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REVIEW

RNA mutations in the moth pheromone gland

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After Darwin, I propose a theory to explain what happened before, i.e. before the tree of life and the emergence of all different organisms. In my view of the beginning of life, RNA mutations have played a central role. This is based on highlight results obtained recently in the female silkworm moth, *Bombyxmori*. Using gene, RNA and protein data, we revealed the occurrence of a high degree of RNA editing in the chemosensory protein (CSP) family specifically expressed in the pheromone gland. Tissue-specificity and high number of RNA and protein variants produced by point and frame shift mutations are in agreement with the hypothesis that RNA mutations are essential for the genesis of proteins with entirely new functions as prerequisite for evolution. In the moth pheromone gland, RNA editing and CSPs are proposed to regulate exocytotic processes required for pheromone biosynthesis and release. Understanding the mechanism of RNA editing controlling CSP expression in the pheromone gland in moths may allow us to better understand specific "genetic" diseases in human, particularly those associated with the common release mechanism of fatty acids and secretory granules in glandular cells.

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"Where do we come from?" This question has threatened humanity since all time. Theory of creation always came from all various religious beliefs and scientific debates. The theory enouncedby Charles Darwin in 1859 happened to be true: "life originates in simple forms and develops with time into more and more complex systems" [1]. According to Darwin's theory, there is clearly no divine creation. "Species from reptiles to birds, plants to insects and mammals to human are not fixed all time but evolve as a result of natural selection" [1]. Modern genetic such as high throughput gene sequencing that reveal genome size and base composition of many various species gives full-support to Darwin's theory, definitely rejecting the theory of "unchangeability" and/or "divine creation" from Richard Owen and his descendants [1-3]. But how far back in history can we trace the birth of life? A recent discovery identifying base nucleotides on meteorite fragments allow us to trace it now back to about 4 000 Mya, where a continuous rain of meteorites impacts the earth [4-5]. RNA was built in space from most rudimentary nucleobase chemicals, but how could one single RNA strand be possibly turned into a cell and why would this happen so particularly on earth?

Earth is rather unstable. There have been forces on earth that broke up enough crust to divide into many continents and lead to the complete extinction of many animal and vegetal species. Continents are still drifting and species extinction is still happening in front of our eyes witnessing new rocks, volcano eruption, earthquakes, typhoons, tsunami flows, climate changes, epidemic diseases and/or virus mutations. There have surely been many times when earth was ready for a drastic event such as the appearance of life to happen. The switch from RNA to cell could be explained by our recent discovery in the silkworm moth Bombyxmori that one single RNA strand does not produce a single type of protein but rather a huge diversity of protein variants to retain multiple functions in a given tissue [6]. RNA built in space at one very long time in the most remote distant past and mutated on the earth surface many thousands Mya over a

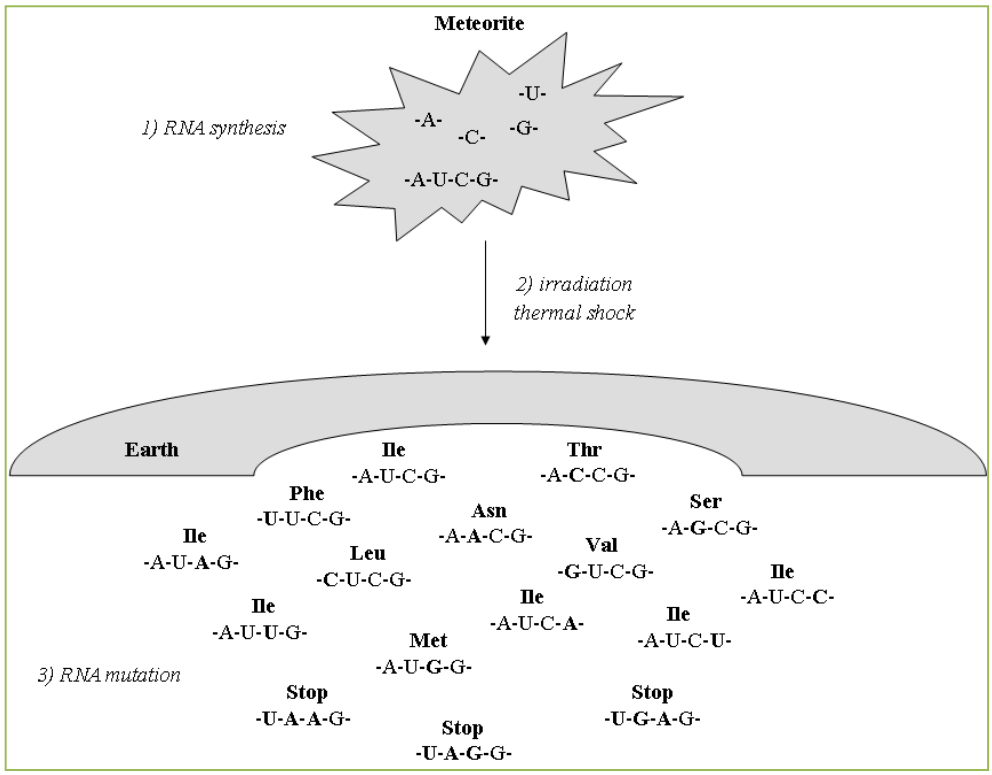

Figure 1. RNA built in space and mutated on earth. The four basic constitutive elements of RNA (adenine, cytosine, guanine and uracil nucleobases) built in space on small stony-iron minerals of meteorites, bringing on earth crust the essence of life (1). An extraordinary event (2) such as an intense radiation, a thermal shock and/or the release of an incredible amount of chemicals of all sorts falling into shale to release gas forced RNA to more flexibility, replication and complete nucleobase re-agency (3). This was prelude to cell formation, tissue differentiation and development of key functional processes such as fat encapsulation and exocytosis.

thermal shock and/or an irradiation peak to produce multifunctional proteins and thereby original cells (Figure 1). There is no doubt that processing of fatty acid and lipid chemicals has been a crucial event during the genesis of cells. Curiously enough, we observe that RNA keeps mutating now and then in very specific tissues such as the moth pheromone gland [6-7].

The finding was made in the insect chemosensory protein (CSP) family, which has been described as being involved in multiple functional systems such as fatty acid transportation, odor recognition, pheromone biosynthesis, insect development, intrinsic tissue regeneration and more recently insecticide resistance in close cooperation with degradative enzymes such as cytochrome P450 oxidases [8-10]. Subtle base replacements are found to occur in the RNA sequence to produce proteins with key amino acid substitution, deleted motifs or prominent C-terminal tail in the group of CSPs from the silkworm moth *B. mori* (BmorCSPs) [6]. This is not observed for one but fourteen BmorCSPs (BmorCSP1, BmorCSP2, BmorCSP3, BmorCSP4, BmorCSP6, BmorCSP7, BmorCSP8, BmorCSP9, BmorCSP11, BmorCSP12, BmorCSP13, BmorCSP14, BmorCSP15 and BmorCSP17). This RNA editing process is not only observed in CSPs but also in the full-amino acid sequences of odor-binding proteins (OBPs) including pheromone binding protein-1 (PBP1), general odorant-binding protein-2 (GOBP2), PBP-related protein-3 (PBP-RP3), protein B1 and sericotropin, suggesting that all various sensory genes including olfactory receptors are subjected to RNA editing (6). These RNA mutations detected in CSPs and OBPs are not related to single nucleotide polymorphisms (SNPs) at genomic level. We sequenced genomic DNA in various individual female moths without finding any SNPs. We found instead a huge amount of RNA-DNA differences (RDDs) particularly expressed in the female pheromone gland and that such tissue-specific RNA mutations resulted in the gland in the synthesis of a huge diversity of protein variants [6]. All of these "subtly modified" proteins have been clearly identified by peptide sequencing [6]. It is very likely that most of all these "subtly modified" protein variants retain very different functions although this still needs to be demonstrated eventually by crystallizing the structure of a truncated CSP protein is form. Very importantly, we have found that RNA editing results in the synthesis of protein is forms varying in size between 7-9 and 12-14 kDa. That most

of them have a totally different N- and/or C-terminus. That mutant CSP forms can also have a very different number of cysteine residues and thereby a very different pattern of disulphide bridges. That specific amino acids (Glycine) are inserted near Cysteine following RNA mutations. That Leucine is replaced by Proline at multiple sites and that a load of mutations occurs mainly on α-helix 1 of the CSP protein [6]. Thus, one single RNA strand can lead through base nucleotide mutations to an extremely large panel of proteins differing by primary and secondary functional structures. This may bring answers to one key question for the appearance of life in the RNA world: how could RNA possibly survive and retain enough diversity for the molecules to create proteins, compartments, cells and later with time various tissues and organisms? The huge diversity of RNA and protein mutants identified in the silkworm tissues has allowed us to enounce the theory of RNA mutations as source of life: "A key element for the appearance of life is that RNA not only produces a large number of 'perfect' copies of itself but also an extremely large number of copies with tiny typo 'mistakes' in the base sequence. RNA concentration is now enough so that replication can take place under any plausible abiotic condition. RNA diversity is now enough so that multiple proteins can be built and eventually with time form membrane or tissue under the same plausible abiotic condition" [11].

Thus, studying clones of CSP mutations may bring answer about the origin of life at the time where the ancestral original RNA molecule created new proteins and thereby new cells (Figure 1). It is very unlikely that all various gene families are subjected to RNA mutations in the extent seen with CSPs. The cell under such conditions of heavy RNA trafficking will certainly explode. However, our results show that RNA editing in CSPs leads to all sorts of combinations like an infinite process [6]. There is up to 44% of mutations on the CSP sequence and more than 78% on the N-terminus [6]. We report not only A>I conversions that require specific adenosine deaminase acting on RNA (ADAR) enzymes and recognition of duplex RNA structures but also many other types of conversions such as T>C, G>A, C>T and G>T that must involve other RNA editing mechanisms than ADARs [6]. CSPs likely represent a very ancestral gene family, which could date back at least to 521 Mya when the first arthropods (trilobites) appeared at the early Cambrian. They are found in most of all insect species as well as in water flea and shrimps (ABH88166, ABH88167 and ABY62738). However, they could well date back to billion years ago when the first form of life appeared. No research has been ever made to identify CSPs in bacteria and/or other ancient and small prokaryotic organisms. This should be definitely done to solve CSP-RNA editing mechanisms in the most primitive living organism [12] and perhaps understand what happened many thousands Mya when the original ancestral RNA molecule landed earth on a meteorite fragment (see Figure 1).

In our study of the silkworm moth, a particularly high degree of RNA editing and CSP peptide variation is found to occur in the female pheromone gland [6], suggesting perhaps that all glandular secretory systems have specifically developed on the basis of RNA and protein mutations.

A gland is more or less an epithelial invagination that turns into connective tissue and differentiate into secretory units. The pheromone gland of the female silkworm moth *B. mori*has only one function, i.e. to produce and secrete pheromone compounds (Bombykol, Bombykal and/or Bombykyl acetate) [13-15]. Our results in *B. mori* suggest that the secretory function of the female pheromone gland strongly depends on RNA editing. The pheromone gland is characterized by a continuous flow of materials (especially fatty acids) from the endoplasmic reticulum where proteins and fats are built, through the Golgi complex where the molecules are processed and eventually modified, and out to various locations including the surface of the plasmic membrane (Figure 2). During this journey, vesicles full of fats and fatty acids are formed, attach to the terminal button end wall of the plasmic membrane, fuse when they come in contact with each other and finally open to release specific pheromone compounds on the top of a dynamic cellular network of all interconnected processes tightly regulated by external environment [16]. Different BmorCSP mutant proteins may well serve at different steps of the exocytotic process necessary for pheromone release in the female moth pheromone gland, each type of CSP being involved in the transport and sequestration of a particular type of fatty acid (Figure 2). For instance, BmorCSP2, BmorCSP6 and BmorCSP11 are the most abundant CSP proteins expressed in the female pheromone gland in *B. mori*. Correlatively, a huge amount of mutations is detected at the level of RNA for these genes in the silkworm moth [6]. The 3D structure of CSPs has been shown to preferentially interact with long hydrophobic fatty acid chains [17]. Strong binding affinity is found between CSPs and fatty acids such as linolenic acid as known pheromone precursor [18]. This suggests that BmorCSP2, BmorCSP6 and BmorCSP11 interact with different fatty acid chains in the moth pheromone biosynthetic pathway and perhaps that variously edited CSP is forms are involved in different steps of the exocytotic process from encapsulation, constitution of secretory pathway and edification of secretory vesicles to docking, priming and release of specific pheromone chemicals at the glandular surface (Figure 2).

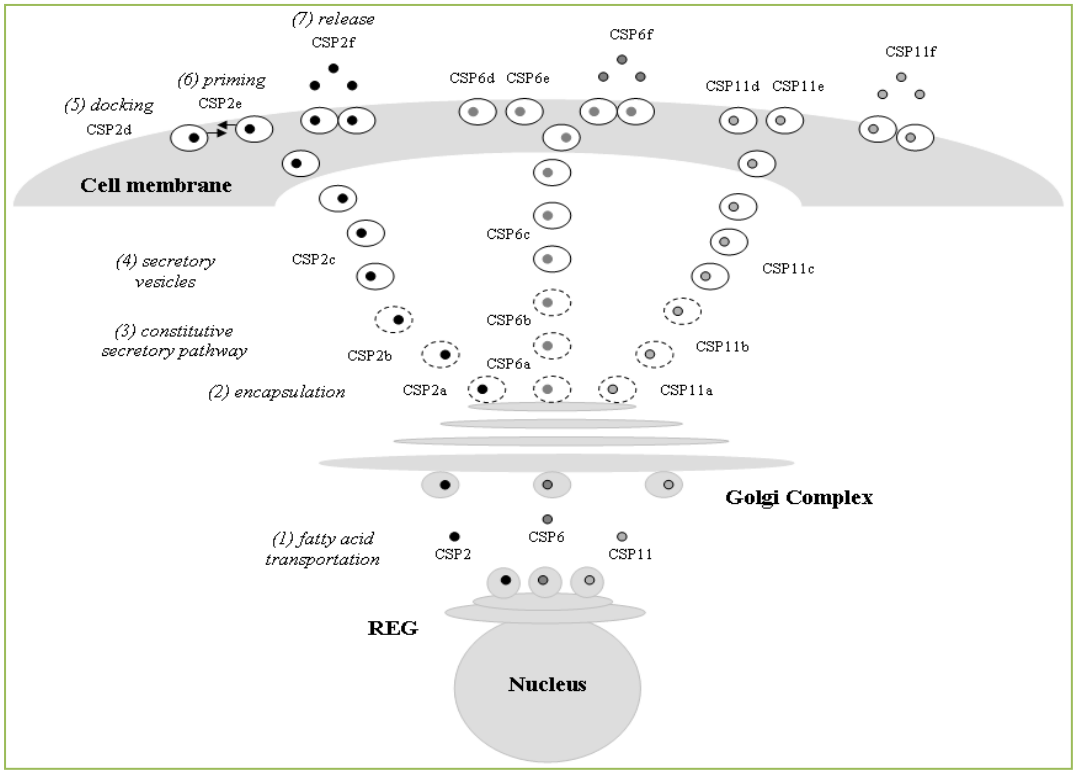

Figure 2. CSP mutants and endocytotic pathways unite the endomembranes of the moth pheromone gland into a complex and dynamic network for pheromone release. Fatty acids and lipids synthesized in the endoplasmic reticulum (REG) are transported by CSPs (CSP2, CSP6 and CSP11) and encapsulated in the membrane of the Golgi Complex [1-2]. Secretory vesicles are constituted and various CSP variants (CSP2a-CSP2c, CSP6a-CSP6c and CSP11a-CSP11c) take part in this process [3-4]. CSP2d, CSP6d and CSP11d variants are involved in the attachment of the vesicle to the cell membrane (docking) (5). CSP2e, CSP6e and CSP11e are rather involved in the attachment of a vesicle to another vesicle (priming) [6]. Other variants such as CSP2f, CSP6f and CSP11f mediate the release of specific pheromone compounds at the glandular surface (7). Dots of different colors represent fatty acids and lipids of different size and shape.

In rats, it has been shown that deficiency in ADAR seriously affects the regulation of exocytosis [19]. Regulatory action of ADAR may affect various mechanisms of intracellular exocytosis such as storing, docking, priming and/or releasing of lipid stocks [20]. This is particularly important for insulin granule biogenesis and insulin secretion, hormone signalling, trafficking of receptors in plasma membranes and release of neurotransmitters at the synaptic level [21-26]. Overshoots of insulin, hormone and/or neurotransmitter are well known to lead to pathological disorders in various physiological systems. Various diseases such as diabetes, obesity as well as specific syndrome brains and neurodegenerative processes have been reported to be associated with dysfunctions in exocytosis [27-29]. Correlatively, various human diseases including obesity, cancer, Alzheimer, Parkinson and Huntington disorder have been reported following dysfunctions in A>I RNA editing [30-37]. It has been proposed than specific ADAR and RNA editing could be used to reverse pathological situations for instance by editing pathogen RNA molecules [11, 38]. This would be a very efficient way to change a mutation on the RNA before it becomes lethal [39, 40]. Following this concept and our discovery in the silkworm, it may be very interesting to investigate the organization of the exocytotic pathway in the moth pheromone gland and to check whether specific CSP mutations affect one of the key steps in this process. It may reveal how specific CSP mutations affect the biosynthesis and release of specific pheromone compounds in insects. It may also help establish new strategies to restore normal conditions in the release of specific hormones and/or neurotransmitters in the various glands and synapses of the human body. Prelude to therapies and treatments against metabolic and neurological diseases, studies on a particular family of insect proteins (Chemosensory Proteins or CSPs) are here envisioned as a strong possibility not only to understand where we come from but also to develop new resources for human health.

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