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Re: Report on the dissertation by Shubhangini Kataruka

The thesis developed by Shubhangini Kataruka addresses an important question at the intersection of developmental and molecular biology. Specifically, Ms. Kataruka explored the contribution of a class of genetic regulators, the microRNAs, in oocytes and early embryos. MicroRNAs are essential for multiple aspects of animal biology, but it was observed early on that they are dispensable for oogenesis and early embryonic development in mice. In fact, it was noticed that most microRNAs are not active in mature oocytes, raising the question of why this is the case. This was the motivation for this thesis.

A number of possible explanations had been put forward, but here, Ms. Kataruka presents a series of elegant, high-quality experiments that provide an answer to this question. With many important discoveries in biology, once the answer is known it seems simple. In this case, the answer is "simply" that microRNAs are at too low concentration relative to their target mRNAs in mature oocytes, to be efficient repressors. During oocyte maturation, this cell expands in volume dramatically and if specific molecules are not produced at sufficient levels and stabilized, they will become diluted. This is what happens to microRNAs in mice. In contrast, the mRNA targets are produced and stabilized to a greater extent, resulting in an unfavourable condition for efficient repression.

To arrive to this conclusion, Ms. Kataruka performed careful quantification of microRNA levels in oocytes, as well as precise repression assays in which defined molecules of reporter targets were used. Repression under conditions found normally in the oocyte is negligible, but under conditions in which target concentration was lowered of microRNA concentration increased, repression became significant. This argued that there is no inherent block of microRNA function in the oocyte but it's a problem of concentration. This was supported by mathematical modelling of microRNA-target interaction and repression.

This finding led to the question of why microRNAs accumulate at lower concentration than mRNAs. Ms. Kataruka measured decay rates for a number of microRNAs and it seems that faster decay rates of microRNAs relative to mRNAs is a major contributor to the observed situation in oocytes. Intriguingly, whereas in mice no microRNA accumulates at high enough concentration, in pig and cow oocytes there are outliers. These microRNAs seem to be somewhat more stable than others, this may explain their higher concentration. MicroRNAs such as these are very interesting to follow up on as they may have been selected to achieve functional repression.

In a second part of the thesis, Ms. Kataruka explores possible differences at the level of Argonaute proteins that might contribute to the low microRNA-mediated repression in oocytes. It had been previously shown that mouse oocytes produce a truncated form of Ago2 and that this may be part of the reason why these cells are not active in microRNA function. However, the arguments above do not support this view. Ms. Kataruka performed a series of well-designed genetic engineering experiments in mice to try to assess possible functions and regulation of this truncated form of Ago2. These experiments revealed that deletion of this form has no functional consequences for development. Moreover, they uncovered regulation by a retroelement, with interesting implications for the evolution of the microRNA pathway in mammals. Finally, the analysis of Ago2 led to finding that rodents use an alternative 5' exon which results in a few amino acid changes at the N-terminus of the protein with consequences for catalytic activity of AGO2. This should not affect microRNA-mediated repression but the cleavage activity associated with endogenous siRNAs, which are functional in oocytes, and therefore has interesting implications.

Overall, this thesis not only reports novel and important findings, but I also enjoyed reading it. The introduction covers relevant aspects of microRNA biology and early development to place the findings in a broader context. The results are well explained and supported with clear figures. The discussion goes deeper in explaining the implications of the work and the new questions it raises.

Based on all this, I congratulate Ms. Kataruka on a very successful PhD thesis.

With best regards,

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