**The Lampbrush Chromosome Debate. February 2017.**

Participants as of 13th February

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**HM to JG with comments in blue added by IS**

In a nutshell, I think you may be overlooking the possibility that transcription on the loops of LBCs has no functional significance whatsoever. Yet it is something that we have all been obsessed by because it’s such an extraordinary phenomenon, especially in newts.

Your current research and the work of other earlier investigators, including myself, represents an all-out attack, using the most sophisticated tools and methods of the 21stC, justified by the perceived need to explain the transcription in the context of the perceived biology of the oocyte and, in doing so, rationalise all the anomalies and peculiarities that we encounter along the way. Indeed, looking back over the c.a.400 publications on LBCs there seems to be only one place and one person who has admitted to doubts and misgivings: none other than the great master HGC in his 1986 book.

Chapter 6 in the book looks at places where there are no LBCs and it’s very hard to find any correlations at all. I recall talking to him about this a lot when he was writing the book. Big genome small genome, big oocyte small oocyte, variations between and within phyla and classes and even families, duration of oogenesis, timing and biology of development, not much seems to make sense. Then when we look at specific situations the plot deepens. I have encountered a few real weirdos in my time but if any of them has correlated with anything it’s been rDNA amplification, not any aspect of genome organisation : Ascaphus, Flectonotus, 2:1 genome sizes in Plethodons, massive egg sizes, gigantic genomes (Necturus), tiny LBCs (how hard is it to justify the almost compulsive transcription of lots of tiny loops on a chicken microchromosome?).

So my present hypothesis is that transcription on the loops of LBCs has no functional significance. It just happens and seems special because it’s in a cell that’s (a) committed to meiosis 1 and (b) dedicated to the mass production of rDNA - and LBCs are breathtakingly beautiful. I most recently found myself rethinking this problem when I was writing my chromomere paper 4 years ago. I subsequently got even more curious after speaking with Elena and Garry and, had we had more time last summer and I could have focussed my mind on LBCs rather than my dodgy knee, I think you and I could have made some interesting progress.

Evolutionary question: what is the selective advantage to having LBCs and/or what might be the disadvantage of having LBCs if they had no functional significance? Don’t know in the first instance and none in the second.

Reading on in your piece you keep making the point that we don’t know what LBCs are doing and all the evidence so far is equivocal. At this advanced stage of our overall knowledge, don’t you think it’s surprising that we still have no hard evidence that any part of a loop transcript is functionally important (I suspect you’re going to correct me on that one).

I guess here we have to distinguish between "functionally important transcripts" and "functionally important transcription". It could be that no functional mRNAs are produced, but maintenance of transcription *per se* is for some reasons important.

The big question: do oocytes really have to have a stockpile of coding RNA to take them through early development and, if they do, how do the animals that have no LBCs get by?

Exactly! this is a very good question, which has no answer. I agree, that just this fact already hints on possible nonconventional function of LBC transcription

At one time I supposed that all animals had LBCs or something like them (mammals have always been excepted but I can’t understand why). I rationalised that if you couldn’t find LBCs then your technique was lousy or you were looking at the wrong time. Xenopus was like that. Nobody bothered with it because its LBCs were horrid little things – until you worked out the right conditions and - Hey Presto! But nevertheless, the variation is wide, from nothing like an LBC to dazzlingly loopy ones like Axolotl.

So you’re aiming to discover what LBCs make and the GV stores that’s useful, why some animals have long loops and others little fluffy ones, what the sequence make-up of a very long loop containing a specific gene is compared to that in a short loop with the corresponding gene. I am watching this space with great interest. My bet is that you find out lots of interesting things along the way but you will eventually conclude that LBCs do not make mRNA to be stored for future embryogenesis and what they do make is largely redundant and recyclable.

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**JG to HM with comments in blue added by IS and comments in red added by HM**

Thanks for your long and thoughtful email about LBCs. Although I don’t agree with you that LBCs have no significance, you have forced me to argue my case more clearly - which I will try to do here. You suggested that "you will eventually conclude that LBCs do not make mRNA to be stored for future embryogenesis and what they do make is largely redundant and recyclable.” I think that both parts of that statement are partially true! Let me clarify.

I think there are really two separate issues. What is a LBC? What functional significance does it have vis-a-vis oogenesis?

I think we should define a LBC on strictly morphological grounds (“I know one when I see one”). A LBC is simply a chromosome that has transcription units (TUs) that are big enough to see by conventional light microscopy. I am, of course, distinguishing a TU from a gene. So what are the factors that make TUs big? A short list is (1) the length of the gene, including its introns, (2) the amount of read-through transcription, (3) the rate of transcription (i.e., number of polymerases per μm), (4) the amount of co-transcriptional splicing and (5) whether the introns are retained all the way to the end of the TU.

This is my favourite part of the discussion! Yes, the length of the gene or TU! And yes, the rate of transcription! Here I speak from a non-meiotic community: one can see a loop only when the transcribed genomic segment is **long** (say, above 50-70 Kb) and **very intensely** transcribed. The latter means a degree of intensity when polymerases sit on a template one after another, as train carriages. As for nascent RNA splicing, I think by now it is quite convincingly shown to be co-transcriptional in many cases and in my view this discussion can be omitted.

I will discuss all of these before tackling the issue of oogenesis. Let me start with (1). Despite enormous differences in C value, the coding regions of genes are remarkably consistent in length among organisms. Thus, the exons of a given gene are essentially the same length for frogs, salamanders, birds, and mammals. So why do the loops (or TUs) vary so much between organisms? Assume for the moment that polymerase packing is the same. Both the introns and the intergenic regions vary enormously with C value. For instance, from what sequence data we now have, we know that the axolotl has one or more truly enormous introns in a number of genes. For this reason alone those loops of the axolotl will be much bigger than those of Xenopus. Downstream transcription is another possible factor, but largely unexplored (except for the histone genes in Notophthalmus). The intergenic regions are longer in organisms with high C value, so downstream transcription in the LBC loops would lead to longer loops in organisms with high C value. In sum, given equal polymerase packing, the loop for gene A in an organism with low C value will be shorter than the corresponding loop in an organism with high C-value because of long introns and long intergenic regions. Without these two factors, high C-values would simply lead to more and/or larger chromomeres. (so western plethodons have retained the same intron lengths as their ancestors but expanded their intergenic regions – or some other widely distributed component of the genome. Different genome sizes, same looking LBCs, different numbers of chromomeres and loops – would need critical re-evaluation) (note by me: Vlad and Macgregor – comparative work on plethodons with different genome sizes).

The above considerations assume equal polymerase loading. We know that loop size for a given organism, such as Xenopus or Ambystoma, varies with age of the oocyte. It is reasonable to assume that these size differences reflect polymerase loading (the number of polymerases per micrometer of B-form DNA). Polymerase loading is the major determinant of the “rate of transcription.” That is, there is reasonable consensus that the rate of polymerase movement varies only within narrow limits. What I am saying is that another major factor affecting loop size is the number of polymerase molecules per μm of DNA.

So, to restate the obvious: loop length reflects the length of the TU and the polymerase packing (transcription rate). Some organisms will have LBCs because they have long TUs and high rates of transcription, whereas others will never have them because the TUs are too short and/or polymerases are scarce (think salamander vs yeast).

A very correct statement!

The second part of the discussion concerns the relationship of LBCs to production and storage of mRNA during oogenesis. The not so obvious conclusion is that an organism with no introns and no downstream transcription – and therefore insignificant LBCs - could transcribe just as much mRNA in the same amount of time as an organism with huge introns and massive downstream transcription – and therefore prominent LBCs. The caveat again is that polymerase packing is the same in the two cases. So size of loops tells us little without information on introns and downstream transcription. That is essentially why I want to do single molecule FISH on LBC loops, as outlined in the grant proposal I sent you.

So we shouldn’t be surprised when two organisms have equal sized eggs and equal time of oogenesis, but one has prominent LBCs and the other has puny ones. The one with big LBCs would, indeed, be making a lot of stuff that is “redundant and recyclable,” as you put it. They are making a lot of introns and perhaps downstream sequences. But both organisms could be making the same amount of mRNA. That this requires a high rate of transcription (polymerase packing) and a long period (days to months) for both of them is indisputable from simple math. That is because both of them have only four copies of the transcribed regions. (that assumes that the fertilised egg really needs lots of mRNA).

This gets us into the issue of why oocytes don’t become polyploid. That one hardly needs discussion: polyploidy is incompatible with meiosis. And it also gets us into the issue of nurse cells. Nurse cells reduce or eliminate the need for high rates of transcription by the oocyte (Drosophila being the extreme).

I thought there are some examples of animals, which have both LBC and nurse cells... Is it true or I am horribly mistaking? Elena?

So to come back to your original conclusion, I would say that **all large oocytes must get mRNA from somewhere to be used during oogenesis or to be stored for future embryogenesis or both.** That mRNA can come from prominent LBCs, small LBCs, nurse cells, or even some combination of these. The size of the LBCs is not the determining factor. So LBCs in the sense of giant loops are dispensable, but high rates of mRNA production are not. (?)

To continue the C-value issue a bit, I am sure you know at least as much about C-values as I do. But there is an interesting table for all animals at:

http://www.genomesize.com/statistics.php

The main point is that C value does not correlate with either evolutionary (C value does certainly correlate with evolutionary history and the consequences of C value change are manifest in all manner of aspects of growth and development – a much more complex issue than you imply) or morphological “complexity.” Thus, if large LBCs tend to occur in organisms with large C values, and vice versa, then small and large LBCs should be found within all the major groups. So, in my opinion, it doesn’t prove anything to point to a group of related organisms and be surprised that some have prominent LBCs and others don’t. The presence or absence of LBCs is not necessarily correlated with the ability to make large amounts of mRNA. This seems contradictory.

Bottom line: what we need to determine (after all these years) is the molecular structure of loops that contain identified genes. Where is the promoter? How long are the introns? Is there downstream transcription? What is the polymerase packing? All this is possible now with single molecule FISH, ChIP-Seq, and deep sequencing of nascent RNA. (and what is happening to the gene transcripts)

Yes, very good points by Joe! I might overlooked the recent LBC papers but I indeed have never seen a clear image showing a loop with marked gene on it. Is a gene always in the beginning of the loop, e.g. at the thin end? Can a gene be in the middle of a loop? Does transcription always starts form a gene promoter? I do not know about single molecular FISH but the transcriptome of diplotene oocytes is probably the most straightforward way. I also do not know what is the best object, Xenopus or chicken - both are sequenced.

Again, excuse my unawareness of recent LBC works, but **how many genes were mapped to LBC loops** all together, including amphibians and birds? I think most of the old reports were focused on repeats.

So there you have my overall thoughts. I value your comments and especially your suggestions for experiments that will test these thoughts.

Joe

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**HM to JG**

Good.  I hoped you would take up the challenge!  I’ll have a further think.  But in the meantime can I ask why a human sperm transforms into LBCs when you put it into a Xenopus oocyte. There is something about that GV environment that simply (or not so simply!) leaves a chromosome no option but to get lampbrushy.

And – and this is an experiment that only you can do since it’s right in your extraordinary range of intricate capabilities – what happens when you put an axolotl sperm into a Xenpus gv?   If you’re right, it should make LBCs with long loops.  Doable!  Actually, I bet you’ve already done it.

Do we assume that the disappointing loops that have been observed but not reported in some amphibians with gigantic genomes and the fact that western plethodons have the same looking LBCs as eastern ones despite the 2:1 genome size difference are all because of inadequately critical LBC technique and uncontrolled observation. A redo of the comparison between the LBCs of P. cinereus and P. vehiculum would be interesting but will probably never be done. I’ll have a look back at our mid-70s records when we were comparing East and West chromomere numbers, which were – in my view - indisputably 60 – 70% higher in the large genomes. I know you have never believed this but take a look again at the 1975 Chromosoma paper. Bigger genomes, more chromomeres, same general sizes of loops (?).  I know this is all a bit vague and circumstantial but your hypothesis is not going to pass the critical evaluation of the world’s expert LBCers without taking note of these kinds of observations.

Keep going.  It’s fun!

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**A E to all with comments in blue added by IS**

I would probably agree with the statement “LBCs provide dispensable maternal mRNA”, but not when the more general terms of informational RNA and early embryogenesis are invoked.

I think that, as lampbrushologists, we are interested in transcription during diplotene. This is why Callan’s chapter on Drosophila sperm Y-chromosome structures was one of my favorite; it went beyond female oocyte LBCs to a broader field of transcription during meiosis 1 prophase and the structures that arise during this stage. We are interested in transcription happening at a critical moment in male and female germ cell development, which has bearings on fertility, embryogenesis and transgenerational epigenetics (more on this at the end).

LBCs stand out among chromosomes because during diplotene they are easily visible and they are beautiful. However, I think that transcription of non-LBCs during diplotene is as important. In that sense, I would vote against a motion stating that “Transcription during diplotene has no functional significance whatsoever in the sense of generating informational RNA for use in early embryogenesis”. In fact, I think that transcription during diplotene is critical.

Since this debate is also intended to challenge some time-honored doctrines I’d like to challenge LBC transcription rates and in doing so propose an explanation for loop length. Transcript density on loops has been considered equivalent to transcription rate. In the LBC literature there has been a general notion that dense transcripts means that there is a lot of transcription happening at a very fast rate. However, I’d like to suggest a somewhat different interpretation in which transcript density reflects a "backlog" of transcripts (termination rates are slower than initiation). LBCs may not be unique due to their intense transcription, but their slow(er) termination rates compared to other meiosis phases. Consequently, this slower termination accumulates RNPs within TUs and forces loops to stretch out the way they do.

Data supporting this interpretation can be found in slow 3H uridine incorporation into transcripts from very long Notophthalmus loops, “The results presented here confirm Gall's observation but go further and show that the overall rate of RNA transcription on the giant loops is genuinely lower than elsewhere, little more than half the average” (Hartley and Callan, 1978). Perhaps the giant (very long) loops in Notophthalmus are a classic example of slow transcription termination leading to transcript backlog, which in turn both reduces isotope incorporation and increases the length of loops (by stretching them out and/or displacing nucleosomes).

This is a very catching hypothesis! I was also thinking along these lines for a while. But then, after lengthy discussions with Dirk Eick (München University) and Patrick Cramer (Max Plank Institute, Gottingen) (http://www.mpibpc.mpg.de/12606063/publications), the gods of the conventional polymerases, I believed them that a rate of Pol-II progression is constant or at least does not change 10x or 100x times between genes and cell types. When Pol-II is initiated, it works with a rate 3.8 Kb/min, no exceptions are known so far. This kills, of course, a very attractive hypothesis that loops of lampbrushes exist for a purpose to have lots of halve-cooked nascent transcripts, which are ready to go. When oocyte receives an appropriate signal, the transcription speeds up and the cell has lots of mRNA to go on.

I recall that we discussed speed of transcription with Michel Bellini but he also did not know at that time how to accurately measure it.

Another possibility is that polymerases cannot proceed because transcripts are not spliced. Having said this, I think it is more probable that absence of splicing would cause an abortion of transcription.

Talking about splicing:

since I was not followed recent LBC publications closely, I am probably missing an important info. But could somebody make a summary of what is known about splicing of lampbrush transcripts? How come that they are processed if most of the known LBC transcripts read from repeats? Are there special LBC splice sites?

Is it possible that the spiral organization of RNP particles along loops (Angelier et al 1984) reflect backlogging instead of RNAP rapidly progressing through a TU?

Thus, the critical factor in determining loop length may be rate of transcription termination. In this sense, western and eastern Plethodons have comparable LBCs despite 2:1 genome size difference because their transcription termination rates are comparable (or more precisely the ratio of initiation and termination is comparable). Likewise, Xenopus loops might be shorter than newts because their transcription termination rates are higher. However when a Xenopus nucleus is injected into a newt GV, the loops get longer (Gall and Murphy 1998). This could be interpreted as Xenopus TUs now engaging with the newt’s slower transcription termination rates which in turn increases loop length. Herbert suggested the opposite experiment and my prediction is that Axolotl (or newt) LBCs will become underwhelming in a Xenopus GV because of the faster termination rates. (An unlikely complication is if the Xenopus termination machinery doesn't mesh well with newt termination sites;  termination rates would still be slow.)

Why would some animals have slower transcription termination rates? Here I’d like to bring in recent studies on transgenerational epigenetics in C. elegans. Although LBCs have not been shown in this nematode, I think that it might provide some elegant and exciting suggestions. Both female and male germ cells make an inventory of “acceptable transcripts”. This process allows the cells to distinguish self from non-self nucleic acids. If a single-copy transgene is introduced, it gets flagged as absent in the “inventory” and is silenced. The inventory is necessarily established in the germline of the prior generation and passed down to progeny, which allows the offspring to catch foreign sequences in their germ cells. In turn, the offspring makes an inventory and passes it along and so forth. Perturbing this mechanism not only affects the surveillance process, but in some cases can also lead to sterility. If interested, you could read more about this in work published by Craig Mello and/or Eric Miska between 2009 and 2015. An accessible article published by HHMI could be found here: <https://www.hhmi.org/sites/default/files/mello_piwi.pdf.> Could it be that reducing transcription termination rates is part of this process?

The type of genome-wide transcription we see in LBCs could be part of the cell making an inventory of “self” transcripts and catching non-self invaders. So even if the LBC contribution to maternal mRNA deposited in a zygote may be dispensible, LBC transcription might be critical for germ cell development, fertility and transgenerational epigenetics. Studying this possibility is now feasible with the increasing number of *Xenopus* (and to a lesser degree newt) transgenic animals.

Once again, I am grateful to be part of this discussion. I do hope that this debate continues and proves fruitful!

With much gratitude,

Ahmed

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**HM to AE**

WOW!  That is GREAT.  Lots of very interesting ideas and good thinking.  It’s probably going to take us a few days to digest and I’m sure we will respond with comments as soon as we have done so.   First thought:  why should transcription rates differ from species to species.  Might this suggest that animals that don’t have LBCs at all have superfast transcription rates.  As an evolutionary biologist I ask what might be the selective advantage of having fast or slow transcription during meiotic prophase?  And I’m delighted that you have brought meiosis into the equation and also the Drosophila Y.

Another question:  what use is actually made of the LBC gene transcripts?  You have come up with one interesting and novel suggestion. I wrote recently to Elena as follows -

Two things were of particular interest to me about Joe’s grant proposal.  First, he made the usual assumption that LBC transcripts must be the primary *raison d’etre* for lampbrush chromosomes.  They are there to make transcripts for the guidance of early embryonic development.  I have always doubted this.  Secondly, and implicit in the first assumption, is the notion that the gene transcripts from loops are important for early development. How?  In the simplest terms, the oocyte fills its cytoplasm with transcripts of hundreds of kinds and somehow they execute a programme of embryonic development from fertilisation through to tadpole (or the equivalent in birds, reptiles etc.).  Do we know anything about transcription or translation or gene expression between fertilisation and, say, gastrula?  I don’t.  And why express a thousand or more genes in oogenesis?  Mick Callan was puzzled by all this, which is why he searched for another genome-wide function and came up with the Master/Slave hypothesis.  And, if there is a carefully proscribed programme or plan of gene expression in the LBC stage, why is it that if you pop a human chromosome into the GV it joins in the programme and starts expressing its genes on LBC loops?  No, sorry, all much too simple.  And how do mammals get through to blastocyst and beyond without the benefit of this preloading with mRNA – unless they are in fact preloaded and ultrafast gv  transcription means no visible LBCs.

I confess that I’m not too confident about this point mainly because I don’t know enough about gene expression in early embryos and there may well be evidence that will rubbish my concerns..

HM

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