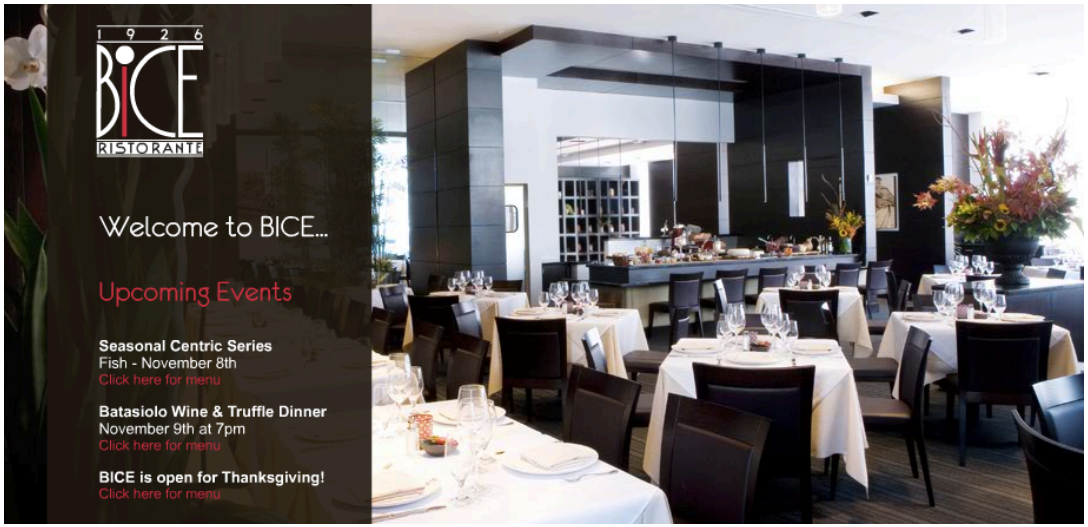
Program Project Grant (PPG)

PPG grant mechanisms are designed to support multidisciplinary research programs with a well-defined central research focus. These grants support a minimum of three interrelated projects that contribute to the program objective. The grants may also include support for common or shared resources (cores and equipment). Interrelationships among component projects are expected to result in a greater contribution to the program goals than if each project were pursued separately.

Budget: up to $1,000,000 / year
Period: up to 5 years
Renewable

Dinner at BiCE

Tuesday November 15, 2010
(during SfN, San Diego)



Stefan Pulst

Ilya Bezprozvanny

& Mee Whi Kim

Tom Otis

& Meera Pratap

Dan Scoles

Adebimpe Kasumu
"Ade"

People we discussed: Marie Francoise, Olaf Reiss, Chris Gomez, Hank Paulsen, Massimo Pandolfo, Yukio Saijo, Erik Jorgensen, Ed Dudek

PPG Working Group Meeting

February 18-20 (Friday – Sunday)

Day 1 Friday:

Friday 9:00 – 11:00, General Introduction:

Pulst	Ataxia & SCAs
Bezprozvanny	Ca^{++}-signaling and neurodegeneration
Otis	Cerebellar physiology
Mee Whi Kim	Structural analysis of PolyQ proteins

Friday 11:00 – 12:30, In-Depth Postdoc Presentations:

Sharan Paul	High-Throughput-Screen
Sharan Paul	Q127 line, biochemical profiling of mice; functional motor analysis genetic interaction of CACNA1A and SCA2 alleles
Sharan Paul	BAC mice
Sharan Paul	iPS cells
Sharan Paul	PC physiology in SCA2 transgenics and knockouts
Sharan Paul	UPDB and movement disorders

Friday 12:30 – 1:30, Lunch:

PPG Working Group Meeting

February 18-20 (Friday – Sunday)

Friday Afternoon:

Detailed discussions on the PPG structure

Project 1: Pulst / Genetic interactions of *ATXN2* and related genes
Project 2: Bezprozvanny / Pathogenic interactions of mutant Ataxin-2 with InsP3R in SCA2
Project 3: Otis / PC electrophysiology of *ATXN2* mutants and intercrosses
Project 4: Kim / Structural analysis of wild type and mutant Ataxin-2
Animal Core: Scoles
Administrative Core: Pulst

Saturday:

Skiing

Progress on a manuscript describing the ATXN2 promoter

Hypothesis

ATXN2 promoter analysis can lead to ways that we can exploit *ATXN2* expression control that might aid in development of therapeutics for SCA2.

Purpose

- To increase understanding on *ATXN2* expression control.
- To accumulate deletion constructs that would allow us to evaluate compounds targeting *ATXN2* expression.

Method

- Clone (ATXN2 promoter)-luciferase-(ATXN2 3'-UTR).
- Modify the construct by creating deletions and evaluate expression…
- …leading to the identification of features important for ATXN2-luc expression.
- Evaluate those features to create a story relevant to SCA2.

Findings

- Multiple inhibitory elements & no evidence for enhancer elements.
- Promoter activity in the 5'-UTR and an ETS consensus sequence (100% match).
- Evidence for one site in the 3'-UTR that supports RNA stability in two cell lines.
- Evidence for site of action by native miRNA in HEK293 cells only.
- Expression in mouse cerebellum higher than other brain regions.

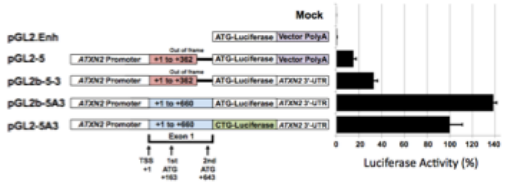
Problems delaying progress

- Assay stopped working… problem resolved last week.
- Relevance of ETS factors…making a story

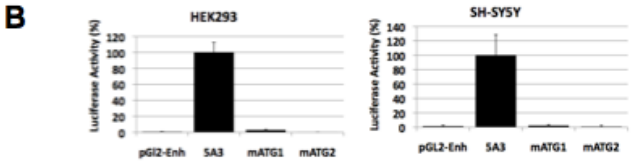
All luciferase assays in our study, as seen from space

(Luciferase assays are incomplete because we had to stop and re-optimize transfection conditions)

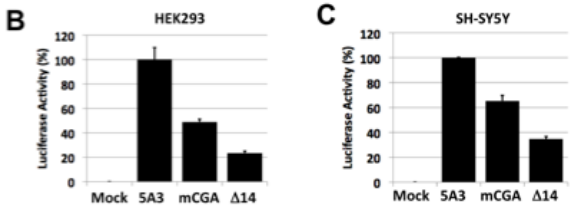
Creating the main construct



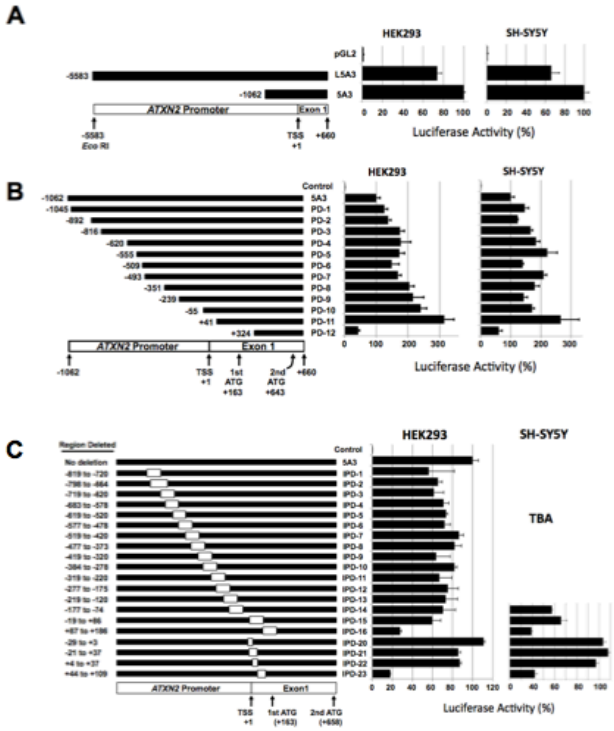
Start Codon mutants



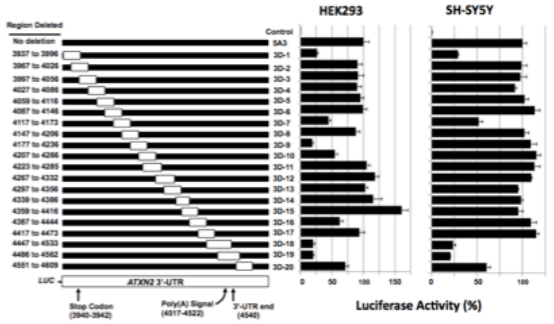
Mutants in ETS region



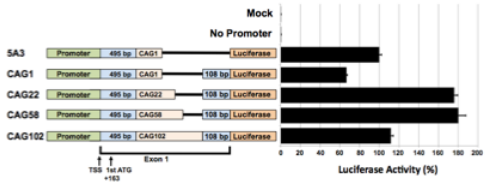
Promoter Region



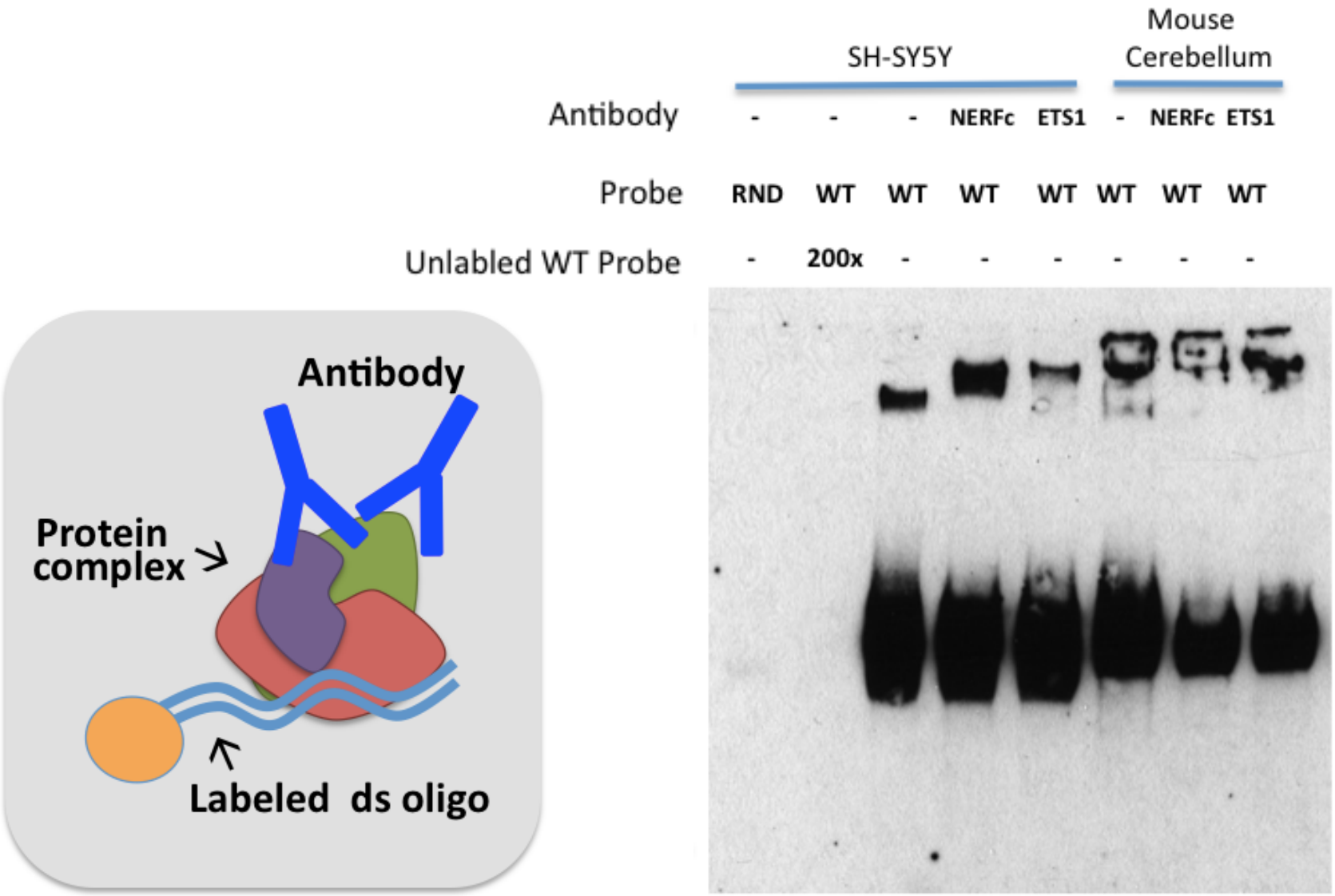
3’-UTR



PolyQs (might put in different ms)



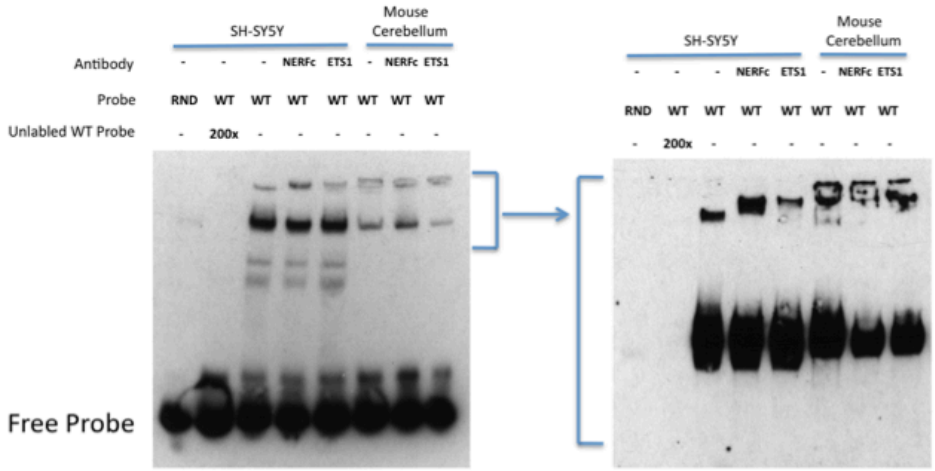
EMSA supershift assays

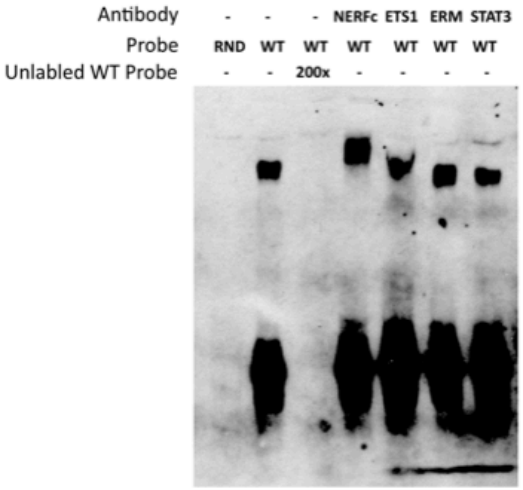


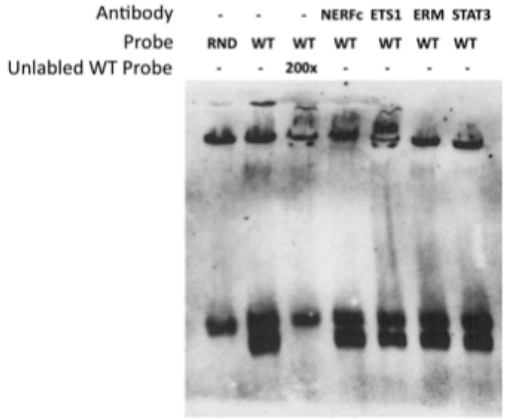
Many of KK's recent EMSA supershift assays

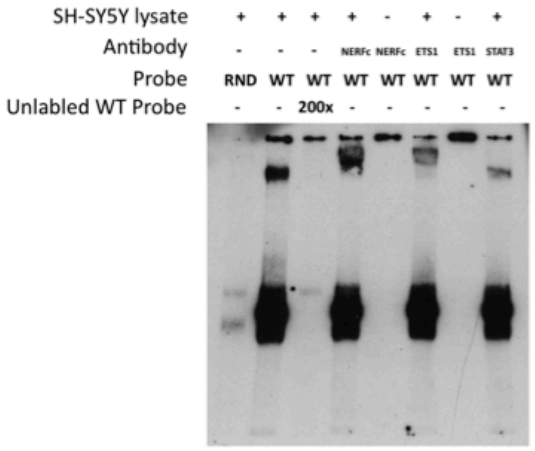
ETS consensus sequence according to B. Graves: CCGGAAGT "proximal redundant"
ETS binding site in *ATXN2*: CCGGAAGT = 100% identity

Note, Barbara Graves published a ChIP-seq experiment (Hollenhorst et al., 2009) showing ETS promoter elements occurring close to the transcription start site (+/- 500 bp) (like in ATXN2) have consensus CCGGAAGT and bind ETS1 ELF1 GABPA (proximal redundant) while those upstream are enhancer elements of sequence CAGGATGT and specifically bind ETS1. CBP (ETS cofactor) which is gaining lots of attention nowadays is more commonly found on the enhancer sites. I saw a lecture yesterday by Jesse Gray (Harvard Postdoc in Michael Greenberg lab) discussing how enhancer TFs bind RNApol-II then the DNA folds and delivers the RNApol-II to the promoter.









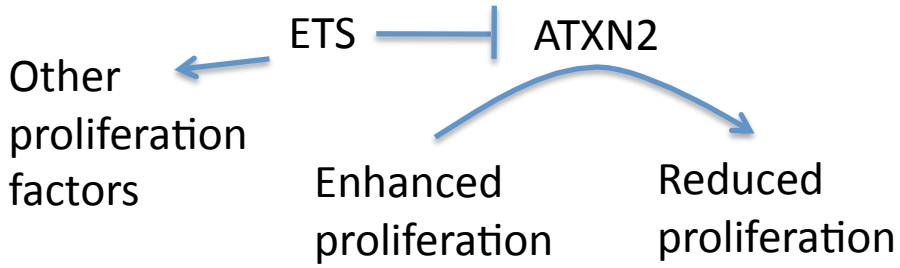
Problem of relevance for ETS factors

Our preliminary data shows that overexpression of ELF2 or ETS1 resulted in reduced ATXN2-luc expression while underexpression of ELF2 or ETS1 resulted in increased ATXN2-luc expression.

This needs verification… but if true you wouldn't want to use ETS factors therapeutically because they are oncogenic (patients would get cancer if you increase their expression).

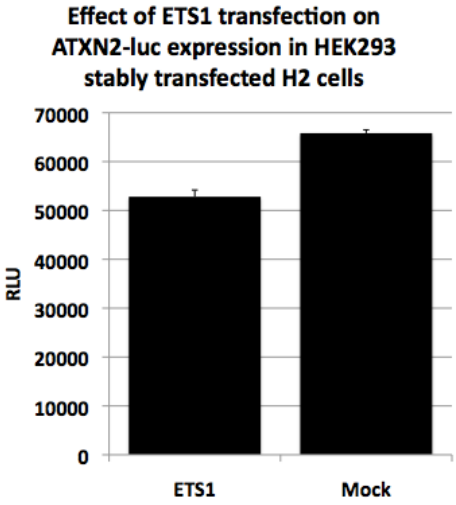
Barbara Graves says it doesn't matter because we are not testing in the correct cell line. This argument would suggest everything we do in HEK293 is irrelevant... ??

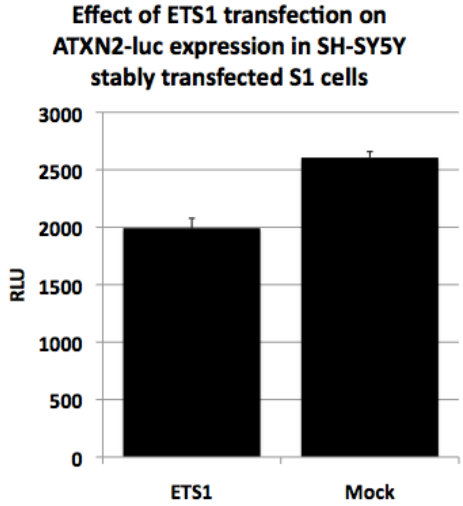
We know ATXN2 expression tends to reduce proliferation, so this could be the model:

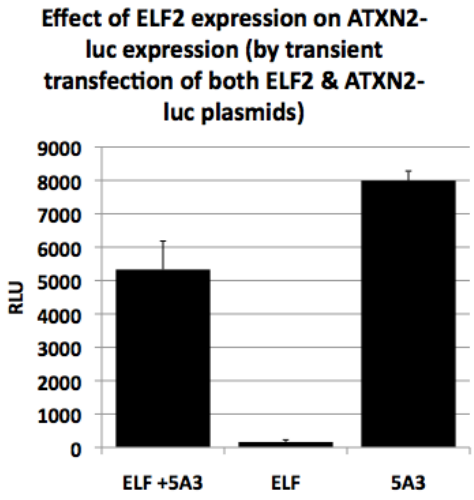


We are seeking other ways to evaluate the other EMSA bands that appear for this probe

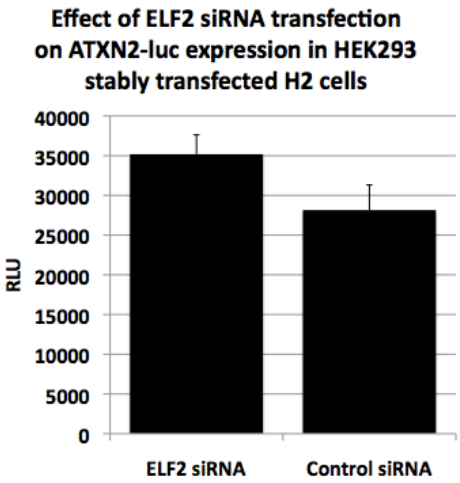
OVER-ESPRESSION OF ETS1 OR ELF2 REDUCES ATXN2-LUC EXPRESSION

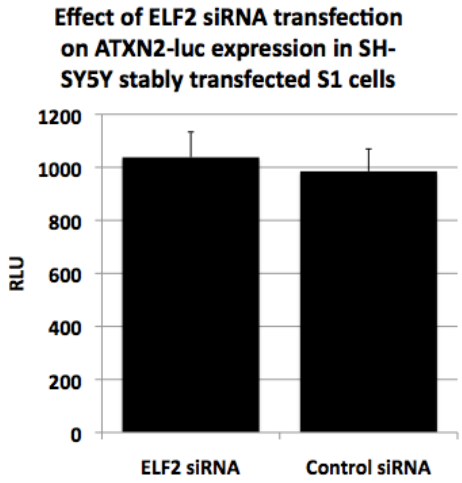






UNDER-EXPRESSION OF ELF2 INCREASES ATXN2-LUC EXPRESSION





HYPOTHESIS:

ETS factors inhibit ATXN2 expression by preventing other factors from binding this very same element to turn up ATXN2-luc expression.