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Conversation between Mitochondria and Nucleus: Role of FEN1 and ING1 in Association with the Ringmaster PCNA

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Abstract

Mitochondria and nucleus maintain collaboration during apoptosis and oncogenesis where two machineries FEN1 and ING1 are playing major role in the theatre of DNA metabolic pathway in concomitant with the metabolic actor PCNA. PCNA, FEN1 and ING1 localized in mitochondria and nucleus by forming a larger protein complex, potentially involved in the regulation of DNA damage repair, apoptosis and cancer. But the mechanism of these proteins migration and coordination between mitochondria and nucleus is unknown. Understanding the signaling across sub-cellular location based on FEN1/ING1/PCNA might lead to interlink the molecular regulation of cell death and immortalization within the mitochondrial and nuclear location.

Keywords: Mitochondria, Nucleus, Apoptosis, FEN1, ING1, PCNA.

1. Introduction

Maintenance of nuclear and mitochondrial genome integrity is critical to multicellular organisms. Nucleus and mitochondria are prime location where a growing number of DNA transacting proteins are found respond to a variety of environmental and cellular stresses. Oncogenesis, ageing and cell death can disrupt the genome either directly by causing DNA damage or indirectly through disruption of normal cellular processes that involve DNA. Critical to the process of maintaining genome integrity in higher organisms are the FEN1, ING1 and PCNA those serves to integrate signals. FEN1 is found in the nucleus and the shortened form of FEN1, FENMIT, localizes to mitochondria to maintain mtDNA integrity [Kazak *et al.* **2013**; Kalifa et al, **2009**; Liu *et al.* **2008**; Szczesny *et al.* **2008**]. FEN1 works nucleus in response to DNA damage and during S-phase of the cell cycle [Zheng *et al.* **2010**]. ING1, an interacting partner of FEN1, translocates to the mitochondria in response to apoptosis inducing stimuli, independent of the cellular p53 pathway.

The ability of ING1 to induce apoptosis in various cancer correlates well with its degree of translocation to the mitochondria after UV treatment [Bose *et al.* **2013**]. ING1 translocate into the nucleolus as a binding partner that interacts with a nucleolar protein CSIG, functions as a novel pro-apoptotic regulator in response to DNA damage [Li *et al.* **2012**]. Moreover, ING1 is localized in the cell nucleus and associated with chromatin modifying enzymes, linking tumor suppression directly

FEN1:Flap endonuclease-1; **ING1**: Inhibitor of growth 1; **PCNA**: Proliferating cell nuclear antigen; **nDNA**: Nuclear DNA; **mtDNA**: Mitochondrial DNA; **EXO**: Exonuclease; **GEN**: Gap endonuclease; **BER**: Base excision repair; **NER**: Nucleotide excision repair **ROS**: Reactive Oxygen Species; **SP**: Short patch; **LP**: Long patch; **Polδ**: DNA polymerase δ; **dsDNA**: Double strand DNA; **RFC**: Replication factor C; **ssDNA**: Single strand DNA; **WRN**: Werner protein; **TNR**: Trinucleotide repeat; **PHD**: Plant homeodomain; **NLS**: Nuclear localization signal; **PIP**: PCNA-interacting protein; **UV**: Ultraviolet; **FENMIT**: FEN1, localizes to mitochondria, mitochondrial targeting; **CSIG**: Cellular senescence-inhibited gene.

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to chromatin regulation [Ludwig *et al.* **2011**]. PCNA is another interacting partner of FEN1 and ING1 involved in DNA replication and damage repair both in cytoplasm and nucleus by forming a larger protein complex potentially involved in the regulation of DNA damage repair. However, the complete mechanism of cross-talk from mitochondria and nucleus is unknown. We briefly describe here the roles of FEN1 and ING1 in association with the ringmaster PCNA in the regulation of DNA repair and apoptosis.

2. Flap endonuclease-1

Apoptosis represents a universal and delicately efficient cellular suicide way indispensable for a variety of typical biological processes ranging from embryonic development to ageing [Sola *et al.* **2006**], and there happens a cross talk between nucleus and mitochondria for maintaining genomic stability [Hong *et al.* **2004**], whereas large number of metabolic actors, exclusively, two tumor suppressor FEN1 [Henneke *et al.* **2003**] and ING1 are playing major role in the theatre of DNA metabolic pathways [Helbing *et al.* **1997**]. FEN1 is a structure-specific metallonuclease which acts on both nDNA and mtDNA [Guo *et al.* **2008**], best known for its FEN, EXO and GEN functions in Okazaki fragment maturation, DNA repair, apoptosis-induced DNA fragmentation, telomere maintenance and LP-BER [Singh *et al.* **2008**]. It is well documented that FEN1 interacts with PCNA and ING1. Elucidating the links in between the regulatory roles of FEN1, PCNA and ING1 regarding mitochondrial and nuclear location and their epigenetical regulations are of important to know.

Base excision repair (BER) is the primary DNA repair pathway that corrects base lesions that arise due to oxidative, alkylation, deamination, and depurinatiation/depyrimidination damage. Such base lesions cause little distortion to the DNA helix structure. BER is important in the maintenance of the nuclear genome and highly proficient in the repair of mtDNA [Bohr *et al.* **2002**]. This is little wonder as mitochondria are the main sites of ROS production, the accumulation of which is positively correlated with aging, and oxidative damage affects replication and transcription of mtDNA and results in a decline in mitochondrial function which in turn leads to enhanced ROS production and further damage to mtDNA that are the primary substrate for BER [Vijg, **2007**]. During DNA replication and SP/LP-BER in mitochondria, the intermediate 5' flap structure is being processed by FEN1 in which helicase activity of hDNA2 converts the nick substrate to flap substrate [Zheng *et al.* **2008**]. FEN activity of FEN1 plays a major role in RNA primer removal in Okazaki fragment maturation; Polδ displaces the RNA/DNA primer to create a flap for FEN1, is assisted by RFC which loads PCNA onto dsDNA [Waga and Stillman, **1998**] and the interaction between FEN1 and PCNA is expedited by post translation methylation [Guo *et al.* **2010**]. The EXO and GEN activities may be important in the resolution of TNR sequence derived secondary structures [Murante *et al.* **1995**], oligonucleosoaml fragmentation of chromosomes in apoptotic cells [Szczesny *et al.* **2008**] and apoptotic DNA fragmentation [Zheng *et al.* **2010**]. FEN1 also interacts with WRN to rescue replication fork [Sharma *et al.* **2004**]. Contrary to PCNA, WRN protein stimulates GEN activity, thereby cleaving the ssDNA region in the duplex DNA molecule [Zheng *et al.* **2005**]. Alliance of FEN1 with Endo G may be a key mechanism to switch FEN1 role from DNA replication and to repair apoptotic DNA fragmentation, which also greatly enhances the GEN and EXO activities, important for disposal of apoptotic DNA [Murante *et al.* **1995**; Szczesny *et al.* **2008**; Zheng *et al.* **2010**]. Meanwhile, FEN1 involves in the formation of PCNA/FEN1 complex for apoptotic degradation where the migration pathway of FEN1 into mitochondria is unknown [Kazak *et al.* **2013**]. How FEN1 phosphorylation influences the FEN1/PCNA interaction and how FEN1 can efficiently bind to PCNA and DNA substrates, and then dissociate from the nuclease reaction are another inevitable question [Branzei and Foiani, **2010**]. There also emerges an irresolute "chicken and egg" question that how FEN1 involves in genome maintenance and apoptotic DNA fragmentation simultaneously.

3. Proliferating cell nuclear antigen

PCNA is one of the central protein molecules responsible for decisions of life and death of the cell [Alenzi et al, **2010**]. This ring-like protein involved in the major DNA replication and repair machinery of the cell [Strzalka and Ziemienowicz, **2011**]. PCNA is a homotrimeric protein that forms a "sliding clamp" to surround damaged DNA [López de Saro, **2009**; Sarmiento *et al.* **2014**]. It serves as a platform to recruit factors that are essential for DNA replication and repair [Manohar and Acharya, **2015**; Trembecka-Lucas *et al.* **2013**]. It has role as the sliding clamp of DNA polymerases of both eukaryotes and prokaryotes [Bruck and O'Donnell, **2001**]. Leading to cell cycle arrest, probably the best known and most studied interaction of PCNA is with p21 [Piccolo and Crispi, **2012**]. Binding of p21 occurs at the same location on PCNA (in the interdomain connector loop) as does the binding of most other PCNA-interacting proteins [Strzalka and Ziemienowicz, **2011**]. When not engaged in DNA replication, PCNA (under the control of p53) commits cells to cell cycle arrest and repair of DNA damage, or,

when repair is not possible, absence or low levels of functional PCNA may drive cells into apoptosis [Mishra *et al.* 2015]. Induction of PCNA transcription in response to radiation and other types of stress is regulated by p53, as p53-binding sites are present in the PCNA gene promoter [Krieg *et al.* 2006]. Low and moderate cellular quantities of p53 positively stimulate transcription of PCNA, while high levels of p53 inhibit PCNA expression [Chen *et al.* 2014]. In response to DNA damage, ING1b can interact with PCNA to promote apoptosis or DNA repair in a p53-independent manner [Satpathy *et al.* 2013; Tallen and Riabowol, 2014, Bose *et al.* 2013]. In addition to PCNA, ING1b is speculated to form complexes with other PCNA-binding partners, such as flap endonuclease (FEN) and DNA (cytosine-5) methyltransferase (MCMT) [Naryzhny, 2008].

4. Inhibitor of growth 1

ING1 is a type II tumour suppressor [He *et al.* 2005], possessing two major splicing isoforms ING1a and ING1b; regulating apoptosis [Helbing *et al.* 1997] and cell proliferation by modulating chromatin structure [Garkavtsev and Riabowol, 1997]. The context of binding ING proteins with PCNA and p53 is to affect DNA replication, repair, apoptosis and senescence respectively. Whereas, the PCNA-interacting-protein (PIP) motif found in INGlb [Scott *et al.* 2001a], mediates an apoptotic response, facilitated via interactions between INGs and p53 in association with ARF, PCNA and its binding partners viz. GADD4 and PAF [Guo *et al.* 2008; Pedeux *et al.* 2005; Gonzalez *et al.* 2006; Simpson *et al.* 2006]. Since PCNA, is critical for DNA replication, repair and interacts with DNA polymerase δ, has implicated ING1b in sensing and responding to DNA damage [Scott *et al.* 2001a]. Perhaps ING1b forms complexes with other PCNA-binding proteins particularly FEN1 [Warbrick, 2000]. Nuclear localization signal (NLS), a highly conserved region, located upstream of the PHD finger region found in ING1, contains three potential NTS, two of which target ING1 to the nucleoli in response to different stresses [Scott *et al.* 2001b], and which interact with Karyopherin-α and -β for nuclear import [Russell *et al.* 2006]. A 14-3-3-binding motif lies in between the PHD and NLS domains, binds ING1b upon phosphorylation of S199, resulting in relocalization of ING1b from nucleus to cytoplasm. Both ING1a and ING1b appear to have opposing tasks and diverse cellular partners, for instance, ING1b binds to PCNA through PIP in response to UV irradiation and induces apoptosis while ING1a does not show any interaction with PCNA under stress conditions [Scott *et al.* 2001a]. As INGlb mediates an apoptotic response but the function of ING1a is presently unidentified [Soliman *et al.* 2008]. Again PIP dissociates from ING proteins or not, once they are recruited to the chromatin is also a mystery.

5. Conclusion

In accordance to the above discussion, there need to identify different pathways that call in FEN1 inside mitochondrial matrix in response to death signals and the mechanism of migration. Additionally the role of phosphorylation in FEN1/PCNA and ING1/PCNA interaction in apoptotic response is also need to address. Understanding the signaling crosstalk between these two organelles during cell death/cancer development in respect to master of the ring PCNA might lead to more options to innovate new therapeutic targets and in a holistic sense it might create an opportunity to interlink cell death and immortalization within different sub cellular location.

6. Conflict of Interest

The Authors declared no conflict of interest.

7. References

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