# The Evolution of T-URF13*:* Does Irreducible Complexity count or not?

## A response to the evangelical DISCOVERY INSTITUTE (and others)

ANDREAS BEYER, HANSJÖRG HEMMINGER & MARTIN NEUKAMM

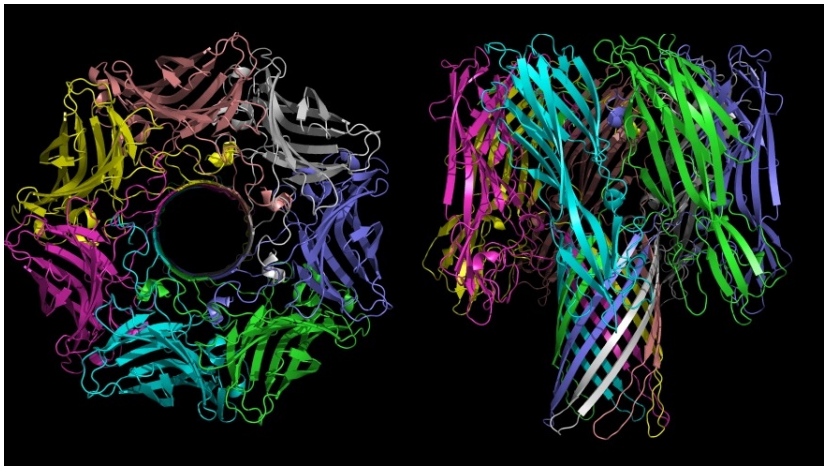

**Fig. 1:** Quaternary structure of a pore-forming membranous protein. The subunits (different colors) form a multimeric complex. The structure of the unique protein URF-13 is quite similar. Image source: Bassophile, Representation of Alpha Haemloysin from Staph Aureus, CC BY-SA 3.0.

The DISCOVERY INSTITUTE, the principal advocate of the Intelligent Design (ID) movement in the US, attempts to invalidate scientific results demonstrating that so-called **irreducibly complex (IC)** biological traits can evolve naturally. According to the ID protagonist Michael BEHE (1996), a system is *by definition* irreducibly complex if it consists of several specifically interacting components that together produce a function such that the removal of any single component leads to the failure of this function. Most biochemical systems are irreducibly complex in that sense. Since they cannot be less complex without losing their function, unguided natural processes based on random mutations and selection cannot build them up gradually, so BEHE. Instead, multiple coordinated mutations would be required *simultaneously*.

BEHE postulates that a stepwise evolution of such systems is too improbable to have happened if more than two coordinated mutations, two specific binding sites among proteins, or two specific interdependencies between the parts of a system must have been established in one fell swoop. Such systems are beyond the “edge of evolution,” BEHE says, and need an intelligent creator. This is the so-called *argument from irreducible complexity* (IC argument).

However, within the last 25 years, ID advocates were faced with more and more

logical arguments *and* empirical data, which made it apparent that their perception of how evolution works is untenable. One intriguing example is the evolution of a mitochondrial gene in the corn plant *Zea mays* named **T-URF13**, which encodes a ligand-gated pore-forming receptor protein called the **URF13** protein (HUNT 2007).

This membrane protein evolved in cultivars bred for male sterility called *Texas cytoplasmatic male-sterile maize* (cms-T maize). Data show that T-URF13 has arisen by rearrangements involving recombination events from two different fragments of the mitochondrial gene coding for **26S rRNA** (see Fig. 2–4). The latter is a special type of non-coding but functional RNA molecule, which is a pivotal component of ribosomes and essential to all cells. In short, male sterility in the cms-T cytotype is linked to the chimeric mitochondrial gene T-URF13 (BOSACCHI et al. 2015).

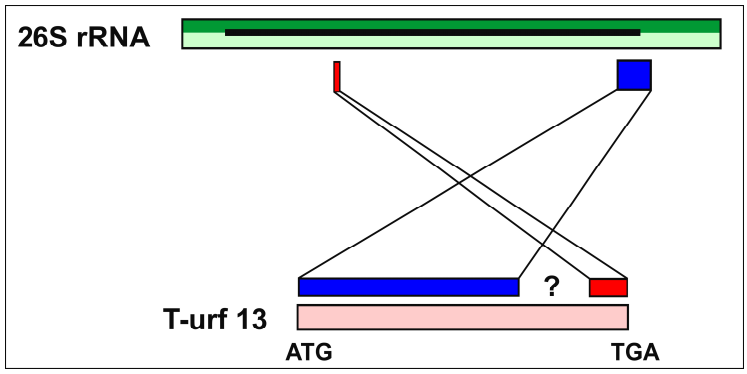


**Fig. 2** shows the origin of the T-URF13 region. This figure shows the mitochondrial 26S rRNA gene in maize, with T-URF13 at the bottom. In between are depictions of the two segments that were pieced together to form T-urf13. Both segments originated from the same strand of 26S rRNA. The question mark denotes a small part of T-URF13 for which no homologous sequence could be identified. Most likely, it arose from non-homologous DNA repair processes during the recombination process. Figure from HUNT (2007).

Remarkably, the URF13 protein comprises various distinct, specifically interacting elements that are functionally independent from each other. All of them are needed to accomplish its specific function:

- The URF13 molecules have specific contact regions that organize themselves into a multimeric protein complex in the membrane (Fig. 1).
- The complex forms an ion channel, switching between two defined conformational shapes that open and close the ion channel. This trait requires *additional, specifically complex* boundary conditions.
- The protein complex comprises a specific binding site for certain signaling molecules that open or close the channel.

URF13 thus displays all the fundamental properties of gated ion channels, which

control, by interacting with other transport proteins, the uptake or excretion of substances into or out of cells. It was found that the sensitivity of cms-T maize to the toxin of the fungus *Bipolaris maydis* is also linked to this property: The toxin binds to the URF13 protein and opens the ion channel, resulting in a massive loss of vital ions such as $Ca^{2+}$ and $NAD^{+}$ (BRAUN et al. 1989). There is some evidence that an analogous mechanism is responsible for male sterility (LEVINGS 1990, p. 947). The URF13 protein specifically reduces mitochondrial activity in the tapetum cells of the anthers. The resulting lack of energy leads to the degeneration of the tapetum cells. This suggests that a signaling molecule inside the tapetum cells also binds to the URF13 protein and opens the ion channel, leading to the loss of energy carriers.

What is the pivotal point? Notably, according to BEHE, those specifically interacting parts and their coordinated binding sites constitute an irreducibly complex system. Intriguingly, the URF13 protein is equivalent, regarding its complexity and functional role, to another irreducible complex three-component system discussed by BEHE (1996, pp. 108–110), see HUNT (2007). However, whereas BEHE asserts that billions of years would not suffice to get such systems naturally, URF13 evolved within a ridiculously short period of less than a hundred years, hardly a blink in evolution.

Accordingly, LENTS (2019) criticized Michael BEHE's new book “Darwin Devolves” and advocated T-URF13 because of “random tinkering.” The natural evolution of such a system from functionally unrelated genes that do not even encode proteins (and never did so in their history) indeed disproves BEHE's IC argument.

## The response of the evangelical DISCOVERY INSTITUTE

MCLATCHIE (2019) tries to invalidate the T-URF13 protein as an example for the evolvability of irreducibly complex systems. He summarizes his refutation in four points (given below in different order):

1. It is exceedingly unlikely that T-URF13 arose *de novo* from unguided mutations. This, in itself, is a *prima facie* reason to consider alternative explanations.
2. The T-URF13 evolutionary claim is a “just so story,” and no demonstration is offered of “textbook” evolutionary mechanisms accounting for its origins.
3. T-URF13 turns out to be a deleterious feature rather than one that is beneficial to the organism. Therefore, the case is possibly consistent with BEHE’s thesis in *Darwin Devolves*.
4. URF13 is a mitochondrial protein regulated by a nuclear gene, suggesting a scheme where T-URF13 did not arise from scratch but arose instead from a “devolutionary” process that broke the regulatory mechanism of some normally useful proteins.

## The flawed probability argument

The main objection against an “unguided” evolutionary origin of T-URF13 refers to the well-known probability argument. The premise is as follows: Neither the phylogenetic timeline nor the limited number of individuals involved seem sufficient (in such a case) for the emergence of an irreducibly complex system since it allegedly requires synchronized, complex mutational events consisting of several independent steps. Yet such evolutionary processes obviously *did* happen!

As we will see, all the data support the finding that the irreducibly complex URF13 protein emerged within a few decades from sequences lacking any genuine protein-coding capability. That supports the thesis that gene sequences that accomplish irreducibly complex functions are not as improbable as BEHE supposes. The immunization tactic of the DISCOVERY INSTITUTE once again aims at disputing the evidence, which refutes their premise, by referencing just the same, obviously flawed, probability argument!

> “Such a combination of events in, to borrow Hunt’s words, ‘one fell swoop’ does indeed seem highly improbable to occur by chance. This is especially the case given that it is estimated that only $10^{40}$ organisms have lived in the entire history of life on Earth … That is too few by a factor of $10^{20}$ for *T-URF13* to evolve *de novo*. And, of course, if we consider the numbers of eukaryotes, or individuals of maize, the number of trials available to generate a complex feature like *T-URF13* is drastically reduced by many orders of magnitude. In fact, surely such a strong improbability should *prima facie* cause us to consider alternative explanations to the one offered by Hunt, namely, that the *T-URF13* ion-gated channel arose by chance ‘in one fell swoop.’” (MCLATCHIE 2019)

This is a circular argument that can be used to reject *all conceivable evidence* for the origin of irreducibly complex systems *a priori!* It is therefore clear that the probabilistic calculations are based on false premises.

Imagine a couple of friends sitting at a table and playing cards. One of the players notes the order in which the cards are dealt. Then the probability of the cards appearing in exactly that order is calculated. The probability turns out to be so small that the friends could have played cards since the big bang without ever being dealt the same card sequence again (KITCHER 2007, p. 93). Nevertheless, the cards were dealt exactly in this order for the very first time! This tells us that the *a priori* occurrence probability of any specific event does not tell us if it actually happened or not. Literally, *any* event can be calculated as improbably as you wish, after the fact. However, since it actually happened, the probability of this specific event equals one. The fallacy of the probability argument is that it presumes the necessity of reaching a *pre-specified* target. This is not the case.

One could object that every conceivable card sequence would serve the players to continue their game, while in biology only a small fraction of all mutations (of all "deals") is actually "allowed" or functional. Nonetheless, there is no need to demand *pre-specified* mutations or pre-defined functions. Instead, there are **countless** potentially functional mutations, which are completely unknown to us. For example, if every thousandth or only every millionth double mutation has a positive effect under certain conditions, the statement that a ***specific*** double mutation occurs with a probability of only $10^{-20}$ becomes meaningless. This historical, unique event will be just one of an unthinkable number of possibilities to benefit the organism.

So, why does the DISCOVERY INSTITUTE keep on overusing the same flawed probability calculations, which correspond to the GIGO concept from computer science (which means "garbage in, garbage out"), for decades?

> "Note that there are at least five discrete evolutionary events that must occur in order for *T-URF13* to arise: First two sections of DNA … must be brought together by random mutation. But this alone is not sufficient to produce a functional gene. The second step is that the atp 6 promoter must be brought into a position to control the gene. Third, fourth, and fifth: The three binding sites needed for *T-URF13* to operate must evolve … Presumably, unless all of these events occur, *T-URF13* cannot function to provide any selectable advantage in certain contexts." (MCLATCHIE 2019)

So what? Again, for two reasons, there is no "probabilistic hurdle" at all:

- BEHE is wrong in assuming that there are *pre-specified* target sequences that evolution has to "work towards." Many completely different sequences could serve to accomplish a particular function. Even when starting from *random sequences*, one or two mutations are often sufficient to gain a particular (or a total new) function (TONKIN et al. 2008; DE KRAKER & GERSHENZON 2011; YONA et al. 2018).
- The respective mutations have to arise neither *simultaneously* nor *in one single organism*. Instead, there is a contemporary, *parallel* accumulation of random mutations in thousands of individuals sharing a gene pool. Then, the different mutations can be combined via sexual recombination in various ways. Such a parallel process tremendously accelerates the evolution of innovations.

**Monte Carlo experiments do show that there is a good chance of producing irreducibly complex systems, even if that would require three, four, or even more "matching" mutations at the same time** (TROTTER et al. 2014). As the authors demonstrate, the evolution of such complex innovations is particularly promising when using genes that are under weak selection pressure, like cryptic genes. The number of cryptic genes in a given genome can be rather high. For instance, the proportion of

endogenous retroviruses in the human genome alone amounts to at least 8%. FINLAY (2006) speaks of 400,000 insertion points—a huge playing field for evolution!

We summarize that probability calculations against evolution are nonsense since we do not know the entire number of viable variants in the protein space:

> "A mathematical argument against evolution requires a detailed knowledge of both the probabilistic and geometric structures of protein space (or possibly genotype space, depending on the context). This knowledge is always lacking in practical situation. When you see a probability calculation in some piece of anti-evolution writing, you can be certain that it is based on biologically unrealistic assumptions." (ROSENHOUSE 2022, p. 160)

## Is the evolutionary explanation a "just-so story"?

The DISCOVERY INSTITUTE claims that homology or sequence similarities are "not enough to establish an evolutionary pathway"; HUNT's mechanistic scenario is a "just-so-story" that did not prove T-URF13 arose through recombinant evolutionary events:

> "[H]ow does Hunt know that *T-URF13* evolved by unguided mutational events from scratch? He doesn't. Or, at the very least, he has not established that *T-URF13* evolved by blind evolution."

> "If an event is observed to have happened that is highly improbable, having taken into account the available probabilistic resources, that in itself is evidence against the chance hypothesis, and it should drive us to investigate other possible options." (MCLATCHIE 2019)

This is a very odd argument, since ID proponents have always demanded evolutionary (breeding) experiments to falsify the IC argument! The moment they are confronted with such an experiment, they pirouette and demand more. But what more? The evolution of mechanisms at a molecular level is **always** reconstructed *indirectly* (by *hypothetico-deductive* reasoning). Therefore, their demand for "more" is either naive empiricist or an immunization strategy. What do they expect—a microscopic film exposure of how the genes fused together? No one sits in a cell nucleus and watches in real time how mutations happen.

Of course, HUNT was able to show that T-URF13 evolved through recombinant processes during the breeding of certain maize varieties. Crucially, T-URF13 is unique to cms-T maize plants (VON ALLMEN et al. 1991; RHOADS et al. 1995; DILL et al. 1997; ARUN et al. 2011; YI et al. 2021). No other sources for T-URF13 except the two mitochondrial DNA sequences we will discuss below have been found. Additionally, the data exclude exceptional pollen transmission of organelles or multiple horizontal gene transfer events as the source of T-URF13 (BOSACCHI et al. 2015).

In other words, if the gene did not just fall out of the blue (and, oddly enough, turned out to be identical for 94% of the ORF[1] length to pre-existing fragments of the mitochondrial genome), we cannot help but state that it arose (*de novo*) from available resources.

What other mechanisms than unguided evolutionary processes could be brought into play? Note that the experiments worked exclusively by selective breeding, that is, by a *Darwinian* mechanism. **T-URF13 is a result neither of "rational design" nor of directed evolution. No intentional processes were involved except the selection of pollen-sterile variants.**

Furthermore, all biochemical mechanisms that are involved in the origination of T-URF13 are well understood.[2] HUNT (2019) correctly mentioned in his response: "Recombination has been studied for decades, and we know enough about the enzymes to know that the attendant chemical mechanisms are all that are needed to promote the genomic shuffling that gave rise to T-URF13." Since we are able to identify the homologs of the T-URF13 sequence in the ancestor's genome, the recombination scenario is currently the only plausible explanation.

## Does T-URF13 originate from a "devolutionary process"?

Could one argue that URF13 admittedly evolved but originated from an *ancestral* pore-forming protein? In that case, the highly specific interdependencies of URF13 would have pre-existed somewhere in the DNA. Therefore, a simple mutation could have restored the receptor gene or altered its activity and specificity. Indeed, the DISCOVERY INSTITUTE prefers speculations about a pre-existing gene that became T-URF13 by some so-called *devolutionary process*, whatever this may mean:

> "One scenario is that the *URF-13* complex is involved in some other process, and that it became broken such that it was no longer properly regulated. Indeed, the first identification of *T-URF13* long pre-dates the sequencing of the maize genome in the early 2000s (Chandler and Brendel, 2002; Schnable *et al*., 2009; Soderlund *et al*., 2009; Gore *et al*., 2009; Vielle-Calzada *et al.* 2009). It thus was impossible to determine whether the *T-URF13* gene had indeed arisen *de novo* 'from scratch,' or whether it was already present and doing something else." (MCLATCHIE 2019)

However, no evidence supports these scenarios. There is no reason in sight to pursue

[1] ORF (Open Reading Frame) is the protein-coding region of a gene.

[2] They even explain the origin of the red-marked intermediate area illustrated in Figs. 3 and 4: When mismatched ends are assembled (via *non-homologous* double-strand break repair mechanisms), the sequence at the junction often changes rather randomly. That is why no significant homologs are found in the databases for this area.

them at all. By now, more than a hundred crop genomes, including mitochondriomes, have been **completely** sequenced and are available in public databases. There is no gene, and there is no sequence resembling T-URF13 except the mentioned 26S rDNA gene (plus the adjacent intergenic region).

In addition, the sequences of thousands of genes coding for rRNA are known from many taxa. Among them is not a single example of any pre-existing protein-coding gene with significant structural similarity to rDNA, with a structure that even remotely resembles T-URF13. Neither is any example known so far of an exaptation of rDNA fragments for coding a membrane protein. This is indeed the first one! In distinct contrast to the claims of the DISCOVERY INSTITUTE, a scenario that proposes the devolution of a pre-existing protein-coding gene is nothing but a just-so story, not supported by any data.

A renewed similarity search (BLAST on May 26th, 2022, carried out by A.B.) confirmed the result. The origin of the sequences coding for T-URF13 was identified without a trace of doubt: the two major fragments form part of the maize gene for the 26S rRNA and the adjacent non-coding region, respectively. There are no better and more specific search hits than these two perfect hits. Moreover, there is no other significant hit than those in 26S rDNAs (see the Appendix!). In addition, in protein databases, there are no significant hits for any part of the URF13 protein apart from URF13 itself.

## Is there a “hidden” protein-coding frame inside the 26S rDNA?

As already mentioned, T-URF13 is a chimera of two parts of the mitochondrial chromosome: an intergenic region that is not transcribed and therefore does not code for a protein (yellow), and part of the 26S rDNA gene encoding the 26S rRNA (green). The latter gene is transcribed into RNA but not translated. That means it also does not code for a protein (PRING & LONSDALE 1989).

Moreover, (26S) rDNA encoding for (26S) rRNA molecules does not contain a protein-coding reading frame—and obviously never did. Why do we know this? If one compares the coding regions (ORFs) of **protein-coding genes** from different species, one repeatedly finds so-called **InDels** (= insertions or deletions) of 3, 6, 9, etc. bases, that is, integer multiples of three. On the other hand, if one compares the coding regions of **genes that code for functional RNA molecules**, particularly the ribosomal RNAs and specifically intergenic regions, one finds InDels that do *not* comply with the “3-step rule”.

The biological explanation for this phenomenon is simple: Tiny insertions and deletions usually produce none or little effect in many regions of ribosomal RNAs, in proteins, and especially in intergenic regions. However, InDels containing 1, 2, 4, 5,

7, 8, 10, etc. bases shift the reading frame of an ORF. From the affected point on, the protein is completely changed and almost always loses its biological function. As a result, such mutations in protein-coding parts (ORFs) are negatively selected.

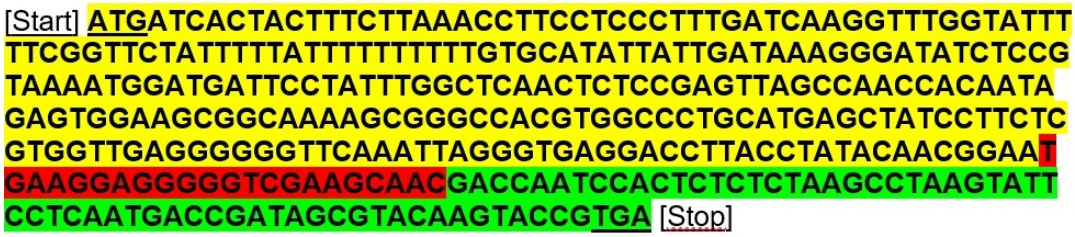


**Fig. 3:** Protein-coding region of the T-URF13 gene.


**Fig. 4:** URF13 protein (amino acids in the IUPAC one-letter code). The colors indicate the origin of the relevant shares:

**Consequently, the fact that rRNAs are *not* subject to the "3-step rule" is direct evidence that such rRNAs and rDNAs never coded for proteins. Therefore, the present state of knowledge is that the highly specific T-URF13 gene evolved from sequences that never coded for proteins in evolutionary history.**

## Do deleterious features invalidate the evolutionary argument?

The paper goes to some lengths to explain that URF13 is not a beneficial but a deleterious feature—a "loss-of-function change"—for the domestic plant. However, the conclusion drawn from this fact amounts to an attempt to mislead readers:

> "It is thus quite telling that, as their flagship example of a new protein complex arising *de novo*, Arthur Hunt, Nathan Lents, and others choose to promote a system that in fact causes harm to the organism that bears it. Surely if there were a better example — one that is functionally advantageous to the organism — then they would be touting that instead." (McLatchie 2019)

In fact, this "loss-of-function change" is not a case of structural *degradation* but a case of structural *formation* caused by **a completely new protein and IC system**. Such formative structures occur, under special conditions, also in the wild. As Hunt (2019b) mentions, male sterility is a feature of so-called gynodioecious plants. In

gynodioecious plants, females are male-sterile, often via mechanisms that recall those that underlie male sterility in cms-T corn. Therefore, such structures can evolve not only by artificial means, but also by natural selection. HUNT (2019b): "To claim that this trait causes harm is to badly misunderstand many aspects of plant biology."

Apart from that, it is impossible to believe that MCLATCHIE does not know that the effect of selective forces does not depend upon a "benefit" in the sense of a quasi-technical optimization but solely upon a fitness benefit. There is simply no causal link between mutations and mutation mechanisms on the one hand and the question of how selection works on them on the other. There is also no link between the complexity of mutational pathways and the question of whether the product is beneficial or deleterious. For the discussion of the probability of an event occurring in evolution, it is completely irrelevant if this event might be labeled "advantageous" or "deleterious" in any "technical" sense.

**Once again, the question of whether a trait can be considered "beneficial" or "harmful" from a functional point of view is independent of the question, how complex it is, and how it arose.** A machine gun can be considered a harmful tool, but surgical instruments are considered beneficial. That does not mean one is developed easier than the other one. The fact that counts is that URF13 is irreducibly complex.

## Does URF13 originate from a signal peptide of *Arabidopsis*?

The institute's final contention is a prime example of argumentative chaos. Below, the argument is quoted in full. Notice, for example, the phrase "an insertion of DNA into a pre-existing gene that was itself a membrane protein for mitochondria." Is there a gene that is concurrently a protein? That is remarkable! Alternatively, look at this: "RLF32_ARATH is a signal peptide for import into mitochondria for a gene in *Arabidopsis*." Experts know that not the plant gene is imported into mitochondria but its product, a protein. However, anyone without expertise will become hopelessly confused. Just savor the whole passage:

> "Proteins embedded in the mitochondrial membrane are synthesized in the cytosol, and therefore they possess an alpha helical a[m]phipathic coil at the N-terminus that is recognized by transport complexes (see this section from the textbook *Molecular Biology of the Cell* for details). It follows that there ought to be a signal peptide for mitochondrial membrane insertion. That implies that what we have is actually an insertion of DNA into a pre-existing gene that was itself a membrane protein for mitochondria and perhaps a channel — and perhaps a regulated channel since it is affected by nuclear genes. A tool called Signal-BLAST, available at the website of the Center for Applied Molecular Engineering (CAME), allows a user to identify signal peptides in a protein sequence. Entering the FASTA file for *T-URF13* yields a result output of a signal peptide, putative cleavage site after AA 35 (by similarity to RLF32_ARATH). RLF32_ARATH is a signal peptide for

> import into mitochondria for a gene in *Arabidopsis*. That protein is involved in cell-cell signaling by way of Ca++ influx. What all this means is that *T-URF13* probably came from a fully functional, pre-existing gene, and did not arise *de novo*." (MCLATCHIE 2019)

The basic argument, disentangled from the chaos, runs as follows: An analytic tool that allows "high-performance signal peptide prediction based on sequence alignment techniques" shows that the mitochondrial gene T-URF13 contains a sequence similar to one coding a signal peptide in the plant *Arabidopsis*. The peptide is needed, so the claim, to transport a mitochondrial membrane protein which is synthesized in the cytosol (the cell body) into the membrane of mitochondria. That must happen because the gene encoding the mitochondrial protein is located in the cell nucleus. Most of the genes coding mitochondrial proteins are indeed located there. In addition, the membrane protein, which contains the signal peptide in question, forms a regulated ion channel. It is said that it regulates the influx of $Ca^{2+}$ ions into the mitochondria of *Arabidopsis*. Therefore, the argument is that T-URF13 could have originated from such an ion channel gene and does not have to be evolved *de novo*.

We can refute the argument easily. HUNT (2019a), for good reasons, has rated it as "jaw-droppingly bad".[3] For one thing, the T-URF13 gene is located not in the nucleus but in the mitochondrial genome. Thus, no import into mitochondria happens, neither for the URF13 protein nor for T-URF13. That is simply a matter of fact. There is no trace of T-URF13 or anything similar in the nuclear genome. So why should a hypothetical predecessor gene code for a signal peptide which transfers cytosol proteins from outside into the mitochondrial membrane? That simply makes no sense.

Secondly, the claim that the analytic tool detects a gene sequence similar to that coding for a signal peptide known from *Arabidopsis thaliana* is dubious. The match is incomplete at best. Strangely enough, the particular *A. thaliana* protein, to which the signal peptide in question belongs, is not transported into mitochondria but into the plasma membrane, so there is something seriously wrong with the whole claim.

Thirdly, there is no significant match between this protein family and URF13. There is even no remotely significant match with **any** protein in the whole protein database (Protein BLAST @ NCBI: https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins). Obviously, whatever the *Arabidopsis* genes are and do, there is no evolutionary connection to T-URF13. However, only experts in molecular genetics realize that the so-called data are fabricated. Everybody else is overwhelmed by the maze of technical jargon. That is the DISCOVERY INSTITUTE's strategy.

[3] Further pseudo-arguments against the T-URF13 example presented by the "Uncommon Descent" guys are debunked by HUNT: https://www.ag-evolutionsbiologie.net/html/2019/T-URF13.html

## Literature

ARUN, V. et al. (2011) Transformation and analysis of tobacco plant var *Petit havana* with T-urf13 gene under anther-specific TA29 promoter. 3 Biotech 1, 73–82.

BEHE, M. (1996) Darwin's black box: The biochemical challenge to evolution. The Free Press.

BOSACCHI, M.; GURDON, C. & MALIGA, P. (2015) Plastid genotyping reveals uniformity of cms-T maize cytoplasms. Plant Physiology 169, 2129–2137.

BRAUN, C.J.; SIEDOW, J.N.; WILLIAMS, M.E. et al. (1989) Mutations in the maize mitochondrial T-urf13 gene eliminate sensitivity to a fungal pathotoxin. PNAS 86, 4435–4439.

DE KRAKER, J.-W. & GERSHENZON, J. (2011) From amino acid to glucosinolate biosynthesis: Protein sequence changes in the evolution of methylthioalkylmalate synthase in *Arabidopsis*. The Plant Cell 23, 38–53.

DILL, C.L.; WISE, R.P. & SCHNABLE, P.S. (1997) Rf8 and Rf* mediate unique T-urf13-transcript accumulation, revealing a conserved motif associated with RNA processing and restoration of pollen fertility in T-cytoplasm maize. Genetics 147, 1367–1379.

FINLAY, G. (2006) Human genetics and the image of God. http://www.cis.org.uk/ireland/documents/God&Science_Paper2_Finlay.pdf

HUNT, A. (2007) On the evolution of Irreducible Complexity. https://pandasthumb.org/archives/2007/05/on-the-evolutio-1.html

HUNT, A. (2019a) T-URF13 and devolution. https://discourse.peacefulscience.org/t/T-URF13-and-devolution/5696

HUNT, A. (2019b) Where gynodioecy is cast out of God's creation https://discourse.peacefulscience.org/t/where-gynodioecy-is-cast-out-of-god-s-creation/5651

KANZAKI, H.; TAKEDA, M. & KAMEYA, T. (1991) Sequence analysis of a mitochondrial DNA fragment isolated from cultured cells of carrot cytoplasmic male-sterile strain. The Japanese Journal of Genetics 66, 719–724. https://www.jstage.jst.go.jp/article/jjg/66/6/66_6_719/_article

KITCHER, P. (2007) Living with Darwin. Evolution, design, and the future of faith. Oxford University Press.

LENTS, N.H. (2019) Behe's last stand: the lion of Intelligent Design roars again. https://www.skeptic.com/reading_room/michael-behes-last-stand-lion-of-intelligent-design-roars-again/

LEVINGS 3rd, C.S. (1990) The Texas cytoplasm of maize: cytoplasmic male sterility and disease susceptibility. Science 250, 942–947.

MCLATCHIE, J. (2019) Does *T-URF13* Refute Irreducible Complexity? A Response to Arthur Hunt. https://evolutionnews.org/2019/04/does-T-URF13-refute-irreducible-complexity-a-response-to-arthur-hunt/

PRING, R.D. & LONSDALE M. (1989) Cytoplasmic male sterility and maternal inheritance of disease susceptibility in maize. Annual Review of Phytopathology 27, 483–502.

RHOADS, D.M.; LEVINGS 3rd, C.S. & SIEDOW, J.N. (1995) URF13, a ligand-gated, pore-forming receptor for T-toxin in the inner membrane of cms-T mitochondria. Journal of Bioenergetics and Biomembranes 27, 437–445.

ROSENHOUSE, J. (2022) The failures of mathematical anti-evolutionism. Cambridge University Press.

TONKIN, C.J.; FOTH, B.J.; RALPH, S.A. et al. (2008) Evolution of malaria parasite plastid targeting sequences. PNAS 105, 4781–4785.

TROTTER, M.V.; WEISSMAN, D.B.; PETERSON, G.I.; PECK, K.M. & MASEL, J. (2014) Cryptic genetic variation can make "Irreducible Complexity" a common mode of adaptation in sexual populations. Evolution 68, S. 3357–3367.

VON ALLMEN, J.-M.; ROTHMANN, W.H.; GENGENBACH, B.G. et al. (1991) Transfer of methomyl and HmT-toxin sensitivity from T-cytoplasm maize to tobacco. Molecular & General Genetics 229, 405–412.

YI, H. et al. (2021) Identification and genetic analysis of two maize CMS-T mutants obtained from out-space-flighted seeds. Genetic Resources and Crop Evolution 68, 1–11.

YONA, A.H.; ALM, E.J. & GORE, J. (2018) Random sequences rapidly evolve into de novo promoters. Nature Communications 9, 1530.

## Appendix: The mysterious PKT5 "gene"

Indeed, a recent BLAST search corroborated the notion that T-URF13 had been composed of two fragments of the mitochondrial genome (mtDNA) of maize. The yellow and green regions (Fig. 3) perfectly match other parts of the mtDNA, as described. The red part finds no match in the maize nuclear genome or mitogenome, corroborating the notion that this part has been created by mitochondrial DNA double-strand repair. The shortness of this part further supports that idea. Furthermore, there are quite a few nearly perfect matches simply by chance in very different organisms, as to be expected by the shortness of this part.

There is, however, a very surprising full-length match with a gene in a male-sterile lineage of carrot, *Daucus carota* (Fig. 5). KANZAKI et al. (1991) named it the *PKT5 gene*. Their paper seems to suggest that very similar (in fact, identical) rearrangements took place independently in maize and carrots to produce male-sterile lines and a full-length match with T-URF13. Since this is not a particularly satisfying explanation, how can this conundrum be explained? Some facts will help:

1. The alignment contains only *two* mismatches, but beyond that, there are *eight* (!) single-base deletions, all of them in *D. carota* PKT5. However, mismatch mutations occur 4–10 times more frequently than insertions or deletions. Moreover, they should have occurred in both genes at about the same rate. Here, however, the green and yellow parts of T-URF13 match perfectly to maize

mitochondrial DNA, whereas all mismatches occur in the carrot "gene." Such characteristics are typical for sequencing errors. This is further substantiated by the fact that all of those deletions would destroy almost half of the open reading frame, creating a substantially changed protein that surely cannot work like the URF13 protein.

2. The similarity of *D. carota* PKT5 to maize mtDNA is almost perfect on the one hand. On the other hand, its similarity to *D. carota* mtDNA is much weaker, as can be seen in the BLAST search. How should a carrot gene arise from the maize mitochondrial genome? The only possibility would be **horizontal gene transfer**, which is also not convincing since there is no further evidence.
3. The experimental approach of KANZAKI et al. is questionable since their hybridization experiment does not make sense. T-URF13 has been assembled from the maize mitochondrial genome and shows, except for the red part, perfect sequence identity. Therefore, it will hybridise with any mitochondrial genome with sufficient sequence similarity. So why even try?
4. To corroborate the mere existence of the carrot PKT5 gene, the presence of the coded protein has to be demonstrated. To our knowledge, this has not happened so far.

```
ATGATCACTACTTTCTTAAACCTTCCTCCCTTTGATCAAGGTTTGGTATTTTTCGGTTCT
ATGATCACTACTTTCTTAAACCTTCCTCCCTTTGATCAAGGTTTGGTATTTTTCGGTTCT

ATTTTTATTTTTTTTTTGTGCATATTATTGATAAAGGGATATCTCCGTAAAATGGATGAT
ATTTTTATTTTTTTTTTGTGCATATTATTGATAAAGGGATATCTCCGTAAAATGGATGAT

TCCTATTTGGCTCAACTCTCCGAGTTAGCCAACCACAATAGAGTGGAAGCGGCAAAAGCG
TCCTATTTGGCTCAACTCTCCGAGTTAGCCAACCACAATAGAGTGGAAGCGGCAAAAGCG

GGCCACGTGGCCCTGCATGAGCTATCCTTCTCGTGGTTGAGGGGGGTTCAAATTAGGGTG
GGCCACGTGGCC-TGCATGAGCTATCCTTCTCGTGGTTGAGGGGGG-TCAA-TTAGGGT-

AGGACCTTACCTATACAACGGAA
AGG-CC-TACCTATACACCGGAT

TGAAGGAGGGGGTCGAAGCAAC
TGAAGGAGGGG-TCGA-GCAAC

GACCAATCCACTCTCTCTAAGCCTAAGTATTCCTCAATGACCGATAGCGTACAAGTACCG
GACCAATCCACTCTCTCTAAGCCTAAGTATTCCTCAATGACCGATAGCGTACAAGTACCG

TGA
TGA
```

**Fig.5:** Alignment between *Zea mays* T-URF13 (top line) and *Daucus carota* PKT5 ORF (bottom line). The colors correspond to Figs. 3 and 4.

This all does not make any sense. By the way, it also does not fit into a design scenario (except you would like to argue, “The designer, in his unfathomable will, can do whatever HE/SHE/IT wants”). Such patterns, however, are typical of artifacts produced experimentally: mix-ups of clones and sequencing errors, which in most cases are never cured in the databases. 1st author (A.B.) experienced such cases during his time in the human genome project, to mention only three examples:

- At that time, a significant portion of clones from cDNA and gDNA libraries were interchanged, meaning the accession or ordering number was assigned to the wrong clone.
- During the assembly of chromosomes from reads, some sequences fall out of alignment for a simple reason. They originated from different species and were erroneously inserted into the processing pipeline.
- Erroneous sequences are produced either by PCR or cloning artifacts or by low-quality sequence runs.

Nowadays, LIMS (laboratory information management systems) have reduced such errors by some orders of magnitude. The respective paper (KANZAKI et al.), however, is from 1991, when such systems did not exist or were in their infancy. It fits well into the scenario that the authors were provided with a cloned version of T-URF13.

In short, obviously, the carrot PKT5 gene simply does not exist.




**06/2022 Last update: November 28th, 2023**