REPORT for MD70 Experiment – 24/7/04

1. Introduction

Previous experiments (Corde et al. Cancer Res. 2003) have shown that synchrotron photoactivation of cis-platinum (PAT-Plat) consists in an excess of DNA single- and double-strand breaks, probably due to an excess of radiation dose delivered to the close vicinity of DNA. However, nature of physical events that deliver excess of energy (Auger electrons, photoelectrons, …) and molecular mechanisms involved in PAT-Plat effect are still to be determined.
DNA is organised in *nucleosomes* (histone proteins H2, H3, H4 + DNA tours) and *linkers* (less condensed DNA held by histone protein H1). Litterature suggests that cis-platinum binds preferentially to linkers and H1 protein (Yaneva et al, 1997, PNAS). During PAT-Plat, Auger and photo-electrons may therefore deliver energy excess from linker to nucleosomes, that may consist in producing a number of small fragments whose length may correspond to DNA rounds in nucleosome (see figure).
There is a number of experimental evidence that increasing concentrations of salt deplete progressively DNA from histone proteins. In this experiment, we proposed to investigate the PAT-Plat effect *in vitro* on DNA depleted from different histones.

2. Sample preparation and irradiation conditions

Equal amounts of cells will be embedded in agarose plugs (neutral matrix) and submitted on ice for 2 h to lysis with different amounts of sodium chloride (0.14M, 0.3M, 1.5M, 2M), known to remove H4 and H3, H2 and H1 histone proteins from DNA, respectively. Thereafter, plugs will be washed and incubated or not in 30 uM cis-platinum to be irradiated at 78.4 keV (30 Gy). After irradiation plugs will be washed and kept at 4C in EDTA 0.2M. Yields of DNA double-strand breaks will be assessed in pulsed-field gel electrophoresis and expressed at fraction of DNA migrating out of the gel (FDM).
By following a set-up already developed in routine at ID17 (see previous proposals), irradiation conditions were applied successfully.

3. Results and published paper

To our knowledge, this experiment was the first example of an in vitro PAT experiment, performed directly onto DNA. Interestingly, DNA depleted from H1 histone exhibited grossly decreased FDM when compared with control FDM obtained in "classical" PAT-Plat conditions (see previous proposals), consistent with our work hypothesis that PAT-Plat effect requires the presence of H1 since cis-platinum binds preferentially to H1 linker protein (Yaneva et al, 1997, PNAS). Furthermore, with regard to FDM provided from irradiated H4-H1, H3-H1 and H2-H1 DNA in presence of cis-platinum, there is no progressive increase of FDM by contrast to that measured after irradiations without cis-platinum.
Altogether, our data show that if cis-platinum cannot bind to H1 linker, the benefit of PAT-Plat is lost. Furthermore, cis-platinum that is not onto DNA is responsible of a minority of DNA breaks created by PAT-Plat.
The great part of these results obtained will take part in a new paper devoted to genetic and biological requirements for optimized PAT-Plat conditions.
We thank ESRF for its trust and help.

4. Work conditions and environment - Conclusions

The assessment of the DNA breaks have been done at the biomedical facility . While the DNA breaks analysis did cause any practical problem, and a very good technical support for the use of the beamline, we had some practical difficulties to perform preparation of DNA plugs in a cell culture room whereas this work is generally performed in a proper molecular biology facility. Like in previous reports (MD40), we hope that, in the next future, ID17 will propose appropriate infrastructures for performing molecular biology experiments in better technical conditions by ensuring the good practices of laboratory and in agreement with the principle of separation of molecular and cellular biology activities.

To this aim, a memorandum have been sent to the scientific direction of ESRF. ESRF and ID17 should be aware of the recent developments of biomedical research areas with its specific technology requirements, in order to welcome more users while covering a multidisciplinary spectrum of research approaches. This will make ESRF ID17 an unique facility.