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Re-criticizing RNA-mediated cell evolution: a radical perspective

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Accomplishment of the unfulfilled; a parenthetical trait of simplicity

Abstract: Genetic inter-communication of the nucleic-organellar dual in eukaryotes is dominated by DNA-directed phenomena. RNA regulatory circuits have also been observed in artificial laboratory prototypes where gene transfer events are reconstructed, but they are excluded from the primary norm due to their rarity. Recent technical advances in organellar biotechnology, genome engineering and single-molecule tracking give novel experimental insights on RNA metabolism not only at cellular level, but also on organismal survival. Here, I put forward a hypothesis for RNA's involvement in gene piece transfer, taken together the current knowledge on the primitive RNA character as a biochemical modulator with model organisms from peculiar natural habitats. It is proposed that RNA molecules of special structural signature and functional identity can drive evolution, integrating the ecological pressure of environmental oscillations into genome imprinting by buffering-out epigenetic aberrancies.

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Introduction

The evolutionary cost for the integrity maintenance of the genetic mosaicism; organellar plus nucleic compartmentalized DNA, in a plant cell is determined by transient and/or chronic oscillations of the sub-cellular electrochemical poise (Lane *et al.* 2013). This ecological adaptation is being synchronized with the movement of genetic material from bioenergetic organelles to the nucleus (Huang *et al.* 2003), under those micro-environmental conditions. The exact molecular nature of the mobile signal that facilitates gene transfer events is under debate so far, although many mechanistic details of such phenomena are well studied (Timmis 2012). The most prominent candidates in such a mediation are DNA molecules, according to a rather complicated artificial reconstruction of gene transfer events in the laboratory (Bock & Timmis 2008). This type of recapitulation has been realized by generation of trans-engineered plants lines (Bock & Timmis 2008) since corresponding reproduction in algae had failed (Lister *et al.* 2003). The potential for RNA-involvement has been characterized as a bizarre biological fact, due to low frequency of RNA-mediated events, under the studied circumstances (Timmis 2012).

Biological problem

In this mini-perspective, the current background on the field is being reviewed, concerning the genetic inter-play between nucleic and organellar genomes. Self-criticizing the existing momentum in the literature, alternative opinions are provided to the scientific community, on why and how the scenario of RNA-mediation in cell evolution and sequentially to the organismal sustainability (Pal 1998) has to be re-considered.

Peculiarity of RNA phenomena in endosymbiotic gene transfer

RNA intermediates have been reported during endosymbiotic gene transfer from mitochondrion to the nucleus (Wischmann & Schuster 1995) but this is not the case with chloroplast (Sheppard *et al.* 2011). The latter peculiarity could likely be explained, due to cryptic regulatory signals, which are located in the plastome (Lloyd & Timmis 2011). Most probably, those genomic elements allow an idiomatic signature in the corresponding transcriptome, having as a result, the necessity of an RNA intermediate to be bypassed. However, a current natural phenomenon provides evidence for reverse mRNA expatriation from nucleus to the chloroplast, by a viroid mediation (Gómez & Pallás 2010). Biologically, this observation is fundamental, denoting the possibility of a non-coding RNA involvement for such trafficking.

New knowledge and technical advances in RNA metabolism

Over the last two years, there has been increasing interest in organellar RNA metabolism (Antonicka & Shoubridge 2015; Schmitz-Linneweber *et al.* 2015) as well as in light stimulus-RNA signalling cross-talk (Cho *et al.* 2014; Petrillo *et al.* 2014). Moreover, breakthrough studies on RNA topology via single-molecule and live-imaging tracking of even naked molecules (Mattick & Clark 2011; Buxbaum *et al.* 2015) gives new insights into RNA localization issues, regarding sites of biogenesis (Eberle & Visa 2014; Shiina & Nakayama 2014) and novel processing pathways in sub-cellular organelles (Germain *et al.* 2013; Lefebvre-Legendre *et al.* 2014). In a laboratory-mediated sense, the generation of small antisense RNAs in plastids could be technically feasible in transplastomic plants (Zhang *et al.* 2015), by triggering functional ancestral remnants of endogenous RNA silencing-*like* pathways. Meanwhile, the elegant Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/Cas9 system is inspired by the natural defence system of archaea and bacteria (Karginov & Hannon 2010) and it is based on short non-coding RNAs (Bhaya *et al.* 2011). It has to do with sequence-specific targeted genome engineering (within the range of a few hundreds of nucleotides) via RNA editing (Bassett *et al.* 2013) and its application in cyanobacteria (Scholz *et al.* 2013) as the progenitors of chloroplasts seems not to be an exception. Conclusively, all the above sophisticated tools can indeed be used to extrapolate probabilities of the RNA-dependency in our case-study.

Redox riboswitch-*like* modules: a growing concept, inspired by photosynthetic organisms

Light intensity acts as a bioenergetic rheostat which modulates the functionality of the genome at the transcriptional level, in transgenic plants undergoing RNA silencing (Kotakis *et al.* 2011). This effect has a consequence on the inter-play between chloroplast and the nucleus. The two sub-genomic loci are coordinated by a transcriptional feedback loop of regulation. Redox-responsive genes that are located in the chloroplast are retrogradely expressed as a function of cellular redox status, which is perturbed, due to RNA silencing activation in the nucleus (Kotakis, unpublished results). One step further on, non-coding RNAs manifest biophysical characteristics that pose a new function: they could orchestrate interactions as *redox* analogues and energetic charge carriers, by cross-linking *genetic* with biochemical modes of action (Kotakis 2015). This constitutes a matter of dual-potency and pleiotropy, unravelling a higher level of dynamic regulation and complexity *in vivo*. This argument can decipher potent RNA mechanisms behind the DNA-based gene transfer and hence, help unmask this poorly understood context.

Democratizing RNA commonalities towards a novel consensus

Overcoming the methodological difficulties, I envision that the theory of ‘redox ∼ non-coding RNAs’ guided cell evolution could now be exemplified under a proper experimental design. It is known that environmental stress induces a higher potential for endosymbiotic gene piece transfer events (Wang *et al.* 2012). Based on retrospective paradigms, endosymbionts from different time-periods of prehistoric life can stand as natural archetypes that integrate a diverse combination of environmental extremities into increased signal conductivity during gene transfer events. Such *vivid factories* (e.g. cryptophytes) from distinct steps of the evolutionary climax (i.e. secondary and tertiary endosymbiosis) may be tested as an excellent experimental template (Moore *et al.* 2012; Archibald 2015). The window of responses becomes broader as well as more pronounced and consequently, acceleration of events takes place in these *natural-type of stochastic bioreactors*, where ‘nucleomorphs’, ‘organellar’ and nucleic sub-genomes co-exist under a regime of strong and multidirectional connectivity, regarding the rates of gene sequences’ flow in-between. In this way, previous phenomenological artefacts could now be clarified, if not falsified by scoring non-coding RNAs divergence in the natural systems mentioned above. The comparative distribution and similarity percentage among (non-coding) RNA sequences originating from different sub-cellular compartments can testify the validity of the newly introduced hypothesis. This is in contrast to obstacles imposed by endogenous limitations of risky biological systems (i.e. stable transgenic lines), coming from laboratory practices.

Concluding remark

Non-coding RNAs are well-known epigenetic regulators, contributing in chromatin methylation state and transgenerational inheritance (Alleman *et al.* 2006; Morris 2015). It is proposed that this class of RNA molecules could also participate in networks of horizontal inheritance (Jheeta 2013) where cytoplasmic genomic elements of prokaryotic origin, are transferred to the main genetic compartment of the eukaryotic host. In parallel, this emerging claim assumes investigation of the non-canonical transcripts’ role to photosynthesis regulation, as well as giving a boost towards detection of atypical RNA species in primordial forms of archean life (Cockell 2014; Jheeta & Joshi 2014). On a visionary scale, changes in redox conditions could influence RNA-based genetic drifts to adapt as a dissipative recruitment in response to environmental variables. This modular valve of compensatory evolution could be harmonized with an adjustable genomic ratchet that is driven by the ecosystem’s selective pressure.

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