



OPINION ARTICLE

# Circulating nucleic acids: a new class of physiological mobile genetic elements [version 1; referees: 2 approved]

Indraneel Mitra

Translational Research Laboratory, Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Navi-Mumbai, India

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**Abstract**

Mobile genetic elements play a major role in shaping biotic genomes and bringing about evolutionary transformations. Herein, a new class of mobile genetic elements is proposed in the form of circulating nucleic acids (CNAs) derived from the billions of cells that die in the body every day due to normal physiology and that act intra-corporeally. A recent study shows that CNAs can freely enter into healthy cells, integrate into their genomes by a unique mechanism and cause damage to their DNA. Being ubiquitous and continuously arising, CNA-induced DNA damage may be the underlying cause of ageing, ageing-related disabilities and the ultimate demise of the organism. Thus, DNA seems to act in the paradoxical roles of both preserver and destroyer of life. This new class of mobile genetic element may be relevant not only to multi-cellular organisms with established circulatory systems, but also to other multi-cellular organisms in which intra-corporeal mobility of nucleic acids may be mediated via the medium of extra-cellular fluid.

**Open Peer Review**

Referee Status:

	Invited Referees	
	1	2
<b>version 1</b> published 30 Sep 2015	 report	 report
<b>1</b> <b>Silvia Gravina</b> , Albert Einstein College of Medicine USA		
<b>2</b> <b>Manchanahalli Rangaswamy Satyanarayana Rao</b> , Jawaharlal Nehru Centre For Advanced Scientific Research India		

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**Corresponding author:** Indraneel Mitra ([indraneel.mitra@gmail.com](mailto:indraneel.mitra@gmail.com))

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## Background

Barbara McClintock published her classic paper on mobile genetic elements (MGEs) in 1950<sup>1</sup>, but it took the scientific community several decades to appreciate the enormity of her discovery. Today, it is recognized that MGEs occur widely in nature in prokaryotes, archaea and eukaryotes and play a major role in shaping their genomes and bringing about evolutionary transformations and adaptation<sup>2-5</sup>. Their bizarre behavior of moving from one part of the genome to another distinguishes them from the functioning of conventional genetic elements.

MGEs belong to two classes *viz.*, intra-genomic and inter-genomic. Intra-genomic MGEs are transposable elements (TEs) or transposons which constitute nearly 50% of the human genome but are variable among species and comprise 1%–5% of prokaryotic genomes<sup>6</sup>. Inter-genomic transposable elements, on the other hand, underlie horizontal or lateral gene transfer (HGT) whereby segments of DNA are transferred from one organism to another<sup>7-9</sup>. Although HGT is known to occur extensively in bacteria and are responsible for development of antibiotic resistance<sup>10</sup>, increasing evidence of HGT between other organisms is coming to light. For example, HGT between prokaryote and eukaryote, eukaryote and eukaryote, eukaryote and prokaryote has been reported<sup>7,11</sup>. Although the initial claims of presence of bacterial DNA in human genome were dismissed as erroneous<sup>12,13</sup>, recent evidence has confirmed the presence of bacteria DNA sequences in about one-third of healthy humans and in greater numbers in cancer cells<sup>14</sup>. A recent analysis of public databases of transcriptome sequences of multiple organisms discovered that human beings have picked up at least 145 genes from other species during the course of evolution<sup>11</sup>. Thus, HGT results in what is called a ‘web of life’ rather than a steadily bifurcating evolutionary tree<sup>8</sup>.

## Circulating nucleic acids as a new class of mobile genetic elements

Based on a recent finding<sup>15</sup>, a new class of mobile genetic elements is proposed *viz.*, circulating nucleic acids, which are produced as a result of normal physiology and operate intra-corporeally or within the body of an organism. Circulating nucleic acids (CNAs) in the form of fragmented DNA and chromatin (DNAfs and Cfs) are known to circulate in blood and are derived from the hundreds of billions of cells that die through apoptosis in the adult human body on a daily basis<sup>16,17</sup>. These fragments have a size range of between 100bp–1000bp, have a half-life of 10–15 minutes and are ultimately removed by the liver<sup>18,19</sup>. The presence of Cfs (nucleosomes) in blood can be detected by a sandwich ELISA assay<sup>15</sup>, but whether naked DNA circulates as such remains an open question since the possibility cannot be excluded that DNAfs isolated from plasma/serum are in fact products of the DNA purification process.

Results of a recent study summarized below have revealed that CNAs can act as mobile genetic elements<sup>15</sup>. DNAfs and Cfs isolated from blood of healthy volunteers and cancer patients are actively taken up by cells in culture whereupon they rapidly accumulate in their nuclei and associate themselves with their chromosomes. The intracellular DNAfs and Cfs trigger a DNA-damage-repair-response (DDR) with up-regulation of multiple pathways of DNA damage and repair that facilitate their integration into host cell genome.

Presence of human DNA in recipient mouse cell chromosomes could be detected by FISH while whole-genome sequencing uncovered tens of thousands of human reads in mouse cells. The integration of DNAfs and Cfs is stable and presence of extraneous DNA was demonstrable in single-cell clones developed from treated cells which had undergone numerous cell divisions. Genomic integration of DNAfs and Cfs results in phosphorylation of H2AX indicative of dsDNA breaks and up-regulation of apoptotic pathways in a proportion of cells. When injected intravenously into mice, DNAfs and Cfs integrate into cells of a variety of organs in the body, activate H2AX and the apoptotic marker active Caspase-3.

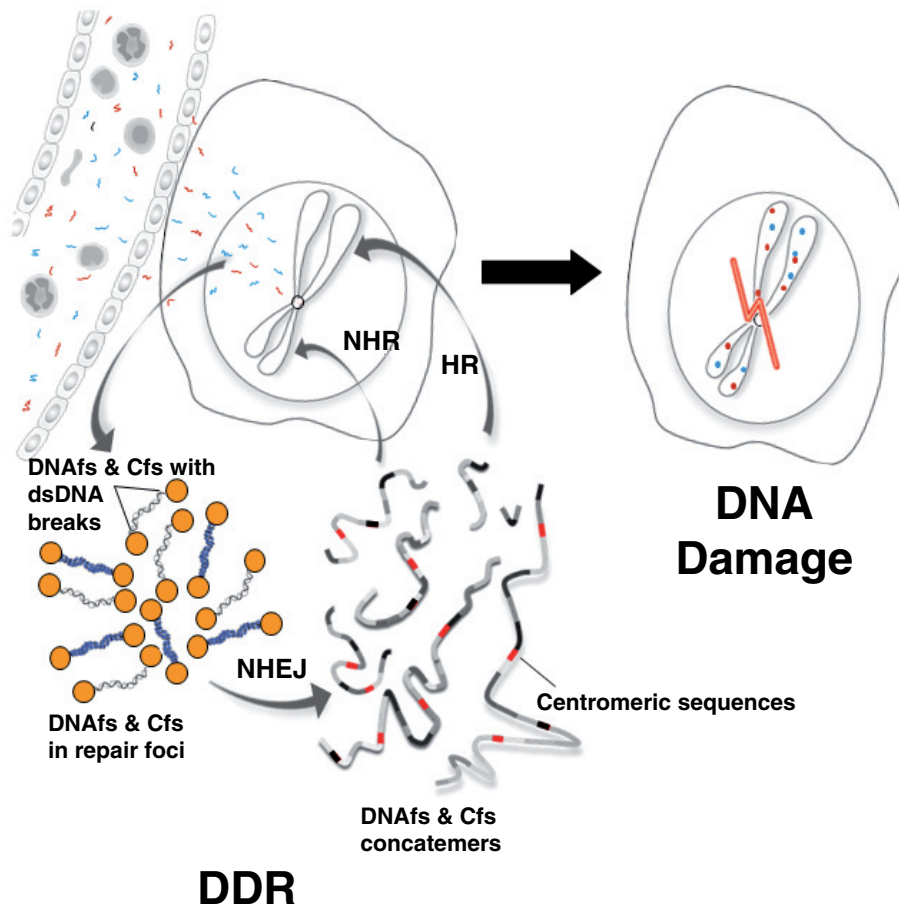
Whether genomic integration of CNAs occurs preferentially in a site-specific manner or is random is not known; but in either case, integration of CNAs would give rise to somatic mutations in the host genome. Since integration of CNAs occurred in all organs of the body examined<sup>15</sup>, it may not be far-fetched to imagine that CNA-integration also occurs in germ cells. Genomic integration of CNAs would lead to DNA rearrangements, translocations and deletions<sup>20</sup> – changes that are hallmarks of ageing, and large DNA rearrangements and cell to cell variations in gene expression are typical of ageing cells<sup>21,22</sup>.

## CNAs integrate into host-cell genomes by a unique mechanism

According to the model depicted in the [Figure](#), CNAs integrate into the genome by a unique mechanism in which activation of DDR plays a central role<sup>15</sup>. When DNAfs and Cfs enter into a cell, the latter mistakenly perceives the intracellular DNAfs and Cfs with dsDNA breaks in their two ends as damaged “self” DNA and activates DDR even before DNA damage has actually occurred. The activated DDR joins up multiple disparate DNAfs and Cfs into long concatemers by non-homologous-end-joining as a part of the repair process. It is the integration into the host cell genomes of the concatemers by homologous or non-homologous recombination that brings about damage to DNA. Thus, paradoxically, the activation of DDR brings about damage to DNA rather than preserving DNA integrity. This model of DNA damage and repair in which DDR precedes DNA damage is the reverse of the classical model based on damage induced by ionizing and UV-radiations and chemicals wherein DDR is activated after DNA damage. It is possible that this model of DNA damage and repair that facilitates CNAs integration may apply to horizontally transferred DNA in other organisms in nature.

## Implications

Although xenobiotics and DNA damaging agents constantly damage human DNA, these are usually transient and do not inflict permanent damage. CNAs, on the other hand, are ubiquitous, physiological and continuously arising, inflicting repeated damage and mutations to the somatic DNA. This naturally suggests that the somatic genome is not stable but remains in a state of turbulence characterized by DNA damage, mutations and rearrangements leading to DNA mosaicism and cell-to-cell variation in genomic structure and function. Indeed, cell-to-cell variations are being increasingly uncovered in the human body and are related to ageing<sup>21,23–25</sup>. The above events and the accompanying genomic instability may give rise to cancerous transformations which is compatible with the steep rise in the incidence of cancer with increasing age<sup>26</sup>. CNAs may also play an etiological



**Figure.** A new mechanistic model of genomic integration of CNAs in which DDR precedes DNA damage. NHEJ = non-homologous end-joining; HR = homologous recombination; NHR = non-homologous recombination. Reproduced with permission from Mitra *et al.*, J Biosci. 2015. **40**: 91–111.

role in several other disease conditions which are characterized by elevated levels of DNAs and Cfs. These include auto-immune disorders<sup>27</sup>, and a host of acute and chronic human pathologies, namely, sepsis<sup>28</sup>, trauma<sup>29</sup>, burns<sup>30</sup>, organ transplantation<sup>31</sup>, diabetes<sup>32</sup>, myocardial infarction<sup>33</sup>, stroke<sup>34</sup> and renal failure<sup>35</sup>.

### Conclusions

CNAs are a new class of physiological, continuously arising intra-corporeal mutagenic agents that might be responsible for ageing, age-related disabilities and ultimately the demise of the organism. Thus, DNA seems to act in paradoxical roles of both preserver and destroyer of life. This new class of intra-corporeal mobile genetic elements may be relevant not only to multi-cellular organisms which have a developed circulatory system, but also to other

multi-cellular organisms in which intra-corporeal mobility of CNAs may be mediated via the medium of extra-cellular fluid.

### Competing interests

No competing interests were disclosed.

### Grant information

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### Acknowledgement

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Current Referee Status:



Version 1

Referee Report 11 November 2015

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**Manchanahalli Rangaswamy Satyanarayana Rao**

Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre For Advanced Scientific Research, Bangalore, India

This article details a new paradigm in cancer genetics based on the recent original article published by the same group. The idea of the origin of the cancer phenotype based on the circulating nucleic acid/ chromatin fragments is really thought provoking and will generate a lot of interest in the cancer researchers. The manuscript is well written summarizing the salient features of their recently published article.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

**Competing Interests:** No competing interests were disclosed.

Referee Report 13 October 2015

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**Silvia Gravina**

Department of Genetics, Albert Einstein College of Medicine, Bronx, NY, USA

In this Opinion Article entitled "[Circulating nucleic acids: a new class of physiological mobile genetic elements](#)", Indraneel Mitra investigated the topic of circulating nucleic acids as a new class of continuously arising DNA damaging agents.

The author discusses a recent work (Mitra *et al.*, 2015) which describes how free nucleic acids not only can enter cell nuclei freely, but they integrate in the host genome and activate proteins of DDR and apoptotic pathways. In particular, in the Opinion Article, the author hypothesizes that cell free DNA continuously arising induced damage can be the cause of aging and age related diseases.

The manuscript is certainly well written and attractive, and discusses a very novel yet important topic in the aging field. My only concern is that in two parts of the manuscript the author jumps to conclusions too quickly, and I would suggest rephrasing certain statements.

More specifically:

- pg2, mid right; “Whether genomic integration of CNAs occurs preferentially in a site-specific manner or is random is not known; but in either case, integration of CNAs would give rise to somatic mutations in the host genome.”

This has to be demonstrated yet. We don't really know if CNA integration is a direct cause of somatic mutations in the host genome. I would suggest making this statement milder (i.e. “Whether genomic integration of CNAs occurs preferentially in a site-specific manner or is random is not known. It is reasonable to speculate that integration of CNAs may give rise to somatic mutations in the host genome.” or “a fascinating hypothesis yet to be tested is that that integration of CNAs may give rise to somatic mutations in the host genome”.)

- Same for pg2 bottom part; “CNAs, on the other hand, are ubiquitous, physiological and continuously arising, inflicting repeated damage and mutations to the somatic DNA”. I would suggest here-as well- a milder statement (i.e., CNAs, on the other hand, are ubiquitous, physiological and continuously arising, inflicting repeated damage and potentially causing mutations to the somatic DNA”).

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

***Competing Interests:*** No competing interests were disclosed.

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