2015 CFAI Special Seminar

Confocal microscopy studies of DNA repair processes in live cells

Inducing low level DNA damage using focused visible light to study recruitment and disengagement of repair factors

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Date / Time : September 29th, 2015 / 16:00-17:00
Venue : The moderate meeting room (事務棟　中会議室)

Modern optical microscopy, including fluorescence confocal microscopy and new super-resolution techniques, open new avenues of research into mechanisms of DNA damage and repair. Microscopy enables studies of dynamics of recruitment and disengagement of repair factors, changes of chromatin structure associated with repair processes, choices of the repair pathway and spatial organisation of repair factors engaged at each damage site, the fate of individual cells following induction of DNA damage, and many more aspects that cannot be accessed directly by standard biochemical assays that embrace and average information from large cell populations.

The precondition for acquiring relevant information regarding mechanisms of DNA repair processes is an ability to induce a very low number of well defined damage events in a selected region of the nucleus, or within a selected gene. So far many laboratories relied in their work on inducing multiple foci of DNA damage by exposing cells to cytotoxic drugs, ionising radiation or UV. Inducing local DNA damage, confined to very small regions of the nucleus, was achieved by exposing cells to photo-sensitisers combined with UV or visible light. In most cases, however, several types of DNA damage were induced in one spot (oxidative, DNA strand breaks, etc.) and the damage was too extensive to be repaired, therefore interpretation of the results of such experiments was complex and cumbersome. Recently a method of inducing individual well defined damage events, using low intensity visible light in the absence of exogenous photosensitisers, has been proposed [1, 4] and successfully used in studies of a number of repair factors, including HP1, 53BP1 and XRCC1 [2,3,4]. I will discuss critical aspects, advantages and limitations of this approach, including the known aspects of the mechanism of damage induction by visible light, the types of damage induced, and the role of photon energy and the light dose. I will also present examples of experiments where induction of individual damage events by low level visible light enabled gaining new information which was difficult to access by other, less specific methods of inducing local DNA damage.

1, Solarczyk KJ, Zarębski M, Dobrucki JW. Inducing local DNA damage by visible light to study chromatin repair. DNA Repair (Amst). 2012 Dec 1;11(12):996-1002.
2, Trembecka-Lucas DO, Szczurek AT, Dobrucki JW. Dynamics of the HP1β-PCNA-containing complexes in DNA replication and repair. Nucleus. 2013 Jan-Feb;4(1):74-82.
3, Trembecka-Lucas DO, Dobrucki JW. A heterochromatin protein 1 (HP1) dimer and a proliferating cell nuclear antigen (PCNA) protein interact in vivo and are parts of a multiprotein complex involved in DNA replication and DNA repair. Cell Cycle. 2012 Jun 1;11(11):2170-5.
4, Solarczyk K, Kordon M, Dobrucki JW. Two stages of XRCC1 recruitment and two classes of XRCC1 foci formed in response to low level DNA damage induced by visible light, or stress triggered by heat shock (under revision).