Année de publication : 2019

Perrine Lavalou, Helene Eckert, Louise Damy, Florian Constanty, Sara Majello, Angelo Bitetti, Antoine Graindorge, Alena Shkumatava (2019 May 3)

Strategies for Genetic Inactivation of Long Noncoding RNAs in Zebrafish.

RNA (New York, N.Y.) : DOI : rna.069484.118

Résumé

The number of annotated long noncoding RNAs (lncRNAs) continues to grow, however their functional characterization in model organisms has been hampered by the lack of reliable genetic inactivation strategies. While partial or full deletions of lncRNA loci disrupt lncRNA expression, they do not permit the formal association of a phenotype with the encoded transcript. Here, we examined several alternative strategies for generating lncRNA null alleles in zebrafish and found that they often resulted in unpredicted changes to lncRNA expression. Removal of the transcriptional start sites (TSSs) of lncRNA genes resulted in hypomorphic mutants due to the usage of either constitutive or tissue-specific alternative TSSs. Deletions of short, deeply conserved lncRNA regions can also lead to overexpression of truncated transcripts. By contrast, a knock-in of a polyadenylation signal enabled complete inactivation of malat1, the most abundant vertebrate lncRNA. In summary, lncRNA null alleles require extensive in vivo validation and we propose insertion of transcription termination sequences as the most reliable approach to generate lncRNA-deficient zebrafish.

Année de publication : 2018

Bitetti A, Mallory AC, Carrieri C, Golini E, Carreño Gutierrez H, Perlas E, Pérez-Rico YA, Tocchini-Valentini GP, Enright AJ, Norton WHJ, Mandillo S, O'Carroll D, Shkumatava A (in press) (2018 Feb 21)

MicroRNA degradation by a conserved target RNA regulates animal behavior

Nat Struct .Mol Biol

Résumé

Année de publication : 2017

Matthew P Davis, Claudia Carrieri, Harpreet K Saini, Stijn van Dongen, Tommaso Leonardi, Giovanni Bussotti, Jack M Monahan, Tania Auchynnikava, Angelo Bitetti, Juri Rappsilber, Robin C Allshire, Alena Shkumatava, Dónal O'Carroll, Anton J Enright (2017 May 14)

Transposon-driven transcription is a conserved feature of vertebrate spermatogenesis and transcript evolution.

EMBO reports : 1231-1247 : DOI : 10.15252/embr.201744059

Résumé

Spermatogenesis is associated with major and unique changes to chromosomes and chromatin. Here, we sought to understand the impact of these changes on spermatogenic transcriptomes. We show that long terminal repeats (LTRs) of specific mouse endogenous retroviruses (ERVs) drive the expression of many long non-coding transcripts (lncRNA). This process occurs post-mitotically predominantly in spermatocytes and round spermatids. We demonstrate that this transposon-driven lncRNA expression is a conserved feature of vertebrate spermatogenesis. We propose that transposon promoters are a mechanism by which the genome can explore novel transcriptional substrates, increasing evolutionary plasticity and allowing for the genesis of novel coding and non-coding genes. Accordingly, we show that a small fraction of these novel ERV-driven transcripts encode short open reading frames that produce detectable peptides. Finally, we find that distinct ERV elements from the same subfamilies act as differentially activated promoters in a tissue-specific context. In summary, we demonstrate that LTRs can act as tissue-specific promoters and contribute to post-mitotic spermatogenic transcriptome diversity.

Année de publication : 2016

Yuvia A Pérez Rico, Valentina Boeva, Allison C Mallory, Angelo Bitetti, Sara Majello, Emmanuel Barillot, Alena Shkumatava (2016 Dec 15)

Comparative analyses of super-enhancers reveal conserved elements in vertebrate genomes.

Genome research : DOI : gr.203679.115

Résumé

Super-enhancers (SEs) are key transcriptional drivers of cellular, developmental and disease states in mammals, yet the conservational and regulatory features of these enhancer elements in non-mammalian vertebrates are unknown. To define SEs in zebrafish and enable sequence and functional comparisons to mouse and human SEs, we used genome-wide histone H3 lysine 27 acetylation (H3K27ac) occupancy as a primary SE delineator. Our study determined the set of SEs in pluripotent state cells and adult zebrafish tissues and revealed both similarities and differences between zebrafish and mammalian SEs. Although the total number of SEs was proportional to the genome size, the genomic distribution of zebrafish SEs differed from that of the mammalian SEs. Despite the evolutionary distance separating zebrafish and mammals and the low overall SE sequence conservation, ~42% of zebrafish SEs were located in close proximity to orthologs that also were associated with SEs in mouse and human. Compared to their non-associated counterparts, higher sequence conservation was revealed for those SEs that have maintained orthologous gene associations. Functional dissection of two of these SEs identified conserved sequence elements and tissue-specific expression patterns, while chromatin accessibility analyses predicted transcription factors governing the function of pluripotent state zebrafish SEs. Our zebrafish annotations and comparative studies show the extent of SE usage and their conservation across vertebrates, permitting future gene regulatory studies in several tissues.

Maximilian Haeussler, Kai Schönig, Hélène Eckert, Alexis Eschstruth, Joffrey Mianné, Jean-Baptiste Renaud, Sylvie Schneider-Maunoury, Alena Shkumatava, Lydia Teboul, Jim Kent, Jean-Stephane Joly, Jean-Paul Concordet (2016 Jul 7)
Evaluation of off-target and on-target scoring algorithms and integration into the guide RNA selection tool CRISPOR.
Genome biology : 148 : DOI : 10.1186/s13059-016-1012-2

Résumé

The success of the CRISPR/Cas9 genome editing technique depends on the choice of the guide RNA sequence, which is facilitated by various websites. Despite the importance and popularity of these algorithms, it is unclear to which extent their predictions are in agreement with actual measurements.

Année de publication : 2015

Allison C Mallory, Alena Shkumatava (2015 Mar 28)
LncRNAs in vertebrates: advances and challenges.
Biochimie : 3-14 : DOI : 10.1016/j.biochi.2015.03.014

Résumé

Beyond the handful of classic and well-characterized long noncoding RNAs (lncRNAs), more recently, hundreds of thousands of lncRNAs have been identified in multiple species including bacteria, plants and vertebrates, and the number of newly annotated lncRNAs continues to increase as more transcriptomes are analyzed. In vertebrates, the expression of many lncRNAs is highly regulated, displaying discrete temporal and spatial expression patterns, suggesting roles in a wide range of developmental processes and setting them apart from classic housekeeping ncRNAs. In addition, the deregulation of a subset of these lncRNAs has been linked to the development of several diseases, including cancers, as well as developmental anomalies. However, the majority of vertebrate lncRNA functions remain enigmatic. As such, a major task at hand is to decipher the biological roles of lncRNAs and uncover the regulatory networks upon which they impinge. This review focuses on our emerging understanding of lncRNAs in vertebrate animals, highlighting some recent advances in their functional analyses across several species and emphasizing the current challenges researchers face to characterize lncRNAs and identify their in vivo functions.

Année de publication : 2012

Igor Ulitsky, Alena Shkumatava, Calvin H Jan, Alexander O Subtelny, David Koppstein, George W Bell, Hazel Sive, David P Bartel (2012 Jun 23)
Extensive alternative polyadenylation during zebrafish development.
Genome research : 2054-66 : DOI : 10.1101/gr.139733.112

Résumé

The post-transcriptional fate of messenger RNAs (mRNAs) is largely dictated by their 3′ untranslated regions (3′ UTRs), which are defined by cleavage and polyadenylation (CPA) of pre-mRNAs. We used poly(A)-position profiling by sequencing (3P-seq) to map poly(A) sites at eight developmental stages and tissues in the zebrafish. Analysis of over 60 million 3P-seq reads substantially increased and improved existing 3′ UTR annotations, resulting in confidently identified 3′ UTRs for >79% of the annotated protein-coding genes in zebrafish. mRNAs from most zebrafish genes undergo alternative CPA, with those from more than a thousand genes using different dominant 3′ UTRs at different stages. These included one of the poly(A) polymerase genes, for which alternative CPA reinforces its repression in the ovary. 3′ UTRs tend to be shortest in the ovaries and longest in the brain. Isoforms with some of the shortest 3′ UTRs are highly expressed in the ovary, yet absent in the maternally contributed RNAs of the embryo, perhaps because their 3′ UTRs are too short to accommodate a uridine-rich motif required for stability of the maternal mRNA. At 2 h post-fertilization, thousands of unique poly(A) sites appear at locations lacking a typical polyadenylation signal, which suggests a wave of widespread cytoplasmic polyadenylation of mRNA degradation intermediates. Our insights into the identities, formation, and evolution of zebrafish 3′ UTRs provide a resource for studying gene regulation during vertebrate development.

Année de publication : 2011

Igor Ulitsky, Alena Shkumatava, Calvin H Jan, Hazel Sive, David P Bartel (2011 Jul 28)

Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution.

Cell : 1537-50 : DOI : 10.1016/j.cell.2011.11.055

Résumé

Thousands of long intervening noncoding RNAs (lincRNAs) have been identified in mammals. To better understand the evolution and functions of these enigmatic RNAs, we used chromatin marks, poly(A)-site mapping and RNA-Seq data to identify more than 550 distinct lincRNAs in zebrafish. Although these shared many characteristics with mammalian lincRNAs, only 29 had detectable sequence similarity with putative mammalian orthologs, typically restricted to a single short region of high conservation. Other lincRNAs had conserved genomic locations without detectable sequence conservation. Antisense reagents targeting conserved regions of two zebrafish lincRNAs caused developmental defects. Reagents targeting splice sites caused the same defects and were rescued by adding either the mature lincRNA or its human or mouse ortholog. Our study provides a roadmap for identification and analysis of lincRNAs in model organisms and shows that lincRNAs play crucial biological roles during embryonic development with functionality conserved despite limited sequence conservation.

Année de publication : 2010

Chanseok Shin, Jin-Wu Nam, Kyle Kai-How Farh, H Rosaria Chiang, Alena Shkumatava, David P Bartel (2010 Jul 13)

Expanding the microRNA targeting code: functional sites with centered pairing.

Molecular cell : 789-802 : DOI : 10.1016/j.molcel.2010.06.005

Résumé

Most metazoan microRNA (miRNA) target sites have perfect pairing to the seed region, located near the miRNA 5′ end. Although pairing to the 3′ region sometimes supplements seed matches or compensates for mismatches, pairing to the central region has been known to function only at rare sites that impart Argonaute-catalyzed mRNA cleavage. Here, we present « centered sites, » a class of miRNA target sites that lack both perfect seed pairing and 3′-compensatory pairing and instead have 11-12 contiguous Watson-Crick pairs to the center of the miRNA. Although centered sites can impart mRNA cleavage in vitro (in elevated Mg(2+)), in cells they repress protein output without consequential Argonaute-catalyzed cleavage. Our study also identified extensively paired sites that are cleavage substrates in cultured cells and human brain. This expanded repertoire of cleavage targets and the identification of the centered site type help explain why central regions of many miRNAs are evolutionarily conserved.

Année de publication : 2009

Alena Shkumatava, Alexander Stark, Hazel Sive, David P Bartel (2009 Feb 26)

Coherent but overlapping expression of microRNAs and their targets during vertebrate development.

Genes & development : 466-81 : DOI : 10.1101/gad.1745709

Résumé

MicroRNAs (miRNAs) are small noncoding RNAs that direct post-transcriptional repression of protein-coding genes. In vertebrates, each highly conserved miRNA typically regulates hundreds of target mRNAs. However, the precise relationship between expression of the miRNAs and that of their targets has remained unclear, in part because of the scarcity of quantitative expression data at cellular resolution. Here we report quantitative analyses of mRNA levels in miRNA-expressing cells of the zebrafish embryo, capturing entire miRNA expression domains, purified to cellular resolution using fluorescent-activated cell sorting (FACS). Focus was on regulation by miR-206 and miR-133 in the developing somites and miR-124 in the developing central nervous system. Comparison of wild-type embryos and those lacking miRNAs revealed predicted targets that responded to the miRNAs and distinguished miRNA-mediated mRNA destabilization from other regulatory effects. For all three miRNAs examined, expression of the miRNAs and that of their predicted targets usually overlapped. A few targets were expressed at higher levels in miRNA-expressing cells than in the rest of the embryo, demonstrating that miRNA-mediated repression can act in opposition

to other regulatory processes. However, for most targets expression was lower in miRNA-expressing cells than in the rest of the embryo, indicating that miRNAs usually operate in concert with the other regulatory machinery of the cell.

Année de publication : 2005

Jochen A Stadler, Alena Shkumatava, William H J Norton, Marlene J Rau, Robert Geisler, Sabine Fischer, Carl J Neumann (2005 May 17)

Histone deacetylase 1 is required for cell cycle exit and differentiation in the zebrafish retina.

Developmental dynamics : an official publication of the American Association of Anatomists : 883-9

Résumé

Histone acetylation is an important epigenetic mechanism for the control of eukaryotic transcription. The histone deacetylase 1 (HDAC1) gene has been implicated in controlling the transcription of core cell cycle regulators, but the in vivo role of HDACs in cell cycle regulation is still poorly understood. Loss of HDAC1 activity causes underproliferation in several contexts during vertebrate development. In contrast, we show here that HDAC1 has the opposite effect in the zebrafish visual system, where loss of HDAC1 activity leads to failure of cells to exit the cell cycle in the retina and in the optic stalk. The effect of HDAC1 on cell cycle exit is cell-autonomous, and loss of HDAC1 in the retina leads to up-regulation of cyclin D and E transcripts. These results demonstrate that the in vivo role of HDAC1 in regulating cell cycle progression is region-specific, as HDAC1 promotes cell cycle exit in the retina but stimulates proliferation in other cellular contexts.

Alena Shkumatava, Carl J Neumann (2005 May 14)

Shh directs cell-cycle exit by activating p57Kip2 in the zebrafish retina.

EMBO reports : 563-9

Résumé

The Hedgehog (Hh) family of signalling proteins control both differentiation and proliferation during animal development. Previous studies have shown that Hh signalling has a stimulatory effect on the cell cycle in several organs by controlling core cell-cycle components. Here, we show that Sonic hedgehog (Shh) signalling has the opposite effect in the zebrafish retina, where it leads to cell-cycle exit, and that this is mediated by transcriptional activation of the cyclin kinase inhibitor p57Kip2. The loss of p57Kip2 activity strongly resembles the Shh mutant eye phenotype, and overexpression of p57Kip2 rescues cell-cycle exit in Shh mutants, indicating that p57Kip2 is both necessary and sufficient to mediate Shh-induced cell-cycle exit in the retina. These findings raise the possibility that stimulation of cell-cycle exit through regulation of core cell-cycle components may be part of a general mechanism required for Hh-directed differentiation.

Jochen A Stadler, Alena Shkumatava, Carl J Neumann (2005 Apr 28)
The role of hedgehog signaling in the development of the zebrafish visual system.
Developmental neuroscience : 346-51

Résumé

The vertebrate visual system is a region of the nervous system that is characterized by relative simplicity, and its development has hence been studied intensively, to serve as a paradigm for the rest of the central nervous system. The zebrafish model organism offers an impressive array of tools to dissect this process experimentally, and in recent years has helped to significantly deepen our understanding of the development of the visual system. A number of these studies have focused on the role of the Hedgehog family of secreted signaling molecules in eye development, and this is the main topic of this review. Hedgehog signaling plays an important role in all major steps of visual system development, starting with the regionalization of the eye primordium into proximal and distal territories, continuing with the control of cellular differentiation in the retina, and ending with the guidance of axonal projections from the retina to the optic centers of the brain.

Année de publication : 2004

Alena Shkumatava, Sabine Fischer, Ferenc Müller, Uwe Strahle, Carl J Neumann (2004 Jul 16)
Sonic hedgehog, secreted by amacrine cells, acts as a short-range signal to direct differentiation and lamination in the zebrafish retina.
Development (Cambridge, England) : 3849-58

Résumé

Neurogenesis in the zebrafish retina occurs in several waves of differentiation. The first neurogenic wave generates ganglion cells and depends on hedgehog (hh) signaling activity. Using transgenic zebrafish embryos that express GFP under the control of the sonic hedgehog (shh) promoter, we imaged the differentiation wave in the retina and show that, in addition to the wave in the ganglion cell layer, shh expression also spreads in the inner nuclear layer. This second wave generates amacrine cells expressing shh, and although it overlaps temporally with the first wave, it does not depend on it, as it occurs in the absence of ganglion cells. We also show that differentiation of cell types found in the inner and outer nuclear layers, as well as lamination of the retina, depends on shh. By performing mosaic analysis, we demonstrate that Shh directs these events as a short-range signal within the neural retina.

Année de publication : 2003

Bernhard Schmierer, Michael K Schuster, Alena Shkumatava, Karl Kuchler (2003 Apr 1)

Activin a signaling induces Smad2, but not Smad3, requiring protein kinase a activity in granulosa cells from the avian ovary.
The Journal of biological chemistry : 21197-203

Résumé

Activin A signaling is an important regulator of ovarian granulosa cell function. The cytosolic signal transducer Smad2 is most highly expressed in chicken granulosa cells (cGC) of preovulatory follicles. Moreover, Smad2 shows predominant nuclear localization in freshly isolated cGC, indicating active Smad signaling in vivo. Primary cGC cultured in vitro require activin A to sustain high Smad2 levels, which otherwise drop dramatically in the absence of activin A. This activin A-dependent Smad2 expression is abrogated by protein kinase A (PKA) inhibitors, suggesting a role for PKA in activin signaling. In the absence of activin A, strong PKA activators such as follicle-stimulating hormone (FSH) and 8-bromo-cyclic AMP fail to elicit Smad2 induction. However, FSH and 8-bromo-cyclic AMP boost activin A-dependent Smad2 up-regulation, giving rise to Smad2 levels similar to expression in vivo levels. Interestingly, the effect is specific for Smad2, since expression of the structurally and functionally closely related Smad3 remains entirely unaffected. Hence, activin A induces Smad2, but not Smad3, to high levels requiring PKA activation. Since Smad2 and Smad3 target distinct yet overlapping sets of TGF-beta/activin-responsive genes, the selective Smad2 induction by FSH/activin A could allow FSH to efficiently modulate the transcriptional readout of activin A signaling in avian granulosa cells.