Année de publication : 2017

Shahad Albadri, Filippo Del Bene, Céline Revenu (2017 Mar 17)

Genome editing using CRISPR/Cas9-based knock-in approaches in zebrafish.

Methods (San Diego, Calif.) : DOI : S1046-2023(16)30283-3

Résumé

With its variety of applications, the CRISPR/Cas9 genome editing technology has been rapidly evolving in the last few years. In the zebrafish community, knock-out reports are constantly increasing but insertion studies have been so far more challenging. With this review, we aim at giving an overview of the homologous directed repair (HDR)-based knock-in generation in zebrafish. We address the critical points and limitations of the procedure such as cutting efficiency of the chosen single guide RNA, use of cas9 mRNA or Cas9 protein, homology arm size etc. but also ways to circumvent encountered issues with HDR insertions by the development of non-homologous dependent strategies. While imprecise, these homology-independent mechanisms based on non-homologous-end-joining (NHEJ) repair have been employed in zebrafish to generate reporter lines or to accurately edit an open reading frame by the use of intron-targeting modifications. Therefore, with higher efficiency and insertion rate, NHEJ-based knock-in seems to be a promising approach to target endogenous loci and to circumvent the limitations of HDR whenever it is possible and appropriate. In this perspective, we propose new strategies to generate cDNA edited or tagged insertions, which once established will constitute a new and versatile toolbox for CRISPR/Cas9-based knock-ins in zebrafish.

Année de publication : 2016

Laura Fontenas, Flavia De Santis, Vincenzo Di Donato, Cindy Degerny, Béatrice Chambraud, Filippo Del Bene, Marcel Tawk (2016 Dec 1)

Neuronal Ndrg4 Is Essential for Nodes of Ranvier Organization in Zebrafish.

PLoS genetics : e1006459 : DOI : 10.1371/journal.pgen.1006459

Résumé

Axon ensheathment by specialized glial cells is an important process for fast propagation of action potentials. The rapid electrical conduction along myelinated axons is mainly due to its saltatory nature characterized by the accumulation of ion channels at the nodes of Ranvier. However, how these ion channels are transported and anchored along axons is not fully understood. We have identified N-myc downstream-regulated gene 4, ndrg4, as a novel factor that regulates sodium channel clustering in zebrafish. Analysis of chimeric larvae indicates that ndrg4 functions autonomously within neurons for sodium channel clustering at the nodes. Molecular analysis of ndrg4 mutants shows that expression of snap25 and nsf are sharply decreased, revealing a role of ndrg4 in controlling vesicle exocytosis. This uncovers a previously unknown function of ndrg4 in regulating vesicle docking and nodes of Ranvier organization, at least through its ability to finely tune the expression of the t-SNARE/NSF machinery.

Thomas O Auer, Filippo Del Bene (2016 Jul 29)
Homology-Independent Integration of Plasmid DNA into the Zebrafish Genome.
Methods in molecular biology (Clifton, N.J.) : 31-51 : DOI : 10.1007/978-1-4939-3771-4_3

Résumé

Targeting nucleases like zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats/CRISPR-associated (CRISPR/Cas) system have revolutionized genome-editing possibilities in many model organisms. They allow the generation of loss-of-function alleles by the introduction of double-strand breaks at defined sites within genes, but also more sophisticated genome-editing approaches have become possible. These include the integration of donor plasmid DNA into the genome by homology-independent repair mechanisms after CRISPR/Cas9-mediated cleavage. Here we present a protocol outlining the most important steps to target a genomic site and to integrate a donor plasmid at this defined locus.

F De Santis, V Di Donato, F Del Bene (2016 Jul 23)
Clonal analysis of gene loss of function and tissue-specific gene deletion in zebrafish via CRISPR/Cas9 technology.
Methods in cell biology : 171-88 : DOI : 10.1016/bs.mcb.2016.03.006

Résumé

In the last few years the development of CRISPR/Cas 9-mediated genome editing techniques has allowed the efficient generation of loss-of-function alleles in several model organisms including zebrafish. However, these methods are mainly devoted to target-specific genomic loci leading to the creation of constitutive knock-out models. On the contrary, the analysis of gene function via tissue- or cell-specific mutagenesis remains challenging in zebrafish. To circumvent this limitation, we present here a simple and versatile protocol to achieve tissue-specific gene disruption based on the Cas9 expression under the control of the Gal4/upstream activating sequence binary system. In our method, we couple Cas9 with green fluorescent protein or Cre reporter gene expression. This strategy allows us to induce somatic mutations in genetically labeled cell clones or single cells, and to follow them in vivo via reporter gene expression. Importantly, because none of the tools that we present here are restricted to zebrafish, similar approaches are readily applicable in virtually any organism where transgenesis and DNA injection are feasible.

Carole Gauron, Francesca Meda, Edmond Dupont, Shahad Albadri, Nicole Quenech'Du, Eliane Ipendey, Michel Volovitch, Filippo Del Bene, Alain Joliot, Christine Rampon, Sophie Vriz (2016 May 10)
Hydrogen peroxide (H2O2) controls axon pathfinding during zebrafish development.

Developmental biology : 133-41 : DOI : 10.1016/j.ydbio.2016.05.004

Résumé

It is now becoming evident that hydrogen peroxide (H2O2), which is constantly produced by nearly all cells, contributes to bona fide physiological processes. However, little is known regarding the distribution and functions of H2O2 during embryonic development. To address this question, we used a dedicated genetic sensor and revealed a highly dynamic spatio-temporal pattern of H2O2 levels during zebrafish morphogenesis. The highest H2O2 levels are observed during somitogenesis and organogenesis, and these levels gradually decrease in the mature tissues. Biochemical and pharmacological approaches revealed that H2O2 distribution is mainly controlled by its enzymatic degradation. Here we show that H2O2 is enriched in different regions of the developing brain and demonstrate that it participates to axonal guidance. Retinal ganglion cell axonal projections are impaired upon H2O2 depletion and this defect is rescued by H2O2 or ectopic activation of the Hedgehog pathway. We further show that ex vivo, H2O2 directly modifies Hedgehog secretion. We propose that physiological levels of H2O2 regulate RGCs axonal growth through the modulation of Hedgehog pathway.

Vincenzo Di Donato, Flavia De Santis, Thomas O Auer, Noé Testa, Héctor Sánchez-Iranzo, Nadia Mercader, Jean-Paul Concordet, Filippo Del Bene (2016 Mar 10)
2C-Cas9: a versatile tool for clonal analysis of gene function.
Genome research : 681-92 : DOI : 10.1101/gr.196170.115

Résumé

CRISPR/Cas9-mediated targeted mutagenesis allows efficient generation of loss-of-function alleles in zebrafish. To date, this technology has been primarily used to generate genetic knockout animals. Nevertheless, the study of the function of certain loci might require tight spatiotemporal control of gene inactivation. Here, we show that tissue-specific gene disruption can be achieved by driving Cas9 expression with the Gal4/UAS system. Furthermore, by combining the Gal4/UAS and Cre/loxP systems, we establish a versatile tool to genetically label mutant cell clones, enabling their phenotypic analysis. Our technique has the potential to be applied to diverse model organisms, enabling tissue-specific loss-of-function and phenotypic characterization of live and fixed tissues.

Timothy W. Dunn, Christoph Gebhardt, Eva A. Naumann, Clemens Riegler, Misha B. Ahrens, Florian Engert, Filippo Del Bene (2016 Feb 3)
Neural Circuits Underlying Visually Evoked Escapes in Larval Zebrafish
Neuron : 89 : 3 : 613-628 : DOI : 10.1016/j.neuron.2015.12.021

Résumé

Escape behaviors deliver organisms away from imminent catastrophe. Here, we characterize

behavioral responses of freely swimming larval zebrafish to looming visual stimuli simulating predators. We report that the visual system alone can recruit lateralized, rapid escape motor programs, similar to those elicited by mechanosensory modalities. Two-photon calcium imaging of retino-recipient midbrain regions isolated the optic tectum as an important center processing looming stimuli, with ensemble activity encoding the critical image size determining escape latency. Furthermore, we describe activity in retinal ganglion cell terminals and superficial inhibitory interneurons in the tectum during looming and propose a model for how temporal dynamics in tectal periventricular neurons might arise from computations between these two fundamental constituents. Finally, laser ablations of hindbrain circuitry confirmed that visual and mechanosensory modalities share the same premotor output network. We establish a circuit for the processing of aversive stimuli in the context of an innate visual behavior.

Kevin Fidelin, Lydia Djenoune, Caleb Stokes, Andrew Prendergast, Johanna Gomez, Audrey Baradel, Filippo Del Bene, Claire Wyart (2016 Jan 12)

State-Dependent Modulation of Locomotion by GABAergic Spinal Sensory Neurons.

Current biology : CB : 3035-47 : DOI : 10.1016/j.cub.2015.09.070

Résumé

The cerebrospinal fluid (CSF) constitutes an interface through which chemical cues can reach and modulate the activity of neurons located at the epithelial boundary within the entire nervous system. Here, we investigate the role and functional connectivity of a class of GABAergic sensory neurons contacting the CSF in the vertebrate spinal cord and referred to as CSF-cNs. The remote activation of CSF-cNs was shown to trigger delayed slow locomotion in the zebrafish larva, suggesting that these cells modulate components of locomotor central pattern generators (CPGs). Combining anatomy, electrophysiology, and optogenetics in vivo, we show that CSF-cNs form active GABAergic synapses onto V0-v glutamatergic interneurons, an essential component of locomotor CPGs. We confirmed that activating CSF-cNs at rest induced delayed slow locomotion in the fictive preparation. In contrast, the activation of CSF-cNs promptly inhibited ongoing slow locomotion. Moreover, selective activation of rostral CSF-cNs during ongoing activity disrupted rostrocaudal propagation of descending excitation along the spinal cord, indicating that CSF-cNs primarily act at the premotor level. Altogether, our results demonstrate how a spinal GABAergic sensory neuron can tune the excitability of locomotor CPGs in a state-dependent manner by projecting onto essential components of the excitatory premotor pool.

Année de publication : 2015

Alessio Paolini, Anne-Laure Duchemin, Shahad Albadri, Eva Patzel, Dorothee Bornhorst, Paula González Avalos, Steffen Lemke, Anja Machate, Michael Brand, Saadettin Sel, Vincenzo Di Donato, Filippo Del Bene, Flavio R Zolessi, Mirana Ramialison, Lucia Poggi (2015 Feb 7)

Asymmetric inheritance of the apical domain and self-renewal of retinal ganglion cell progenitors depend on Anillin function.
Development (Cambridge, England) : 832-9 : DOI : 10.1242/dev.118612

Résumé

Divisions that generate one neuronal lineage-committed and one self-renewing cell maintain the balance of proliferation and differentiation for the generation of neuronal diversity. The asymmetric inheritance of apical domains and components of the cell division machinery has been implicated in this process, and might involve interactions with cell fate determinants in regulatory feedback loops of an as yet unknown nature. Here, we report the dynamics of Anillin – an essential F-actin regulator and furrow component – and its contribution to progenitor cell divisions in the developing zebrafish retina. We find that asymmetrically dividing retinal ganglion cell progenitors position the Anillin-rich midbody at the apical domain of the differentiating daughter. anillin hypomorphic conditions disrupt asymmetric apical domain inheritance and affect daughter cell fate. Consequently, the retinal cell type composition is profoundly affected, such that the ganglion cell layer is dramatically expanded. This study provides the first in vivo evidence for the requirement of Anillin during asymmetric neurogenic divisions. It also provides insights into a reciprocal regulation between Anillin and the ganglion cell fate determinant Ath5, suggesting a mechanism whereby the balance of proliferation and differentiation is accomplished during progenitor cell divisions in vivo.

Antonio Mazzocca, Francesco Dituri, Flavia De Santis, Addolorata Filannino, Chiara Lopane, Regina C Betz, Ying-Yi Li, Naofumi Mukaida, Peter Winter, Cosimo Tortorella, Gianluigi Giannelli, Carlo Sabbà (2015 Jan 16)
Lysophosphatidic acid receptor LPAR6 supports the tumorigenicity of hepatocellular carcinoma.
Cancer research : 532-43 : DOI : 10.1158/0008-5472.CAN-14-1607

Résumé

The aberrant processes driving hepatocellular carcinoma (HCC) are not fully understood. Lysophosphatidic acid receptors (LPAR) are commonly overexpressed in HCC, but their contributions to malignant development are not well established. In this report, we show that aberrant expression of LPAR6 sustains tumorigenesis and growth of HCC. Overexpression of LPAR6 in HCC specimens associated with poor survival in a cohort of 128 patients with HCC. We took a genetic approach to elucidate how LPAR6 sustains the HCC tumorigenic process, including through an expression profiling analysis to identify genes under the control of LPAR6. RNAi-mediated attenuation of LPAR6 impaired HCC tumorigenicity in tumor xenograft assays. Expression profiling and mechanistic analyses identified Pim-3 as a pathophysiologically relevant LPAR6 target gene. In nonmalignant cells where LPAR6 overexpression was sufficient to drive malignant character, Pim-3 was upregulated at the level of transcription initiation through a STAT3-dependent mechanism. A further analysis of HCC clinical specimens validated the connection between overexpression of LPAR6 and

Pim-3, high proliferation rates, and poorer survival outcomes. Together, our findings establish LPAR6 as an important theranostic target in HCC tumorigenesis.

Année de publication : 2014

Thomas O Auer, Karine Duroure, Jean-Paul Concordet, Filippo Del Bene (2014 Nov 13)
CRISPR/Cas9-mediated conversion of eGFP- into Gal4-transgenic lines in zebrafish.
Nature protocols : 2823-40 : DOI : 10.1038/nprot.2014.187

Résumé

Here we present a protocol for the conversion of eGFP-transgenic zebrafish lines into lines expressing Gal4 from the same locus. This conversion allows the in-depth analysis of the former eGFP-expressing cell population; with the Gal4-upstream activating sequence (UAS) system, diverse UAS transgenes can be transactivated. Site-specific targeting of the gene encoding eGFP is achieved using the clustered regularly interspaced short palindromic repeats/CRISPR-associated 9 (CRISPR/Cas9) system. A single-guide RNA (sgRNA) that targets eGFP is injected into embryos together with a donor vector containing an optimized version of Gal4 (KalTA4) to trigger integration of the donor into the targeted eGFP genomic location. To enable screening for successful integration events, injection is performed in a UAS:RFP transgenic background; fish showing mosaic eGFP-to-RFP conversion are raised to adulthood. The progeny of these adult fish are then screened for stable germline transmission, and converted progeny are used to generate stable lines. We have been able to generate two stably converted transgenic lines within 4 months.

Thomas O Auer, Tong Xiao, Valerie Bercier, Christoph Gebhardt, Karine Duroure, Jean-Paul Concordet, Claire Wyart, Maximiliano Suster, Koichi Kawakami, Joachim Wittbrodt, Herwig Baier, Filippo Del Bene (2014 Oct 6)
Deletion of a kinesin I motor unmasks a mechanism of homeostatic branching control by neurotrophin-3.
eLife : DOI : 10.7554/eLife.05061

Résumé

Development and function of highly polarized cells such as neurons depend on microtubule-associated intracellular transport, but little is known about contributions of specific molecular motors to the establishment of synaptic connections. In this study, we investigated the function of the Kinesin I heavy chain Kif5aa during retinotectal circuit formation in zebrafish. Targeted disruption of Kif5aa does not affect retinal ganglion cell differentiation, and retinal axons reach their topographically correct targets in the tectum, albeit with a delay. In vivo dynamic imaging showed that anterograde transport of mitochondria is impaired, as is synaptic transmission. Strikingly, disruption of presynaptic activity elicits upregulation of Neurotrophin-3 (Ntf3) in postsynaptic tectal cells. This in turn promotes exuberant branching

of retinal axons by signaling through the TrkC receptor (Ntrk3). Thus, our study has uncovered an activity-dependent, retrograde signaling pathway that homeostatically controls axonal branching.

Thomas O Auer, Filippo Del Bene (2014 Apr 8)
CRISPR/Cas9 and TALEN-mediated knock-in approaches in zebrafish.
Methods (San Diego, Calif.) : 142-50 : DOI : 10.1016/j.ymeth.2014.03.027

Résumé

The targeted introduction of mutations utilizing sequence specific transcription activator-like effector nucleases (TALENs) and the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) 9 system (RNA-guided nucleases, RGNs) has revolutionized reverse genetic approaches in numerous model organisms. In zebrafish, both systems were successfully applied to generate loss-of-function alleles by targeting open reading frames or deletion and inversion of whole chromosomal regions. In addition to the production of these loss-of-function alleles, genomic engineering by insertion of short sequences utilizing single stranded DNA oligonucleotides as templates for homology based repair was made possible, enabling effective insertion of loxP sites or tags for protein coding genes. Recent studies based on homologous recombination and non-homologous end joining have also broadened the repertoire for genome editing. These approaches allow the targeted insertion of open reading frames or even whole donor vectors. In this review we summarize the use of TALENs and RNA-guided nucleases in the field of zebrafish genetics with a special focus on knock-in approaches.

Année de publication : 2013

Thomas O Auer, Karine Duroure, Anne De Cian, Jean-Paul Concordet, Filippo Del Bene (2013 Oct 31)
Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair.
Genome research : 142-53 : DOI : 10.1101/gr.161638.113

Résumé

Sequence-specific nucleases like TALENs and the CRISPR/Cas9 system have greatly expanded the genome editing possibilities in model organisms such as zebrafish. Both systems have recently been used to create knock-out alleles with great efficiency, and TALENs have also been successfully employed in knock-in of DNA cassettes at defined loci via homologous recombination (HR). Here we report CRISPR/Cas9-mediated knock-in of DNA cassettes into the zebrafish genome at a very high rate by homology-independent double-strand break (DSB) repair pathways. After co-injection of a donor plasmid with a short guide RNA (sgRNA) and Cas9 nuclease mRNA, concurrent cleavage of donor plasmid DNA and the selected chromosomal integration site resulted in efficient targeted integration of donor

DNA. We successfully employed this approach to convert eGFP into Gal4 transgenic lines, and the same plasmids and sgRNAs can be applied in any species where eGFP lines were generated as part of enhancer and gene trap screens. In addition, we show the possibility of easily targeting DNA integration at endogenous loci, thus greatly facilitating the creation of reporter and loss-of-function alleles. Due to its simplicity, flexibility, and very high efficiency, our method greatly expands the repertoire for genome editing in zebrafish and can be readily adapted to many other organisms.

Christoph Gebhardt, Herwig Baier, Filippo Del Bene (2013 Jun 21)
Direction selectivity in the visual system of the zebrafish larva.
Frontiers in neural circuits : 111 : DOI : 10.3389/fncir.2013.00111

Résumé

Neural circuits in the vertebrate retina extract the direction of object motion from visual scenes and convey this information to sensory brain areas, including the optic tectum. It is unclear how computational layers beyond the retina process directional inputs. Recent developmental and functional studies in the zebrafish larva, using minimally invasive optical imaging techniques, indicate that direction selectivity might be a genetically hardwired property of the zebrafish brain. Axons from specific direction-selective (DS) retinal ganglion cells appear to converge on distinct laminae in the superficial tectal neuropil where they serve as inputs to DS postsynaptic neurons of matching specificity. In addition, inhibitory recurrent circuits in the tectum might strengthen the DS response of tectal output neurons. Here we review these recent findings and discuss some controversies with a particular focus on the zebrafish tectum's role in extracting directional features from moving visual scenes.