Decentralized Clocks and Direct Photoreception: The Zebrafish as an Integrative Model for Circadian Biology

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Abstract

Circadian rhythms are fundamental, endogenously generated biological mechanisms that have evolved to synchronize organisms with the diurnal cycles resulting from Earth' s rotation. The zebrafish (Danio rerio) has emerged as a premier vertebrate model for investigating these rhythms due to its unique combination of features: optical transparency during embryonic and larval stages, rapid external development, genetic tractability, and high evolutionary conservation of core circadian clock genes and neural pathways with humans. A particularly distinctive trait is the intrinsic photosensitivity and autonomous circadian oscillatory capacity found in cells throughout the zebrafish body, including peripheral organs and tissues. This provides an unparalleled system for dissecting the coordinated and independent regulation of central and peripheral clocks. This review systematically synthesizes recent advances in zebrafish circadian research, focusing on the molecular architecture of the core clock, the redundant multi-tissue light-input pathways, and the clock' s precise regulation of key physiological processes such as neural function, cardiovascular activity, metabolism, immunity, and reproduction. We further discuss current limitations and challenges, including the mapping of relevant neural circuits, understanding inter-tissue communication, and conducting lifespan-wide investigations. Finally, we outline promising future directions, such as leveraging emerging technologies for circuit analysis, exploring the impact of environmental disruptors, and advancing translational medical applications.

Full Text

Preamble

Decentralized Clocks Direct Photoreception: Zebrafish Integrative Model Circadian Biology Chun-jiao Wen-bo Da-long College Animal Science Technology, Anhui Agricultural University, Hefei 230036, *Corresponding authors:

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Abstract

Circadian rhythms fundamental, endogenously generated biological mechanisms evolved synchronize organisms diurnal cycles resulting Earth' s rotation. zebrafish Danio rerio emerged premier vertebrate model investigating these rhythms unique combination features: optical transparency during embryonic larval stages, rapid external development, genetic tractability, evolutionary conservation circadian clock genes neural pathways humans. particularly distinctive trait intrinsic photosensitivity autonomous circadian oscillatory capacity found cells throughout zebrafish body, including peripheral organs tissues. provides unparalleled system dissecting coordinated independent regulation central peripheral clocks. review systematically synthesizes recent advances zebrafish circadian research, focusing molecular architecture clock, redundant multi-tissue light-input pathways, clock' s precise regulation physiological processes neural function, cardiovascular activity, metabolism, immunity, reproduction. further discuss current limitations challenges, including mapping relevant neural circuits, understanding inter-tissue communication, conducting lifespan-wide investigations.

Finally, outline promising future directions, leveraging emerging technologies circuit analysis, exploring impact environmental disruptors, advancing translational medical applications.

Keywords

Zebrafish; Circadian rhythm; Decentralized Clocks; Peripheral oscillator; Photoentrainment; Disease model

1. Introduction

Circadian rhythms constitute universal endogenous timekeeping system enables organisms align expression, cellular physiology, organ function predictable daily environmental cycles, thereby maintaining homeostasis optimizing fitness Disruption intrinsic rhythmicity whether lifestyle factors shift environmental perturbations, genetic mutations strongly associated spectrum human disorders, including

sleep disturbances, metabolic syndrome, cardiovascular diseases, neuropsychiatric conditions, cancer Therefore, deciphering molecular basis, regulatory networks, physiological outputs circadian rhythms central question basic biology holds profound implications translational medicine public health.

Among model organisms circadian research, zebrafish occupy crucial evolutionary experimental niche, bridging between invertebrate models Drosophila melanogaster mammalian systems mouse Unlike Drosophila which utilize distinct clock genes regulatory logic, mammals, where circadian control centralized hypothalamic suprachiasmatic nucleus (SCN), zebrafish exhibit degree conservation clock genes Clock pathways humans.

Concurrently, possess unique biological features expand scope inquiry.

These include external fertilization, transparent embryos permitting real-time visualization rhythmic processes, rapid organogenesis within hours post-fertilization, robust genetic toolkit manipulation CRISPR/Cas9, transgenesis, morpholino knockdown defining characteristic zebrafish apart decentralized circadian regulatory architecture contrast mammals, where peripheral tissues exhibit dampened autonomy heavily synchronization, virtually zebrafish cells classical photoreceptive tissues retina pineal gland peripheral organs liver, heart, intestine, cultured lines harbor intrinsic circadian oscillators possess autonomous photosensitivity allows zebrafish integrate light signals directly central peripheral levels, offering powerful model dissect independent functions synchronization mechanisms distributed clocks [11]. circadian oscillator built around conserved transcription-translation feedback (TTFL). loop, CLOCK/BMAL heterodimers activate transcription genes, resulting PER/CRY proteins subsequently inhibit CLOCK/BMAL activity, establishing ~24-hour cycle Teleost-specific genome duplication further endowed zebrafish multiple paralogs clock genes, particularly within family, which undergone subfunctionalization mediate specialized

roles rhythm generation, light entrainment, tissue-specific regulation molecular oscillator complemented redundant, multi-tiered light-input system encompassing retina, deep-brain photoreceptors, peripheral tissue photoreceptors, ensuring robust environmental signal detection precise phase calibration [17]. fundamental energy sensory input, light enables image perception through mediates non-image-forming (NIF) functions. zebrafish genome encodes opsin genes, providing prototype understanding functions light [18]. sophisticated circadian network governs array physiological processes zebrafish, including sleep-wake cycles, learning memory, cardiovascular function, energy metabolism, immune responses, reproduction, gut-microbiota interactions, which evolutionarily conserved humans example, disruption zebrafish clock genes recapitulates human disease phenotypes sleep fragmentation, metabolic dysregulation, cardiovascular defects, providing valuable insights disease mechanisms [26].

Despite significant progress, critical knowledge persist. intracellular signaling cascades linking light perception opsins, Cryptochromes, other photoreceptors

clock machinery fully elucidated; neural circuits mediating communication between central pacemaker telencephalon) peripheral tissues remain incompletely mapped; dynamics circadian rhythms across zebrafish lifespan, embryogenesis aging, fully characterized review comprehensively synthesizes recent progress zebrafish circadian rhythm research. begin molecular mechanisms oscillator, followed detailed

analysis

multi-tissue light-input pathways. summarize circadian regulation physiological systems address existing limitations challenges.

Finally, outline future research directions harness emerging technologies resolve unanswered questions promote translational applications. provide consolidated overview zebrafish model circadian research inform novel approaches exploring evolution, function, dysfunction biological clocks.

2. Core

Architecture Multisystem Regulation Zebrafish Circadian Clock

2.1 A

Highly Conserved Molecular Oscillator circadian oscillator zebrafish constructed around highly homologous mammalian system.

CLOCK proteins dimerize E-box motifs (CACGTG) promoters genes drive their transcription [15].

Newly synthesized proteins complexes cytoplasm, undergo phosphorylation kinases translocate nucleus, where directly inhibit CLOCK/BMAL activity, repressing their transcription [30]. rhythmic degradation PER/CRY proteins, primarily ubiquitin-proteasome system night, releases inhibition, allowing cycle activation begin [31]. primary reinforced secondary stabilizing loops.

REV-ERB represses, while activates, transcription through competitive binding elements (ROREs) promoter [32].

Notably, RORaa shown directly regulate expression RORE, adding layer cross-regulation Additionally, D-box elements promoters clock-controlled genes bound transcription factors (e.g., Dbp). expression these factors dually controlled clock acute light exposure, providing direct conduit photic input shape circadian outputs [35]. distinctive genomic feature zebrafish presence multiple paralogs nearly clock genes,

result

teleost-specific genome duplication. zebrafish genome contains clock genes clock1a clock1b genes bmal1 bmal2 three genes, least genes.

Functional analyses revealed extensive subfunctionalization. example, cry1a cry1b cry3a cry3b retain transcriptional repressor activity participate light-dependent phase resetting, Cry1a acting photoreceptor undergoing light-induced conformational changes Figure contrast, motifs necessary CLOCK/BMAL interaction transcriptional repression [16]; enriched cones function non-circadian light

detection, magnetoreception [37]. expanded diversified genetic repertoire enhances regulatory flexibility provides natural system probing structure-function relationships within circadian machinery.

Circadian Molecular Regulatory Diagram Zebrafish figure illustrates transcription-translation feedback (TTFL) auxiliary regulatory network zebrafish circadian clock.

Clock (clock1a/1b) (bmal1/2) heterodimers, which E-box (CACGTG) elements activate transcription (per1/2/3) (cry1a/1b/3a/3b) genes.

Cytoplasmic Per/Cry complexes, which phosphorylated kinases, translocate nucleus inhibit Clock/Bmal activity, resulting 24-hour oscillation.

Per/Cry rhythmically degraded ubiquitin-proteasome system relieve inhibition.

Auxiliary loops involve Rev-Erb (e.g., roraa) competitively regulating RORE, whereas family) mediates light-core clock coupling through D-box elements.

2.2 Redundant

Efficient Photoreceptive Input Systems Light, fundamental energy sensory input, profoundly shaped Earth [18].

Zebrafish possess remarkable capacity light detection, supported redundant light-input pathways cellular molecular levels, ensuring reliable environmental signal capture circadian system Figure

Schematic Diagram Zebrafish Retinal Photoreceptors, Brain Photoreceptors, Peripheral Photoreceptors their Roles Circadian Photoentrainment figure depicts three light-sensing systems mediate zebrafish circadian photoentrainment light-driven behaviors. zebrafish retina directly integrates light signals biological rhythms intrinsic molecular clock, enabling regulation behavioral rhythms.

Distributed deep-brain photoreceptors non-visual light perception system essential phase entrainment.

Zebrafish possess decentralized circadian system, peripheral tissues/organs sustaining autonomous oscillations responding directly light photopigments signaling.

Retinal Pathway: retina zebrafish serves sensory organ intrinsic circadian system maintains rhythmic cycles.

Unlike mammals, which depend retinal-SCN pathway centralized clock regulation, zebrafish retina integrates light signals directly biological rhythms inherent molecular clocks [10]. integration enables retina regulate visual function behavioral rhythms independently [38]. retinal circadian

clock system responds external light equally importantly, sustains intrinsic rhythms absence light, through multiple feedback loops These feedback mechanisms driven clock genes, including clock bmal1 which regulate expression genes crucial maintaining daily rhythm visual function [39].

Specifically, CLOCK/BMAL1 complex regulates expression long-wavelength opsin mRNA, exhibiting circadian rhythm levels afternoon trough early morning [39].

Notably, rhythmic expression persists constant darkness, indicating retina' s autonomous circadian nature [39]. addition, light exposure directly induces expression light-responsive clock genes cry1a which subsequently influence retinal systemic circadian rhythms, facilitating synchronization environmental light-dark cycles Studies shown mutations

result

phase delays zebrafish locomotor rhythms, lengthened cycles, reduction visual sensitivity contrast sensitivity [42].

Retinal circadian rhythms impact visual processing through modulation neurotransmitter systems, notably dopamine melatonin During daylight, dopamine release retina promotes function, enhancing visual sensitivity color discrimination [43].

Furthermore, dopamine regulates transmission visual signals altering electrical coupling between photoreceptors bipolar horizontal cells [43]. night, increased melatonin secretion shifts retinal function towards rod-dominated, low-light vision [44]. manner, retina adjusts photoreceptor activity response specific visual demands night.

Mutations per1b cause damage dopaminergic cells retina, resulting dopamine deficiency decreased contrast sensitivity [45].

These findings emphasize critical retinal clocks dopamine maintaining visual perception behavioral rhythms [45]. addition regulating visual function, retinal clock genes development visual system [46]. example, transcription factor NeuroD regulated clock genes after photoreceptor differentiation, contributing rhythmic expression phototransduction genes [46].

Moreover, circadian rhythms regulate synaptogenesis

along retinal-tectal pathway, further underscoring involvement retinal clocks development visual neural circuits [47]. effects retinal rhythms extend beyond local visual functions, influencing systemic behavioral rhythms [38]. under constant

darkness, zebrafish exhibit clear circadian rhythms visual sensitivity, faster recovery visual responses morning, better suited rapid visual tasks, slower recovery night [48]. retinal clock adjusts dominance photoreceptors modulates visual sensitivity, ensuring optimal performance under varying light conditions [43]. larvae, light regulates rhythmic phagocytosis retinal outer segment retinal pigment epithelium (RPE), thereby preventing accumulation harmful compounds photoreceptors Furthermore, zebrafish larvae undergo disassembly synaptic ribbons night, exhibiting "night blindness" energy-saving mechanism Retinal rhythms synchronize central nervous system through neural projections, influencing behavioral responses zebrafish [17].

Pathways retina optic tectum hypothalamus regulate circadian changes behaviors visual escape responses, optokinetic responses (OKR), visual motor responses (VMR) [17].

Although larvae lacking retinal ganglion cells still maintain basic locomotor rhythms, retina plays essential adjusting phase behavioral rhythms, particularly during subjective night, behavioral phase advancement occurs Brain Photoreceptors: network composed multiple neural pacemakers light-responsive regions zebrafish brain expresses clock genes regions including ventral thalamic nuclei, periventricular (PGZ), dorsal nucleus ventral telencephalic (Vd), hypothalamus, torus longitudinalis (TL), preglomerular nuclei, valvula cerebelli.

Among these regions, ventral thalamic nuclei, receive retinal input zebrafish, while hypothalamus, preglomerular nuclei, valvula cerebelli directly photosensitive vitro clock genes multiple brain regions zebrafish, including telencephalon, diencephalon, mesencephalon, optic tectum, pituitary, rhombencephalon, photoinduced [54].

Therefore, appears retinofugal inputs necessary clock expression. zebrafish brain

contain various types photosensitive cells, photosensitive neurons widely distributed non-image-forming brain regions, thalamus, hypothalamus, preoptic area. contain different photopigments, including opsins, cryptochrome flavin-containing oxidases Among family non-visual opsins, TMT-opsin expressed broadly neural non-neural tissues, paralleling clock expression [53].

VAL-opsin subtypes thalamus distinctly regulated: valopa rhythm endogenous, whereas valopb light-modulated [55].

Furthermore, larvae lacking pineal glands, *opn4m-1* mediates phototaxis, behavior absent mutants rescued *opn4m-1* overexpression domain [56].

Melanopsin double mutants exhibit reduced daytime locomotor activity, requiring sustained melatonin inhibition restore normal activity levels [57]. absence discrete zebrafish utilize distributed deep-brain photoreceptive centers [58]. pineal gland, photosensitive neuroendocrine organ, generates robust circadian rhythms melatonin secretion Rhythmic melatonin detectable post-fertilization originates differentiating pineal before retinal photoreceptors become functional [61]. mutants lacking forebrains, pineal maintains rhythmic expression clock

melatonin synthesis genes, confirming autonomy pacemaker Larvae without pineal glands exhibit blunted locomotor responses darkness, underscoring their behavioral [64].

Collectively, these findings highlight complex non-visual light perception system essential phase entrainment specific light-driven behaviors.

However, complete molecular cascades linking deep-brain photoreceptor activation clock remain fully defined.

Peripheral Photoreceptors: Unlike SCN-centric model mammals, zebrafish exhibit decentralized circadian system Their peripheral organs (e.g., heart, liver), tissues (e.g., skin, muscle, intestine), embryonic lines sustain endogenous, undamped circadian oscillations vitro multiple cycles without central input Moreover, these tissues respond directly light-dark cycles adjust their circadian phase. autonomy stems widespread expression photopigments associated signaling components peripheral tissues [69]. zebrafish genome encodes opsin genes visual, non-visual), providing

molecular basis tissue-specific light detection Figure example, opn6b highly expressed heart, whereas specific skin.

Among family non-visual opsins, zebrafish-specific TMT-opsin subfamily shares homology known photopigments; vitro studies confirmed activation light initiation protein signaling, supporting photopigment [71].

Despite widespread expression opsins these non-retinal tissues, light-sensitive physiological functions these opsins remain fully elucidated. distribution photoproteins various tissues zebrafish figure shows distribution opsin classes zebrafish mouse, representative vertebrate species.

Presence marked absence Opsins classified major groups: visual opsins (green), cone-like non-visual opsins (blue), opn3/tmt opsins (purple), rgr/rrh/opn5 opsins (yellow), opsins (black), opsins (red). (Figure adapted [70]) addition opsins, cryptochromes flavin-containing oxidases candidate photoreceptive proteins. zebrafish, proteins possess multiple isoforms.

Cry1a functions ultraviolet/blue light photoreceptor directly communicate molecular oscillator [72]. cry2b shows high-amplitude

rhythmic expression across tissues, underpinning peripheral light perception [73].

Notably, light induce hydrogen peroxide production, which activates pathway [74]. involve light-responsive flavoenzymes absorb near-UV/blue light promote generation [75].

Light-responsive D-box enhancers serve nuclear targets reactive oxygen species (ROS), antioxidant enzyme catalase modulate clock expression regulating levels [76].

Thus, signaling complement classical pathways sophisticated circadian regulatory network.

2.3 Circadian

Orchestration Multi-System Physiology Neural Rhythmicity:

Zebrafish display clear diurnal sleep-wake patterns, consolidated night daytime activity Figure Genetic ablation clock bmal1 causes severe sleep fragmentation arrhythmic locomotion, modeling human circadian sleep disorders. cellular level, clock regulates synaptic homeostasis optic tectum, where synapse number strength increase during wakefulness pruned during sleep process dependent intact sleep-wake cycles [47].

Learning memory fluctuate diurnally; enhanced avoidance learning specific phases correlates expression brain.

Disruption rhythms impairs hippocampal-like O-GlcNAcylation rhythms downregulates nuclear leading cognitive deficits [22].

Anxiety-like behaviors circadian variation, suggesting model disorders. example, protein kinase (prkcaa), which linked neuropsychiatric disorders, modulates morning expression immediate early genes; attenuates their normal nocturnal repression [77].

Circadian Clock Regulatory Network Zebrafish Multi-System Physiology figure centers zebrafish illustrate regulation multi-system physiological functions circadian clock. functional modules involved include: nervous system, encompassing sleep-wake cycles, learning memory, synaptic plasticity; cardiovascular system, including heart rate, cardiac output, vascular development regeneration; metabolic system, covering lipid glucose metabolism detoxification; reproductive system, involving spermatogonial differentiation fertilization, ovarian reserve tumorigenesis, maternal clock inheritance; intestinal system, comprising epithelial renewal microbiota homeostasis. associations between these functional modules zebrafish reflect coordinated control circadian clock multiple physiological systems.

Cardiovascular Homeostasis: molecular clock plays specific roles within cardiovascular tissue Figure occurrence adverse cardiovascular events exhibits circadian rhythmicity.

Heart cardiac output under strict circadian control [78]. bmal2 mutants heart rhythms develop bradycardia reduced stroke volume, directly linking clock cardiovascular function [51].

Vascular development regeneration exhibit daily rhythms.

Clock genes endothelial cells rhythmically regulate other angiogenic factors.

Circadian disruption severely impairs developmental angiogenesis adult regeneration [79].

Notably, bmal1 knockdown inhibited vessel formation, whereas knockdown accelerated vessel formation, revealing antagonistic effects.

Mechanistically, BMAL1 binds E-boxes promoter, deficiency compromises Notch inhibition-induced sprouting, highlighting crosstalk between circadian developmental pathways [19].

Hypoxia-inducible factors (HIFs) their targets, VEGF, clock-modulated.

Circadian disruption developing zebrafish impairs hypoxic responses, alters erythropoiesis, disrupts

vascular patterning, and increases mortality [80 –82].

Hepatic Rhythmicity: liver exemplifies peripheral clock-metabolism crosstalk.

Transcriptomic analyses revealed circadian oscillations hundreds metabolic genes involved lipogenesis, gluconeogenesis, detoxification Figure Mutations clock genes induce hepatic steatosis, dyslipidemia, glucose intolerance, insulin resistance, modeling metabolic syndrome observed shift workers Constant darkness reduces metabolic immune expression induces fatty liver [84].

Melatonin exerts beneficial metabolic effects increasing satiety signals liver suppressing Igf-I expression [85]. roraa mutants downregulation fatty oxidation genes transporters fabp2 which direct RORaa targets signaling axis, positioning RORaa integrator circadian metabolic regulation [33]. factors targets exhibit light- clock-dependent rhythms, aligning detoxification capacity daily environmental exposure [23].

Immune Rhythmicity: Innate immune responses under circadian regulation Figure [24]. migratory efficiency zebrafish neutrophils injury sites peaks during modulated immune cell-intrinsic clock systemic glucocorticoid signaling.

Inflammatory cytokine expression (e.g., *il-1 oscillates, indicating preprogramming inflammatory pathway clock molecular clock component Clock1a regulates rhythmic recruitment behavior neutrophils modulating antioxidant response through* nfe212a/duox* pathway [88]. per1b mutations distinct effects: required neutrophil bactericidal activity, driving production enhancing infection-induced hmgb1a expression clear bacteria [89].

Cry-binding domain essential regulation. Conversely, Cry1a-deficient neutrophils increased bactericidal activity hmgb1a expression [90].

Conserved BMAL1 motifs hmgb1a promoter constrain induction light phase.

Mutating BMAL1 motif impairs light-dependent priming bactericidal activity, indicating light optimizes neutrophil function circadian timer. contrast, per1b mutation downregulates subsequent phosphorylation, reducing activation proinflammatory expression [91].

immune system regulated circadian rhythm. figure illustrates diurnal variation neutrophils model bacterial infection injury zebrafish larvae. clock1a regulates

neutrophil migration coordinating rhythmic expression nfe212a genes control reactive oxygen species (ROS) level.

Light-regulated increases reactive oxygen species (ROS) production bacterial killing zebrafish neutrophils controlling Hmgb1 expression.

Reproductive Rhythmicity: Circadian rhythmicity inherent feature reproductive system.

Circadian clock-controlled retinoic signaling plays significant spermatogonial differentiation fertilization Figure Sertoli cells, circadian clock regulates synthesis receptor expression binding E-box elements aldh1a2 rarga genes [92].

After diffuses spermatogonia, inhibits transcriptional repressor Zbtb16a promote

spermatogonial differentiation. Meanwhile, signaling upregulates expression sperm surface fusion factor Izumo1, enhancing sperm fertilization capacity without affecting sperm count [92].

Circadian disruption, either global Sertoli cell-specific disruption clock1a bmal1 temporal perturbation desynchronization clock1a expression,

result

arrested spermatogonial differentiation reduced fertilization [92].

Clock genes regulate ovarian function [93]. Loss-of-function mutations premature depletion ovarian reserve, resulting declined reproductive capacity. addition, circadian rhythm disruption disturbs reproductive hormone levels ultimately induces ovarian tumorigenesis zebrafish Embryos inherit maternal clock products through oogenesis initiate their rhythms [96].

Furthermore, preconceptional circadian rhythm disruption impairs ovarian function female offspring, specifically manifested compromised follicular development, reduced oocyte quality, decreased embryonic developmental potential, impairment closely associated abnormal lipid metabolism disruption ovarian immune microenvironment offspring Homeostasis: zebrafish intestine functions peripheral clock tissue autonomous regulatory capacity. intrinsic pacemaker directly photoentrained precise daily mitosis, regulating cycle genes modest effects cyclins Figure proliferation apoptosis intestinal epithelial cells expression nutrient absorption genes systemically clock-regulated [98].

Notably, composition function commensal microbiota exhibit diurnal fluctuations synchronized clock.

Disruption clock genes constant light alters microbial composition, increasing opportunistic bacteria reducing probiotics, leading dysbiosis [99]. makes zebrafish ideal model studying "circadian clock-gut-brain axis." example, constant light larvae elevates cortisol, inhibits intestinal peristalsis, creates stress-induced constipation model characterized inflammation, impaired neural activity, dysregulated aquaporin/VIP expression [99].

Probiotic supplementation, particularly Bifidobacterium longum alleviates these phenotypes reducing cortisol, modulating inflammation, restoring

motility neural activity 100], demonstrating functional crosstalk within circadian-gut-microbiota network.

3. Future

Perspectives Compared classical circadian models, zebrafish distinguished their unique combination vertebrate-level physiological complexity, optical accessibility, distributed photoreceptive capacity. presence decentralized photoreceptors further enhances theoretical value model. mammals, peripheral clocks typically regarded subordinate oscillators indirectly regulated central pacemaker. zebrafish, however, peripheral tissues function primary light-responsive units capable autonomous entrainment. distributed sensory architecture suggests relationship between central peripheral clocks strictly follow hierarchical structure, instead dynamically modulated across tissues depending environmental conditions, developmental stage, metabolic state. framework redefines central question chronobiology.

Whereas previous research focused central clock imposes order passive peripheral oscillators, future investigations shift toward understanding multiple semi-autonomous oscillators achieve coordinated coherence. brain integrate temporal signals peripheral tissues, peripheral clocks collectively constrain central timing? enabling direct experimental manipulation central peripheral photoreceptive units within organism, zebrafish provide powerful platform address these questions.

Future studies integrating tissue-specific optogenetic control, longitudinal whole-body imaging, network modeling reveal vertebrate circadian systems operate adaptive, multi-node temporal networks rather rigid hierarchical structures. insights could reshape understanding central peripheral clocks coordinate under physiological conditions their uncoupling contributes disease.

Exploring clock genes holds profound significance disease treatment, their value extends beyond circadian rhythm regulation encompass complex organismal interactions multi-dimensional functional roles.

Increasing evidence supports existence bidirectional communication between biological clocks physiological systems, tight coupling among clocks across organs.

Intestinal clocks

influence sleep-wake cycle temporally maintaining homeostasis glutamatergic neurons hypothalamic nuclei, participate regulating brain cognitive processes, whereas defects biological clocks exert significant negative impact cognitive performance [101]. parallel, synergistic interaction between microbiota liver biological clocks attracted considerable attention [102]. host' s glucose demand,

microbiota activate hepatic gluconeogenesis through extracellular vesicles, production these extracellular vesicles exhibits circadian rhythmicity related host' s nutritional status, which further confirms synergistic regulatory network biological clocks among systems [103].

Notably, addition their function circadian rhythm regulation, clock genes possess significant "non-canonical functions" participate numerous pathways outside traditional circadian control.

CLOCK reshape neuronal connectivity networks, abnormal expression important contributing factors neuropsychiatric disorders autism schizophrenia [104].

BMAL1 exhibits diverse functions: deletion cause pluripotent cells partially reverse totipotent-like state, process independent CLOCK involvement achieved synergistically inhibiting activation MERVL genes TRIM28 [105]. field vascular diseases, regulatory BMAL1 re-evaluated; relevant studies revealed pathogenic vascular calcification suggested targeting dysregulated circadian rhythm factors serve novel therapeutic strategy preventing diabetic vascular calcification [105]. discovery these non-canonical functions opened avenues understanding pathogenesis complex diseases cognitive disorders developing targeted therapeutic regimens.

Furthermore, research development small-molecule drugs targeting clock regulatory pathways achieved phased progress. compounds capable regulating activity BMAL1/CLOCK complex entered preclinical research phase expected combination chronochemotherapy, enabling precision therapy "targeted temporal" [106, 107].

Although chronotherapy mediated clock genes achieved numerous breakthroughs clinical applications, still faces challenges translating basic research clinical practice.

First, regulatory network clock genes extremely complex: there significant differences expression rhythms regulatory mechanisms clock genes across different tissues disease states, clock regulatory mechanisms diseases fully elucidated, limiting development targeted chronotherapeutic regimens. future, development multi-omics technologies genomics, transcriptomics, metabolomics, popularization wearable devices (which monitor rhythmic indicators physiological parameters real-time), clock research chronotherapy towards precise, personalized, systematically integrated direction. excellent model circadian rhythm biology research, zebrafish unique advantages mechanism elucidation, screening, regimen validation. leveraging zebrafish model deeply analyze inter-system regulatory mechanisms non-canonical functions clock genes, identify regulatory targets different diseases individuals, provide precise theoretical support development chronotherapeutic regimens.

Meanwhile, using zebrafish model rapidly verify effectiveness clock gene-targeted drugs rationality chronotherapeutic administration schedules strongly support improving therapeutic efficacy, reducing adverse reactions, enhancing patients'

quality life, ushering "temporal precision systematic synergy" disease treatment.

4. Conclusion

Zebrafish circadian research established comprehensive framework encompassing molecular mechanisms, multi-tiered photic inputs, systemic physiological regulation.

While conserving canonical architecture vertebrates, zebrafish uniquely combine genomic expansion (e.g., subfunctionalized paralogs), tripartite redundant photoreceptive system, autonomous peripheral photoreception collectively illuminating evolutionary adaptability functional sophistication biological clocks. model yielded profound insights circadian networks orchestrate neural plasticity, cardiovascular dynamics, metabolic flux, immune vigilance, reproductive function,

host-microbiome symbiosis, providing indispensable platform investigate "clock-organ-environment" interactions model human diseases rooted circadian disruption.

Despite these advances, fundamental questions remain critically, intracellular signaling cascades coupling photoreception oscillator molecular identity synchronizing between central peripheral clocks.

Addressing these through interdisciplinary approaches deepen understanding circadian biology catalyze development novel diagnostics, therapeutics, preventive strategies spectrum human disorders.

Owing unparalleled combination genetic tractability, optical accessibility, physiological relevance, evolutionary position, zebrafish poised remain cornerstone model advancing foundational chronobiology emerging precision medicine.

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Conflict Interest authors declare conflicts interest.

References

1. Fang, Chavan, LiWang, Golden, Synchronization circadian clock environment tracked time. e2221453120. 2. Patke, Young, Axelrod, Molecular mechanisms physiological importance circadian rhythms. (2020).
2. Crosby, implications mammalian cellular circadian entrainment. (2025) 4. Vatine, Vallone, Gothilf, Foulkes, swim!

Zebrafish circadian clock. Letters (2011).

5. N

sslein-Volhard, Dahm, Zebrafish: Practical Approach (Oxford University Press, 2002). 6. Kimmel, Ballard, Kimmel, Ullmann, Schilling, Stages embryonic development zebrafish. (1995). 7. Sun, Generation Endogenous Promoter-Driven Luciferase Reporter System Using CRISPR/Cas9 Investigating Transcriptional Regulation Clock BMAL1.

Biomedicines (2022). 8. Moore, Whitmore, Circadian rhythmicity light sensitivity zebrafish brain. e86176 (2014). 9. Whitmore, Foulkes, Sassone-Corsi, Light directly organs cells culture vertebrate circadian clock.

Nature (2000). 10. Steindal, Whitmore, Zebrafish Circadian Clock Entrainment Importance Broad Spectral Light Sensitivity.

Front Physiol (2020). 11. Junko, Light-Dependent Regulation Circadian Clocks Vertebrates.

Chronobiology Science Biological Structure (IntechOpen, 2019). 12. Miyamura, CLOCK:BMAL-independent circadian oscillation zebrafish Cryptochrome1a gene.

Pharm (2009).

13. Cahill, Clock mechanisms zebrafish. Tissue (2002). 14. Tamai, Young, Whitmore, Light signaling zebrafish circadian clock Cryptochrome (2007). 15. Ishikawa, Hirayama, Kobayashi, Todo, Zebrafish represses transcription mediated CLOCK-BMAL heterodimer without inhibiting binding Genes Cells (2002). 16. Sacksteder, Kimmey, Immunity, Infection, Zebrafish Clock.

Infect Immun e00588-21. 17. Baier, Scott, Visual Systems Zebrafish.

Neurosci (2024). 18. Shi, Light, opsins, life: Mammalian photophysiological functions beyond image perception.

Neuron (2025). 19. Jensen, Clock controls angiogenesis.

Cycle (2013). 20. Suppermpool, Lyons, Broom, Rihel, Sleep pressure modulates single-neuron synapse number zebrafish.

Nature (2024). 21. Costa, Current State Modeling Human Psychiatric Disorders Using Zebrafish. (2023). 22. Park, E.-S.

I.-O. Light-Dependent Circadian Rhythm Governs O-GlcNAc Cycling Influence Cognitive Function Adult Zebrafish.

JOURNAL PINEAL RESEARCH (2024).

23. Carmona-Anto

anzas, Migaud, Santi, Vera, Light- clock-control genes involved detoxification.

Chronobiology International (2017). 24. Mazur, Effects light circadian clock antiviral immune response zebrafish.

Shellfish Immunol (2023). 25. Peyric, Moore, Whitmore, Circadian Clock Regulation Cycle Zebrafish Intestine. e73209 (2013). 26. Zhdanova, Aging circadian system zebrafish effects melatonin sleep cognitive performance.

Brain (2008). 27. Wang, Light-regulated microRNAs shape dynamic expression zebrafish circadian clock.

Genetics e1011545 (2025).

28. D

Agostino, circadian rhythmicity knockout zebrafish larvae. iScience (2022). 29. Krylov, Izvekov, Pavlova, Pankova, Osipova, Circadian rhythms zebrafish (Danio rerio) behaviour sources their variability.

BIOLOGICAL REVIEWS (2021).

30. Smadja

Storz, Casein kinase activity: element zebrafish circadian timing system. e54189 (2013). 31. Ben-Shlomo, Kyriacou, Circadian rhythm entrainment flies mammals.

Biochem Biophys (2002). 32. Emery, Reppert, rhythmic Neuron (2004). 33. Yang, Direct regulation Roraa: insights circadian metabolic interplay zebrafish.

Cell. (2025).

34. Flores, Hall, Jury, Crosier, Crosier, zebrafish retinoid-related orphan receptor (ror) family.

Patterns (2007). 35. Mracek, Regulation Genes Reveals Central D-Box Enhancer Light-Dependent Expression. e51278 (2012). 36. Wang, Comparative genomic

analysis

teleost genes. Genetica (2009). 37. Balay, Widen, Waskiewicz,

Analysis

zebrafish cryptochrome2 expression photoreceptors.

Patterns (2020). 38. Li, Circadian Vision Zebrafish:

Molecule Neural Network Behavior. Rhythms (2019). 39. Li, CLOCK Required Maintaining Circadian Rhythms Opsin Expression Photoreceptor Cells. (2008). 40. Okano, photoperiodic measurement served biphasic expression Cryptochrome1ab zebrafish (2020). 41. Steindal, Whitmore, Circadian Clocks Learned Biology (2019). 42. Ruggiero, Period Regulator Multiple Tissue-Specific Circadian Functions.

Front Neurosci (2021). 43. Bhoi, Goel, Ribelayga, Mangel, Circadian clock organization retina: clock components pathways visual function.

Retin (2023).

44. McMahon, Iuvone, Tosini, Circadian Organization Mammalian Retina:

Regulation Physiology Diseases. Retin (2014). 45. Nie, Effects circadian clock protein Per1b zebrafish visual functions.

Chronobiology International (2018). 46. Laranjeiro, Whitmore, Transcription factors involved retinogenesis co-opted circadian clock following photoreceptor differentiation.

Development (2014). 47. Du, X.-F. Circadian regulation developmental synaptogenesis hypocretinergic system.

Commun (2023). 48. Zang, Circadian regulation vertebrate photoreceptor function. eLife e68903. 49. Partinen, Nevala, mies, Ihalainen, Nymark, Phagocytosed Photoreceptor Outer Segment Particles Within Retinal Pigment Epithelium Diurnal Rhythmicity Variation Between Subtypes Larval Zebrafish.

FASEB e70853 (2025). 50. Kwon, Freeman, Phagocytosis Retinal Pigment Epithelium:

Recognition, Resolution, Recycling. Front Immunol (2020). 51. Emran, Dowling, Larval zebrafish their photoreceptors

night. Communicative & Integrative Biology 3 , 430 –432 (2010).

52. Chaigne, Contribution opn4xa function circadian photoentrainment diurnal zebrafish.

Genet e1011172 (2024). 53. Northcutt, Wullimann, Visual System Teleost Fishes:

Morphological Patterns Trends. Sensory Biology Aquatic Animals Atema, Popper, Tavolga, (Springer, York, 1988).

54. Organization

physiology zebrafish nervous system. Physiology (Academic Press, 2010). 55. Hang, Kitahashi, Parhar, Brain area-specific diurnal photic regulation

val-opsinA val-opsinB genes zebrafish.

Neurochem (2015). 56. Fernandes, Brain Photoreceptors Control Light-Seeking Behavior Zebrafish Larvae.

Current Biology (2012). 57. Dekens, Fontinha, Gallach, Pfluegler, Tessmar-Raible, Melanopsin elevates locomotor activity during state diurnal zebrafish.

REPORTS (2022). 58. Begemann, Neumann, A.-M. Oster, Regulation function extra-SCN circadian oscillators brain.

Physiol (Oxf) e13446 (2020).

59. Ortiz-Plac

Salido, Melatonin Interplay Physiology Disease-The Fountain Eternal Youth Revisited.

Biomolecules (2025). 60. Cahill, Circadian regulation melatonin production cultured zebrafish pineal retina.

Brain Research (1996). 61. Kazimi, Cahill, Development circadian melatonin rhythm embryonic zebrafish.

Developmental Brain Research (1999). 62. Noche, P.-N., Goldstein-Kral, Glasgow, Liang, Circadian rhythms pineal organ persist zebrafish larvae ventral brain.

NEUROSCIENCE (2011). 63. Appelbaum, Zebrafish arylalkylamine-N-acetyltransferase genes targets regulation circadian clock.

Endocrinol (2006).

64. Wexler, Zebrafish Dark-Dependent Behavior Requires Phototransduction Pineal Gland.

Pineal e70021 (2024). 65. Pando, Pinchak, Cermakian, Sassone-Corsi, cell-based system recapitulates dynamic light-dependent regulation vertebrate clock.

Proceedings National Academy Sciences (2001). 66. Whitmore, Foulkes, Sassone-Corsi, Zebrafish Clock rhythmic expression reveals independent peripheral circadian oscillators.

Neurosci (1998). 67. Kaneko, Hernandez-Borsetti, Cahill, Diversity zebrafish peripheral oscillators revealed luciferase reporting.

Proceedings National Academy Sciences (2006). 68. Idda, Circadian timing injury-induced proliferation zebrafish. e34203 (2012). 69. Cermakian, Sassone-Corsi, Environmental stimulus perception control circadian clocks.

Current Opinion Neurobiology (2002). 70. Davies, extended family novel vertebrate photopigments widely expressed displays diversity function.

Genome (2015). 71. Moutsaki, Teleost multiple tissue (tmt) opsin: candidate photopigment regulating peripheral clocks zebrafish?

Molecular Brain Research (2003). 72. Michael, Fribourgh, Gelder, Partch, Animal Cryptochromes:

Divergent Roles Light Perception, Circadian Timekeeping Beyond.

Photochem Photobiol (2017).

analysis

zebrafish photolyase/cryptochrome family: types cryptochromes present zebrafish.

Genes Cells (2000). 74. Hirayama, Sassone-Corsi, Circadian control reduction/oxidation pathway:

Catalase represses light-dependent clock expression zebrafish. (2007). 75. Hockberger, Activation flavin-containing oxidases underlies light-induced production mammalian cells. (1999). 76. Pagano, Evolution shapes responsiveness D-box enhancer element light reactive oxygen species vertebrates.

Scientific Reports (2018). 77. Hu, Dysfunction Prkcaa Links Social Behavior Defects Disturbed Circadian Rhythm Zebrafish.

INTERNATIONAL JOURNAL MOLECULAR SCIENCES (2023). 78. Fong, Daily rhythms heartbeat intrinsic zebrafish heart. (2021). 79. Jensen, Opposing effects circadian clock genes bmal1 period2 regulation VEGF-dependent angiogenesis developing zebrafish. (2012). 80. Pelster, Multiplicity hypoxia-inducible transcription factors their connection circadian clock zebrafish.

Physiol Biochem (2015). 81. Sato, Takeda, roles HIF-1 signaling cardiovascular diseases.

Cardiol (2023).

82. Kopp, Bringing obesity light: Rev-erb central player light-induced adipogenesis zebrafish? (Lond) (2016). 83. Xu, Y.-X.

Exposure zebrafish embryos sodium propionate disrupts circadian behavior glucose metabolism-related development.

Ecotoxicol Environ (2022).

84. Effect

environment intestinal flora expression genes related liver metabolism zebrafish (Danio rerio).

Comparative Biochemistry Physiology Toxicology Pharmacology (2021). 85. Piccinetti, Melatonin peripheral circuitries: insights appetite

and metabolism in Danio rerio. Zebrafish 10 , 275 -282 (2013).

86. Ren, Wang, Melatonin regulates rhythmic migration neutrophils zebrafish.

Journal Pineal Research (2015). 87. Narasimamurthy, Circadian clock protein cryptochrome regulates expression proinflammatory cytokines. (2012). 88. Chen, Circadian clock1a coordinates neutrophil recruitment nfe212a/duox-reactive oxygen species pathway zebrafish.

Reports (2023). 89. Ren, D.-L. Circadian genes period1b period2 differentially regulate inflammatory responses zebrafish.

Shellfish Immunol (2018). 90. Du, light-regulated circadian timer optimizes neutrophil bactericidal activity boost daytime immunity.

Immunol eadn3080 (2025).

91. Ren, D.-L., Wang, X.-B. Circadian period1b regulates proinflammatory cytokine expression through signalling zebrafish.

Shellfish Immunol (2018). 92. Liu, circadian clock orchestrates spermatogonial differentiation fertilization regulating retinoic signaling vertebrates.

National Science Review nwae456 (2025). 93. Zheng, Loss-of-function mutations circadian rhythm regulator Per1/Per2 premature ovarian insufficiency Reprod (2019). 94. Khan, Artificial Light Night (ALAN), alarm ovarian physiology: study possible chronodisruption zebrafish (Danio rerio).

Science Total Environment (2018). 95. Zheng, Loss-of-function mutations circadian rhythm regulator Per1/Per2 premature ovarian insufficiency.

Reprod (2019). 96. Delaunay, Thisse, Marchand, Laudet, Thisse, Inherited Functional Circadian Clock Zebrafish Embryos.

Science (2000). 97. Guan, Maternal circadian disruption before pregnancy impairs ovarian function female offspring mice.

Science Total Environment (2023). 98. Stokes, Circadian Clock BMAL1 Coordinates Intestinal Regeneration.

Gastroenterol Hepatol (2017). 99. Lee, Effect Probiotics Stress-Associated Constipation Model

Zebrafish (Danio rerio) Larvae. Int J Mol Sci 25 , 3669 (2024).

100. Graves, Chronic early stress alters neuroimmune profile functioning developing zebrafish Brain Behav Immun Health (2023).

101. Guo, Intestinal clock shapes sleep-wake cycle sustaining glutamine homeostasis.

Metabolism 2423-2437.e6 (2025). 102. Chen, Intestinal Clock Promotes Cognitive Memory Through Adenosine Signaling.

Advanced Science e04526 (2025). 103. Tan, microbiota-derived extracellular vesicles exhibit diurnal regulation activate hepatic gluconeogenesis.

Metab (2025). 104. Liu, Human CLOCK enhances neocortical function.

Neurosci. 1716-+ (2025). 105. He, Bmal1 Regulates Vascular Calcification Noncanonical Circadian Pathway.

Arteriosclerosis, Thrombosis, Vascular Biology (2026) 106. Li, ISX-9 potentiates CaMKII -mediated BMAL1 activation enhance circadian amplitude.

Commun (2022). 107. He, small molecule Nobiletin targets molecular oscillator enhance circadian rhythms protect against metabolic syndrome.

Metab (2016).

Note: Figure translations are in progress. See original paper for figures.