Tunicate fast evolving genomes, a treasure for new discoveries?

Tunicates are organisms characterized by a fast rate of genomic and developmental evolution. Some fast evolving evolutionary changes include loss of synteny, fast changes in cis-regulatory sequences, and loss of several key regulatory developmental genes (Satou et al., 2008; Denoeud et al., 2010), such as several central or posterior Hox genes involved in AP patterning of metazoans (Ikuta and Saiga, 2005) and *Gbx* involved in the establishment of the midbrain-hindbrain boundary in vertebrates (Wada et al. 2003). Genome studies of miRNA prevalence in several species of tunicates (i.e. *Oikopleura*, *Ciona* and *Didemnum*) have revealed many losses of conserved miRNA families as well as many gains of unique miRNAs among recently divergent lineages in the tunicates when compared to other groups of chordates (Fu et al., 2008; Velandia et al. 2016). Thus, relaxed constraints in the evolution of genomes and developmental trajectories in the tunicates may have been responsible for the plethora of reproductive strategies, morphologies, and life histories observed in the group (Holland, 2014).

let-7 was the first miRNA in tunicates proposed to have a regulatory role in development:

The first microRNA (miRNA) ever reported in any tunicate was let-7, which was first detected in *Ciona robusta* and *Herdmania curvata* (Pasquinelli & Reinhart et al., 2000). A previous study the same year in *C. elegans* had shown that small RNA let-7 (21nt) was required for late larval to adult developmental transition (Reinhart et al, 2000). Small RNA let-7 was then shown to also be differentially expressed during the development of many distantly related animal taxa, but was not detected in Porifera, Ctenophora, Cnidaria, and Acoelomorpha, suggesting that let-7 was involved in the regulation of late temporal transitions during development or in the evolution of complex life histories in the Nephrozoa (Pasquinelli & Reinhart et al., 2000; Pasquinelli et al., 2003).

miRNAs and moRNAs in development:

Both MicroRNAs (miRNAs or miRs) as well as MicroRNA offset RNA (moRNAs or moRs) are developmentally regulated as shown during *C. robusta* development(Shi et al., 2009). In spite of the considerably higher abundance of miRs and miRs\* in cells than their corresponding abundance of moRs, all three small RNA types have been shown to have regulatory roles for gene expression. Although a vast majority of miRNAs remain to be studied, there are already many cases of well-studied miRNAs (including many that are mentioned in this chapter that have been studied in tunicates) that are known to target mRNAs, modulate their levels of expression, and affect developmental processes both in plants and animals (Zhao et al. 2018). Only recently two studies demonstrated for the first time that two moRs (viral moR-rR1-3-5p and moR-21 could also modulate gene expression, and were not merely the byproduct of miRNA biogenesis (Umbach et al 2010, Zhao et al. 2016).

Neuronal fate determination and regulation by miR-124:

The miRNA miR-124 is expressed in the nervous system of many animals, including *Drosophila* (Aboobaker et al., 2005), *C. elegans* (Clark et al., 2010) and humans (Sempere et al., 2004). As was first observed by *in vitro* studies of mouse brain cells, low expression of miR-124 was related to neural stem cell maintenance, whereas high expression of miR-124 induced the differentiation of neuronal cell types (Cheng et al., 2009). A regulative role of miR-124 in non-neural vs. neural fate decisions was further investigated by embryonic experiments *in vivo* (Chen et al. 2011) and by theoretical and *in silico* modeling analyses in *Ciona robusta* (Chen et al. 2014). These studies showed that miR-124 promotes nervous system development by feedback interactions with Notch signaling. During nervous system development of *Ciona robusta*, cells in the dorsal and ventral midline epidermis of the teilbud embryo either take an epidermal sensory neuron (ESN) or peripheral nervous system (PNS) fate, a decision mediated by lateral inhibition using a classical model of feedback loop regulation of Notch-Delta signaling in neighboring cells (Collier et al. 1996; Chen et al. 2014). Cells that take an ESN fate showed low expression of miR-124 presumably by Notch inhibition, whereas cells that take a PNS fate expressed high levels of miR-124, which in the latter case it was shown to target and repress non-neuronal genes (e.g. neuronal repressors SCP1 and PTBP1) downstream of Notch signaling (Chen et al. 2011). In addition, expression of miR-124 in larval epidermal cells was sufficient for ectopic neural specification, which resembled mis-expression experiments using Pou4, an important transcription factor for sensory neuron specification (Chen et al. 2011; Tang et al. 2013). Whereas miR-124 targeting to SCP1 is thought to have evolved in the vertebrates+tunicates, miR targeting to PTBP1 may be conserved among bilaterians except for ecdysozoans (Chen et al. 2011) suggesting that the miRNA regulatory logic in lateral inhibition models of Notch-Delta signaling may have broader implications in other organisms yet to be studied (Chen et al. 2014). The research team also showed that miR-124 acted at the gastrula stage and targeted other non-neural genes such as muscle determinant *Macho-1* and notochord determinant *Brachyury* to allow for ectodermal fate specification (Chen et al. 2011).

Muscle development and the polycistronic miR-1/miR-133 cluster:

A well-studied case of miRNA regulation in muscle development is the miR-1/miR-133 polycistronic cluster. Whereas miR-1 promotes differentiation of muscle, miR-133 promotes proliferation of muscle precursors (Chen et al, 2006). In the chordates, these two miRNAs are encoded in an antisense direction in a relatively close localization (3-11 kb apart) within the gene mind bomb 1 (MIB1), and transcribed as a single primary (i.e. polycistronic) transcript. Except for *Drosophila* and ambulacrarians (i.e. echinoderms and hemichordates), a close proximity of these two miRNAs has been documented in most animal taxa suggesting some form of functional regulatory constraint of a condensed miR-1/miR-133 cluster for the bilaterians (Campos-Paysaa et al., 2011). During *Ciona robusta* development, the polycistronic transcription can be detected in the nuclei of presumptive tail muscle cells from the gastrula stage onward, and its transcription is regulated by an 850 bp sequence upstream of the transcipt start site (Kusakabe et al. 2013). Differential expression of the two miRNAs in muscle tissues was only detected in the adult, where body wall muscle expressed similar levels of miR-1 and miR-133 and heart muscle expressed significantly higher levels of miR-1 (Kusakabe et al. 2013).

miRNA expression during oral siphon (OS) regeneration:

Three stages of regeneration have been proposed that reconstruct main events of regeneration that match expected expression profiles in the corresponding timeframes (Knapp, et al. 2013). The three phases correspond to: i. *wound Healing,* ii. *transition,* and iii*. re-development*. Using miRNA-mRNA transcriptional profiling using a correlation network, differential expression of mRNAs was correlated to miRNA profiles during the three regeneration windows mentioned above in *Ciona robusta* oral siphon regeneration (Spina et al. 2017). In the first phase, i.e. *wound healing*, miRNA target clusters of miR 4178b-5p and miR 4\_20211 were found to be correlated to the differential expression of genes involved in the following GO term functional classifications: immune response, stress response and apoptosis. In the second phase, i.e. *transition*, miR 4008c-5p, miR 4123-5p, miR 4178-5p, miR 2\_15911, miR 4\_20211, and miR 11\_7539 were correlated and known to target Wnt, TGFb and MAPK pathway genes that may be regulating the proliferative state characteristic of this particular timeframe. In the third phase, i.e. *re-development*, miR4008c-5p, miR 10\_4533, and miR 11\_6940 known to target ECM peptidase inhibitors are correlated with the characteristic extracellular matrix remodeling that occurs at the final phase of regeneration and which resembles the original developmental processes. In contrast other miRs were found expressed throughout the regenerative process. MiRNA miR 10\_4533 known to target IGF and IGFb was found expressed presumably regulating the proliferation of progenitors. Also miR-9 was found expressed throughout regeneration and is known to be essential for neural development and function, presumably by targeting and regulating genes involved in cytoskeleton and cell cycle functions (Galderisi et al., 2003; McBeath et al., 2004), instead of targeting Notch or Hes-1 (Spina et al. 2017).

miRNA expression during *Oikopleura dioica* development:

A most thorough study of the miRNA repertoire expressed during development has been published for the larvacean *Oikopleura dioca* (Fu et al. 2008). Using a miRNA array approach with 55 candidate miRNAs and 10 developmental stages for analyses, some general patterns of miRNA occurrence emerged. MicroRNAs were expressed throughout the life cycle of the animal, and were deposited in eggs as maternal determinants for early zygotes. Expression of zygotic miRNAs, such as miR-1487 and miR-1488, was observed starting on the blastula stage (1.5h post fertilization). Most miRNAs analyzed showed developmental regulation (for specific miRNAs that were differentially expressed at each stage see Fu et al, 2008), except for some such as miR-1497 that was expressed throughout all stages (Fu et al. 2008). From this study, the first sex specific miRNAs were revealed: miR-1478 was expressed day 6 females in the oocytes, whereas miR-1487/88 were expressed in day 6 males. Interestingly, the compact genomes of *O. dioica* showed one single copy of most miRNA loci, except for miR-1490a, miR-1493, miR-1497d, and miR-1504 that were in two copies (Fu et al. 2008).

REFS:

**Aboobaker, A. A., Tomancak, P., Patel, N., Rubin, G. M. and Lai, E. C. (2005). Drosophila microRNAs exhibit diverse spatial expression patterns during embryonic development. Proc. Natl. Acad. Sci. USA 102, 18017-18022.**

**Campo-Paysaa F, Sémon M, Cameron RA, Peterson KJ, Schubert M. microRNA**

**complements in deuterostomes: origin and evolution of microRNAs. Evol Dev. 2011**

**Jan-Feb;13(1):15-27. doi: 10.1111/j.1525-142X.2010.00452.x. PubMed PMID:**

**21210939.**

**Chen,J.F., et al.2006. Theroleof microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nat. Genet. 38: 228–233.**

**Chen JS, Pedro MS, Zeller RW. miR-124 function during Ciona intestinalis**

**neuronal development includes extensive interaction with the Notch signaling**

**pathway. Development. 2011 Nov;138(22):4943-53. doi: 10.1242/dev.068049. PubMed**

**PMID: 22028027.**

**Chen JS, Gumbayan AM, Zeller RW, Mahaffy JM. An expanded Notch-Delta model**

**exhibiting long-range patterning and incorporating MicroRNA regulation. PLoS**

**Comput Biol. 2014 Jun 19;10(6):e1003655. doi: 10.1371/journal.pcbi.1003655.**

**eCollection 2014 Jun. PubMed PMID: 24945987; PubMed Central PMCID: PMC4063677.**

**Clark, A. M., Goldstein, L. D., Tevlin, M., Tavare, S., Shaham, S. and Miska, E. A. (2010). The microRNA miR-124 controls gene expression in the sensory nervous system of Caenorhabditis elegans. Nucleic Acids Res. 38, 3780-3793.**

**Collier JR, Monk NA, Maini PK, Lewis JH (1996) Pattern formation by lateral inhibition with feedback: A mathematical model of Delta-Notch intercellular signalling. Journal of Theoretical Biology 183: 429–446**

**Denoeud F, Henriet S, Mungpakdee S, et al. 2010. Plasticity of animal genome architecture unmasked by rapid evolution of a pelagic tunicate. Science 330:1381–1385.**

**Fu X, Adamski M, Thompson EM. 2008. Altered miRNA repertoire in the simplified chordate, Oikopleura dioica. Mol Biol Evol 25:1067–1080**

**Galderisi, U., Jori, F.P., Giordano, A. (2003). Cell cycle regulation and neural differentiation. Oncogene 22, 5208–5219. doi:10.1038/sj.onc.1206558**

**Holland, Linda Z. Genomics, evolution and development of amphioxus and tunicates: The Goldilocks principle, 2014**

**Lee, R. C., Feinbaum, R. L.&Ambros,V. The C. elegans heterochronic gene lin-4 encodes smallRNAs with antisense complementarity to lin-14. Cell 75, 843–854 (1993)**

**Knapp, D., Schulz, H., Rascon, C.A., Volkmer, M., Scholz, J., Nacu, E., Le, M., Novozhilov, S., Tazaki, A., Protze, S., Jacob, T., et al.** (2013). Comparative Transcriptional Profiling of the Axolotl Limb Identifies a Tripartite Regeneration- Specific Gene Program. PLOS ONE 8, e61352. doi:10.1371/journal.pone.0061352

Kusakabe R, Tani S, Nishitsuji K, Shindo M, Okamura K, Miyamoto Y, Nakai K,

Suzuki Y, Kusakabe TG, Inoue K. Characterization of the compact bicistronic

microRNA precursor, miR-1/miR-133, expressed specifically in Ciona muscle

tissues. Gene Expr Patterns. 2013 Jan-Feb;13(1-2):43-50. doi:

10.1016/j.gep.2012.11.001. Epub 2012 Nov 16. PubMed PMID: 23159539.

**McBeath, R., Pirone, D.M., Nelson, C.M., Bhadriraju, K., Chen, C.S. (2004). Cell Shape, Cytoskeletal Tension, and RhoA Regulate Stem Cell Lineage Commitment. Dev. Cell 6, 483–495. doi:10.1016/S1534-5807(04)00075-9**

**Pasquinelli AE, McCoy A, Jiménez E, Saló E, Ruvkun G, Martindale MQ, Baguñà J.**

**Expression of the 22 nucleotide let-7 heterochronic RNA throughout the Metazoa: a**

**role in life history evolution? Evol Dev. 2003 Jul-Aug;5(4):372-8. PubMed PMID:**

**12823453.**

Reinhart, B. et al. The 21-nucleotide let-7 RNA regulates developmental timing in C. elegans. Nature 403, 901–906 (2000)

Satou S, Mineta S, Ogasawara M, et al. 2008. Improved genome assembly and evidence‐based global gene model set for the chordate Ciona intestinalis: new insight into intron and operon

populations. Genome Biol 9:R152.

Sempere, L. F., Freemantle, S., Pitha-Rowe, I., Moss, E., Dmitrovsky, E. and Ambros, V. (2004). Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biol. 5, R13.

Joyce Tang W, Chen JS, Zeller RW. Transcriptional regulation of the peripheral

nervous system in Ciona intestinalis. Dev Biol. 2013 Jun 15;378(2):183-93. doi:

10.1016/j.ydbio.2013.03.016. Epub 2013 Mar 30. PubMed PMID: 23545329.

Umbach JL, Strelow LI, Wong SW, Cullen BR. Analysis of rhesus rhadinovirus microRNAs expressed in virus-induced tumors from infected rhesus macaques. Virology. 2010;405: 592–599. pmid:20655562

Wada S, Tokuoka M, Shoguchi E, et al. 2003. A genomewide survey of developmentally relevant genes in Ciona intestinalis. II. Genes for homeobox transcription factors. Dev Genes Evol 213:222–234.

Zhao J, Schnitzler GR, Iyer LK, Aronovitz MJ, Baur WE, Karas RH.

MicroRNA-Offset RNA Alters Gene Expression and Cell Proliferation. PLoS One. 2016

Jun 8;11(6):e0156772. doi: 10.1371/journal.pone.0156772. eCollection 2016. PubMed

PMID: 27276022; PubMed Central PMCID: PMC4898817.

Zhao Y, Cong L, Lukiw WJ. Plant and Animal microRNAs (miRNAs) and Their

Potential for Inter-kingdom Communication. Cell Mol Neurobiol. 2018

Jan;38(1):133-140. doi: 10.1007/s10571-017-0547-4. Epub 2017 Sep 6. Review.

PubMed PMID: 28879580