SISSA PhD Course in Functional and Structural Genomics
academic year 2018-19, admission exam - spring session, written test
SISSA Main building, room 04
May 3rd, 2018

QUESTIONS

(1) Mice harboring a truncating nonsense mutation in the middle of X-gene cds display a selective reduction of the number of astrocytes residing in their neocortex. Neurons and oligodendrocytes are unaffected. Please propose possible *cellular* mechanisms underlying this phenomenon as well as a simple experimental strategy to discriminate among them. Suggest how would you prove that the transcription factor encoded by the wt X-gene keeps the astrocyte number sufficiently low and propose a simple molecular mechanism accounting for these data.

(2) Tail-DNA PCR-genotyping of three putative transgenic founder mice, A, B and C, confirmed the presence of the intended transgene. Subsequent qRT-PCR profiling of a small sample of their hindlimb skin at postnatal day 20 showed that this transgene was robustly expressed, as expected. The three mice (and their transgenic descendants) were repeatedly backcrossed to wildtype individuals, to propagate the transgene in vivo. At each generation, transgenic progenies were recognized by tail-DNA PCR. Disappointingly, "founder A" did not generate any F1 PCR-positive progenies. Unexpectedly, "founder C" F3 transgenic descendant, selected by tail-DNA PCR, showed no skin transgene expression. Please provide possible explanations for these problems and - when possible - suggest how fixing/preventing them.

(3) Is autophagy involved in neurodegenerative diseases?

(4) List and describe which neurodegenerative diseases share molecular neuropathological mechanisms similar to prion diseases

(5) Mouse mutants A and B are homozygous for two distinct synonimous mutations, falling within the cds of the same voltage-gated channel subunit, within codons x and y, respectively. Immunofluoresce analysis of wild type brains by a monoclonal antibody which specifically recognizes this subunit gives an intense signal, exactly where expected. The same assay, run on the two mutant brains, A and B, does not give any signal at all. Western blot analysis of neocortical tissue lysates gives a strong, specific band at the right position in case A, a faint band in case B. Please propose possible mechanisms accounting for these results and suggest an experimental strategy to distinguish among them.

(6) Our mutant mouse models harbor only one functional copy of an evolutionarily conserved gene expressed in all main neural neocortical cell types. This leads to a dramatic increase of neocortical excitability. We intend to reconstruct molecular mechanisms resulting in this phenotype. For this purpose, we want to start from transcriptome fluctuations, triggered by a standard pharmacological treatment in each main neural cell type of mixed dissociated cultures (glutamatergic neurons, gabaergic neurons, astrocytes, oligodendrocytes), at different tme

points. Because of presumptive fast dynamics of phenomena investigated, RNA from distinct cell types should be harvested as fast as possible. Different approaches can be conceived.... how would you proceed?

(7) Why do proteins involved in neurodegenerative diseases misfold?

(8) Current pharmacological treatments for neurodegenerative diseases.

(9) Induced pluripotent stem cells: derivation, maintenance, in vivo correlates, fields of use.

(10) Advantages of single cell transcriptomics versus conventional cell population transcriptomics.

(11) Analyzing sequencing data mapped on the genome from DNA of a single human postmortem brain you find that, in a unique specific genomic location, the reads mapped display the presence of 3 different nucleotides: A, T and C. The A results to be present in about 48% of the reads, the T is present in 47% of reads while the C is present only in 5% of reads mapping on that position. The coverage of the region is very high and all the reads with errors were removed; therefore these nucleotides are real and their frequencies are not an error and/or an artifact. How can you explain this result? What A, T and C represent?

(12) Split reads and discordant reads. Explain what they are, why are they useful and which kind of variations they can identify.

(13) Why is comparative genomics useful in the identification of potentially functional non-coding regions? What is the function of many of the conserved non-coding regions?

(14) Explain the reasons why transposable elements are believed to be at the basis of the birth of many long non-coding RNAs.

(15) The gene XYZ is transcribed from the locus xyz in a fish species living in two different places: the Indian Ocean and the Southern Ocean. The sequence of the transcript for the gene XYZ is almost identical between fishes from the two places; there is only a single nucleotide difference: in position 39 of the transcript the species from the Indian Ocean has a G while the species from the Southern Ocean has an A. However, when researchers sequence the genome for the xyz locus both the groups of fishes show to have an A in that position. How can you explain this difference at the RNA level and not in DNA?

(16) Describe the life-cycle of autonomous and non-autonomous non-LTR retrotransposons.

(17) Conditional control of transgene expression.

(18) Viral vectors.