Why do cells in cultures stop dividing even in the presence of growth factors and fresh medium? What is the mechanism that “counts” cell divisions? How would you attempt to circumnavigate this issue? Describe a strategy/experiment that can be used or help in understanding this problem.

At each division there is a progressive shortage of the cell’s telomere which is a cap, a DNA sequence (same sequence repeated few times)- protecting the chromosome at the end of each chromosome. Eventually when the telomeres are gone the cell stops dividing (Hayflick’s limit).

In human, a cell can divide 25-50 times before stopping. To circumvent this problem, we can bypass the checkpoint mechanisms checking the

The enzyme telomerase slows down this clock by reversing telomere shortening.

Telomerase has its own RNA molecules which it uses to bind to the last telomere

sequence on the chromosome, add a new telomere repeat sequence, realign the

telomere with the template and this process is eventually reproduced. We can use CRISPR/cas9 technology to study telomere shortening and lengthening in some cancers, telomere and telomerase editing.

A new mutation in the APP gene has been found associated with early onset of Alzheimer’s Disease. What model would you choose and why to study the impact of such mutation on APP metabolism?

Amyloid precursor protein (APP) is processed sequentially by the β-site APP cleaving enzyme and γ-secretase to generate amyloid β (Aβ) peptides, one of the hallmarks of Alzheimer's disease. In the APP hypothesis, there is an increase of bet amyloid production and decrease of amyloid breakdown: APP starts accumulating creating senile plaques and neurofibrillary tangles which block neurotransmitter in synapses. Having an animal model to study the impact of the mutation of the APP could provide an insight to link it to AD. We can CRISPR/Cas 9 raplace this gene in a mice with the mutated version and express it using Cre recombinase. Then we can perform CAT scan inaging to study brain atrophy, structure abnormalities, and submit the mice to behavioral tests compared to Wild Type mice to asses motor, and sensory functions, learning and memory , social beahviors , anxiety and depression behaviors.

Propose a model to study cellular senescence and apply this model to the study of aging.

In this model we propose to evaluate the effect oof calory restriction on aging process. We have two groups of fish nothobranchius Furzeri which is a fish which has a life span of 3-7 months. The first group have young and older fish and the same for the second group. Within each group, we divide the groups into 3 groups: one which are fed 3 times a day, regular portions, another one which experiences intermittent fasting: 18 hours-2 hours feeding and within these 2 hours only twice fed with regular portions and the last group which are fed only one regular portion of food. We keep monitoring these fish until the fish naturally pass away.

When developing a genetic modified organism or cell line what are the 3 main ways and relative techniques that can be used to change gene function? Provide examples (bullet points are ok).

The three main ways and relative technique that can be used to change a gene function are:

siRNA

CRISPR/cas9

TALENs

Knock In: the targeted gene is replaced with gene of interest

Knock Out: the targeted gene is deleted

Transgenetic mice: a new gene is added

What would you choose as a model of peripheral regeneration and why?

Axons can be regenerated in the PNS under favorable conditions. I will select as animal model mice with neurotmesis as even it is a more involved and difficult surgery and needs a microsurgery to reattach the stumps, it allows an easier study when comparing different experiment groups. With this animal model we will cut the sciatic nerve as it is the longest nerve in the nervous system. We will then use optogenetics as we want to selectively stimulate dorsal root ganglion (DRG) nerves. An experimental group of mice will be injected with AAV-Chr2 targeting the DRG neurons which will be stimulated upon light activation. We will divide our mice into two groups the optogenetically stimulated mice and wild-type mice which will undergo the same surgery. We will follow an established schedule to stimulate the DRG neurons and monitor the experiment group and control group looking for the expression of neurotrophic factors in the experiment groups (BDNF, NGF). After few months we will submit the two groups to motor and sensory tests and compare the performances of the two groups on these tests.

Provide an example of a genetic-encoded probe to study cellular function and discuss how you would apply that to cells in culture.

We want to investigate mutation of Huntington ’ s disease which is a genetic disease caused by a mutation of the Huntington gene and how this affects calcium signaling. We use Camgaroo, GEI probe which was specifically designed to measure calcium concentration levels. It is a protein obtained by fusing YFP with calmodulin binding domain which is the protein binding Ca2+. We will inject AAV-Camgaroo virus in the cells with modified HTT gene using CRISPR/Cas 9 technique and control cells. Upon light excitation, when calcium is expressed, the probe becomes fluorescent and emit on a specific wavelength. We then measure Ca2+ levels and determine if HTT mutated gene modifies calcium signaling.

In order to improve regeneration in the CNS what would you target, why and how?

CNS neurons after injury nerves are severed or damaged and need to regenerate. I will use transgenic factors with engineered neurotrophic factors such BNDF or NGF and overexpress in the mice for the research to increase neuronal survival.

How would you study neuronal activity in the brain in vivo? Elaborate and provide an example of an experiment.