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Mouse embryonic stem (ES) cells are self-renewing cells and that maintain pluripotency to differentiate into all types of cells. Their pluripotency is properly maintained in the presence of Leukemia Inhibitory Factor (LIF) in conventional culture condition without feeder cells. *Oct3/4* is known to play an important role in mouse ES cells to maintain pluripotency (Niwa, 2007). To analyze the molecular mechanism behind self-renewal of ES cells and identify new candidate genes as downstream targets of Oct3/4, we previously manipulated Oct3/4 expression level in ES cells and carried out expression profiling by microarray analysis (Matoba et al., 2006). Forced-expression of *Tbx3*, which is one of these candidate genes, in mouse ES cells allow to form stem cell colonies in the culture media without LIF as found in the case of Nanog. To confirm the maintenance of pluripotency in these cells forming stem cell colonies, we used reversible *Tbx3* expression system, which is based Cre-loxP system, and chimeric mice analysis (Ogawa et al., 2007). In the result, these cells maintained by forced expression of *Tbx3* without LIF were capable to produce chimeric mice. These result suggest that Tbx3 is involved in molecular network for maintain the pluripotency of mouse ES cells. Now, we try to examine the effect of forced expression of Tbx3 on self-renewal of human ES cells.

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doi:10.1016/j.ydbio.2007.03.575

Program/Abstract # 276
Influence of brain environment on proliferation of neuronal progenitors

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Neuronal progenitors (NP) might be used in therapy of neurodegenerative diseases in ageing with neuronal loss linked to amyloidosis-β, e.g. in Down's syndrome (DS), however, the influence of the brain environment altered by the ageing and disease on neurogenesis is not clear. Recently we found that amyloid-β peptide (Aβ) impairs development of human NP in culture. Now we tested how proliferation of NP is affected by extracts from hippocampus–the neurogenic area in adult brain–prepared from 3- and 9-month-old mice: control and APP-transgenic, that are a model of amyloidosis-β. NP were isolated from 3-day-old control mice and cultured for 24 h or 72 h with hippocampus extracts (20 mg proteins per ml). Proliferation of NP was tested by the BrdU incorporation test and with Ab Ki67. Development of amyloidosis-β and neurogenesis *in situ* in hippocampus were estimated by WB with Abs against Musashi, Sox2 and Ab. Preliminary results show that proliferation of NP in short cultures was stimulated by extracts from young control and young and old transgenic mice, i.e. without and with amyloidosis-β, respectively. In prolonged cultures all extracts except for those from transgenic mice with amyloidosis-β reduced NP proliferation. The results suggest that age- and disease-associated alterations in local environment influence neurogenesis in hippocampus. The supportive influence of the factors present locally on NP proliferation is reduced in ageing while hippocampus affected with amyloidosis-β contains factors that prompt neurogenesis in ageing hippocampus. Further studies on NP maturation may explain the lack of spontaneous neuroregeneration in AD and DS. Supported by NYS OMRDD.

doi:10.1016/j.ydbio.2007.03.576

Program/Abstract # 277
Histamine affects cell proliferation, apoptosis and differentiation of cerebro cortical neural stem cells

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Histamine (HA) is one of the first neurotransmitters to appear in the developing central nervous system (CNS). Between rat embryonic days (E) 13–18, HA and its forming enzyme L-histidine decarboxylase are detected in the mesencephalon. By E15, histaminergic fibers reach the cerebral cortex. HA concentration is several-fold higher during development than in mature CNS. This rise correlates in time with high rates of neurogenesis. We performed *in vitro* experiments with E14 neural stem cells (NSC) from rat cerebral cortex to study the effect of HA on proliferation, differentiation and cell death. Cells were proliferated in the presence of FGF-2, followed by 6 days of differentiation. In order to know if NSC express HA receptors, RNA was isolated, and histaminergic receptors were amplified by RT-PCR in both proliferative and differentiated cells. Treatments with increasing amounts of HA (100 nM up to 1 mM) showed, by cresyl violet and BrdU incorporation assays, an increase on cell proliferation, and decreased apoptotic cell number as measured by TUNEL. In contrast, HA increased, in a concentration-dependent manner ($EC_{50}=13\pm0.2$ mM), apoptotic cell death after differentiation. Immunocytochemical experiments showed a significant increase in the number of MAP2-positive neurons from 9% to 21%, and a decrease of GFAP-positive glial cells from 37% to 22% after differentiation. Our data show that NSC express HA receptors, that HA regulates cell number during both

proliferative and differentiative conditions and that this biogenic amine also affects cell fate *in vitro*.

Supported by DGAPA (IN226703), UNAM.

doi:10.1016/j.ydbio.2007.03.577

Program/Abstract # 278
Embryonic stem cell-derived precursors but not neurosphere cells efficiently differentiate to dopaminergic neurons in the embryonic midbrain

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Neural Precursor Cells (NPCs) have proven to be a source of the dopaminergic neurons population affected during Parkinson's disease. However, dopaminergic neuron differentiation in vitro is relatively low and the identification of NPCs with high plasticity to generate dopaminergic neurons remain to be done. Here, we demonstrate that NPCs isolated from the embryonic midbrain produced many mature neurons and site-specifically differentiated to dopaminergic neurons after reintegration into the ventral midbrain. However, midbrain NPCs expanded in vitro as neurospheres produced few neurons in the midbrain and did not differentiate to dopaminergic neurons. By contrast, NPCs derived from Embryonic Stem Cells (ESCs) strongly differentiated to mature neurons in the embryonic midbrain. Surprisingly, neuralization was not required for abundant neuronal differentiation of ESCs-derived precursors after integration into the embryonic midbrain. More importantly, neurogenic ESCs-derived precursors generated many dopaminergic neurons exclusively when located at the site of endogenous dopaminergic neuron differentiation in the midbrain. These data indicate that neurosphere culture causes dramatic changes in the differentiation potential of neurogenic NPCs, while ESCs-derived precursors efficiently respond to midbrain neurogenic signals and differentiate to dopaminergic neurons.

Supported by IMPULSA/UNAM, DGAPA/UNAM and Conacyt.

doi:10.1016/j.ydbio.2007.03.578

Program/Abstract # 279
Regulation of progesterone and estrogen α receptors expression during differentiation of mouse embryonic stem cells to dopamine neurons

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Embryonic stem cells (ESC) possess the ability to differentiate into nerve cells, including dopamine (DA) neurons, which upon transplantation revert the motor signs of Parkinsonian animals. Ovarian steroid hormones estradiol and progesterone (P), play important roles in development and reproduction in mammals. Parkinson's disease is more frequent in men than in women. This suggests that sexual hormones could play a role in DA neuron survival and differentiation. We investigated the expression pattern of P and estrogen (E) receptors at the protein level during the 5-stage protocol of DA neuronal differentiation of mouse ESC, by Western blot and immunocytochemistry. The expression of the transcription factor Oct-4 indicated that ESC were in a pluripotent state in stage 1. The neural stem cell marker nestin was expressed on stage 4, and neurons positive for tyrosine hydroxylase (TH) were detected at the end of the procedure. P receptor isoforms A and B content augmented in stage 5 relative to stage 1, and E receptor a suffered a reduction when neural precursors and DA neurons were present. In addition, we found that 92% of DA neurons expressed PR and only 19% of these neurons co-expressed TH and E receptor a. We also found that 100 nM estradiol increased the number of TH+ cells. These results show that estradiol influence DA differentiation of ESC.

doi:10.1016/j.ydbio.2007.03.579

Program/Abstract # 280
Differentiation of aldynoglia from multipotential neural precursors. Microarrays analysis

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CNS in mammals exhibits well defined regions in which axonal reparation arise spontaneously. In those areas, a particular glial phenotype, the aldynoglia, promotes neuronal growth and regeneration. The prototype of this central glia is the ensheating cells from the olfactory bulb. Since factors that regulate the production of new cells from multipotential neural precursors (MNP) have only recently started to be recognized, we are interested in the differentiation mechanisms of the aldynoglia phenotype from the MNP. In this work we induced *in vitro* the differentiation of embryonic MNP towards the aldynoglia phenotype. For the analysis of the genetic expression, we hybridized microarrays between undifferentiated MNP and MNP that were differentiated in vitro for 24 h. Less than 2% of the 5,000 analyzed genes modified its mRNA expression in a significant level (>2.5 times) after the differentiation was accomplished. We analyzed 84 genes in detail. In differentiated MNP cells 61 genes increased their expression, 65% are genes related directly to cellular differentiation processes, principally ARF3, cytokeratin18, EGAP, nucleophosmin1, prolactin receptor, HNF4a, BMPrIa, Mash1 and TNF2. Only 23 genes diminished their expression with the differentiation, mainly cell cycle regulatory proteins as cyclin D1, and others related with the leave of the undifferentiated phenotype, mainly fancc, POU3f3 and Sam68. The results of these analyses are a starting point to the study of the genes involved in the induction of the differentiation of aldynoglia from MNP.

doi:10.1016/j.ydbio.2007.03.580