

Measuring all-protein concentration in DNA repair foci by FLIM

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On average one cell suffers up to tens of thousands of DNA lesions per day, induced by endogenous and environmental factors [1-3]. Induction of DNA damage leads to the recruitment of repair factors and formation of a DNA repair focus. It is reasonable to expect that the numerous repair factors that are recruited to a damaged site transiently create a distinct local microenvironment surrounding a DNA lesion. Despite the seemingly stable nature of repair foci (DSB repair foci may last for hours, and SSB for several minutes or longer), repair proteins recruited to DNA damage are dynamic, continuously exchanging with the surrounding nucleoplasm throughout the repair process. Thus, an intriguing question arises as to the actual total concentration of all proteins residing in a repair focus and the role of high molecular crowding in creating an environment conducive to effective repair.

Using fluorescence lifetime imaging (FLIM), we demonstrated that repair foci that are formed in response to DNA breaks are much more densely packed with proteins than the surrounding nucleoplasm [4]. According to our data the local concentration of all proteins (i.e., the residing and recruited ones) in double- and single-strand DNA repair foci can be even 2.2 times higher than that in the surrounding nucleoplasm, which brings them close to the achievable maximum concentration. We hypothesize that a microenvironment characterized by such a high protein concentration may facilitate the formation of protein condensates, resulting in the stabilization of repair complexes.

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