inactivation of pSTAT3 (Ozawa et al., 2004; Zhang et al., 2004).

Immunofluorescence imaging of untreated retina showed that at PN1 there are high levels of pSTAT3, but at PN3 these levels begin to decrease and rhodopsin begins to be expressed. This pattern continues until P7 when pSTAT3 was expressed at very low levels and rhodopsin was highly expressed (Zhang et al., 2004). Furthermore, PN1 mouse retina in explant culture treated with CNTF show decreased rhodopsin expression and increased pSTAT3 expression, yet rhodopsin expression returns two days after CNTF withdrawal from media. This coincided with a decrease in pSTAT3. A Cre-loxP system was used under a retina-specific promoter to knockout STAT3 function in the retina. When these knockout retinas were treated with CNTF at PN1 for 5 days there was no observable pSTAT3 and rhodopsin levels were high – much like wild type, untreated retina (Ozawa et al., 2004). These results were reproduced when STAT3 function was removed using electroporation or adenoviral transduction of a dominant-negative form of STAT3 (Ozawa et al., 2004; Zhang et al., 2004). This suggests that CNTF modulates rod photoreceptor differentiation in a STAT3 dependent manner.

The STAT3 inhibitor SOCS3 also plays a role in rod photoreceptor development. Protein levels of SOCS3 increased in the mouse retina greatly between E18 and PN1 and its expression during the first two weeks of development is located in the outer nuclear layer, which develops into the photoreceptor layer. Those cells that express rhodopsin also express SOCS3, suggesting that it negatively regulates STAT3 in the early postnatal retina. Indeed, there was rhodopsin expression in PN1 retinal explants electroporated with *SOCS3* cDNA and treated with CNTF for 7 days, whereas explants not electroporated were sensitive to the CNTF and did not express rhodopsin. Additionally, removing SOCS3 at E12 results in a decrease in the number of rhodopsin-positive cells as early as PN3 (Ozawa et al., 2007). Thus, inhibition of STAT3 by SOCS3 is required for development of rod photoreceptors.

Compared to rod photoreceptors, there is little published describing the activity of STAT3 in cones. However, CNTF has been shown to induce green opsin in cultured embryonic chick retina in a time dependent manner, although whether STAT3 is required for this activity is not known (Fuhrmann et al., 1995; Xie and Adler, 2000).

While CNTF was used to block rod differentiation, it had the opposite effect on Müller cell differentiation. Explant culture experiments with PN1 retina treated with CNTF for 6 days increased the number of cells expressing the Müller cell markers glial fibrillary acidic protein (GFAP) and cellular retinal binding protein (CRALBP). LIF, but not EGF, had the same effect as CNTF on GFAP expression in the 6-day explant culture model and the Jak2 inhibitor AG490 was sufficient to reduce the number of GFAP- and CRALBP- expressing cells back to control levels. After 3 days in culture with CNTF, immunofluorescence was used to visualize the location of STAT3 phosphorylation. Control retinas expressed pSTAT3 in the ganglion cell layer and those retinas treated with CNTF had stronger labeling in ganglion cells in addition to the inner retina where nascent Müller cells are located. BrdU incorporation was used to determine the effect CNTF had on biasing proliferating cells to a Müller fate. PN1 retina explants were pulsed with BrdU and treated with CNTF for 6 days. By day 6 in the control group only 1.7% of BrdU+ cells stained with GFAP and 3% stained with CRALBP. Treatment with CNTF increased the percentage of BrdU+/GFAP+ cells to 13% and BrdU+/CRALBP+ to 20%. The increased labeling of both markers indicates an increase in the number of Müller cells. These data suggest that activation of the STAT3 pathway pushes proliferating progenitors in the inner retina towards a Müller cell phenotype (Goureau et al., 2004).

Canonically Jak2 and SOCS3 regulate STAT3 activation, but other molecules can modulate pSTAT3 as well. It was determined that IGF1 and PMA can affect retinal development when they were shown to decrease pSTAT3 in early postnatal retina and induce rod differentiation compared to control (Pinzon-Guzman et al., 2010, 2011). PKC isoforms β1 and γ were shown to be mediators of this effect. Retinas treated with either IGF1 or PMA and incubated with a PKC-β1-selective inhibitor contained fewer rhodopsin+ cells than those treated with IGF1 or PMA alone. PKC-β1 inhibition did not decrease the number of cells to control levels, whereas using a pan PKC inhibitor did recapitulate the control results. Opsin expression was examined over the first two weeks postnatal in mice lacking PKC-γ. Without PKC-γ, opsin was expressed less than control until PN7, but is similar to control by PN13.

Culturing PKC-γ knockout retina with PKC-β1 inhibitor for 8 days completely abrogated opsin expression. PKC isoforms affect rhodopsin expression through a mechanism that alters pSTAT3 levels, since tyrosine phosphorylated STAT3 is increased in retina explants from PKC-γ knockout retina and further increased when a PKC-β1 inhibitor is added to the culture media (Pinzon-Guzman et al., 2011). These results suggest that the STAT3 and PKC pathways interact to modulate rod photoreceptor development.

Further investigation of the pathways downstream of IGF1 and PMA was conducted using the pan tyrosine phosphatase inhibitor sodium orthovanadate. Pretreatment of explant cultured retina with sodium orthovanadate prior to incubation with IGF1 or PMA prevented the decrease of pSTAT3 normally caused by these molecules. Using another, more selective tyrosine phosphatase inhibitor identified the Shp1/2 phosphatases as being downstream of IGF1 and PMA. Inhibiting Shp1/2 blocked the ability of IGF1 or PMA to decrease pSTAT3 and completely inhibited rhodopsin expression (Pinzon-Guzman et al., 2014). These experiments give insight into a mechanism, other than the canonical pathway, in which STAT3 can be inactivated and rhodopsin expression enhanced.

1.2.2 Response to injury

Besides the role for STAT3 in retinal development, it also responds to injury in the adult retina. Cone photoreceptor function loss 1 (cpf1) mice have a loss-of-function mutation in Pde6c, a protein required for light transduction in cones. This causes degeneration of cones starting at three weeks postnatal. These retinas showed an increase in CNTF, LIF, STAT3, and SOCS3 gene expression by postnatal week four. It is hypothesized that CNTF, LIF, and STAT3 are increased in order to block apoptosis of the degrading cones. SOCS3 expression is likely increased because it is a gene target of STAT3. Although these genes are mostly expressed in Müller cells in adults, a population of cones also expressed these STAT3 pathway proteins (Schaeferhoff et al., 2010). A unique study was undertaken to examine the use of CNTF for military men and women who were exposed to damaging amounts of laser

emission. Researchers targeted cones as the cell population of interest and utilized a mouse model constitutively expressing GFP in cones. Intravitreal CNTF injection after laser-induced damage increased the number of GFP positive cells compared to control over the course of eight weeks. The mechanism of this effect was not interrogated, but it likely involves STAT3 activation (Aslam et al., 2013). These studies indicate that STAT3 may play a major role in cone photoreceptors responding to injury.

Besides laser injury, STAT3 is activated in response to retinal inflammation caused by lipopolysaccharide (LPS) injection. Adult mice received an intraperitoneal injection of LPS and retinas were examined after 8 hours. Increased phosphorylation of STAT3 was found in RGCs, the INL, and in a few photoreceptors. By 16 hours post injection many photoreceptors expressed phosphorylated STAT3 (Ozawa et al., 2008). Expression of the STAT3 inhibitor SOCS3 was also increased in the inner nuclear and photoreceptor layers by 8 hours post injection. To examine the effect that STAT3 signaling and LPS inflammation had on visual function three metrics were observed: amount of rhodopsin protein, outer segment length, and ERG recording. SOCS3 was deleted in retina under the retina specific α-Cre promoter and eight hours after LPS injection, rhodopsin levels were unchanged in wild type retina but significantly lower in SOCS3 knockout (KO) retina. After 48 hours the wild-type retinas saw a 50% decrease in rhodopsin expression, whereas rhodopsin was nearly absent in retinas lacking SOCS3. Similarly, LPS decreased outer segment length by 48 hours in wild type mice and the effect was more severe in KO retina. Rhodopsin is expressed in the outer segments and the decline in rhodopsin expression is likely the cause of the small outer segments. Rhodopsin is required for phototransduction and consequently the amplitude of the ERG a- and b-waves were decreased in the SOCS3 KO retina compared to wild type after LPS treatment. As was expected, after LPS treatment more phosphorylated STAT3 was observed in retina lacking SOCS3 than wild type. This observation was first seen after 8 hours and persisted for 10 days. Directly activating STAT3 with IL-6 in adult retina explant cultures decreased rhodopsin protein levels. It was found that after IL-6 treatment, more ubiquitin was bound to rhodopsin, leading to its degradation. These data show that in response to retinal inflammation, pSTAT3

levels are increased which lead to degradation of rhodopsin, decreased outer segment lengths, and impaired visual function (Ozawa et al., 2008).

GFAP is expressed in activated Müller cells after injury. Adult mouse retina treated with CNTF and LIF induced GFAP expression in Müller cells through a pathway requiring STAT3 phosphorylation (Wang et al., 2002). To observe whether pSTAT3 and GFAP were induced after mechanical injury, adult mouse optic nerves were crush lesioned and the retina examined. Injury was found to induce endogenous LIF expression and subsequently increase pSTAT3 and GFAP levels in Müller cells three days after lesioning. The response still occurred in CNTF knockout animals, suggesting that LIF alone was the endogenous STAT3 activator secreted after injury. CNTF and LIF double knockouts confirmed that LIF was required for the activation of pSTAT3 and GFAP in Müller cell after optic nerve lesion (Kirsch et al., 2010). In addition to mechanical injury, continuous subtoxic light exposure can increase pSTAT3 expression (Peterson et al., 2000). These data provide evidence that pSTAT3 is linked to activated GFAP in Müller cells in response to injury or stress.

As glaucoma is a leading cause of blindness, understanding the gene changes associated with the disease can yield useful information for prevention or treatment. A rat model of glaucoma was used to investigate gene expression changes between control retina and retina injected with saline to increase intraocular pressure. Many genes exhibited expression changes including STAT3 whose cDNA levels were increased in the treated retina. Immunofluorescence images of treated retina confirmed the increase in STAT3 protein as well as phosphorylated STAT3 in glaucomatous tissue (Wang et al., 2010). Although the role of endogenously activated STAT3 in retina with glaucoma hasn't been examined, it likely promotes survival of RGCs in response to axonal degradation.

The optic nerve head (ONH) is the location where ganglion cells exit the retina to become the optic nerve. It is this area that is damaged during glaucoma and may lead to blindness. A rat model was used that mimicked glaucoma by raising the intraocular pressure (IOP) through injection of sterile saline from a bag hanging above the eye for 60 minutes. After the needle was removed, normal pressure and

blood flow returned to the eye. The Jak inhibitor AG490 was used to investigate the role of STAT3 on axon bundles at the optic nerve. After the transient increase in IOP, axon bundles were lost in vehicle treated eyes and the remaining axons were malformed. Treatment with AG490 saved axons from death and allowed them to retain their normal morphology. Similarly, AG490 prevented loss of the RGC layer after increased IOP. Inhibiting STAT3 phosphorylation may be beneficial in treating glaucoma (Wong et al., 2014).

Not only did AG490 protect neurons, it also was able to affect astrocytes. Astrocytes at the ONH were morphologically different following ischemia-perfusion injury; they were rounder and thicker than their non-injured counterparts. GFAP, a marker of reactive astrocytes, was also raised after IOP injury, but AG490 decreased GFAP expression. Concurrently, phosphorylation of STAT3 was increased in these astrocytes and AG490 injection maintained astrocytes in a more elongated conformation and kept pSTAT3 at the same level as control (Wong et al., 2014). A similar study examined rat optic nerve astrocytes after an acute increase in IOP. They saw the same morphologic changes, increased pSTAT3 and GFAP expression. Nestin, a marker of undifferentiated astrocytes, increased after IOP and its expression colocalized to those cells expressing STAT3 (Zhang et al., 2013). These data suggest that STAT3 is activated in response to increased IOP and is responsible for alterations in axon and astrocyte morphology at the optic nerve.

1.2.3 Neural protection

Activation of STAT3 can have protective effects and researchers have investigated which ligands and receptors were responsible for STAT3 stimulation in this context. Mice were exposed to bright light to precondition them before exposing them to severely bright light. This preconditioning light exposure increased expression of several cytokines including LIF and OSM. It also protected photoreceptors from death and allowed them to retain function as measured by electroretinogram. Use of the LIFR antagonist LIF05 blocked the phosphorylation of STAT3 and subsequent protection of photoreceptors that follows

(Chollangi et al., 2009). The role of the gp130 receptor was investigated using genetic manipulation. Using a Cre-loxP system, gp130 was removed in the whole retina, only photoreceptors, or only Müller cells. A decrease in pSTAT3 immunofluorescence after LIF treatment indicated that gp130 was effectively blocked in the given cell types. Bright light preconditioning was used to determine the requirement of gp130 in the various cell types to respond to injury. While control retina had normal morphology when preconditioned, those with gp130 removed in the whole retina or in only photoreceptors displayed morphology of retina without preconditioning; they suffered severe photoreceptor loss. Müller cells in which gp130 was removed responded like control retina to the preconditioning treatment and were protected from damage. This suggests that gp130 is required for the endogenous protective effect of preconditioning with bright light in photoreceptors but is unnecessary in Müller cells (Ueki et al., 2009).

In Müller cells STAT3 has been shown to have a protective effect against various stressors (Zhang et al., 2010). In this way Müller cells are similar to astroyctes in the brain where pretreatment with LIF protects cells from reactive oxygen species-induced death by increasing pSTAT3 (Lapp et al., 2014). It was observed that LIF protected photoreceptors from light damage by phosphorylating STAT3. Retina treated with LIF performed better after being exposed to bright light than PBS-treated retina and photoreceptors were protected from cell death. During this protection, pSTAT3 was induced in Müller cells as well as in the nuclei of photoreceptors (Rhee et al., 2013; Ueki et al., 2008). Not only does exogenous LIF have this effect, but endogenous LIF also protects photoreceptors from degeneration after exposure to light damage (Bürgi et al., 2009). The VPP mouse is a model of human autosomal dominant retinitis pigmentosa, which is generated through mutation of the murine opsin gene (Naash et al., 1993). In this model photoreceptor loss begins between PN15 and PN28. Coincident with photoreceptor degradation is LIF expression in a subset of Müller cells. Mice with this mutation and that are also homozygous null for *LIF* have increased photoreceptor loss and fail to activate pSTAT3 and or show increased GFAP (Joly et al., 2008). In addition to LIF, OSM also stimulates pSTAT3 in Müller cells. A

rat strain with retinal degeneration was protected from photoreceptor loss by injection of OSM (Xia et al., 2011). These data suggest that endogenous and exogenous stimulation of pSTAT3 in Müller cells plays a role in photoreceptor protection.

The previous experiments suggest a model where a non-IL-6 family cytokine is secreted that initiates a response in Müller cells. After stimulation Müller cells induce pSTAT3 and GFAP expression and likely secrete an IL-6 ligand. It is then the secreted IL-6 family ligand that is responsible for photoreceptor protection.

Similar to its action in Müller cells, pSTAT3 has a protective effect in RGCs. Dexamethasone increases hydrogen peroxide production and induces apoptosis; however, treatment of a rat RGC cell line with IL-10 protected cells against apoptosis induced by dexamethasone. Interrogation of the mechanism of this protection showed that STAT3, but not Akt, was phosphorylated after IL-10 treatment (Boyd et al., 2003). The involvement of STAT3 signaling in RGCs was also investigated in a mouse model of glaucoma. Mouse intraocular pressure was elevated through laser-induced venous photocoagulation, which led to cell death in RGCs two weeks after laser treatment. Intravitreal injection of CNTF into the eye one day after laser treatment significantly reduced the amount of RGC loss through activation of STAT3 (Ji et al., 2004). A similar role for STAT in RGCs was seen after glutamate excitotoxicity or ischemia. PSTAT3 levels were elevated 24 hours post-ischemia and injection of an adenovirus containing constitutively active STAT3 was sufficient to decrease cell death in primary cultured RGCs exposed to ischemic conditions. Constitutive pSTAT3 also rescued RGCs from glutamate toxicity (Zhang et al., 2008).

Another experiment used myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis (MOG-EAE) to induce apoptosis of RGCs and cause optic neuritis in adult rats. It was discovered that when CNTF was intravitreally injected on the same day as MOG-EAE that visual function as measured by electroretinography was less decreased after 7 days. Interrogation of causative pathways revealed that both pSTAT3 and pMAPK were increased following a 7-day CNTF

injection course; CNTF was intravitreally injected 0, 4, and 7 days after MOG immunization. Expression of the anti-apoptotic protein and STAT3 target Bcl-2 was also increased 8 to 18 hours after the last CNTF injection on day 7, suggesting that STAT3 plays a protective role in RGCs (Maier et al., 2004).

A similar effect on protection and function can be seen when others used a different IL-6 family ligand, OSM, after optic nerve crush and observed RGCs. Mouse retina underwent optic nerve crush lesioning for 4 seconds and were then treated with OSM, PBS, or were untreated. Two weeks later it was observed that there was a greater density of RGCs in the OSM-treated group than either the PBS or untreated group. At only 8 days after injury the electrophysiological activity of RGCs was monitored via pattern ERG (PERG). Eyes treated with OSM or CNTF performed better than untreated retina; however, all groups performed equally poorly by 15 days post-injury. Immunofluorescent staining one hour after OSM treatment showed pSTAT3 in Müller cells, but not RGCs suggesting an indirect mechanism by which OSM protects RGC survival and electrophysiological properties (Xia et al., 2014). Although no reports describe the same experiments using LIF, because OSM binds to the LIFR it is likely that using LIF instead of OSM would have similar effects.

Because of STAT3's function in retinal protection, several clinical trials have examined the benefit of CNTF in retinal disease patients. In order to deliver CNTF, clinicians utilized an encapsulated cell implant that contained ARPE19 cells transfected with the human *CNTF* gene. The wall of the implant was semipermeable and allowed for secretion of CNTF out of the capsule and into the vitreous. In a Phase 1 clinical trial, the implant secreted CNTF properly and there were no adverse events due to the implant or CNTF (Sieving et al., 2006). Subsequent studies have examined the efficacy of CNTF treatment in patients with retinitis pigmentosa, geography atrophy in age-related macular degeneration, or CNGB3 achromatopsia. Patients suffering from CNGB3 achromatopsia showed increased retina thickness, but visual acuity, rod function, and chromatic discrimination were unchanged (Zein et al., 2014). Increased outer nuclear layer thickness and cone density were seen in retinitis pigmentosa patients, but those individuals exhibited no increase in visual function compared to sham treated patients (Pilli et al., 2014;

Talcott et al., 2011). The most promising results were in patients with geography atrophy in age-related macular degeneration. They saw increased outer nuclear layer thickness like the others and showed better visual acuity as measured by a Snellen chart, yet no changes were observed in electroretinogram recordings (Zhang et al., 2011).

Like in the neural and glial cell types of the retina, RPE cells can also be protected by STAT3. The Wnt/β-catenin ligand Wnt3a protected APRE19 cells grown in a polarized monolayer from H_2O_2-induced reactive oxygen species damage. Without Wnt3a treatment cells were significantly less viable than their control counterparts not exposed to H_2O_2. Pretreatment with Wnt3a decreased the effectiveness of H_2O_2 on cell death. Concurrent with cell protection was increased pSTAT3 expression and localization of pSTAT3 with β-catenin. Transfection with siRNA targeted to STAT3 completely eliminated the ability of Wnt3a to protect ARPE19 cells from H_2O_2-induced cell death (Fragoso et al., 2012). This evidence shows that Wnt activation can lead to activation of STAT3 to protect RGCs from reactive oxygen species-induced death.

1.2.4 Regeneration

Beyond response to injury and neural protection, activating STAT3 can induce cell regeneration. Injection of CNTF into zebrafish prior to light damage enhanced Müller cell proliferation and protected photoreceptors from apoptosis. A STAT3 morpholino blocked its expression and showed that CNTF stimulates Müller cell proliferation through STAT3, but that the protection of photoreceptors did not require STAT3. This process was mediated through MAPK (Kassen et al., 2009). These experiments show that in the zebrafish, STAT3 signaling induces Müller cells to re-enter the cell cycle and begin proliferating, but does not directly enhance photoreceptor survival. Some research has provided evidence for Müller cell dedifferentiation in mammals, but has not implicated STAT3 in the process (Liu et al., 2013; Reyes-Aguirre et al., 2013).

Zebrafish were exposed to damaging light and retinas examined several times over the following 96 hours. It was observed that rhodopsin and green opsin expression began decreasing at 16 hours and that photoreceptor morphology changed, suggesting cell death. At the same time a marker of proliferation, PCNA, began being expressed in the inner nuclear layer. By 96 hours, rhodopsin and green opsin expression was nearly lost and PCNA expression had expanded to the outer nuclear layer. Phosphorylated STAT3 expression started at 16 hours post-injury, peaked at 31 hours, and was nearly absent by 96 hours. STAT3 expression was localized to Müller cells; however, only a subset of Müller cells that expressed STAT3 also expressed PCNA (Kassen et al., 2007). In addition to STAT3, it was found that Ascl1a and Lin28a are required for Müller cell proliferation, but STAT3 was necessary for maximal proliferation (Nelson et al., 2012). This process involved the production of TNFα, which stimulated pSTAT3. Knockdown of TNFα production with a morpholino diminished Müller cell proliferation but did not alter photoreceptor apoptosis (Nelson et al., 2013). Therefore, STAT3 activation can directly contributed to proliferation of Müller cells in response to injury.

In addition to cell survival and maintenance of function, STAT3 can activate neurite growth in RGCs after trauma. The optic nerve or lens of adult mice and rats were crushed and RGCs cultured. Animals that received CNTF injection after the injury had increased neurite outgrowth compared to those injected with vehicle through a mechanism that involved pSTAT3 and pERK (Müller et al., 2009, 2007). The same is true when the ligand was switched to LIF or IL-6 (Leibinger et al., 2009, 2013a, 2013b). Expression of a constitutively active form of STAT through adeno-associated virus induction also produced greater axonal growth following optic nerve injury, suggesting that the effects of IL-6, CNTF, and LIF are through STAT3 (Pernet et al., 2013).

The previous studies examined exogenous administration of STAT3 ligands, but studying zebrafish RGCs after severing the optic nerve showed increased levels of *LIF* mRNA 3 days post-injury and decreased levels of *CNTF* mRNA. The increase in *LIF* mRNA coincided with an increase in LIF protein and phosphorylated STAT3 in the ganglion cell layer and inner nuclear layer. When LIF

morpholinos were injected at the site of optic nerve injury, the level of activated STAT3 was decreased. To test neurite outgrowth, optic nerves were cut, morpholinos injected, and retina removed and cultured after 3 days. Explants were cultured for 5 days and the number of cells displaying neurite outgrowth was counted. Ablation of LIF protein decreased the number of neurite growing cells compared to morpholino control or injury alone. These experiments show that after optic nerve injury endogenous LIF levels are increased to activate STAT3 and induce neurite outgrowth from RGCs (Ogai et al., 2014).

While STAT3 induces axonal regeneration, the STAT3 negative regulator SOCS3 reduces it in zebrafish and mice. When the zebrafish optic nerve was lesioned and retina cultured in media containing a SOCS3a morpholino, RGC axon lengths and pSTAT3 levels were significantly increased compared to control (Elsaeidi et al., 2014). The same relationship between SOCS3 and axon regeneration was seen in SOCS3 KO mouse retina. Furthermore, those SOCS3 KO retina cultured in CNTF after optic nerve injury exhibited even greater axon regeneration length compared to SOCS3 KO without CNTF treatment (Smith et al., 2009; Sun et al., 2011). These data suggest that SOCS3 inhibits axon regeneration in RGCs after optic nerve injury at that SOC3 may be targeted to improve healing post-injury.

1.2.5 Immunity

The STATs have functions in immunology in multiple tissues, including the retina. The role of T helper cells in retinal ganglion cell death was investigated. A model of ischemia reperfusion was performed in mouse retina by increasing the intraocular pressure for 60 minutes and then allowing normal pressure to return. This was first done in C57Bl/6 and severe combined immunodeficiency (SCID) mice and it was observed that the wild-type mice suffered more severe cell loss than the SCID mice. The T helper (Th2) cell pathway was implicated after STAT6 knockout retina showed similar results as the retina from SCID mice. Interestingly, STAT6 knockout retina had increased STAT3 expression compared to wild-type retina and ischemia caused Y705 phosphorylation of STAT3. A similar pattern was observed

for STAT1. These data suggest that in retina lacking STAT6, and thus a Th2 response, STAT3 may have a protective effect (Huang et al., 2013b).

STAT3 was shown to illicit an immune response in a model of uveitis called experimental autoimmune uveoretinitis (EAU). It was discovered that Th17 cells are found in response to EAU, the differentiation of which from naïve T-cells requires STAT3. A conditional knockout system was used to create $CD4^+$ T-cells lacking STAT3. EAU was induced by injecting interphotoreceptor retinoid-binding protein (IRBP) in complete Freunds's adjuvant (CFA) into wild-type mice. As is typical, these mice exhibited photoreceptor loss and immune cell infiltration into the retina by 14 days post-injection; however, those mice lacking $CD4^+$ STAT3 did not show the EAU phenotype. Moreover, their lymph nodes and spleen contained lower levels of Th17 compared to wild-type mice. When IRBP-specific T-cells were purified from wild-type mice with EAU and transplanted into $CD4^+$ STAT3 KO mice, the EUA phenotype was observed in the STAT3 KO mice (Liu et al., 2008). This indicates that STAT3 is required for Th17 production in EAU. A small molecule was later used to inhibit STAT3 and was able to reproduce the STAT3 KO results, giving support to the possibility of inhibiting STAT3 as a therapy for uveitis (Yu et al., 2012). This pathway was further investigated and it was shown that STAT3 binds to the miR-155 locus and enhances its expression. Mice lacking miR-155 are resistant to EAU, similar to mice with STAT3 KO $CD4^+$ T-cells. It was found that miR-155 is expressed in Th17 cells and that without STAT3 miR-155 is not expressed in response to EAU (Escobar et al., 2013). What role miR-155 is playing in these cells is not yet known.

STAT3 also regulates innate immunity in the RPE. Activation of toll-like receptor 3 (TLR3) by pathogens induces an inflammatory response and can protect the RPE against oxidative stress. ARPE19 cells were treated with paraquat to induce reactive oxygen species formation, which led to a 40% loss of cells. Application of the TLR3 agonist poly(I:C) reduced cell death to only 20%. The target of poly(I:C) was selective for TLR3 as siRNA against the receptor blocked the protective effect of poly(I:C). Cells treated with poly(I:C) and paraquat showed increased pSTAT3 nuclear localization; however, cells

treated with paraquat alone failed to activate STAT3. Knockdown of STAT3 protein with siRNA confirmed that STAT3 was required for protection by poly(I:C) against reactive oxygen species since those cells exhibited the same level of cell loss as wild-type cells treated with paraquat alone (Patel and Hackam, 2013). While the previous experiments were performed in a cell line, the role of TLR3 *in vivo* was also examined. Paraquat was injected into the retina in the subretinal space near the RPE and photoreceptor layer. This injection caused photoreceptor death, but a combined injection of paraquat and poly(I:C) decreased cell loss as measured by optical coherence tomography. As before, STAT3 siRNA was used to show that STAT3 was required for the TLR3-mediated protection. Ablating STAT3 negated the protective effects of poly(I:C) on photoreceptor layer width and visual acuity of the animal as measured by optokinetics (Patel and Hackam, 2014).

Ultraviolet rays from the sun cause cell damage and one group investigated UVB damage on ARPE19 cells. They saw that UVB exposure induced rapid STAT3 phosphorylation that lasted until 24 hours post-exposure. Tannic acid is a known protector against UVB damage and preincubation of cells in tannic acid drastically decreased STAT3 activation after UVB exposure. In response to UVB light mRNA for *complement factor B*, which is a part of the innate immune system, is increased; however, this up-regulation is blocked by tannic acid treatment. Jak inhibition with AG490 or STAT3 inhibition with siRNA showed that *complement factor B* mRNA expression required STAT3 activation (Chou et al., 2012).

Age-related macular degeneration is a leading cause of blindness and it is exacerbated by choroidal neovascularization (CNV), which is characterized in part by neovascularization of the retina by choroidal blood vessels passing through the RPE. A laser is used to produce a mouse model of CNV and examine the molecular mechanism of the disease. After CNV induction, IL-6 protein and mRNA levels were increased in the isolated RPE-choroid complex. Elevated IL-6 levels increased the severity of neovascularization as an IL-6R blocking antibody decreased the amount of CNV volume as measured using fluorescence confocal microscopy. Since IL-6 stimulates STAT3, STAT3 localization was

investigated and found in macrophages and endothelial cells. Blockade of the IL-6 receptor inhibited STAT3 phosphorylation and decreased the number of macrophages as measured by the number of cells expressing the F4/80 epitope (Izumi-Nagai et al., 2007). A separate study revealed that hyperglycemic condition increase CNV and further activates pSTAT3 (Li et al., 2012).

Just like in other tissues, STAT3 activates the immune response in the retina, specifically to stressors like ultraviolet or reactive oxygen damage and retinal diseases.

1.2.6 Phototransduction

RPE65 is a protein located in RPE cells that converts all-*trans*-retinol to 11-*cis*-retinal during the visual cycle. Adult mice that underwent intravitreal injection of LIF showed constant STAT3 activation starting 2 days post-injection and lasting until 9 days post-injection. Concurrently there was a significant decline in *RPE65* mRNA and protein levels during the same time period. Isomerase activity of RPE65 was decreased in LIF treated retina as measured by the conversion of all-*trans*-[^{3}H]retinol to 11-*cis*-[^{3}H]retinol. This suggests that the RPE65 protein remaining in LIF treated retina is insufficient to mimic that of control retina. Knocking out STAT3 and gp130 in RPE cells and treating retina with LIF failed to decrease RPE65. While the gp130 receptor is required in the RPE for this effect, whether injected LIF or a cell-secreted ligand binds to gp130 is unknown. These data suggest that in untreated, wild-type retina STAT3 levels remain low in RPE cells to allow the phototransduction process to occur without hindrance (Chucair-Elliott et al., 2012).

Chapter 2

Materials and Methods

2.1 Animals

C57Bl/6J and RD1 mice were purchased from Jackson Laboratory and housed on a 12-hour/12-hour light/dark cycle with *ad libitum* access to rodent chow and water. Retinas were removed at embryonic day 16.5 for explant culture or at embryonic days 14.5, 17.5, and postnatal days 1, 2, 4, 7, 13, and 30 for either protein or RNA extraction. Experiments on animals were conducted in accordance with Institutional Animal Care And Use Committee and the Pennsylvania State University College of Medicine guidelines.

2.2 Retinal isolation and culture

Whole retina were dissected from embryos by removing the sclera and lens and cultured in UltraCULTURE™ serum-free media (Lonza Rockland Inc.) supplemented with gentamycin antibiotic (10 µg/mL) (Zhang et al., 2002). Each retina was cultured in 1mL of media in a 24-well culture dish incubated at 37°C with a 5% CO_2 balanced air atmosphere. Every other day 0.50 mL of media was replaced with fresh media.

2.3 Western blot

Retinas were lysed in CytoBuster™ Protein Extraction Reagent (EMD Milipore) supplemented with EDTA, protease inhibitor cocktail (Thermo Fisher Scientific Inc.), and PhosSTOP phosphatase inhibitor cocktail tablet (F. Hoffman-La Roche Ltd) per the package instructions. Cell lysates were then sonicated for 10 seconds on ice to ensure complete disruption of cytoplasmic and nuclear membranes. Protein concentration was measured using the DC™ Protein Assay (Bio-Rad Laboratories Inc.).

Approximately 20 µg of protein was separated through an Any kD™ Criterion™ TGX™ gel (Bio-Rad Laboratories Inc.) and transferred to a 0.45 µm nitrocellulose membrane (Bio-Rad Laboratories Inc.) using a Trans-blot® SD Semi-Dry Transfer Cell (Bio-Rad Laboratories Inc.). The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline and 10% Tween-20 for one hour and incubated overnight at 4°C in primary antibody diluted in 3% nonfat dry milk. Secondary antibody conjugated to horseradish peroxidase was incubated with the membrane for 2 hours at room temperature and protein detected with SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific Inc.). STAT3 D3Z2G rabbit monoclonal primary antibody (1:1000), which detects both α and β isoforms, was purchased from Cell Signaling Technology and anti-rabbit secondary antibody (1:3000) was purchased from Jackson ImmunoResearch Laboratories Inc.

2.4 RNA extraction and polymerase chain reaction

RNA was extracted from one embryonic retina per sample or two postnatal retinas per sample using a QIAshredder homogenization column and RNeasy Mini Kit (QIAGEN) per the package instructions. A SuperScript™ First-Strand Synthesis SuperMix (Life Technologies) was used to create cDNA and polymerase chain reaction (PCR) performed using a Bio-Rad T-100 Thermal Cycler to verify the presence of both STAT3 isoforms. Primers: fwd 5'-ACCAACATCCTGGTGTCTCC-3', rev 5'-TTATTTCCAAACTGCATCAATGA-3'. Tm = 61°C. STAT3α = 233 bp, STAT3β = 183 bp.

2.5 Quantitative PCR

Bio-Rad iQ SYBR® Green Supermix was used to quantitate cDNA of selected genes. A Bio-Rad CFX96 Real-Time System with C1000 Touch Thermal Cycler attached to a computer running Bio-Rad CFX Manager Version 3.1 was used for the qPCR. Primers: GAPDH QuanTitect (Qiagen), Vimentin QuantiTect (Qiagen), Rho; fwd 5'- TGGCTGGTCCAGGTACATC-3', rev 5'-GGATTCGTTGTTGACCTCAG-3', Pde6b; fwd 5'- AGGATGTGGCAGAGTGTCC-3', rev 5'-

GGATTCGTTGTTGACCTCAG-3', Pitnm3; fwd 5'-CTGCTCGGAGGCTTTCTC-3', rev 5'-CAGAGGACTTGAGGAACTCG-3'. Tm = 55°C.

2.6 Array data collection

A microarray chip was constructed that contained 12, 280 primer sets for genes expressed in the retina. Each primer set amplified cDNA from two biological and two technical replicates of retinal cDNA. The microarray was analyzed using the maSigPro R package (Conesa et al., 2006) with default parameters in 3 different ways: 1. time course analysis of the case/control ratio with 4 total replicates as input; 2. multiple series time course analysis of the cases vs. controls using 4 total replicates as input; 3. multiple series time course analysis of the cases vs. controls using 2 biological replicates of averaged 2 technical replicates. Changes in gene expression were considered statistically significant at a p-value < 0.05.

2.7 Cluster and tree view analysis

Gene Cluster 3.0 was used to produce Hierarchical clustering of the genes and Java TreeView Version 1.1.6r4 produced the heat map image.

Chapter 3

STAT3 isoforms α and β are differentially expressed in the retina

3.1 Rationale

It is known that *STAT3* mRNA can be spliced into two isoforms, yielding proteins with different gene targets and functions (Maritano et al., 2004). In the developing retina there is STAT3 expression in the embryo, which declines during the first week after birth and then reappears in the adult (Zhang et al., 2003). In the adult retina, STAT3 is activated after injury, whereas it controls retinal development in the embryo and PN1-PN7 retina. This observation prompted the question whether STAT3 isoforms were differentially expressed in the retina throughout development.

3.2 Results

To test the gene expression patterns of STAT3 isoforms RT-PCR was performed on E14.5, PN1, PN4, PN7, PN30, and adult RD1 retina as well as liver from an adult wild-type mouse. RD1 mice carry a mutation in the Pde6b gene causing photoreceptor degeneration by three weeks after birth (LaVail and Sidman, 1974). The primers used were designed to visualize both the α and β isoforms by including the 50 base region excised in the β isoform within the PCR product (Maritano et al., 2004). STAT3α appears as a strong band at 233 bases and STAT3β appears as a weaker band at 183 bases. The PCR products clearly show STAT3α expression in all samples tested and β expression in all but the E14.5 retina sample; however, STAT3α bands are much stronger than the STAT3β bands (Figure 5).

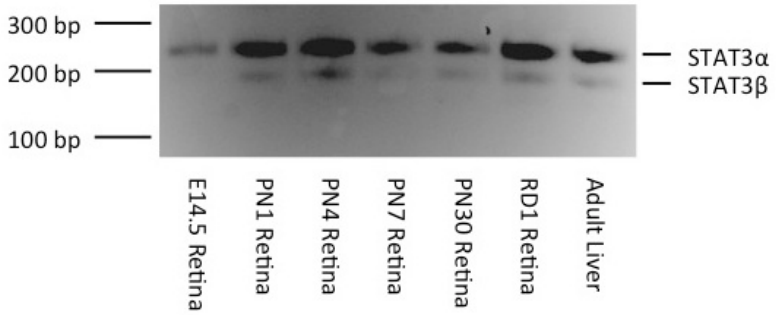

Figure 5: *STAT3* isoform mRNA expression. Shown are RT-PCR products of *STAT3α* and *β* isoforms in E14.5, PN1, PN4, PN7, PN30, RD1 retina, and adult wild-type liver.

The next step was to verify if both messages were produced as protein. A monoclonal antibody that recognizes an epitope shared by both STAT3 isoforms was used to probe a western blot membrane containing whole cell lysates from E17.5, PN2, PN4, PN13, PN30 retina, and HeLa cells as a positive control. Histones were visualized using a Coomassie gel stain as loading control. As expected the HeLa lysate contains both STAT3α and STAT3β proteins; however, the amount of STAT3β is much less than that of STAT3α. By comparison, all of the retina samples tested contained STAT3α, but none expressed STAT3β (Figure 6).

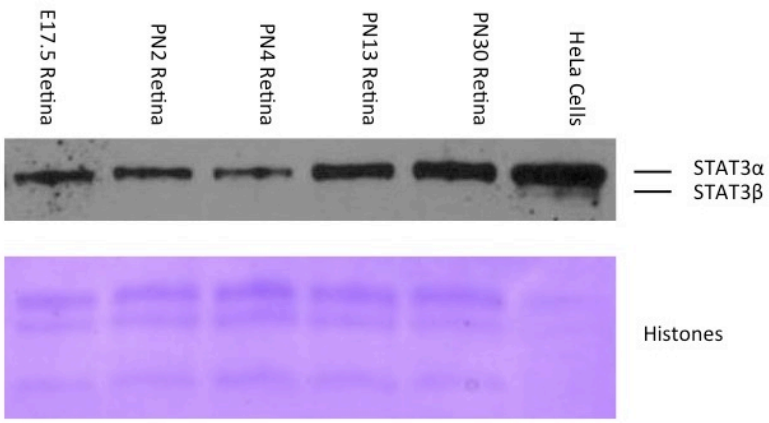

Figure 6: STAT3 isoform protein expression. Western blot shows STAT3α, but not STAT3β, in all retina samples. Histones visualized by Coomassie gel stain for loading control.

3.3 Discussion

It has been shown before that STAT3 is expressed in the retina during development when it controls rod differentiation and in the adult when it performs several functions including responding to injury. Until now researchers hadn't examined which isoforms were expressed in the retina during these stages. STAT3α and β are not redundant as previous studies have shown that they up-regulated expression of a different set of genes, so these experiments sought to determine the expression of STAT3α and β isoforms in the mouse retina (Maritano et al., 2004). *STAT3α* was the primary message being expressed with *STAT3β* mRNA being weakly or not expressed, depending on the age of the retina. Although the STAT3β nucleotide length is shorter than that of STAT3α, and therefore intercalates less ethidium bromide, it is not believed to be sufficient to explain the difference in band strength. Moreover, when protein was examined no STAT3β was visible in the retina lysate, whereas STAT3α was visible in all retinal lysates including those of the RD1 retina. The appearance of STAT3 in RD1 samples reveals that STAT3 is localized to cells other than photoreceptors, which agrees with previous research showing

STAT3 expression in Müller cells in the adult retina and not in the photoreceptor layer. For the first time, research here shows that STAT3α, and not STAT3β, is expressed in the adult and perinatal retina.

Chapter 4

CNTF stimulation diminishes rod-specific gene expression and enhances Müller glia-specific gene expression

4.1 Rationale

Activating STAT3 with CNTF is known to block rod photoreceptor development and premature inactivation of pSTAT3 with PMA or IGF1 can increase rod development (Ozawa et al., 2004; Pinzon-Guzman et al., 2010; Zhang et al., 2004)I . Besides affecting rod photoreceptors CNTF treatment also alters the number of bipolar and Müller cells (Ezzeddine et al., 1997). A microarray was used to determine which retinal genes exhibited expression changes when embryonic retinas were treated with CNTF.

4.2 Results

To investigate the genes affected during CNTF treatment of the developing retina, retinas from E16.5 retina were removed and cultured for 1, 3, 6, or 10 days in the constant presence of CNTF. RNA was extracted and expression data was generated using a custom microarray containing primers for over 12, 000 genes present in the retina. The fold change in gene expression between CNTF and control was calculated for each time point and the data were analyzed using the maSigPro software package for R. Genes that displayed significant expression fold change were labeled biologically relevant if the change was at least 1.5 fold ($\log_2$). Figure 7 shows a heat map of gene expression for all of the genes. From the heat map it is apparent that a subset of the genes increase expression and a subset of the genes decrease expression after CNTF treatment. A full list of the genes whose expression changed with CNTF treatment can be found in Appendix B.

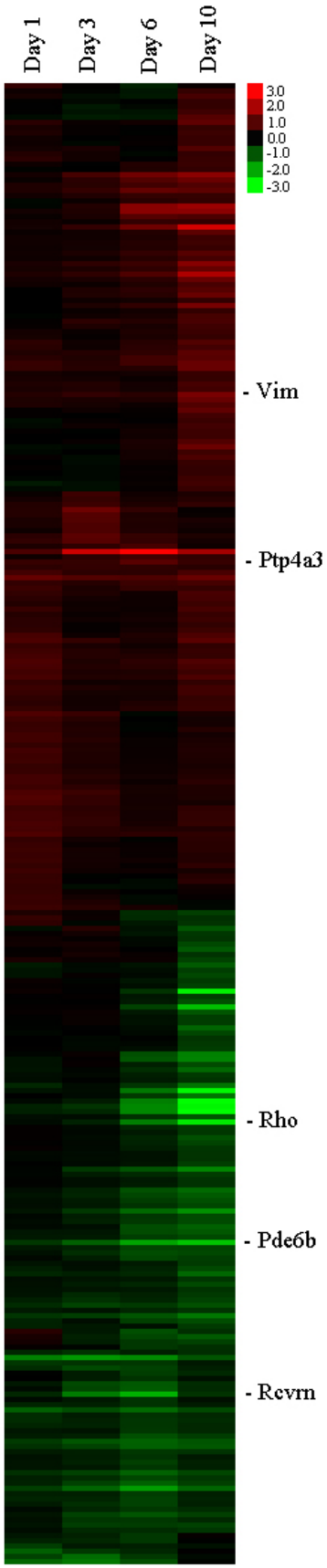
Day 1
Day 3
Day 6
Day 10
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
- Vim
- Ptp4a3
- Rho
- Pde6b
- Rcvrn

Figure 7: Microarray heat map. A heat map shows the expression patterns of genes in retina cultured with CNTF compared to control explant retina. Fold change values have been $\log_2$ transformed. Red indicates increased expression; green indicates decreased expression. The location of select genes has been notated.

To understand what these gene changes meant in the context of different retinal cell types, the list of genes that significantly changed expression was compared to a list of retina cell-type specific genes. Figure 8 shows a summary of the findings. The cell type with the greatest number of genes changed was rod photoreceptors. Twenty rod photoreceptor genes significantly changed, while Müller cell genes, which had the next largest number of changed genes, only had five. Many of the rod genes like rhodopsin, Pde6b, and recoverin were decreased; however, Ptp4a3, Aldoa, and Hmgb2 exhibited increased expression. Some rod genes were highly altered, like rhodopsin and Pde6b who changed over 2-fold. Those Müller cell genes that changed expression were increased with CNTF treatment. Bipolar and amacrine cells each contained two genes whose expression decreased in the CNTF group. The cone photoreceptor cell type showed two genes increasing expression and three genes decreasing expression. Neither ganglion cells nor horizontal cells displayed changes in their cell-type specific gene expression.

Quantitative RT-PCR was performed to confirm the expression data from the microarray. Rhodopsin, Pde6b, Pitnm3, and vimentin expression levels were tested with qRT-PCR and the results confirmed those from microarray (Figure 8).

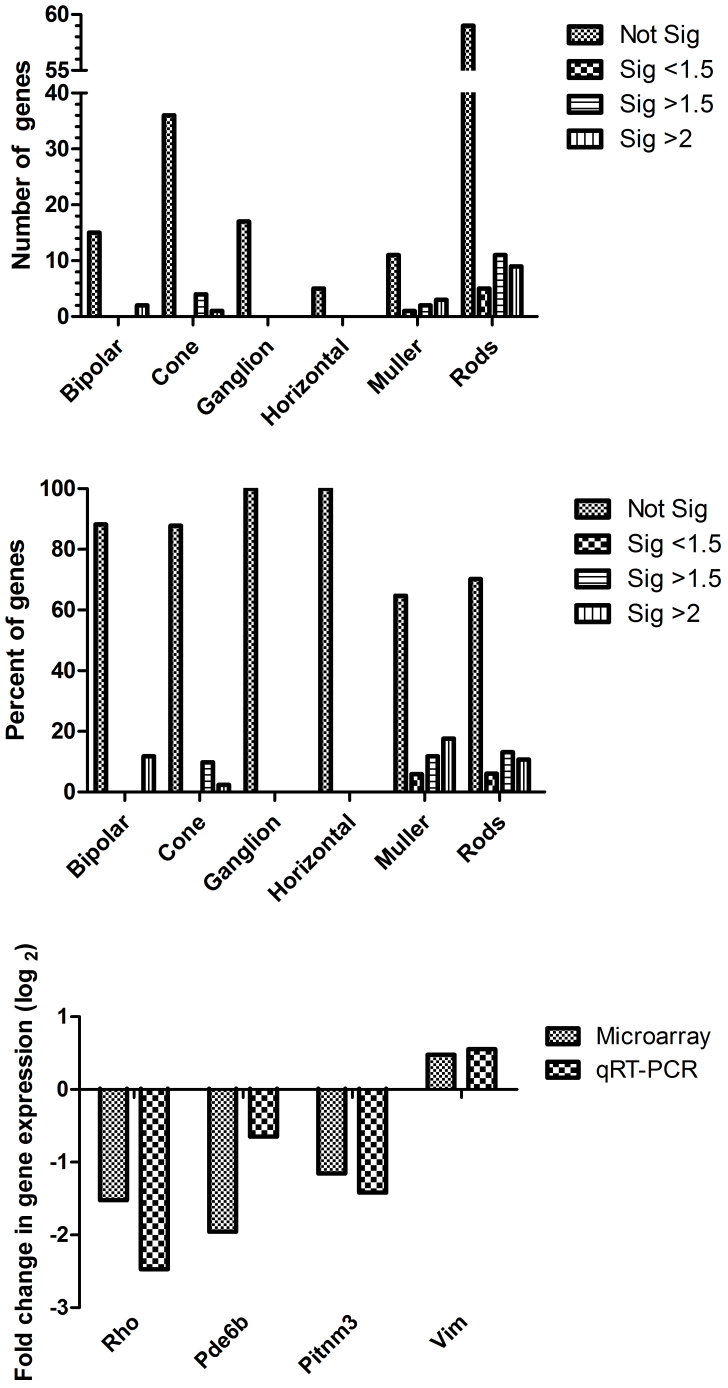
Number of genes
60
55
40
30
20
10
0
Bipolar
Cone
Ganglion
Horizontal
Muller
Rods
Not Sig
Sig <1.5
Sig >1.5
Sig >2
Percent of genes
100
80
60
40
20
0
Bipolar
Cone
Ganglion
Horizontal
Muller
Rods
Not Sig
Sig <1.5
Sig >1.5
Sig >2
Fold change in gene expression (log 2)
1
0
-1
-2
-3
Rho
Pde6b
Pitnm3
Vim
Microarray
qRT-PCR

Figure 8: Cell-type specific gene changes. Histograms show either the number of genes or percentage of genes that were significantly changed with CNTF treatment. Groups are segregated by genes whose changes are statistically significant but less than 1.5-fold, significantly different and over 1.5 but less than 2, and significantly different and greater than 2-fold change. Another histogram shows expression of select genes as analyzed via microarray and qRT-PCR.

4.3 Discussion

The effects of CNTF treatment on STAT3 activation during retinal development and on retina cell specification have been studied previously. They have observed a switch in cell identity, wherein CNTF causes a decrease in rod photoreceptors and an increase in bipolar, Müller, and amacrine cell numbers. The current work begins to examine the genetic events that prelude the cell-type specific changes observed with CNTF treatment. Of the over 12, 000 genes examined, CNTF significantly changes the expression of only 2%. That a small percentage of genes are changed is evidence that CNTF produces a selective effect on gene expression. It also shows that a relatively small number of genes control cell identify in the retina.

Chapter 5

General discussion

STAT3 is important in immunologic processes, but also has roles outside the immune system, such as in the retina. At E17.5 activated STAT3 is found in the inner retinal layer, but switches to the outer retinal layer by PN1 (Zhang et al., 2003). Both cell populations are undifferentiated at this time. By PN7 pSTAT3 expression is absent in the outer and inner nuclear layers and can be seen in a very small number of ganglion cells (Zhang et al., 2003). During PN1 to PN7 it controls differentiation of rod photoreceptors and may impact Müller glia development (Ozawa et al., 2004; Rhee et al., 2004; Zhang et al., 2004). Although STAT3 protein levels are negligible after the first week of birth, they reappear by 8 weeks postnatal in Müller glia and retinal ganglion cells (Zhang et al., 2003). At this age STAT3 provides protection against injury or stress and is activated in response to these stimuli in order to prevent cell death and retinal degeneration.

STAT3 has two isoforms and they are known to have non-overlapping function. In mouse embryonic fibroblasts STAT3α alone activates SOCS3 transcription after Il-6 treatment, while STAT3β has unique targets including the anti-apoptotic gene Phlda1 (Maritano et al., 2004; Ng et al., 2012). The current research sought to examine the expression patterns of α and β isoforms in retina of different ages to begin to understand if the different isoforms could account for the different functions in the perinatal and mature retina. It was found that both the α and β isoforms of *STAT3* were expressed in the retina at various ages; however, only the α isoform was made into protein. This indicates that STAT3α is responsible for both the developmental and the adult functions of STAT3 in the retina. At first glance the roles that STAT3 plays in the developing and mature retina may seem unrelated, but maintaining cells in an undifferentiated state and protecting cells from death are not very different as both require transcription of proliferation and anti-apoptotic genes. And so the pertinent question remaining is: are the

STAT3 gene targets during development and in the adult retina different? If so, what allows STAT3 to preferentially bind the appropriate genes at the appropriate time?

During retinal development, treating PN1 retina with CNTF stimulates pSTAT3, blocks rhodopsin expression and rod photoreceptor differentiation, and promotes differentiation of bipolar, amacrine, and Müller glia cells. Research here provided insight into the changes in gene expression that underlie the phenotypic changes associated with CNTF treatment. The cell type with the largest number of changed genes was rod photoreceptors; three genes were increased and 17 genes showed decreased expression with CNTF treatment. Expression patterns of 5 Müller glia genes were increased and bipolar and amacrine cells each had two cell-type specific genes down regulated. These data describe a mechanism by which CNTF affects differentiation of several retina-specific cell types through altering specific gene expression in those cells. It is reasonable to suspect that the transcription factor responsible for CNTF's ability to alter the expression of these genes is STAT3; however, more experiments are required to test this hypothesis.

Another question requiring attention is how many of the genes whose expression was changed by CNTF contain a STAT3 binding site in its promoter. Published ChIP-Seq data exists for STAT3 in E14 mouse embryonic stem cells (accession number GSM288353), H3K4me2 marks in PN1 and PN7 retina, and DNase1 hypersensitivity sites in newborn and one week old retina (John et al., 2011; Popova et al., 2012; Sabo et al., 2004, 2006). Each gene that exhibited expression change was queried using the UCSC Genome Browser and with the accompanying ChIP-Seq data (Karolchik et al., 2004; Kent, 2002; Kent et al., 2002, 2005, 2010; Mouse Genome Sequencing Consortium et al., 2002; Raney et al., 2014). If the gene promoter contained a STAT3 binding site and open chromatin (as evidence by H3K4me2 marks or DNase1 hypersensitivity site), then it was labeled as having an active STAT3 binding site. Using these criteria, 34% of the genes analyzed did have an active STAT3 binding site, and 66% did not. Because this process will likely incur type I and type II errors, the percentages may not accurately reflect what is seen in the retina *in vivo*. What we can say is that there are distinct populations of genes altered by CNTF

treatment, those that are bound by STAT3 and those that are not bound by STAT3. More experiments are required to understand whether the genes changed by CNTF, but not bound by STAT3, are altered by other pathways (e.g. MAPK) or by STAT3 in a trans-regulatory mechanism.

Appendix A

Extracellular ligands, receptors, and intracellular molecules in the STAT3 pathway

Extracellular Ligands		
Molecule	**Role**	**Reference**
Cardiotrophin 1	Activator	
Cardiotrophin-like cytokine	Activator	(Uemura et al., 2002)
Ciliary neurotrophic factor	Activator	(Wishingrad et al., 1997)
Colony-stimulating factor 1	Activator	(Novak et al., 1995)
Epidermal growth factor	Activator	(Zhong et al., 1994)
Granulocyte colony-stimulating factor	Activator	(Tian et al., 1994)
Hepatocyte growth factor	Activator	(Boccaccio et al., 1998)
Interferon α	Activator	(Raz et al., 1994)
Interferon γ	Activator	(Raz et al., 1994)
Interleukin 5	Activator	(Caldenhoven et al., 1995)
Interleukin 6	Activator	(Wegenka et al., 1993)
Interleukin 9	Activator	(Demoulin et al., 1996)
Interleukin 10	Activator	(Finbloom and Winestock, 1995)
Interleukin 11	Activator	(Mahboubi et al., 2000)
Interleukin 22	Activator	(Xie et al., 2000)
Leptin	Activator	(Vaisse et al., 1996)
Leukemia inhibitory factor	Activator	(Wegenka et al., 1993)
Neuropoietin	Activator	(Derouet et al., 2004)
Oncostatin M	Activator	(Kortylewski et al., 1999)
Platelet-derived growth factor	Activator	(Raz et al., 1994)
TNFα	Activator	(Guo et al., 1998)
Wnt3a	Activator	(Fragoso et al., 2012)

Receptors		
Molecule	**Role**	**Reference**
CNTFR	Signal propagation	(Elson et al., 2000)
EGFR	Signal propagation	(Rubin Grandis et al., 2000)
Eyk	Signal propagation	(Zong et al., 1996)
gp130	Signal propagation	(Lütticken et al., 1994)
IL-6Rα	Signal propagation	(Kishimoto, 1994)
IL-10R	Signal propagation	(Lai et al., 1996)
IL-11R	Signal propagation	(Baumann et al., 1996a)
Leptin receptor	Signal propagation	(Baumann et al., 1996b)
LIFR	Signal propagation	(Stahl et al., 1995)
OSMR	Signal propagation	(Hermanns et al., 1999)
PDGFR	Signal propagation	(Vignais et al., 1996)
Ros	Signal propagation	(Zong et al., 1998)

Intracellular Molecules		
Molecule	**Role**	**Reference**
Hes1	Activator	(Kamakura et al., 2004)
14-3-3 zeta	Blocks S727 dephosphorylation	(Zhang et al., 2012)
PIAS3	Inactivator	(Chung et al., 1997a)
SOCS3	Inactivator	(Starr et al., 1997)
c-Met	Kinase	(Boccaccio et al., 1998)
CK2	Kinase	(Piazza, 2006)
ERK	Kinase	(Chung et al., 1997b)
Fes	Kinase	(Nelson et al., 1998)
Jak2	Kinase	(Guschin et al., 1995)
JNK	Kinase	(Turkson et al., 1999)
PERK	Kinase	(Meares et al., 2014)
Src	Kinase	(Cao et al., 1996)
Crm-1	Nuclear export	(Bhattacharya and Schindler, 2003)
Ran	Nuclear export	(Cimica et al., 2011)
Importin-α	Nuclear import	(Ma and Cao, 2006)
Importin-β	Nuclear import	(Cimica et al., 2011)
LMW-DSP2	Phosphatase	(Sekine et al., 2006)
PTP-Meg2 (PTPN9)	Phosphatase	(Yuan et al., 2010)
Protein Phosphatase 2A	Phosphatase	(Woetmann et al., 1999)
PTEN	Phosphatase	(Sun and Steinberg, 2002)
PTP1B	Phosphatase	(Lund et al., 2005)
PTPLAD2	Phosphatase	(Zhu et al., 2014)
PTPRD	Phosphatase	(Veeriah et al., 2009)
PTPRT	Phosphatase	(Zhang et al., 2007)
Shp-1 (PTPN6)	Phosphatase	(Han et al., 2006)
Shp-2 (PTPN11)	Phosphatase	(Gunaje and Bhat, 2001)
TC-PTP	Phosphatase	(Yamamoto et al., 2002)
TC45	Phosphatase	(Hoeve et al., 2002; Kim et al., 2010)
EZI	Transcriptional activator	(Nakayama et al., 2002)
p300	Transcriptional activator	(Nakashima et al., 1999)
Cyclin D1	Transcriptional repressor	(Bienvenu et al., 2001)
Grim-19	Transcriptional repressor	(Lufei et al., 2003)
MyoD	Transcriptional repressor	(Kataoka et al., 2003)
pERK2	Transcriptional repressor	(Jain et al., 1998)
Tip60	Transcriptional repressor	(Xiao et al., 2003)

Appendix B

Retina genes whose expression changed with CNTF treatment

Up-regulated			
9930104L06Rik	Eif1	Ms11	Rps25
Acap2	Esd	Ndufa4l2	Rps6
Actb	Ext2	Nfic	Rpsa
Actg1	Fam3b	Nnat	Rras
Adcy1	Fam65a	Nup133	Sf3b1
Agtpbp1	Fau	Olfm1	Shd
Aldh1a1	Frmd8	Pacsin1	Slc1a3
Aldoa	Glul	Paqr3	Snhg11
B2m	Gnai2	Pdcd6	Snora33
BB283400	Gnb2l1	Pdpn	Sparc
Bex2	Gpc1	Pea15a	Stk11
C2cd4d	Gpi1	Pfn2	Syt11
Capns1	H19	Plcb4	Tk2
Cd9	H3f3b	Pom121	Tmsb10
Cdh11	Hmgb2	Ppdpf	Tmsb4x
Cdh2	Hnrnpa3	Ppia	Tnfaip8
Cdk2ap1	Hnrnpr	Ppp1r12c	Tubb5
Cdk4	Hsd17b4	Prdx1	Txnrd1
Cfl1	Irf2bpl	Prdx6	Vim
Ckb	Itm2a	Ptma	Wdr89
Cpsf7	Kcnb1	Rab5c	Zbtb7a
Cryab	Kcne1l	Rac3	
Crym	Kdr	Rangap1	
Ctsl	Kmt2e	Rn45s	
D430019H16Rik	Krt73	Rpl17	
Dcaf12	Lancl2	Rpl23a	
Ddx6	Ldlrap1	Rpl29	
Dkk3	Lepr	Rpl3	
Dst	Macf1	Rpl35	
E2f6	Map6	Rpl37a	
Eef1a1	Mest	Rpl5	
Eef2	Mfge8	Rpl9	
Ehmt2	Mfsd6	Rps11	

Down-regulated	
1110002L01Rik	Neurog2
2210018M11Rik	Ntm
2510039O18Rik	Numb
2610034M16Rik	Opn1sw
2610100L16Rik	Pcif1
Aak1	Pcmt1
Ado	Pdc
AK208460	Pde6b
Angpt1	Pitpnm3
Arxes2	Ppp1r3f
Cdc42se2	Prr14
Cyp51	Pygb
Dhx8	Rangrf
Dnajb6	Rcvrn
Ebp1	Rhbdl3
Elfn1	Rho
Epb4.1l1	Rprd1a
Fam213a	Rtbdn
Gfra1	Rtn4r12
Gjc2	Sash1
Gnat1	Sgtb
Gngt1	Slc9a5
Gpc3	Snta1
Gzmm	Sox11
Hapln4	Tbc1d12
Itsn1	Tpd52
Kcnj9	Tspan5
Kcnma1	Ube2g2
Kirrel	Usp33
Lingo3	Vegfc
Lrp4	
Myef2	
Nefl	

References

Akira, S. (1999). Functional roles of STAT family proteins: lessons from knockout mice. Stem Cells *17*, 138–146.

Alvarez, J.V., Febbo, P.G., Ramaswamy, S., Loda, M., Richardson, A., and Frank, D.A. (2005). Identification of a Genetic Signature of Activated Signal Transducer and Activator of Transcription 3 in Human Tumors. Cancer Res. *65*, 5054–5062.

Anchan, R.M., Reh, T.A., Angello, J., Balliet, A., and Walker, M. (1991). EGF and TGF-alpha stimulate retinal neuroepithelial cell proliferation in vitro. Neuron *6*, 923–936.

Aslam, S.A., Davies, W.I., Singh, M.S., Charbel Issa, P., Barnard, A.R., Scott, R.A., and MacLaren, R.E. (2013). Cone photoreceptor neuroprotection conferred by CNTF in a novel in vivo model of battlefield retinal laser injury. Invest Ophthalmol Vis Sci *54*, 5456–5465.

Basso, E., Fante, L., Fowlkes, J., Petronilli, V., Forte, M.A., and Bernardi, P. (2005). Properties of the Permeability Transition Pore in Mitochondria Devoid of Cyclophilin D. J. Biol. Chem. *280*, 18558–18561.

Baumann, H., Wang, Y., Morella, K.K., Lai, C.F., Dams, H., Hilton, D.J., Hawley, R.G., and Mackiewicz, A. (1996a). Complex of the soluble IL-11 receptor and IL-11 acts as IL-6-type cytokine in hepatic and nonhepatic cells. J. Immunol. Baltim. Md 1950 *157*, 284–290.

Baumann, H., Morella, K.K., White, D.W., Dembski, M., Bailon, P.S., Kim, H., Lai, C.F., and Tartaglia, L.A. (1996b). The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. Proc. Natl. Acad. Sci. U. S. A. *93*, 8374–8378.

Bhattacharya, S., and Schindler, C. (2003). Regulation of Stat3 nuclear export. J Clin Invest *111*, 553–559.

Bienvenu, F., Gascan, H., and Coqueret, O. (2001). Cyclin D1 Represses STAT3 Activation through a Cdk4-independent Mechanism. J. Biol. Chem. *276*, 16840–16847.

Boccaccio, C., Andò, M., Tamagnone, L., Bardelli, A., Michieli, P., Battistini, C., and Comoglio, P.M. (1998). Induction of epithelial tubules by growth factor HGF depends on the STAT pathway. Nature *391*, 285–288.

Boengler, K., Hilfiker-Kleiner, D., Heusch, G., and Schulz, R. (2010). Inhibition of permeability transition pore opening by mitochondrial STAT3 and its role in myocardial ischemia/reperfusion. Basic Res. Cardiol. *105*, 771–785.

Bourillot, P.Y., Aksoy, I., Schreiber, V., Wianny, F., Schulz, H., Hummel, O., Hubner, N., and Savatier, P. (2009). Novel STAT3 target genes exert distinct roles in the inhibition of mesoderm and endoderm differentiation in cooperation with Nanog. Stem Cells *27*, 1760–1771.

Boyd, Z.S., Kriatchko, A., Yang, J., Agarwal, N., Wax, M.B., and Patil, R.V. (2003). Interleukin-10 receptor signaling through STAT-3 regulates the apoptosis of retinal ganglion cells in response to stress. Invest Ophthalmol Vis Sci *44*, 5206–5211.

Bürgi, S., Samardzija, M., and Grimm, C. (2009). Endogenous leukemia inhibitory factor protects photoreceptor cells against light-induced degeneration. Mol Vis *15*, 1631–1637.

Butturini, E., Carcereri de Prati, A., Chiavegato, G., Rigo, A., Cavalieri, E., Darra, E., and Mariotto, S. (2013). Mild oxidative stress induces S-glutathionylation of STAT3 and enhances chemosensitivity of tumoural cells to chemotherapeutic drugs. Free Radic. Biol. Med. *65*, 1322–1330.

Butturini, E., Darra, E., Chiavegato, G., Cellini, B., Cozzolino, F., Monti, M., Pucci, P., Dell'Orco, D., and Mariotto, S. (2014). S-Glutathionylation at Cys328 and Cys542 Impairs STAT3 Phosphorylation. ACS Chem. Biol.

Caldenhoven, E., Dijk, T. van, Raaijmakers, J.A.M., Lammers, J.-W.J., Koenderman, L., and Groot, R.P. de (1995). Activation of the STAT3/Acute Phase Response Factor Transcription Factor by Interleukin-5. J. Biol. Chem. *270*, 25778–25784.

Cao, X., Tay, A., Guy, G.R., and Tan, Y.H. (1996). Activation and association of Stat3 with Src in v-Src-transformed cell lines. Mol. Cell. Biol. *16*, 1595–1603.

Carpenter, R.L., and Lo, H.-W. (2014). STAT3 Target Genes Relevant to Human. Cancers *6*, 897–925.

Carraro, G., El-Hashash, A., Guidolin, D., Tiozzo, C., Turcatel, G., Young, B.M., De Langhe, S.P., Bellusci, S., Shi, W., Parnigotto, P.P., et al. (2009). miR-17 family of microRNAs controls FGF10-mediated embryonic lung epithelial branching morphogenesis through MAPK14 and STAT3 regulation of E-Cadherin distribution. Dev. Biol. *333*, 238–250.

Chauhan, D., Kharbanda, S.M., Ogata, A., Urashima, M., Frank, D., Malik, N., Kufe, D.W., and Anderson, K.C. (1995). Oncostatin M induces association of Grb2 with Janus kinase JAK2 in multiple myeloma cells. J Exp Med *182*, 1801–1806.

Chollangi, S., Wang, J., Martin, A., Quinn, J., and Ash, J.D. (2009). Preconditioning-induced protection from oxidative injury is mediated by leukemia inhibitory factor receptor (LIFR) and its ligands in the retina. Neurobiol Dis *34*, 535–544.

Chou, W.-W., Wang, Y.-S., Chen, K.-C., Wu, J.-M., Liang, C.-L., and Juo, S.-H.H. (2012). Tannic acid suppresses ultraviolet B-induced inflammatory signaling and complement factor B on human retinal pigment epithelial cells. Cell. Immunol. *273*, 79–84.

Chucair-Elliott, A.J., Elliott, M.H., Wang, J., Moiseyev, G.P., Ma, J.-X., Politi, L.E., Rotstein, N.P., Akira, S., Uematsu, S., and Ash, J.D. (2012). Leukemia Inhibitory Factor Coordinates the Down-regulation of the Visual Cycle in the Retina and Retinal-pigmented Epithelium. J. Biol. Chem. *287*, 24092–24102.

Chung, C.D., Liao, J., Liu, B., Rao, X., Jay, P., Berta, P., and Shuai, K. (1997a). Specific inhibition of Stat3 signal transduction by PIAS3. Science *278*, 1803–1805.

Chung, J., Uchida, E., Grammer, T.C., and Blenis, J. (1997b). STAT3 serine phosphorylation by ERK-dependent and -independent pathways negatively modulates its tyrosine phosphorylation. Mol Cell Biol *17*, 6508–6516.

Cimica, V., Chen, H.C., Iyer, J.K., and Reich, N.C. (2011). Dynamics of the STAT3 transcription factor: nuclear import dependent on Ran and importin-β1. PLoS One *6*, e20188.

Conesa, A., Nueda, M.J., Ferrer, A., and Talón, M. (2006). maSigPro: a method to identify significantly differential expression profiles in time-course microarray experiments. Bioinformatics *22*, 1096–1102.

Darnell, J.E., Kerr, I.M., and Stark, G.R. (1994). Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science *264*, 1415–1421.

Dauer, D.J., Ferraro, B., Song, L., Yu, B., Mora, L., Buettner, R., Enkemann, S., Jove, R., and Haura, E.B. (2005). Stat3 regulates genes common to both wound healing and cancer. Oncogene *24*, 3397–3408.

Demoulin, J.B., Uyttenhove, C., Roost, E.V., DeLestré, B., Donckers, D., Snick, J.V., and Renauld, J.C. (1996). A single tyrosine of the interleukin-9 (IL-9) receptor is required for STAT activation, antiapoptotic activity, and growth regulation by IL-9. Mol. Cell. Biol. *16*, 4710–4716.

Derouet, D., Rousseau, F., Alfonsi, F., Froger, J., Hermann, J., Barbier, F., Perret, D., Diveu, C., Guillet, C., Preisser, L., et al. (2004). Neuropoietin, a new IL-6-related cytokine signaling through the ciliary neurotrophic factor receptor. Proc. Natl. Acad. Sci. U. S. A. *101*, 4827–4832.

Do, D.V., Ueda, J., Messerschmidt, D.M., Lorthongpanich, C., Zhou, Y., Feng, B., Guo, G., Lin, P.J., Hossain, M.Z., Zhang, W., et al. (2013). A genetic and developmental pathway from STAT3 to the OCT4-NANOG circuit is essential for maintenance of ICM lineages in vivo. Genes Dev. *27*, 1378–1390.

Elliott, J., Cayouette, M., and Gravel, C. (2006). The CNTF/LIF signaling pathway regulates developmental programmed cell death and differentiation of rod precursor cells in the mouse retina in vivo. Dev Biol *300*, 583–598.

Elsaeidi, F., Bemben, M.A., Zhao, X.F., and Goldman, D. (2014). Jak/STAT signaling stimulates zebrafish optic nerve regeneration and overcomes the inhibitory actions of socs3 and sfpq. J Neurosci *34*, 2632–2644.

Elson, G.C.A., Lelièvre, E., Guillet, C., Chevalier, S., Plun-Favreau, H., Froger, J., Suard, I., de Coignac, A.B., Delneste, Y., Bonnefoy, J.-Y., et al. (2000). CLF associates with CLC to form a functional heteromeric ligand for the CNTF receptor complex. Nat. Neurosci. *3*, 867–872.

Escobar, T., Yu, C.-R., Muljo, S.A., and Egwuagu, C.E. (2013). STAT3 Activates miR-155 in Th17 Cells and Acts in Concert to Promote Experimental Autoimmune Uveitis. Invest. Ophthalmol. Vis. Sci. *54*, 4017–4025.

Ezzeddine, Z.D., Yang, X., DeChiara, T., Yancopoulos, G., and Cepko, C.L. (1997). Postmitotic cells fated to become rod photoreceptors can be respecified by CNTF treatment of the retina. Development *124*, 1055–1067.

Finbloom, D.S., and Winestock, K.D. (1995). IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1 alpha and STAT3 complexes in human T cells and monocytes. J Immunol *155*, 1079–1090.

Fink, K., and Grandvaux, N. (2013). STAT2 and IRF9: Beyond ISGF3. JAK-STAT *2*, e27521.

Fragoso, M.A., Patel, A.K., Nakamura, R.E.I., Yi, H., Surapaneni, K., and Hackam, A.S. (2012). The Wnt/β-Catenin Pathway Cross-Talks with STAT3 Signaling to Regulate Survival of Retinal Pigment Epithelium Cells. PLoS ONE *7*.

Fu, X.Y., Kessler, D.S., Veals, S.A., Levy, D.E., and Darnell, J.E. (1990). ISGF3, the transcriptional activator induced by interferon alpha, consists of multiple interacting polypeptide chains. Proc. Natl. Acad. Sci. U. S. A. *87*, 8555–8559.

Fuhrer, D.K., and Yang, Y.C. (1996). Complex formation of JAK2 with PP2A, P13K, and Yes in response to the hematopoietic cytokine interleukin-11. Biochem Biophys Res Commun *224*, 289–296.

Fuhrmann, S., Kirsch, M., and Hofmann, H.D. (1995). Ciliary neurotrophic factor promotes chick photoreceptor development in vitro. Development *121*, 2695–2706.

Gao, H., Bryzgalova, G., Hedman, E., Khan, A., Efendic, S., Gustafsson, J.-A., and Dahlman-Wright, K. (2006). Long-term administration of estradiol decreases expression of hepatic lipogenic genes and improves insulin sensitivity in ob/ob mice: a possible mechanism is through direct regulation of signal transducer and activator of transcription 3. Mol. Endocrinol. Baltim. Md *20*, 1287–1299.

Geiszt, M., Witta, J., Baffi, J., Lekstrom, K., and Leto, T.L. (2003). Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense. FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol. *17*, 1502–1504.

Giorgetti-Peraldi, S., Peyrade, F., Baron, V., and Van Obberghen, E. (1995). Involvement of Janus kinases in the insulin signaling pathway. Eur J Biochem *234*, 656–660.

Gough, D.J., Corlett, A., Schlessinger, K., Wegrzyn, J., Larner, A.C., and Levy, D.E. (2009). Mitochondrial STAT3 Supports Ras-Dependent Oncogenic Transformation. Science *324*, 1713–1716.

Goureau, O., Rhee, K.D., and Yang, X.-J. (2004). Ciliary neurotrophic factor promotes muller glia differentiation from the postnatal retinal progenitor pool. Dev. Neurosci. *26*, 359–370.

Gunaje, J.J., and Bhat, G.J. (2001). Involvement of tyrosine phosphatase PTP1D in the inhibition of interleukin-6-induced Stat3 signaling by alpha-thrombin. Biochem. Biophys. Res. Commun. *288*, 252–257.

Guo, D., Dunbar, J.D., Yang, C.H., Pfeffer, L.M., and Donner, D.B. (1998). Induction of Jak/STAT Signaling by Activation of the Type 1 TNF Receptor. J. Immunol. *160*, 2742–2750.

Guschin, D., Rogers, N., Briscoe, J., Witthuhn, B., Watling, D., Horn, F., Pellegrini, S., Yasukawa, K., Heinrich, P., Stark, G.R., et al. (1995). A major role for the protein tyrosine kinase JAK1 in the JAK/STAT signal transduction pathway in response to interleukin-6. EMBO J. *14*, 1421.

Han, Y., Amin, H.M., Franko, B., Frantz, C., Shi, X., and Lai, R. (2006). Loss of SHP1 enhances JAK3/STAT3 signaling and decreases proteosome degradation of JAK3 and NPM-ALK in ALK+ anaplastic large-cell lymphoma. Blood *108*, 2796–2803.

Hennig, A.K., Peng, G.H., and Chen, S. (2013). Transcription coactivators p300 and CBP are necessary for photoreceptor-specific chromatin organization and gene expression. PLoS One *8*, e69721.

Hermanns, H.M., Radtke, S., Haan, C., Leur, H.S.-V. de, Tavernier, J., Heinrich, P.C., and Behrmann, I. (1999). Contributions of Leukemia Inhibitory Factor Receptor and Oncostatin M Receptor to Signal Transduction in Heterodimeric Complexes with Glycoprotein 130. J. Immunol. *163*, 6651–6658.

Hertle, D., Schleichert, M., Steup, A., Kirsch, M., and Hofmann, H.D. (2008). Regulation of cytokine signaling components in developing rat retina correlates with transient inhibition of rod differentiation by CNTF. Cell Tissue Res *334*, 7–16.

Hicks, D., and Courtois, Y. (1992). Fibroblast growth factor stimulates photoreceptor differentiation in vitro. J Neurosci *12*, 2022–2033.

Hoeve, J. ten, Ibarra-Sanchez, M. de J., Fu, Y., Zhu, W., Tremblay, M., David, M., and Shuai, K. (2002). Identification of a Nuclear Stat1 Protein Tyrosine Phosphatase. Mol. Cell. Biol. *22*, 5662–5668.

Horvath, C.M. (2000). STAT proteins and transcriptional responses to extracellular signals. Trends Biochem Sci *25*, 496–502.

Huang, G., Yan, H., Ye, S., Tong, C., and Ying, Q.L. (2013a). STAT3 phosphorylation at tyrosine 705 and serine 727 differentially regulates mouse ES cell fates. Stem Cells.

Huang, P., Huo, Y., Lou, L.X., Li, H., Barnstable, C.J., Zhang, C., and Zhang, S.S. (2013b). CD4 positive T helper cells contribute to retinal ganglion cell death in mouse model of ischemia reperfusion injury. Exp. Eye Res. *115*, 131–139.

Ichiba, M., Nakajima, K., Yamanaka, Y., Kiuchi, N., and Hirano, T. (1998). Autoregulation of the Stat3 gene through cooperation with a cAMP-responsive element-binding protein. J. Biol. Chem. *273*, 6132–6138.

Ihle, J.N. (1995). Cytokine receptor signalling. Nature *377*, 591–594.

Izumi-Nagai, K., Nagai, N., Ozawa, Y., Mihara, M., Ohsugi, Y., Kurihara, T., Koto, T., Satofuka, S., Inoue, M., Tsubota, K., et al. (2007). Interleukin-6 Receptor-Mediated Activation of Signal Transducer and Activator of Transcription-3 (STAT3) Promotes Choroidal Neovascularization. Am. J. Pathol. *170*, 2149–2158.

Jain, N., Zhang, T., Fong, S.L., Lim, C.P., and Cao, X. (1998). Repression of Stat3 activity by activation of mitogen-activated protein kinase (MAPK). Oncogene *17*, 3157–3167.

Ji, J.Z., Elyaman, W., Yip, H.K., Lee, V.W., Yick, L.W., Hugon, J., and So, K.F. (2004). CNTF promotes survival of retinal ganglion cells after induction of ocular hypertension in rats: the possible involvement of STAT3 pathway. Eur J Neurosci *19*, 265–272.

John, S., Sabo, P.J., Thurman, R.E., Sung, M.-H., Biddie, S.C., Johnson, T.A., Hager, G.L., and Stamatoyannopoulos, J.A. (2011). Chromatin accessibility pre-determines glucocorticoid receptor binding patterns. Nat. Genet. *43*, 264–268.

Joly, S., Lange, C., Thiersch, M., Samardzija, M., and Grimm, C. (2008). Leukemia inhibitory factor extends the lifespan of injured photoreceptors in vivo. J Neurosci *28*, 13765–13774.

Kamakura, S., Oishi, K., Yoshimatsu, T., Nakafuku, M., Masuyama, N., and Gotoh, Y. (2004). Hes binding to STAT3 mediates crosstalk between Notch and JAK-STAT signalling. Nat Cell Biol *6*, 547–554.

Karolchik, D., Hinrichs, A.S., Furey, T.S., Roskin, K.M., Sugnet, C.W., Haussler, D., and Kent, W.J. (2004). The UCSC Table Browser data retrieval tool. Nucleic Acids Res. *32*, D493–D496.

Kassen, S.C., Ramanan, V., Montgomery, J.E., T Burket, C., Liu, C.G., Vihtelic, T.S., and Hyde, D.R. (2007). Time course analysis of gene expression during light-induced photoreceptor cell death and regeneration in albino zebrafish. Dev Neurobiol *67*, 1009–1031.

Kassen, S.C., Thummel, R., Campochiaro, L.A., Harding, M.J., Bennett, N.A., and Hyde, D.R. (2009). CNTF induces photoreceptor neuroprotection and Müller glial cell proliferation through two different signaling pathways in the adult zebrafish retina. Exp Eye Res *88*, 1051–1064.

Kataoka, Y., Matsumura, I., Ezoe, S., Nakata, S., Takigawa, E., Sato, Y., Kawasaki, A., Yokota, T., Nakajima, K., Felsani, A., et al. (2003). Reciprocal Inhibition between MyoD and STAT3 in the Regulation of Growth and Differentiation of Myoblasts. J. Biol. Chem. *278*, 44178–44187.

Kelley, M.W., Turner, J.K., and Reh, T.A. (1994). Retinoic acid promotes differentiation of photoreceptors in vitro. Development *120*, 2091–2102.

Kent, W.J. (2002). BLAT—The BLAST-Like Alignment Tool. Genome Res. *12*, 656–664.

Kent, W.J., Sugnet, C.W., Furey, T.S., Roskin, K.M., Pringle, T.H., Zahler, A.M., and Haussler, and D. (2002). The Human Genome Browser at UCSC. Genome Res. *12*, 996–1006.

Kent, W.J., Hsu, F., Karolchik, D., Kuhn, R.M., Clawson, H., Trumbower, H., and Haussler, D. (2005). Exploring relationships and mining data with the UCSC Gene Sorter. Genome Res. *15*, 737–741.

Kent, W.J., Zweig, A.S., Barber, G., Hinrichs, A.S., and Karolchik, D. (2010). BigWig and BigBed: enabling browsing of large distributed datasets. Bioinformatics *26*, 2204–2207.

Kidder, B.L., Yang, J., and Palmer, S. (2008). Stat3 and c-Myc genome-wide promoter occupancy in embryonic stem cells. PloS One *3*, e3932.

Kim, D.J., Tremblay, M.L., and Digiovanni, J. (2010). Protein tyrosine phosphatases, TC-PTP, SHP1, and SHP2, cooperate in rapid dephosphorylation of Stat3 in keratinocytes following UVB irradiation. PloS One *5*, e10290.

Kim, E., Kim, M., Woo, D.-H., Shin, Y., Shin, J., Chang, N., Oh, Y.T., Kim, H., Rheey, J., Nakano, I., et al. (2013). Phosphorylation of EZH2 Activates STAT3 Signaling via STAT3 Methylation and Promotes Tumorigenicity of Glioblastoma Stem-like Cells. Cancer Cell *23*, 839–852.

Kirsch, M., Fuhrmann, S., Wiese, A., and Hofmann, H.D. (1996). CNTF exerts opposite effects on in vitro development of rat and chick photoreceptors. Neuroreport *7*, 697–700.

Kirsch, M., Schulz-Key, S., Wiese, A., Fuhrmann, S., and Hofmann, H. (1998). Ciliary neurotrophic factor blocks rod photoreceptor differentiation from postmitotic precursor cells in vitro. Cell Tissue Res *291*, 207–216.

Kirsch, M., Trautmann, N., Ernst, M., and Hofmann, H.D. (2010). Involvement of gp130-associated cytokine signaling in Müller cell activation following optic nerve lesion. Glia *58*, 768–779.

Kishimoto, T. (1994). Signal transduction through homo- or heterodimers of gp130. Stem Cells Dayt. Ohio *12 Suppl 1*, 37–44; discussion 44–45.

Kortylewski, M., and Yu, H. (2008). Role of Stat3 in suppressing anti-tumor immunity. Curr. Opin. Immunol. *20*, 228–233.

Kortylewski, M., Heinrich, P.C., Mackiewicz, A., Schniertshauer, U., Klingmüller, U., Nakajima, K., Hirano, T., Horn, F., and Behrmann, I. (1999). Interleukin-6 and oncostatin M-induced growth inhibition of human A375 melanoma cells is STAT-dependent and involves upregulation of the cyclin-dependent kinase inhibitor p27/Kip1. Oncogene *18*, 3742–3753.

Koukos, G., Polytarchou, C., Kaplan, J.L., Morley-Fletcher, A., Gras-Miralles, B., Kokkotou, E., Baril-Dore, M., Pothoulakis, C., Winter, H.S., and Iliopoulos, D. (2013). MicroRNA-124 regulates STAT3 expression and is down-regulated in colon tissues of pediatric patients with ulcerative colitis. Gastroenterology *145*, 842–852.e2.

Lai, C.F., Ripperger, J., Morella, K.K., Jurlander, J., Hawley, T.S., Carson, W.E., Kordula, T., Caligiuri, M.A., Hawley, R.G., Fey, G.H., et al. (1996). Receptors for interleukin (IL)-10 and IL-6-type cytokines use similar signaling mechanisms for inducing transcription through IL-6 response elements. J. Biol. Chem. *271*, 13968–13975.

Lapp, D.W., Zhang, S.S., and Barnstable, C.J. (2014). Stat3 mediates LIF-induced protection of astrocytes against toxic ROS by upregulating the UPC2 mRNA pool. Glia *62*, 159–170.

LaVail, M.M., and Sidman, R.L. (1974). C57BL-6J mice with inherited retinal degeneration. Arch. Ophthalmol. *91*, 394–400.

Leibinger, M., Müller, A., Andreadaki, A., Hauk, T.G., Kirsch, M., and Fischer, D. (2009). Neuroprotective and axon growth-promoting effects following inflammatory stimulation on mature retinal ganglion cells in mice depend on ciliary neurotrophic factor and leukemia inhibitory factor. J Neurosci *29*, 14334–14341.

Leibinger, M., Andreadaki, A., Diekmann, H., and Fischer, D. (2013a). Neuronal STAT3 activation is essential for CNTF- and inflammatory stimulation-induced CNS axon regeneration. Cell Death Dis *4*, e805.

Leibinger, M., Müller, A., Gobrecht, P., Diekmann, H., Andreadaki, A., and Fischer, D. (2013b). Interleukin-6 contributes to CNS axon regeneration upon inflammatory stimulation. Cell Death Dis *4*, e609.

Levy, D.E., Kessler, D.S., Pine, R., Reich, N., and Darnell, J.E. (1988). Interferon-induced nuclear factors that bind a shared promoter element correlate with positive and negative transcriptional control. Genes Dev. *2*, 383–393.

Li, X., Cai, Y., Wang, Y.-S., Shi, Y.-Y., Hou, W., Xu, C.-S., Wang, H.-Y., Ye, Z., Yao, L.-B., and Zhang, J. (2012). Hyperglycaemia Exacerbates Choroidal Neovascularisation in Mice via the Oxidative Stress-Induced Activation of STAT3 Signalling in RPE Cells. PLoS ONE *7*.

Liedtke, W., Leman, E.E., Fyffe, R.E.W., Raine, C.S., and Schubart, U.K. (2002). Stathmin-Deficient Mice Develop an Age-Dependent Axonopathy of the Central and Peripheral Nervous Systems. Am. J. Pathol. *160*, 469–480.

Lillien, L., and Cepko, C. (1992). Control of proliferation in the retina: temporal changes in responsiveness to FGF and TGF alpha. Development *115*, 253–266.

Lim, C.P., and Cao, X. (2006). Structure, function, and regulation of STAT proteins. Mol Biosyst *2*, 536–550.

Lin, J.X., and Leonard, W.J. (2000). The role of Stat5a and Stat5b in signaling by IL-2 family cytokines. Oncogene *19*, 2566–2576.

Liu, B., Hunter, D.J., Rooker, S., Chan, A., Paulus, Y.M., Leucht, P., Nusse, Y., Nomoto, H., and Helms, J.A. (2013). Wnt signaling promotes Müller cell proliferation and survival after injury. Invest. Ophthalmol. Vis. Sci. *54*, 444–453.

Liu, L., McBride, K.M., and Reich, N.C. (2005). STAT3 nuclear import is independent of tyrosine phosphorylation and mediated by importin-α3. Proc. Natl. Acad. Sci. U. S. A. *102*, 8150–8155.

Liu, L., Li, H., Li, J., Zhong, H., Zhang, H., Chen, J., and Xiao, T. (2011). miR-125b suppresses the proliferation and migration of osteosarcoma cells through down-regulation of STAT3. Biochem. Biophys. Res. Commun. *416*, 31–38.

Liu, X., Lee, Y.S., Yu, C.-R., and Egwuagu, C.E. (2008). Loss of STAT3 in CD4+ T Cells Prevents Development of Experimental Autoimmune Diseases. J. Immunol. *180*, 6070–6076.

Lufei, C., Ma, J., Huang, G., Zhang, T., Novotny-Diermayr, V., Ong, C.T., and Cao, X. (2003). GRIM-19, a death-regulatory gene product, suppresses Stat3 activity via functional interaction. EMBO J. *22*, 1325–1335.

Lund, I.K., Hansen, J.A., Andersen, H.S., Møller, N.P.H., and Billestrup, N. (2005). Mechanism of protein tyrosine phosphatase 1B-mediated inhibition of leptin signalling. J. Mol. Endocrinol. *34*, 339–351.

Lütticken, C., Wegenka, U.M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., Harpur, A.G., Wilks, A.F., Yasukawa, K., and Taga, T. (1994). Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. Science *263*, 89–92.

Ma, J., and Cao, X. (2006). Regulation of Stat3 nuclear import by importin alpha5 and importin alpha7 via two different functional sequence elements. Cell Signal *18*, 1117–1126.

Ma, J., Zhang, T., Novotny-Diermayr, V., Tan, A.L., and Cao, X. (2003). A novel sequence in the coiled-coil domain of Stat3 essential for its nuclear translocation. J Biol Chem *278*, 29252–29260.

Mahboubi, K., Biedermann, B.C., Carroll, J.M., and Pober, J.S. (2000). IL-11 Activates Human Endothelial Cells to Resist Immune-Mediated Injury. J. Immunol. *164*, 3837–3846.

Maier, K., Rau, C.R., Storch, M.K., Sättler, M.B., Demmer, I., Weissert, R., Taheri, N., Kuhnert, A.V., Bähr, M., and Diem, R. (2004). Ciliary neurotrophic factor protects retinal ganglion cells from secondary cell death during acute autoimmune optic neuritis in rats. Brain Pathol *14*, 378–387.

Maritano, D., Sugrue, M.L., Tininini, S., Dewilde, S., Strobl, B., Fu, X., Murray-Tait, V., Chiarle, R., and Poli, V. (2004). The STAT3 isoforms α and β have unique and specific functions. Nat. Immunol. *5*, 401–409.

Masuhara, M., Sakamoto, H., Matsumoto, A., Suzuki, R., Yasukawa, H., Mitsui, K., Wakioka, T., Tanimura, S., Sasaki, A., Misawa, H., et al. (1997). Cloning and characterization of novel CIS family genes. Biochem. Biophys. Res. Commun. *239*, 439–446.

Meares, G.P., Liu, Y., Rajbhandari, R., Qin, H., Nozell, S.E., Mobley, J.A., Corbett, J.A., and Benveniste, E.N. (2014). PERK-Dependent Activation of JAK1 and STAT3 Contributes to Endoplasmic Reticulum Stress-Induced Inflammation. Mol. Cell. Biol. *34*, 3911–3925.

Minamoto, S., Ikegame, K., Ueno, K., Narazaki, M., Naka, T., Yamamoto, H., Matsumoto, T., Saito, H., Hosoe, S., and Kishimoto, T. (1997). Cloning and functional analysis of new members of STAT induced STAT inhibitor (SSI) family: SSI-2 and SSI-3. Biochem. Biophys. Res. Commun. *237*, 79–83.

Miyakoshi, M., Yamamoto, M., Tanaka, H., and Ogawa, K. (2014). Serine 727 phosphorylation of STAT3: an early change in mouse hepatocarcinogenesis induced by neonatal treatment with diethylnitrosamine. Mol Carcinog *53*, 67–76.

Mouse Genome Sequencing Consortium, Waterston, R.H., Lindblad-Toh, K., Birney, E., Rogers, J., Abril, J.F., Agarwal, P., Agarwala, R., Ainscough, R., Alexandersson, M., et al. (2002). Initial sequencing and comparative analysis of the mouse genome. Nature *420*, 520–562.

Müller, A., Hauk, T.G., and Fischer, D. (2007). Astrocyte-derived CNTF switches mature RGCs to a regenerative state following inflammatory stimulation. Brain *130*, 3308–3320.

Müller, A., Hauk, T.G., Leibinger, M., Marienfeld, R., and Fischer, D. (2009). Exogenous CNTF stimulates axon regeneration of retinal ganglion cells partially via endogenous CNTF. Mol Cell Neurosci *41*, 233–246.

Murano, T., Okamoto, R., Ito, G., Nakata, T., Hibiya, S., Shimizu, H., Fujii, S., Kano, Y., Mizutani, T., Yui, S., et al. (2014). Hes1 promotes the IL-22-mediated antimicrobial response by enhancing STAT3-dependent transcription in human intestinal epithelial cells. Biochem Biophys Res Commun *443*, 840–846.

Naash, M.I., Hollyfield, J.G., al-Ubaidi, M.R., and Baehr, W. (1993). Simulation of human autosomal dominant retinitis pigmentosa in transgenic mice expressing a mutated murine opsin gene. Proc. Natl. Acad. Sci. *90*, 5499–5503.

Nakashima, K., Yanagisawa, M., Arakawa, H., Kimura, N., Hisatsune, T., Kawabata, M., Miyazono, K., and Taga, T. (1999). Synergistic Signaling in Fetal Brain by STAT3-Smad1 Complex Bridged by p300. Science *284*, 479–482.

Nakayama, K., Kim, K.-W., and Miyajima, A. (2002). A novel nuclear zinc finger protein EZI enhances nuclear retention and transactivation of STAT3. EMBO J. *21*, 6174–6184.

Nelson, C.M., Gorsuch, R.A., Bailey, T.J., Ackerman, K.M., Kassen, S.C., and Hyde, D.R. (2012). Stat3 defines three populations of Müller glia and is required for initiating maximal müller glia proliferation in the regenerating zebrafish retina. J Comp Neurol *520*, 4294–4311.

Nelson, C.M., Ackerman, K.M., O'Hayer, P., Bailey, T.J., Gorsuch, R.A., and Hyde, D.R. (2013). Tumor necrosis factor-alpha is produced by dying retinal neurons and is required for Muller glia proliferation during zebrafish retinal regeneration. J Neurosci *33*, 6524–6539.

Nelson, K.L., Rogers, J.A., Bowman, T.L., Jove, R., and Smithgall, T.E. (1998). Activation of STAT3 by the c-Fes Protein-tyrosine Kinase. J. Biol. Chem. *273*, 7072–7077.

Neophytou, C., Vernallis, A.B., Smith, A., and Raff, M.C. (1997). Müller-cell-derived leukaemia inhibitory factor arrests rod photoreceptor differentiation at a postmitotic pre-rod stage of development. Development *124*, 2345–2354.

Ng, D.C.H., Lin, B.H., Lim, C.P., Huang, G., Zhang, T., Poli, V., and Cao, X. (2006). Stat3 regulates microtubules by antagonizing the depolymerization activity of stathmin. J. Cell Biol. *172*, 245–257.

Ng, I.H.W., Ng, D.C.H., Jans, D.A., and Bogoyevitch, M.A. (2012). Selective STAT3-α or -β expression reveals spliceform-specific phosphorylation kinetics, nuclear retention and distinct gene expression outcomes. Biochem. J. *447*, 125–136.

Nishinakamura, R., Matsumoto, Y., Matsuda, T., Ariizumi, T., Heike, T., Asashima, M., and Yokota, T. (1999). Activation of Stat3 by cytokine receptor gp130 ventralizes Xenopus embryos independent of BMP-4. Dev. Biol. *216*, 481–490.

Novak, U., Harpur, A.G., Paradiso, L., Kanagasundaram, V., Jaworowski, A., Wilks, A.F., and Hamilton, J.A. (1995). Colony-stimulating factor 1-induced STAT1 and STAT3 activation is accompanied by phosphorylation of Tyk2 in macrophages and Tyk2 and JAK1 in fibroblasts. Blood *86*, 2948–2956.

Oates, A.C., Wollberg, P., Pratt, S.J., Paw, B.H., Johnson, S.L., Ho, R.K., Postlethwait, J.H., Zon, L.I., and Wilks, A.F. (1999). Zebrafish stat3 is expressed in restricted tissues during embryogenesis and stat1 rescues cytokine signaling in a STAT1-deficient human cell line. Dev. Dyn. *215*, 352–370.

Ogai, K., Kuwana, A., Hisano, S., Nagashima, M., Koriyama, Y., Sugitani, K., Mawatari, K., Nakashima, H., and Kato, S. (2014). Upregulation of leukemia inhibitory factor (LIF) during the early stage of optic nerve regeneration in zebrafish. PloS One *9*, e106010.

Ohnishi, S., Laub, F., Matsumoto, N., Asaka, M., Ramirez, F., Yoshida, T., and Terada, M. (2000). Developmental expression of the mouse gene coding for the krüppel-like transcription factor KLF5. Dev. Dyn. *217*, 421–429.

O'Shea, J.J. (1997). Jaks, STATs, cytokine signal transduction, and immunoregulation: are we there yet? Immunity *7*, 1–11.

Ozawa, Y., Nakao, K., Shimazaki, T., Takeda, J., Akira, S., Ishihara, K., Hirano, T., Oguchi, Y., and Okano, H. (2004). Downregulation of STAT3 activation is required for presumptive rod photoreceptor cells to differentiate in the postnatal retina. Mol Cell Neurosci *26*, 258–270.

Ozawa, Y., Nakao, K., Shimazaki, T., Shimmura, S., Kurihara, T., Ishida, S., Yoshimura, A., Tsubota, K., and Okano, H. (2007). SOCS3 is required to temporally fine-tune photoreceptor cell differentiation. Dev Biol *303*, 591–600.

Ozawa, Y., Nakao, K., Kurihara, T., Shimazaki, T., Shimmura, S., Ishida, S., Yoshimura, A., Tsubota, K., and Okano, H. (2008). Roles of STAT3/SOCS3 pathway in regulating the visual function and ubiquitin-proteasome-dependent degradation of rhodopsin during retinal inflammation. J. Biol. Chem. *283*, 24561–24570.

Patel, A.K., and Hackam, A.S. (2013). Toll-like receptor 3 (TLR3) protects retinal pigmented epithelium (RPE) cells from oxidative stress through a STAT3-dependent mechanism. Mol. Immunol. *54*, 122–131.

Patel, A.K., and Hackam, A.S. (2014). A novel protective role for the innate immunity Toll-Like Receptor 3 (TLR3) in the retina via Stat3. Mol. Cell. Neurosci. *63*, 38–48.

Peng, G.H., and Chen, S. (2007). Crx activates opsin transcription by recruiting HAT-containing co-activators and promoting histone acetylation. Hum Mol Genet *16*, 2433–2452.

Pernet, V., Joly, S., Jordi, N., Dalkara, D., Guzik-Kornacka, A., Flannery, J.G., and Schwab, M.E. (2013). Misguidance and modulation of axonal regeneration by Stat3 and Rho/ROCK signaling in the transparent optic nerve. Cell Death Dis *4*, e734.

Peterson, W.M., Wang, Q., Tzekova, R., and Wiegand, S.J. (2000). Ciliary neurotrophic factor and stress stimuli activate the Jak-STAT pathway in retinal neurons and glia. J Neurosci *20*, 4081–4090.

Piazza, F.A. (2006). Multiple myeloma cell survival relies on high activity of protein kinase CK2. Blood *108*, 1698–1707.

Pilli, S., Zawadzki, R.J., and Telander, D.G. (2014). The dose-dependent macular thickness changes assessed by fd-oct in patients with retinitis pigmentosa treated with ciliary neurotrophic factor. Retina Phila. Pa *34*, 1384–1390.

Pinzon-Guzman, C., Shaomin Zhang, S., and Barnstable, C.J. (2010). Protein kinase C regulates rod photoreceptor differentiation through modulation of STAT3 signaling. Adv Exp Med Biol *664*, 21–28.

Pinzon-Guzman, C., Zhang, S.S., and Barnstable, C.J. (2011). Specific protein kinase C isoforms are required for rod photoreceptor differentiation. J Neurosci *31*, 18606–18617.

Pinzon-Guzman, C., Xing, T., Zhang, S.S.-M., and Barnstable, C.J. (2014). Regulation of Rod Photoreceptor Differentiation by STAT3 Is Controlled by a Tyrosine Phosphatase. J. Mol. Neurosci. 1–8.

Popova, E.Y., Xu, X., DeWan, A.T., Salzberg, A.C., Berg, A., Hoh, J., Zhang, S.S., and Barnstable, C.J. (2012). Stage and gene specific signatures defined by histones H3K4me2 and H3K27me3 accompany mammalian retina maturation in vivo. PloS One *7*, e46867.

Ramage, L., Junne, T., Hahne, K., Lithgow, T., and Schatz, G. (1993). Functional cooperation of mitochondrial protein import receptors in yeast. EMBO J. *12*, 4115.

Ramana, C.V., Gil, M.P., Schreiber, R.D., and Stark, G.R. (2002). Stat1-dependent and -independent pathways in IFN-γ-dependent signaling. Trends Immunol. *23*, 96–101.

Raney, B.J., Dreszer, T.R., Barber, G.P., Clawson, H., Fujita, P.A., Wang, T., Nguyen, N., Paten, B., Zweig, A.S., Karolchik, D., et al. (2014). Track data hubs enable visualization of user-defined genome-wide annotations on the UCSC Genome Browser. Bioinformatics *30*, 1003–1005.

Ray, S., Sherman, C.T., Lu, M., and Brasier, A.R. (2002). Angiotensinogen gene expression is dependent on signal transducer and activator of transcription 3-mediated p300/cAMP response element binding protein-binding protein coactivator recruitment and histone acetyltransferase activity. Mol Endocrinol *16*, 824–836.

Ray, S., Boldogh, I., and Brasier, A.R. (2005). STAT3 NH2-terminal acetylation is activated by the hepatic acute-phase response and required for IL-6 induction of angiotensinogen. Gastroenterology *129*, 1616–1632.

Raz, R., Durbin, J.E., and Levy, D.E. (1994). Acute phase response factor and additional members of the interferon-stimulated gene factor 3 family integrate diverse signals from cytokines, interferons, and growth factors. J. Biol. Chem. *269*, 24391–24395.

Reyes-Aguirre, L.I., Ferraro, S., Quintero, H., Sánchez-Serrano, S.L., Gómez-Montalvo, A., and Lamas, M. (2013). Glutamate-induced epigenetic and morphological changes allow rat Müller cell dedifferentiation but not further acquisition of a photoreceptor phenotype. Neuroscience *254*, 347–360.

Rhee, K.D., Goureau, O., Chen, S., and Yang, X.J. (2004). Cytokine-induced activation of signal transducer and activator of transcription in photoreceptor precursors regulates rod differentiation in the developing mouse retina. J Neurosci *24*, 9779–9788.

Rhee, K.D., Nusinowitz, S., Chao, K., Yu, F., Bok, D., and Yang, X.J. (2013). CNTF-mediated protection of photoreceptors requires initial activation of the cytokine receptor gp130 in Müller glial cells. Proc Natl Acad Sci U A *110*, E4520–E4529.

Rubin Grandis, J., Zeng, Q., and Drenning, S.D. (2000). Epidermal growth factor receptor--mediated stat3 signaling blocks apoptosis in head and neck cancer. The Laryngoscope *110*, 868–874.

Sabo, P.J., Hawrylycz, M., Wallace, J.C., Humbert, R., Yu, M., Shafer, A., Kawamoto, J., Hall, R., Mack, J., Dorschner, M.O., et al. (2004). Discovery of functional noncoding elements by digital analysis of chromatin structure. Proc. Natl. Acad. Sci. U. S. A. *101*, 16837–16842.

Sabo, P.J., Kuehn, M.S., Thurman, R., Johnson, B.E., Johnson, E.M., Cao, H., Yu, M., Rosenzweig, E., Goldy, J., Haydock, A., et al. (2006). Genome-scale mapping of DNase I sensitivity in vivo using tiling DNA microarrays. Nat. Methods *3*, 511–518.

Schaeferhoff, K., Michalakis, S., Tanimoto, N., Fischer, M.D., Becirovic, E., Beck, S.C., Huber, G., Rieger, N., Riess, O., Wissinger, B., et al. (2010). Induction of STAT3-related genes in fast degenerating cone photoreceptors of cpfl1 mice. Cell Mol Life Sci *67*, 3173–3186.

Schindler, C., and Darnell, J.E. (1995). Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. Annu Rev Biochem *64*, 621–651.

Schindler, C., Fu, X.Y., Improta, T., Aebersold, R., and Darnell, J.E. (1992). Proteins of transcription factor ISGF-3: one gene encodes the 91-and 84-kDa ISGF-3 proteins that are activated by interferon alpha. Proc. Natl. Acad. Sci. U. S. A. *89*, 7836–7839.

Sekine, Y., Tsuji, S., Ikeda, O., Sato, N., Aoki, N., Aoyama, K., Sugiyama, K., and Matsuda, T. (2006). Regulation of STAT3-mediated signaling by LMW-DSP2. Oncogene *25*, 5801–5806.

Sieving, P.A., Caruso, R.C., Tao, W., Coleman, H.R., Thompson, D.J.S., Fullmer, K.R., and Bush, R.A. (2006). Ciliary neurotrophic factor (CNTF) for human retinal degeneration: Phase I trial of CNTF delivered by encapsulated cell intraocular implants. Proc. Natl. Acad. Sci. U. S. A. *103*, 3896–3901.

Smith, P.D., Sun, F., Park, K.K., Cai, B., Wang, C., Kuwako, K., Martinez-Carrasco, I., Connolly, L., and He, Z. (2009). SOCS3 deletion promotes optic nerve regeneration in vivo. Neuron *64*, 617–623.

Snyder, M., Huang, X.-Y., and Zhang, J.J. (2008). Identification of Novel Direct Stat3 Target Genes for Control of Growth and Differentiation. J. Biol. Chem. *283*, 3791–3798.

Stahl, N., Farrugella, T.J., Boulton, T.G., Zhong, Z., Jr., J.E.D., and Yancopoulos, G.D. (1995). Choice of STATs and Other Substrates Specified by Modular Tyrosine-Based Motifs in Cytokine Receptors. Science *267*, 1349–1353.

Starr, R., Willson, T.A., Viney, E.M., Murray, L.J.L., Rayner, J.R., Jenkins, B.J., Gonda, T.J., Alexander, W.S., Metcalf, D., Nicola, N.A., et al. (1997). A family of cytokine-inducible inhibitors of signalling. Nature *387*, 917–921.

Stephanou, A., Knight, R.A.L., and D, S. (2009). STAT1 and STAT3: Opposing roles in Cell Death and Cell Cycle Regulation. JAK-STAT Pathway in Disease (Texas: Landes Bioscience).

Sun, S., and Steinberg, B.M. (2002). PTEN is a negative regulator of STAT3 activation in human papillomavirus-infected cells. J. Gen. Virol. *83*, 1651–1658.

Sun, F., Park, K.K., Belin, S., Wang, D., Lu, T., Chen, G., Zhang, K., Yeung, C., Feng, G., Yankner, B.A., et al. (2011). Sustained axon regeneration induced by co-deletion of PTEN and SOCS3. Nature *480*, 372–375.

Takeda, K., Noguchi, K., Shi, W., Tanaka, T., Matsumoto, M., Yoshida, N., Kishimoto, T., and Akira, S. (1997). Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality. Proc Natl Acad Sci U A *94*, 3801–3804.

Talcott, K.E., Ratnam, K., Sundquist, S.M., Lucero, A.S., Lujan, B.J., Tao, W., Porco, T.C., Roorda, A., and Duncan, J.L. (2011). Longitudinal Study of Cone Photoreceptors during Retinal Degeneration and in Response to Ciliary Neurotrophic Factor Treatment. Invest. Ophthalmol. Vis. Sci. *52*, 2219–2226.

Tian, S.S., Lamb, P., Seidel, H.M., Stein, R.B., and Rosen, J. (1994). Rapid activation of the STAT3 transcription factor by granulocyte colony-stimulating factor. Blood *84*, 1760–1764.

Turkson, J., Bowman, T., Adnane, J., Zhang, Y., Djeu, J.Y., Sekharam, M., Frank, D.A., Holzman, L.B., Wu, J., Sebti, S., et al. (1999). Requirement for Ras/Rac1-Mediated p38 and c-Jun N-Terminal Kinase Signaling in Stat3 Transcriptional Activity Induced by the Src Oncoprotein. Mol. Cell. Biol. *19*, 7519–7528.

Ueki, Y., Wang, J., Chollangi, S., and Ash, J.D. (2008). STAT3 activation in photoreceptors by leukemia inhibitory factor is associated with protection from light damage. J Neurochem *105*, 784–796.

Ueki, Y., Le, Y.Z., Chollangi, S., Muller, W., and Ash, J.D. (2009). Preconditioning-induced protection of photoreceptors requires activation of the signal-transducing receptor gp130 in photoreceptors. Proc Natl Acad Sci U A *106*, 21389–21394.

Uemura, A., Takizawa, T., Ochiai, W., Yanagisawa, M., Nakashima, K., and Taga, T. (2002). CARDIOTROPHIN-LIKE CYTOKINE INDUCES ASTROCYTE DIFFERENTIATION OF FETAL NEUROEPITHELIAL CELLS VIA ACTIVATION OF STAT3. Cytokine *18*, 1–7.

Ushijima, R., Sakaguchi, N., Kano, A., Maruyama, A., Miyamoto, Y., Sekimoto, T., Yoneda, Y., Ogino, K., and Tachibana, T. (2005). Extracellular signal-dependent nuclear import of STAT3 is mediated by various importin alphas. Biochem Biophys Res Commun *330*, 880–886.

Vaisse, C., Halaas, J.L., Horvath, C.M., Darnell, J.E., Stoffel, M., and Friedman, J.M. (1996). Leptin activation of Stat3 in the hypothalamus of wild–type and ob/ob mice but not db/db mice. Nat. Genet. *14*, 95–97.

Veeriah, S., Brennan, C., Meng, S., Singh, B., Fagin, J.A., Solit, D.B., Paty, P.B., Rohle, D., Vivanco, I., Chmielecki, J., et al. (2009). The tyrosine phosphatase PTPRD is a tumor suppressor that is frequently inactivated and mutated in glioblastoma and other human cancers. Proc. Natl. Acad. Sci. *106*, 9435–9440.

Vignais, M.L., Sadowski, H.B., Watling, D., Rogers, N.C., and Gilman, M. (1996). Platelet-derived growth factor induces phosphorylation of multiple JAK family kinases and STAT proteins. Mol. Cell. Biol. *16*, 1759–1769.

Villasenor, A., Wang, Z.V., Rivera, L.B., Ocal, O., Asterholm, I.W., Scherer, P.E., Brekken, R.A., Cleaver, O., and Wilkie, T.M. (2010). Rgs16 and Rgs8 in embryonic endocrine pancreas and mouse models of diabetes. Dis. Model. Mech. *3*, 567–580.

Wakahara, R., Kunimoto, H., Tanino, K., Kojima, H., Inoue, A., Shintaku, H., and Nakajima, K. (2012). Phospho-Ser727 of STAT3 regulates STAT3 activity by enhancing dephosphorylation of phospho-Tyr705 largely through TC45. Genes Cells *17*, 132–145.

Wang, D.Y., Ray, A., Rodgers, K., Ergorul, C., Hyman, B.T., Huang, W., and Grosskreutz, C.L. (2010). Global Gene Expression Changes in Rat Retinal Ganglion Cells in Experimental Glaucoma. Invest. Ophthalmol. Vis. Sci. *51*, 4084–4095.

Wang, Y., Smith, S.B., Ogilvie, J.M., McCool, D.J., and Sarthy, V. (2002). Ciliary neurotrophic factor induces glial fibrillary acidic protein in retinal Müller cells through the JAK/STAT signal transduction pathway. Curr Eye Res *24*, 305–312.

Wegenka, U.M., Buschmann, J., Lütticken, C., Heinrich, P.C., and Horn, F. (1993). Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. Mol. Cell. Biol. *13*, 276–288.

Wegrzyn, J., Potla, R., Chwae, Y.-J., Sepuri, N.B.V., Zhang, Q., Koeck, T., Derecka, M., Szczepanek, K., Szelag, M., Gornicka, A., et al. (2009). Function of Mitochondrial Stat3 in Cellular Respiration. Science *323*, 793–797.

Wen, Z., and Darnell, J.E. (1997). Mapping of Stat3 serine phosphorylation to a single residue (727) and evidence that serine phosphorylation has no influence on DNA binding of Stat1 and Stat3. Nucleic Acids Res *25*, 2062–2067.

Wen, Z., Zhong, Z., and Darnell, J.E. (1995). Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. Cell *82*, 241–250.

Wishingrad, M.A., Koshlukova, S., and Halvorsen, S.W. (1997). Ciliary Neurotrophic Factor Stimulates the Phosphorylation of Two Forms of STAT3 in Chick Ciliary Ganglion Neurons. J. Biol. Chem. *272*, 19752–19757.

Woetmann, A., Nielsen, M., Christensen, S.T., Brockdorff, J., Kaltoft, K., Engel, A.-M., Skov, S., Brender, C., Geisler, C., Svejgaard, A., et al. (1999). Inhibition of protein phosphatase 2A induces serine/threonine phosphorylation, subcellular redistribution, and functional inhibition of STAT3. Proc. Natl. Acad. Sci. *96*, 10620–10625.

Wong, M., Li, Y., Li, S., Zhang, S., Li, W., Zhang, P., Chen, C., Barnstable, C.J., Zhang, S.S., Zhang, C., et al. (2014). Therapeutic Retrobulbar Inhibition of STAT3 Protects Ischemic Retina Ganglion Cells. Mol. Neurobiol.

Wurster, A.L., Tanaka, T., and Grusby, M.J. (2000). The biology of Stat4 and Stat6. Oncogene *19*, 2577–2584.

Xia, X., Li, Y., Huang, D., Wang, Z., Luo, L., Song, Y., Zhao, L., and Wen, R. (2011). Oncostatin M protects rod and cone photoreceptors and promotes regeneration of cone outer segment in a rat model of retinal degeneration. PLoS One *6*, e18282.

Xia, X., Wen, R., Chou, T.-H., Li, Y., Wang, Z., and Porciatti, V. (2014). Protection of Pattern Electroretinogram and Retinal Ganglion Cells by Oncostatin M after Optic Nerve Injury. PLoS ONE *9*, e108524.

Xiao, H., Chung, J., Kao, H.-Y., and Yang, Y.-C. (2003). Tip60 is a co-repressor for STAT3. J. Biol. Chem. *278*, 11197–11204.

Xie, H.Q., and Adler, R. (2000). Green cone opsin and rhodopsin regulation by CNTF and staurosporine in cultured chick photoreceptors. Invest Ophthalmol Vis Sci *41*, 4317–4323.

Xie, M.-H., Aggarwal, S., Ho, W.-H., Foster, J., Zhang, Z., Stinson, J., Wood, W.I., Goddard, A.D., and Gurney, A.L. (2000). Interleukin (IL)-22, a Novel Human Cytokine That Signals through the Interferon Receptor-related Proteins CRF2–4 and IL-22R. J. Biol. Chem. *275*, 31335–31339.

Yamamoto, T., Sekine, Y., Kashima, K., Kubota, A., Sato, N., Aoki, N., and Matsuda, T. (2002). The nuclear isoform of protein-tyrosine phosphatase TC-PTP regulates interleukin-6-mediated signaling pathway through STAT3 dephosphorylation. Biochem. Biophys. Res. Commun. *297*, 811–817.

Yan, S., Zhou, C., Zhang, W., Zhang, G., Zhao, X., Yang, S., Wang, Y., Lu, N., Zhu, H., and Xu, N. (2008). beta-Catenin/TCF pathway upregulates STAT3 expression in human esophageal squamous cell carcinoma. Cancer Lett. *271*, 85–97.

Yanagi, Y., Masuhiro, Y., Mori, M., Yanagisawa, J., and Kato, S. (2000). p300/CBP acts as a coactivator of the cone-rod homeobox transcription factor. Biochem Biophys Res Commun *269*, 410–414.

Yang, J., Chatterjee-Kishore, M., Staugaitis, S.M., Nguyen, H., Schlessinger, K., Levy, D.E., and Stark, G.R. (2005). Novel Roles of Unphosphorylated STAT3 in Oncogenesis and Transcriptional Regulation. Cancer Res. *65*, 939–947.

Yang, J., Liao, X., Agarwal, M.K., Barnes, L., Auron, P.E., and Stark, G.R. (2007). Unphosphorylated STAT3 accumulates in response to IL-6 and activates transcription by binding to NF?B. Genes Dev. *21*, 1396–1408.

Yang, J., Huang, J., Dasgupta, M., Sears, N., Miyagi, M., Wang, B., Chance, M.R., Chen, X., Du, Y., Wang, Y., et al. (2010). Reversible methylation of promoter-bound STAT3 by histone-modifying enzymes. Proc. Natl. Acad. Sci. U. S. A. *107*, 21499–21504.

Yoo, J.-Y., Huso, D.L., Nathans, D., and Desiderio, S. (2002). Specific Ablation of Stat3β Distorts the Pattern of Stat3-Responsive Gene Expression and Impairs Recovery from Endotoxic Shock. Cell *108*, 331–344.

Yu, C.-R., Lee, Y.S., Mahdi, R.M., Surendran, N., and Egwuagu, C.E. (2012). Therapeutic Targeting of STAT3 (Signal Transducers and Activators of Transcription 3) Pathway Inhibits Experimental Autoimmune Uveitis. PLoS ONE *7*, e29742.

Yuan, T., Wang, Y., Zhao, Z.J., and Gu, H. (2010). Protein-tyrosine Phosphatase PTPN9 Negatively Regulates ErbB2 and Epidermal Growth Factor Receptor Signaling in Breast Cancer Cells. J. Biol. Chem. *285*, 14861–14870.

Yuan, Z.L., Guan, Y.J., Chatterjee, D., and Chin, Y.E. (2005). Stat3 dimerization regulated by reversible acetylation of a single lysine residue. Science *307*, 269–273.

Al Zaid Siddiquee, K., and Turkson, J. (2008). STAT3 as a target for inducing apoptosis in solid and hematological tumors. Cell Res *18*, 254–267.

Zein, W.M., Jeffrey, B.G., Wiley, H.E., Turriff, A.E., Tumminia, S.J., Tao, W., Bush, R.A., Marangoni, D., Wen, R., Wei, L.L., et al. (2014). CNGB3-Achromatopsia Clinical Trial With CNTF: Diminished Rod Pathway Responses With No Evidence of Improvement in Cone Function. Invest. Ophthalmol. Vis. Sci. *55*, 6301–6308.

Zhang, C., Li, H., Liu, M.G., Kawasaki, A., Fu, X.Y., Barnstable, C.J., and Shao-Min Zhang, S. (2008). STAT3 activation protects retinal ganglion cell layer neurons in response to stress. Exp Eye Res *86*, 991–997.

Zhang, J., Chen, F., Li, W., Xiong, Q., Yang, M., Zheng, P., Li, C., Pei, J., and Ge, F. (2012). 14-3-3ζ interacts with stat3 and regulates its constitutive activation in multiple myeloma cells. PloS One *7*, e29554.

Zhang, K., Hopkins, J.J., Heier, J.S., Birch, D.G., Halperin, L.S., Albini, T.A., Brown, D.M., Jaffe, G.J., Tao, W., and Williams, G.A. (2011). Ciliary neurotrophic factor delivered by encapsulated cell intraocular implants for treatment of geographic atrophy in age-related macular degeneration. Proc. Natl. Acad. Sci. *108*, 6241–6245.

Zhang, S., Li, W., Wang, W., Zhang, S.S., Huang, P., and Zhang, C. (2013). Expression and activation of STAT3 in the astrocytes of optic nerve in a rat model of transient intraocular hypertension. PloS One *8*, e55683.

Zhang, S.S., Wei, J.Y., Li, C., Barnstable, C.J., and Fu, X.Y. (2003). Expression and activation of STAT proteins during mouse retina development. Exp Eye Res *76*, 421–431.

Zhang, S.S., Wei, J., Qin, H., Zhang, L., Xie, B., Hui, P., Deisseroth, A., Barnstable, C.J., and Fu, X.Y. (2004). STAT3-mediated signaling in the determination of rod photoreceptor cell fate in mouse retina. Invest Ophthalmol Vis Sci *45*, 2407–2412.

Zhang, S.S., Li, H., Huang, P., Lou, L.X., Fu, X.Y., and Barnstable, C.J. (2010). MAPK signaling during Müller glial cell development in retina explant cultures. J Ocul Biol Infor *3*, 129–133.

Zhang, S.S.-M., Fu, X.-Y., and Barnstable, C.J. (2002). Tissue culture studies of retinal development. Methods *28*, 439–447.

Zhang, X., Guo, A., Yu, J., Possemato, A., Chen, Y., Zheng, W., Polakiewicz, R.D., Kinzler, K.W., Vogelstein, B., Velculescu, V.E., et al. (2007). Identification of STAT3 as a substrate of receptor protein tyrosine phosphatase T. Proc. Natl. Acad. Sci. *104*, 4060–4064.

Zhong, Z., Wen, Z., and Darnell, J.E. (1994). Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science *264*, 95–98.

Zhou, X., Smith, A.J., Waterhouse, A., Blin, G., Malaguti, M., Lin, C.Y., Osorno, R., Chambers, I., and Lowell, S. (2013). Hes1 desynchronizes differentiation of pluripotent cells by modulating STAT3 activity. Stem Cells *31*, 1511–1522.

Zhu, S., Wang, Z., Zhang, Z., Wang, J., Li, Y., Yao, L., Mei, Q., and Zhang, W. (2014). PTPLAD2 is a tumor suppressor in esophageal squamous cell carcinogenesis. FEBS Lett. *588*, 981–989.

Zong, C., Yan, R., August, A., Darnell, J.E., and Hanafusa, H. (1996). Unique signal transduction of Eyk: constitutive stimulation of the JAK-STAT pathway by an oncogenic receptor-type tyrosine kinase. EMBO J. *15*, 4515–4525.

Zong, C.S., Zeng, L., Jiang, Y., Sadowski, H.B., and Wang, L.-H. (1998). Stat3 Plays an Important Role in Oncogenic Ros- and Insulin-like Growth Factor I Receptor-induced Anchorage-independent Growth. J. Biol. Chem. *273*, 28065–28072.