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A single formula to describe radiation-induced protein relocalization: Towards a mathematical definition of individual radiosensitivity

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HIGHLIGHTS

- A number of stress response proteins relocalize in nucleus as identifiable foci.
- We propose a single formula to describe appearance/disappearance kinetics of foci.
- The parameters of the Bodgi's function allows to define radiosensitivity.

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ABSTRACT

Immunofluorescence with antibodies against DNA damage repair and signaling protein is revolutionarising the estimation of the genotoxic risk. Indeed, a number of stress response proteins relocalize in nucleus as identifiable foci whose number, pattern and appearance/disappearance rate depend on several parameters such as the stress nature, dose, time and individual factor. Few authors proposed biomathematical tools to describe them in a unified formula that would be relevant for all the relocalizable proteins. Based on our two previous reports in this Journal (Foray et al., 2005; Gastaldo et al., 2008), we considered that foci response to stress is composed of a recognition and a repair phase, both described by an inverse power function provided from a Euler's Gamma distribution. The resulting unified formula called "Bodgi's function" is able to describe appearance/disappearance kinetics of nuclear foci after any condition of genotoxic stress. By applying the Bodgi's formula to DNA damage repair data from 45 patients treated with radiotherapy, we deduced a classification of human radiosensitivity based on objective molecular criteria, notably like the number of unrepaired DNA double-strand breaks and the radiation-induced nucleo-shuttling of the ATM kinase.

1. Introduction

To date there is increasing evidence that unrepaired DNA damage are responsible for cell lethality and tissue radiosensitivity, and that misrepaired DNA damage are linked to genomic instability and cancer proneness (Jeggo and Lobrich, 2007; Joubert et al., 2008). Immunofluorescence technique that allows the detection of individual DNA damage and protein relocalization via appropriate antibodies is upsetting the estimation of the genotoxic risk, notably that linked to ionizing radiation exposure (Rothkamm and Lobrich, 2003). Indeed, some DNA damage repair and signaling proteins have been shown to relocalize after genotoxic stress as discrete nuclear foci, which facilitates their quantification and provides information about the spatial distribution of the early biophysical events at the origin of DNA damage (Fig. 1). After stress, nuclear foci generally appear and disappear at rates that depend on numerous parameters such as dose, post-stress time and individuals (Bekker-Jensen and Mailand, 2010; Bekker-Jensen et al., 2006; FitzGerald et al., 2009; Franchitto and Pichierri, 2002; Maser et al., 1997; Mirzoeva and Petrini, 2001; Neumaier et al., 2012; Rothkamm and Lobrich, 2003; Scully et al., 1997; Stewart et al., 2003). The quantification of the radiation-induced

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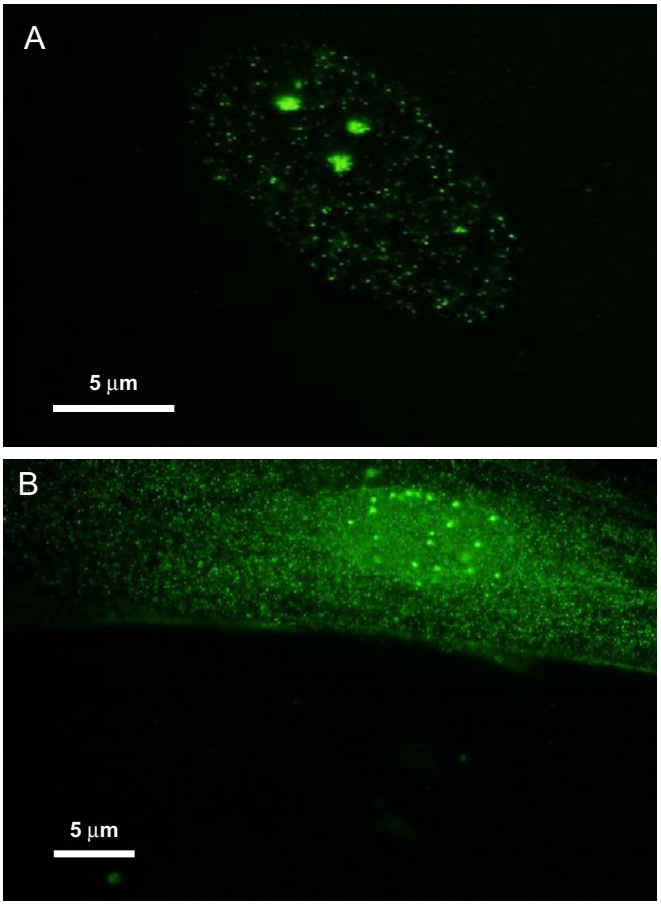

Fig. 1. Representative examples of immunofluorescence images of human fibroblasts labeled by γ-H2AX (A) or pATM (B) antibodies. Nuclear foci are visible in nucleus with both antibodies while cytoplasmic staining is only observed with pATM marker.

nuclear foci is also at the basis of biological dosimetry that may be useful in case of nuclear accident or estimation of the dose after medical exposure response (Jakob and Durante, 2012; Kinner et al., 2008; Testard and Sabatier, 1999).

It was shown that immunofluorescence with antibodies against the phosphorylated forms of the variant H2AX histone (γH2AX) allows the detection of DNA double-strand breaks (DSB), the key-DNA damage of the radiation response (Jakob and Durante, 2012; Kinner et al., 2008; Rothkamm and Lobrich, 2003). The γH2AX foci reflect the radiation-induced DSB that are recognized by the major mammalian DSB repair pathway, the non-homologous end-joining (NHEJ). While γH2AX foci were found to be an interesting biomarker of the radiation response, they present a variety of patterns whose biological significance is not fully understood yet (Costes et al., 2010; Neumaier et al., 2012). Some other proteins like 53BP1, MDC1, MRE11, etc., phosphorylated generally, show radiation-induced relocalization as nuclear foci but with different choreography: appearance in 1 min to some hours, disappearance in some min to several hours (Bekker-Jensen and Mailand, 2010; Bekker-Jensen et al., 2006; FitzGerald et al., 2009; Franchitto and Pichierri, 2002; Maser et al., 1997; Mirzoeva and Petrini, 2001; Neumaier et al., 2012; Rothkamm and Lobrich, 2003; Scully et al., 1997; Stewart et al., 2003) (see also Section 5). Despite the intensive use of immunofluorescence, there are only a few biomathematical studies of the kinetics of appearance/disappearance of nuclear foci (Jakob and Durante, 2012; Kinner et al., 2008; Lisby and Rothstein, 2009; Lisby et al., 2004; Neumaier et al., 2012; Rothkamm and Lobrich, 2003). Unified models describing the nuclear foci choreography would however help in establishing molecular models of radiosensitivity. In two previous papers, we provided evidence that the repair rate of *individual* DNA damage is time-independent whereas *a population* of DNA damage is time-dependent and obeys the Gamma probability distribution (Foray et al., 2005; Gastaldo et al., 2008). Here, we propose a unified formula that describes kinetics of appearance/disappearance of nuclear foci relevant for any protein involved in the major DSB repair and signaling pathways. This model permits to establish temporal correlations between different downstream and upstream actors of radiation response and to quantify the radiosensitivity risk.

2. The model

2.1. Main principles

In response to any DNA breaking agent, DNA damage repair and signaling proteins relocalize as nuclear immunofluorescence foci by generally obeying two kinetic phases:

- *The foci appearance phase:* during which the number of foci increases and reaches its maximum at a rate, value and post-stress time that depend on many parameters like dose and individual factors. Such phase may possibly be preceded by the nucleo-shuttling of some proteins and leads to DNA damage recognition;
- *The foci disappearance phase:* during which the number of foci decreases up to a residual value, at a rate that depends on many parameters like dose and individual factors. The rate of foci disappearance is not necessarily linked to the rate of foci appearance. Such phase is generally interpreted as repair of DNA damage.

Hence, the total number of DNA damage revealed by nuclear foci observed by immunofluorescence, assessed at a given post-stress time t after a single dose D, $N(t,D)$, obeys the following equation:

$$\frac{dN(t)}{dt} = (K_{rec} - K_{rep})N \quad (1)$$

where K_{rec} is the DNA damage recognition rate and K_{rep} is the DNA damage repair rate.

Throughout this model, we considered each DNA damage *taken individually* (*microscopic approach*) and characterized by constant transition rates k. Thereafter, we considered *the DNA damage subpopulations* with time-dependent transition rates K (*macroscopic approach*) (Foray et al., 2005; Gastaldo et al., 2008). We deliberately chose to take DSB induced by X- or gamma-rays as an example. However, our model is relevant for other genotoxic stress and types of DNA damage (data not shown) (Foray et al., 2005; Gastaldo et al., 2008).

2.2. Induction of DSB

The number of DSB physically induced by X-rays (or gamma-rays) assessed immediately after irradiation (*i.e.* without effect of repair), $N_{ind}(D)$, is linearly dose-dependent and is about 40 (37 ± 5) DSB per Gy per human diploid cell in our hands (Foray et al., 1997; Joubert et al., 2008). Hence, in the frame of *microscopic* view, the DSB induction rate k is assumed to be *constant*. In the case of X-rays (or gamma-rays) irradiations that are not targeted (*e.g.* microirradiation), all the cells receive the same dose. Consequently, in the frame of *macroscopic* view, there is a single population of cells with a constant DSB induction rate $K_{ind} = k_{ind}$.