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POSTER SESSIONS

CSI-01 – Cell Cycle & Checkpoints

SUN-001
Adaptations of the DNA replication licensing process in mouse embryonic stem cells

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Mouse Embryonic Stem Cells (mESCs) have the ability to proliferate and self-renew indefinitely in culture and, when stimulated, to differentiate towards all three germ layers. They exhibit an unusual cell cycle which consists mainly of S phase cells, a short G1 phase and a lack of major checkpoint responses. Maintaining genome stability is pivotal for embryonic stem cells, as they give rise to all mature cell types. In order for committed cells to maintain genome integrity, DNA replication is restricted to only once per cell cycle. This is accomplished through the assembly onto chromatin of multisubunit protein complexes which license DNA for a subsequent round of replication. Replication licensing takes part in G1 phase and consists of the loading of the hexameric MiniChromosome Maintenance 2-7 (MCM2-7) complex onto chromatin, a step which is dependent on the licensing factor Cdt1. We previously showed that the loading of MCM2-7 onto chromatin takes place in two waves in live mammalian cells: upon mitotic exit and at the G1/S phase transition.

We are interested to understand the molecular mechanisms which ensure genome stability in mESCs, and govern DNA replication licensing, and how these are compared to differentiated and cancer cells. We show that mESCs have a very short G1 phase and move to S-phase with high synchrony following mitotic arrest. During S-phase, replication factories are visualized by immunofluorescence against the replication protein PCNA. They show characteristic early, middle and late S-phase localization, reminiscent of replication factory dynamics in differentiated and cancer cells. Cdt1 is specifically detected during the G1 phase of the mESC cell cycle and degraded following entry to S-phase. MCM2 and MCM7 can be immunodetected onto chromatin following mitotic exit and in S phase, and exhibit differential localization along the cell cycle. Following DNA damage by UV-irradiation in G1, Cdt1 is rapidly proteolysed, while changes in chromatin-loaded MCM proteins are evident. When mESCs are irradiated during mitosis, Cdt1 degradation is delayed until entry into G1, suggesting the Cdt1 is protected from proteolysis in mitosis. mESCs irradiated during mitosis or in G1 fail to progress to S-phase. We will be using functional live-cell imaging to assess licensing in mESCs by Fluorescence Recovery After Photobleaching (FRAP) of the GFP-tagged MCM protein subunits, through the cell cycle of uncommitted cells, or when cells are moving towards differentiation, with a concomitant lengthening of G1 phase.

Keywords: DNA replication licensing, mouse embryonic stem cells.

SUN-002
Analysis of the mechanism of DNA damage and replication arrest-induced histone mRNA decay

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Histone mRNA decay (HD) is the process which ensures that histone mRNA is rapidly degraded following completion of DNA replication at the end of S-phase. Strict coordination between histone protein production and DNA replication is essential for the correct packaging of newly replicated DNA, as imbalances can lead to deleterious effects such as genomic instability. Interestingly, histone mRNA stability is controlled by the presence of a stem-loop structure at the 3' end of histone mRNA, and a protein, HBP/SLBP (hairpin/stem loop binding protein) which specifically binds to this structure, plays a major role in histone mRNA metabolism. Importantly, previous studies have shown HD is also one functional target of an intra S-phase checkpoint activated when DNA synthesis is inhibited, ensuring that histone mRNA is rapidly destroyed when global DNA replication is blocked. Consistent with this, replication stress-induced histone mRNA decay is blocked in the presence of inhibitors of the PIKK family of checkpoint protein kinases, implicating PIKK family members in the regulation of this process. Therefore, we aim to utilise a proteomic approach to to identify HBP/SLBP-associated proteins and post-translational status in order to elucidate the detailed mechanism of checkpoint activated HD. We have established isogenic cell lines stably expressing functional, tagged HBP/SLBP by using the Flp-In™ T-Rex™ Expression system. Our results indicate that isogenic Flp-In HeLa cell lines inducibly expressing tagged forms of SLBP under the control of a doxycycline promoter are a useful model system for the molecular analysis of SLBP function during replication stress.

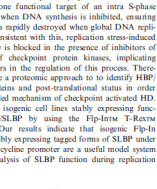

Fig. 1.

Keywords: hairpin/stem loop binding protein (HBP/SLBP), histone mRNA decay, Intra S-phase checkpoint.

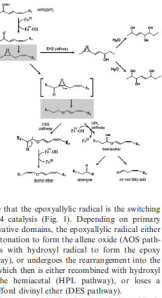

Fig. 1.

mutagenesis indicate that the epoxyallylic radical is the switching point of the CYP74 catalysis (Fig. 1). Depending on primary sequences of conservative domains, the epoxyallylic radical either undergoes the deprotonation to form the allene oxide (AOS pathway), or recombines with hydroxyl radical to form the epoxy alcohol (EAS pathway), or undergoes the rearrangement into the vinyl ether radical, which then is either recombined with hydroxyl radical to afford the hemiacetal (HPL pathway), or loses a hydrogen atom to afford divinyl ether (DES pathway).

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Keywords: Catalytic mechanisms, site-directed mutagenesis, The CYP74 enzymes.

WED-077
Synergistic effect of minerals and low-intensity electromagnetic field on fibroblasts proliferation

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Background: The beneficial actions of warm crystals and low-intensity electromagnetic field (EMF) on human body are well known. A resonance environment that includes at least one mineral / crystal will support the transition of electromagnetic radiation in a favorable frequency range for repairing immediately adjacent tissue. Crystals can transmit vibrational energies that have biological effects. Such a device would be beneficial for facial skin (counteracting effect on wrinkles), creating stability for dental implants and generally by stimulating the regenerative capacity.

The aim of this study was to investigate the influence on cell proliferation of different mineral compositions (aragonite, topaz) placed at the interface between the EMF generating device and outside the culture dish. The beneficial effect is more remarkable as there is no direct contact with the target cells.

Method and results: The cellular model is based on human dermal fibroblasts Hs27; they are the predominant cells to synthesize extracellular matrix, with special importance both in the medical and cosmetic fields.

CSIV-04 – Modelling biological processes

The electronic apparatus used is based on a device for generating a sinusoidal frequency with precision and stability.

In the action area of EMF, above and below the plate culture were applied various types of mineral powders (topaz and aragonite). On the same culture plate a "control" area was preserved, kept only under the influence of the electromagnetic field. Also, another control culture plate was prepared in the same conditions, but unexposed either to EMF or minerals influences. Fibroblasts were exposed to EMF and minerals for 3 days, 2 h per day. After this, the cells viability was measured using MTS assay. In the case of cells exposed both to minerals and EMF the proliferation rate was higher (7–12%) and extreme statistically significant than in the case of cells exposed only to EMF (5–10%), compared to unexposed control.

Conclusions: Although the beneficial effects of EMF and crystals are well known, official recognition and full acceptance requires more solid experimental data. The proposed method is non-invasive and risk-free because it stimulates the physiological mechanism of healing, opening opportunities for further exploitation in regenerative medicine.

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Keywords: EMF, fibroblasts, MTS assay.

WED-079
TDP-43 inclusion bodies formed in bacteria are structurally amorphous, non-amyloid and inherently toxic to neuroblastoma cells

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Accumulation of ubiquitin-positive, tau- and a-synuclein-negative intracellular inclusions of TDP-43 in the central nervous system represents the major hallmark correlated to amyotrophic lateral sclerosis and frontotemporal lobar degeneration with ubiquitin-positive inclusions. Such inclusions have variably been described as amorphous aggregates or more structured deposits having an amyloid structure. Following the observations that bacterial inclusion bodies generally consist of amyloid aggregates, we have overexpressed full-length TDP-43 and C-terminal TDP-43 in *E. coli*, purified the resulting full-length and C-terminal TDP-43 containing inclusion bodies (FL and Ct TDP-43 IBs) and subjected them to biophysical analyses to assess their structure/morphology. We show that both FL and Ct TDP-43 aggregates contained in the bacterial IBs do not bind amyloid dyes such as thioflavin T and Congo red, possess a disordered secondary structure, as inferred using circular dichroism and infrared spectroscopies, and are susceptible to proteinase K digestion, thus possessing none of the hallmarks for amyloid. Moreover, atomic force microscopy revealed an irregular structure for both types of TDP-43 IBs and confirmed the absence of amyloid-like species after proteinase K treatment. Cell biology experiments showed that FL TDP-43 IBs were able to impair the viability of cultured neuroblastoma cells when added to their extracellular medium and, more modestly, when transfected into their cytosol, where they are at least in part ubiquitinated and phosphorylated. These data reveal an inherently high propensity of TDP-43 to form amorphous aggregates, which possess, however, an inherently high ability to cause cell dysfunction. This indicates that a gain of toxic function caused by TDP-43 deposits is effective in TDP-43 pathologies, in addition to possible loss of function mechanisms originating from the cellular mistrafficking of the protein.

Keywords: None.