

# Kroll lab overview: Regulating cell fate, proliferation, and differentiation in the developing nervous system

## Summary

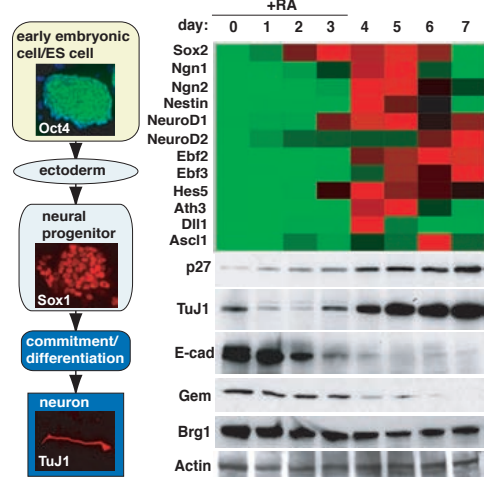
We are interested in understanding how embryonic and embryonic stem (ES) cells regulate self-renewal, lineage commitment, and differentiation and are studying these questions in the context of vertebrate neural development. Stepwise transcriptional cascades convert pluripotent embryonic cells into neurons: multipotent neural precursors form, commit to neuronal lineages, and then undergo cell cycle withdrawal and neuronal differentiation. We use both mouse ES cells and mouse and *Xenopus* embryos to study these processes.

A major focus of our work is on chromatin regulatory proteins, including the SWI-SNF and Polycomb complexes and the novel protein Geminin. These complexes critically regulate transcription to control self-renewal and differentiation in multiple cell contexts. They also regulate cell cycle progression and maintain genome integrity and their dysregulation is a pivotal aspect of multiple human malignancies. Our current efforts include:

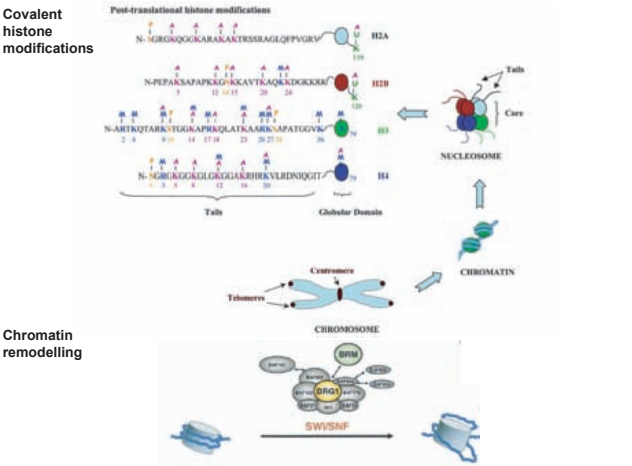
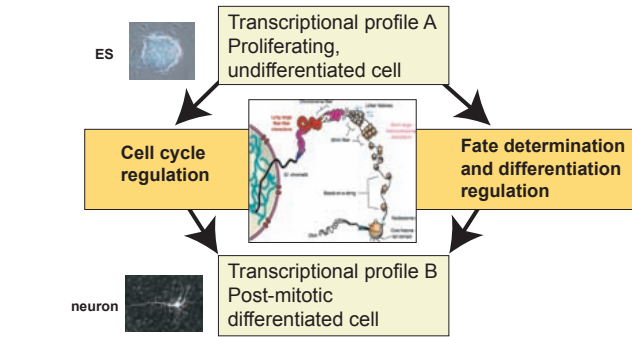
**Transcriptional regulatory networks in neural development.** 1. identifying direct targets and regulatory enhancers used by several key bHLH transcription factors to regulate neuronal commitment, 2. determining how neural precursor-specific gene expression is transcriptionally regulated in early embryos and ES cells, and 3. Using genomic and function-based screens to isolate novel regulators of neurogenesis.

**Gem, SWI-SNF, and Polycomb interplay regulating transcription in embryonic and ES cells.** We are defining how interactions between these complexes regulate transcription by defining direct transcriptional targets, composition of the protein complexes involved, and mechanisms of action at the chromatin level. We are also assessing how this is integrated with control of cell cycle progression and genome stability/euploidy, to determine how this occurs in normal cells and define its contributions to malignancy.

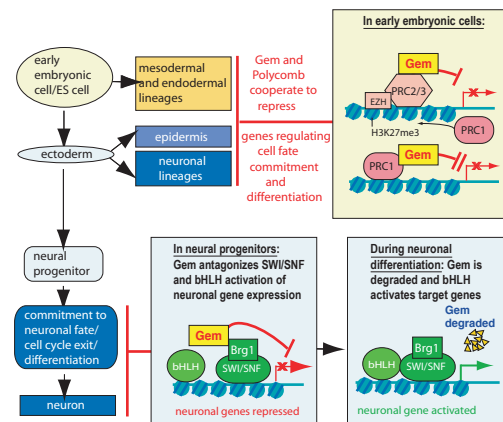
## Making embryonic and ES cells into neurons



## Regulation of chromatin structure: a central hub for controlling and integrating cell state changes during differentiation



## Model: interactions between the Polycomb and SWI/SNF complexes and the novel Geminin protein regulate transcription to control neural cell fate and differentiation



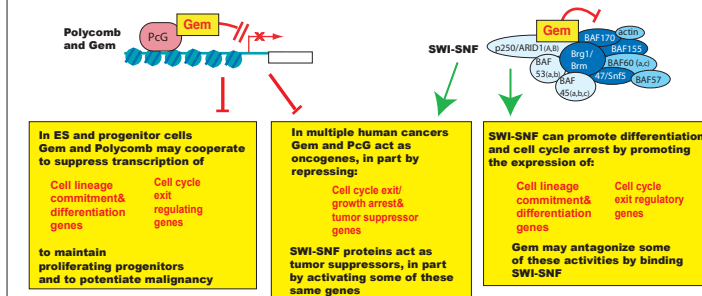
## Experimental questions and goals:

- What is the composition of the Gem-Polycomb and Gem-SWI/SNF complexes in ES and precursor cells?
- What is the mechanism through which Gem regulates transcription at the chromatin level?
- What are the transcriptional targets of Gem, SWI/SNF and PcG in regulating neural fate and neuronal differentiation?
- What are the roles of Gem-PcG and Gem-SWI/SNF interactions in regulating neural cell fate and differentiation?

## Balancing proliferation and differentiation in normal development and in cancer.

## Model: Gem, Polycomb and SWI-SNF interact to coordinate transcriptional control of cell fate and differentiation with cell cycle control

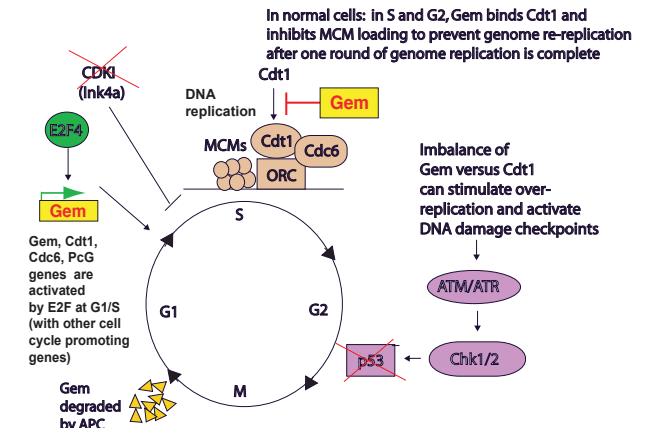
In normal development:	proliferation and self-renewal	growth arrest and differentiation
In malignancy:	oncogenic activities	tumor suppressor activities



## Experimental questions and goals:

- What specific roles do Gem-Polycomb and Gem-SWI/SNF interactions play in regulating the cell cycle, self-renewal and arrest, in normal developmental contexts and in malignancy?
- What are key transcriptional targets of Gem, SWI/SNF and PcG in normal cell cycle control and in malignancy?

## Geminin's double life: In addition to regulating transcription, Geminin maintains the fidelity of DNA replication and genome stability by antagonizing the DNA replication initiation regulator Cdt1

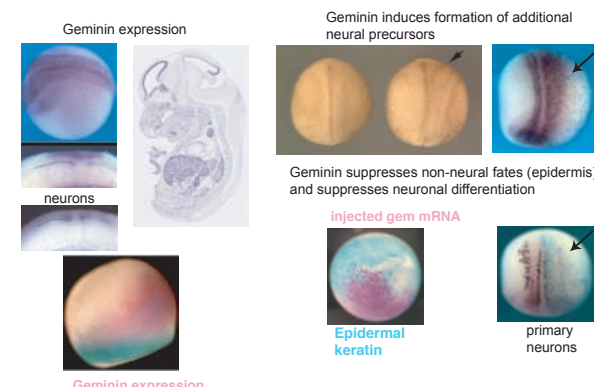


## Experimental questions and goals:

- In malignancy, cells with imbalances of Gem versus Cdt1 levels may re-replicate their DNA and become aneuploid/acquire genomic instability. In combination with other mutational events (inactivation of checkpoints like p53 and ARF as shown) could these cells escape arrest and apoptosis to contribute to malignancy?
- Are Gem's roles in regulating both replication and transcription linked through Gem's effects on chromatin structure?

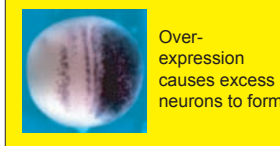
## What is Geminin?

- Novel nuclear protein
- Regulates transcription to control cell fate and differentiation and regulates DNA replication
- Binds the replication initiation protein Cdt1 and the chromatin regulatory Polycomb and SWI-SNF complexes
- Highly expressed in proliferating ES and progenitor cells and down-regulated before differentiation



## Defining transcriptional regulatory networks controlling neural development

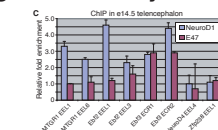
### Neurogenin and NeuroD Key bHLH transcription factors regulating neural development



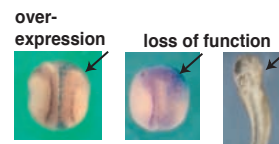
### What are their direct transcriptional targets?

Arrays identify direct targets involved in regulation of neurogenesis-example:

Ngn and NeuroD bind regulatory elements in target genes in embryonic brain

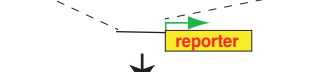
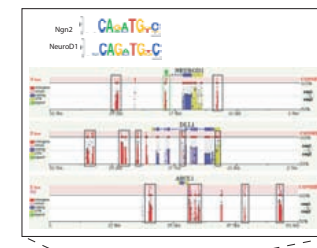


### Identifying additional novel genes required for neural development: example



What regulatory element signature allows Ngn and NeuroD to recognize their targets involved in neural development?

Cross-species locus comparisons, whole genome computational screens functional assays in mouse ES and *Xenopus*



transgenic expression from regulatory element

endogenous expression of gene

