Science AMA Series: I am Alain Laederach, I study how our genetics affect the folding of our mRNAs and lead to disease, AMA!

Alain$_{L}aederach^{1}andr/ScienceAMAs^{1}$

[1]Affiliation not available

April 17, 2023

the WINNOWER

REDDIT

Science AMA Series: I am Alain Laederach, I study how our genetics affect the folding of our mRNAs and lead to disease, AMA!

ALAIN_LAEDERACH R/SCIENCE

ABSTRACT

[removed]

READ REVIEWS

WRITE A REVIEW

CORRESPONDENCE:

DATE RECEIVED:
September 11, 2015

DOI:
10.15200/winn.144188.86814

ARCHIVED:
September 10, 2015

CITATION:
Alain_Laederach , r/Science , Science AMA Series: I am Alain Laederach, I study how our genetics affect the folding of our mRNAs and lead to disease, AMA!, *The Winnower* 2:e144188.86814 , 2015 , DOI: 10.15200/winn.144188.86814

With CRISPR-RNA technology, and the talk of it being a new age for genetic modification in pre-existing organisms, and not just editing embryonic genomes and allowing them to develop. How is this actually done? Surely you'd need to go around to every single cell in someones body and edit that gene? I may be missing something but I struggled to understand it when I was told in lectures it had already been used on great apes.

Pdonger

CRISPR-Cas is indeed all the rage, and it is an extremely cool technology, I agree. On a basic level, the technology allows you to target double strand breaks (at the DNA level) using a guide RNA. Then using homology directed repair, you can insert new DNA into the genome.

You are absolutely correct that if you wanted to edit someone's genome, the only possible time to do it would be at the germline (single cell stage). If you were able to deliver the CRISPR-Cas system, using a virus for example, only infected cells (and their offspring) would be edited. This could still be very cool therapeutically, if you can target particular cell types. But yes, we could potentially edit an early stage embryo, but any kind of treatment using this technology would only change as many cells as you could get to.

small differences in our genetic code, especially those in the 99% of our genome that are non-coding

How small is a "small difference" when it comes to effecting RNA? Are we talking single nucleotides or does it have to be a massive mutation to make a difference? Can you please explain the process of how something that's non-coding still alters the RNA and inevitably the protein? Thanks!

Girthanthaclops

This question is a very basic one we are trying to answer in my lab, so thanks for asking. I admit I was not being very precise in my language when I said small difference. One thing I have realized in looking at human genetics, is that we are all far more identical than different, which is really cool. As mentioned below, we've shown that single nucleotide variants, which is the "smallest" mutation we can imagine can lead to cancers including retinoblastoma. It is really all a question of where the mutation

is, not necessarily how significant it is.

The thing to remember with RNA is that after it is transcribed from DNA, a lot of potential regulatory steps can still affect it. The first is splicing, where the introns (which are non-coding are removed). Mutations can affect this, one genetic disease where splicing is altered is Spinal Muscular Atrophy. After the mRNA is spliced it is exported and then must be translated into proteins. I am particularly interested in this step, as the efficiency with which an mRNA is translated depends on Untranslated regions (UTRs) that are either 5' or 3' of the coding sequence. It is at this level that we think mutations in the RB1 gene are causing retinoblastoma in individuals.

So yes, in general the protein level needs to be affected to get a phenotypic effect, but there are multiple post-transcriptional mechanisms that are directly regulated by RNA.

You mention a second layer of regulation.... are you looking at how genetics effects sub-RNA proteins (snRNA, siRNA) and how this relates to malformation and disease?

bazooka_matt
The second layer here I am referring to is the folding of the RNA. Since RNA is single stranded, and G can pair with C, A with U, it will fold into complex structures. These structures are often recognized specifically by RNA binding proteins. Thus if a mutation alters the fold of the RNA, by altering the base-pairing pattern, it may affect the RNA's affinity for crucial binding partners, affecting post-transcriptional regulation.

Hi there Dr. Laederach and welcome to the reddit,

While your work is highly interesting, do you find it difficult to get people outside of research interested in your basic biology studies? Do you find it easy/difficult to communicate with the lay audience when talking about something as 'basic', complicated, and descriptive as mRNA?

What do you think are the largest misconceptions of mRNA that the lay audience may have? The academic/scientific audience?

Thanks you!

gammadeltat
This is an excellent question, and I admit I was a little nervous even suggesting this AMA (would I be the first scientist who no one would ask questions too!). Fortunately it looks like I have my work cutout for me, this is quite fun.

It was interesting, as when I was writing the news and views and working on this feature with a reporter, I struggled with how best to communicate the scientific excitement about the field. It's definitely less cool than dinosaurs, or the mars rover pictures. It's also a lot more complicated, and can seem frustrating to the lay audience as I think in this field, we're just starting to figure out what we don't know. Thus I do find it challenging, and I'm still trying to figure out the best way to explain to people why I am so excited about mRNAs!

As for mRNAs and the lay field, I'm not sure most people are even aware of how central these molecules are to all life. Everyone has heard of DNA and the double helix, but I think RNA is a little like calculus, something complicated you may learned about it Biol 101 and hope to forget!

What do you think will be the next big thing in the RNA and gene expression fields in the next 10 years? Along the same line, if I'm currently in university what would be the biggest skills I should obtain now to prepare myself to be marketable and a good scientist later down the road (other than being hardworking and curious). Thanks!

psf73

I suspect which RNAs are being expressed, and their relative changes in expression profiling under different stress will be great biomarkers. One area of significant growth are exRNA, or extracellular RNAs which are likely signaling molecules as well as potential biomarkers. If we can get the problem of delivering RNA-like molecules figured, RNA therapeutics will be a paradigm shift.

Do you know of any evidence of small non-coding RNAs that can directly bind proteins and inhibit their action? Or do you believe that such an idea is far-fetched? Like miRNAs that act directly on proteins, but not linked through complementary gene sequence.

waterwheel

Well, the best examples are aptamers, these are artificial RNAs that are evolved by a process known as SELEX. These adopt specific structures and can bind a very wide range of protein interfaces. It's not clear the extent to which nature selects for aptamers as well, but it is amazing how specific you can tune RNA binding to proteins.

How do you think our previous knowledge of signaling pathways (by knockdown/knockout/genetic mutation) will be affected by our increased understanding of protein expression control by non-coding RNAs? Are scientists doing genetic manipulations taking into account effects of mutations on lncRNAs and other factors when they do these manipulations currently?

Thanks!

nctiger13

That's the million dollar question. I really don't know how much we are going to have to revisit our assumptions. However one thing is very clear, and it will be fascinating to see how this works out. One thing scientists have realized with CRISPR-Cas is that it is not quite as specific as we would like on a genome-wide scale. However, it turns out many of the genetic manipulations we do, e.g. making a knockout mouse, have this same problem. Thus it could be that a lot of our understanding of phenotype is the result of these "off-target" effects.

Please excuse me if I do not articulate as well as the doctors and graduate students on this subreddit. I have a basic understanding of cell biology and microbiology but with studying mRNA and how diseases can eventually occur from the replication through gene expression of the mRNA how far in the future do you think when physicians will screen patients at different intervals of life (5, 10, 15 yrs, etc.) to check for the probability of patients mRNA to contribute to turn into said disease?

Would this be something you could screen once in someones life for, or with mRNA being a constant process would it be more appropriate to do multiple screening throughout ones life?

Which specific cell in the body do you look at do determine if they would have to capability to produce a disease?

Can every cell in the body produce a disease if the mRNA is damaged?

Now for some personal questions!

At what point in your life did you know that studying RNA and how it can lead to diseases was what you wanted to pursue?

What is your favorite food?

Thank you in advance for answering questions on /r/science!

J_k_h
This is an interesting point and this will allow me to remind everyone of a fundamental aspect of our genetics. Since we originate as a single cell, our genetics (in the classical sense, i.e. the code in our genome is the same for every cell). As such, your genome will be the same when you are a fetus or a grandfather! It is now possible to isolate fetal cells from a mother's blood, and basically if you sequenced that genome, that information will be the same for the rest of your life.

For me what is interesting about RNA is that it does change, i.e. which RNAs get expressed in the cell, how they are processed will change with age, stress, hormone levels, and each of our RNAs will react differently to these stimuli.

One area where RNA expression is already having a direct effect in the clinic is in oncology. My colleague Chuck Perou looks at mRNA expression signatures in tumors to predict how they will react to certain drugs, this works especially well in breast cancer.

As for when I got into RNA, it was kind of an accident, I got a post-doc in a lab that had just started working on RNA at the time, and the rest is history.

As for my favorite food, good question, there is pretty much no food I don't really like. I however am not fond of Cilantro, and it turns out I have a good excuse, when I genotyped myself it turns out I am heterozygous for the allele that makes it taste like soap!

What diseases have you found linked to perturbations of RNA structure? Are any of those diseases linked to non-coding RNAs that are interfering with protein translation, and are those non-coding RNAs derived from non-coding portions of the genome?

It seems to me that the 99% of our genome that is non-coding was initially thought of as junk that simply got carried along through time, but we're realizing very rapidly that that area of our DNA might be playing an essential regulatory role in protein expression. Is that an accurate view of where we stand?

Thanks!

whogonnastopmenow
Hi and thanks for your question. So far, the three diseases my lab has identified as directly caused by RNA structure are hyperferritinemia cataract syndrome, retinoblastoma, and cartilage hair hypoplasia. If these aren't too familiar to you, it's because they are exceedingly rare. The latter however, may be familiar to quite a few redditors, Verne Troyer has CHH. It is associated with a non-coding RNA known as RMRP.

In both hyperferritinemia and retinoblastoma, these are private variants (i.e. mutations that occur only in one individual and their descendants) that map to the 5' UTR of thoses genes. RMRP is a ncRNA, that is associated with many RBPs and assists in mitochondrial splicing.

You are correct that 98-99% of our genome is non-coding but a significant portion of it is transcribed (estimates range from 75-95%). So it is likely that a non-coding mutation may end up in a ncRNA in some cell type. Exactly why our cells have are transcribing so much of our genomes is not yet clear, but given all the RNA is doing in terms of gene regulation, I definitely think it is important to keep investigating these non-coding RNAs.

Since almost all living things use the structurally stable DNA to store information and the most conserved RNAs across the domains of life have very conserved structures (rRNAs, tRNAs..), do you think that wobbly, structurally diverse mRNAs are maybe a hazard for the cell? Are we

evolving toward having only very structurally defined RNAs?

credopuedo
Excellent question, I like your use "structurally diverse" to describe mRNAs. I actually think the classic RNAs, like tRNA, rRNA, snRNAs are in fact the exception, not the rule. It's actually quite difficult to get an RNA to fold to a single structure. What I've found with nature is that generally evolution is quite happy with "good enough." Most of the time, a diverse structural ensemble is sufficient to do what is needed, so that is what we observed. Thus, I don't think we're going to evolve more rRNAs anytime soon!

Which class of non-coding RNA (lncRNA, circRNA, etc.) do you find the most interesting/exciting?

Another completely unrelated question, what kinds of software/techniques does your lab use to determine folding? I've seen a lot of articles that use SHAPE, and I've always been interested in how it predicts folding.

BeerandBiochemistry
I've actually gotten a lot more interested in viral RNAs recently, these can be used. We've been studying alpha viruses recently, and those are over 10kb. Obviously everyone is very excited about lncRNAs these days as they are new, but I like to remind everyone in my lab that 95% of the RNA in all our cells is the good old ribosome. It is the most conserved sequence in all clades of life, and literally makes proteins. How cool is that, we're all made by RNA!

For hyperferritinemia cataract syndrome, retinoblastoma, and cartilage hair hypoplasia), have you found that the diseases are caused by mRNA directly (for example by altering gene expression or resisting degredation), or by mis-translation of the mRNA? Additionally, when you say that a change in the structure of mRNA can have effects, is this the same concept as when proteins change structure, which alters their function and can lead to either aggregation or losses/gains of function?

catson4
So far, we've focused on translation as that is a relatively straightforward mechanism to assay experimentally using something like a luciferase assay. mRNA degradation can also be assayed, but those experiments I find are a little more involved. We're certainly interested in looking for RNA stability changes too, and are excited about new technologies including BRIC-Seq that should allow us to measure these effects quantitatively in a high-throughput way.

Thank you for discussing your research here!

Has anyone found examples of single nucleotide changes that don't consistently cause conformational changes, but instead cause instability, similar to temperature-sensitive mutations or the protein structure instability seen in heritable prion diseases?

neurobeegirl
That is an excellent question. RNA folding is a little different from protein folding. First, proteins fold due to a hydrophobic collapse of the core, RNAs don't have such a core. What is really interesting about RNA however, is that it has far fewer side chains than proteins (4 in RNA, as opposed to ~20 for proteins). As such, each nucleotide has many more potential partners to pair with than amino acids in proteins. This means RNAs tend have many more potential alternative conformations. My lab is interested in this phenomenon, and in retinoblastoma, it seems that mutliple conformations are required for regulatory function. These are "collapsed" into single conformations by single variants, it's really quite surprising.

RNA nucleotides can be modified enzymatically from the original A, C, G, or U, and this can affect the folding and function of the RNA molecule. Do these modifications ever appear in mRNA, and if so, do they affect folding/misfolding related to disease?

jeargle
This is an excellent point, I believe what you are referring to is RNA editing, the most common form of it is A to I editing. What is interesting is the I mimics G in it's base-pairing, so you are correct it has the potential to affect RNA structure. Unfortunately we have yet to find an example of this, but I can tell you my lab is very interested in finding an example of this.

Hi Dr. Laederach! Can mRNAs have structural functions in organisms that live in extreme environments--thermal vents and such?

credopuedo
Yes, absolutely. In fact there are organisms that have thermosensors in their 5' UTRs, these are called RNA thermometers. Another win for RNA!

Hey Dr. Laederach, thanks for doing this AMA! I'm a bio student down the road at Elon University. I think your work is really fascinating; regulation of gene expression has always intrigued me because of its potential medical and the idea that people are more than just their DNA.

Anyways, just a quick question: do you have any idea how RNA structure affects ncRNA and its regulation of transcription?

goalposthead4525
Well it can clearly cause disease, one really good example is cartialge hair hypoplasia where a ncRNA is mutated and leads to dwarfism. The interesting thing is that not all mutations to the RNA cause disease, so these molecules are really doing something.

Keep studying Biology, it's a great field, and consider applying to our BBSP graduate program when you graduate.

Can alternative mRNA folding affect splice site selection, for example as a mechanism to explain why some mutations, particularly those far from the actual splice site, might cause splicing errors.

whatsherface
Yes, the classic example is in Spinal Muscular Atrophy, where intronic mutations can cause the phenotype. In my lab we've been studying alternative splicing in Chronic Obstructive Pulmonary Disease (COPD) and we think we have a neat story there, but that paper is still under review so I'll be able to say more when it gets accepted.

DNA and RNA are wonderful structures...My questions are they photo sensitive in nature and...what would happen if they are put in a altogether different solution do they evolve out.

etimejumper
Yes, it is an interesting point, for me most recently one of the most intersting novel of functions of RNA is mimicing fluorescent proteins, for example the spinach and broccoli RNAs, fold into complex structures and are able to fluoresce.