

Medusozoa: Combined Research Evidence Packet

Supporting material for the working manuscript · 18 September 2026

This packet joins the supplied literature synthesis to the completed Aurelia time-course analysis. It records the supported findings, their limits, the source crosswalk, and how to reproduce the calculations. It is not an additional biological study or an independent replication.

1. Central question and current answer

Question: Can a brief, naturally realistic mechanical or electrical input produce a measurable internal change after the input stops, and does that change affect wound closure, tissue reorganization, or genuine regrowth?

Current answer: Published experiments establish several components: mechanical regulation of organizer activity, electrical control and withdrawal-associated regrowth, distinct requirements for closure versus organ outgrowth, and wound-associated molecular contributions to regeneration. The new calculations add a timed, early repair-cell profile in Aurelia. The complete sequence for a separate natural cue remains unestablished in the located evidence. [1] [2] [3] [17] [31]

This is not a measured absence of such a mechanism. It identifies the comparisons and variables that are still missing.

2. What is newly added to the earlier synthesis

The earlier synthesis proposed a future analysis of the Aurelia deposit. That analysis is now complete and included in the new manuscript; the original synthesis is preserved as a dated input.

WIC denotes the authors' wound-induced-cell cluster.

Measurement	Newly quantified result	Boundary
Captured WIC share	5.246% at day 0; 38.341% at day 0.5; 36.136% at day 1; 0.663% at day 2	Relative captured composition, not absolute tissue abundance
Captured WIC count	Maximum 10,767 at day 1, versus 8,082 at day 0.5	Total cell capture differs between stages
Within-WIC 20-gene mean	Sampled maximum at day 0.5	Descriptive signature, not validated pathway activity
Plg-annotated gene-Aco20833	Within-WIC mean maximum at day 0; all-cell mean maximum at day 0.5	Gene annotation remains a candidate
Late WIC expression	21, 41, 23, and 4 cells at days 4, 5, 6, and 7	Sparse cells are not independent animal replicates

The paper describes proportional enrichment during blastema formation, while the deposited atlas's numerical fractional maximum is at 0.5 dpa and remains high at day 1. The compilation retains exact sampled times and flags that phase wording rather than silently assigning different stage labels. [3] [31]

3. How to read the evidence categories

- **Published experimental result:** an observation or intervention in the cited primary study; its organism, exposure, and endpoint still matter.
- **Preprint experimental result:** a publicly reported experiment that was not peer reviewed as of the stated source check.
- **Descriptive secondary analysis:** a calculation from the same authors' public data, not

- independent replication or a new animal experiment.
- **Component or external comparison:** a relevant physical or biological result that does not establish the complete mechanism in jellyfish.
- **Computational inference:** an inferred trajectory, communication network, or model prediction rather than direct tracking of the claimed process.
- **Unresolved connection:** the required comparison is absent from the located evidence; this is not an experimentally refuted or instrument-limited result.

The evidence ledger below is the source of the companion CSV. A claim classified as a published result should not be widened beyond its supported inference.

4. Claim-by-claim evidence ledger

M01: Mechanical stretch can affect organizer activity

- **Category:** Published experimental result
- **Organism context:** Freshwater Hydra tissue spheroids
- **Supported inference:** Suppressing osmotic inflation impairs normal Wnt3/organizer outcomes; sustained Wnt3 or organizer tissue can bypass the usual mechanical requirement.
- **Not established:** Persistence after a completed alternative pulse; transfer to normal medium restores continuing exposure.
- **Sources:** [1]

M02: Alternative deformations and muscle forces affect mechanical behavior

- **Category:** Published experimental result
- **Organism context:** Hydra tissue mechanics and Aurelia body shape
- **Supported inference:** Microaspiration measures deformation; repeated strain events and contraction-dependent shape changes extend mechanical evidence.
- **Not established:** A single finite external cue producing post-off regenerative effects; modeled strain-to-morphogen coupling is not direct measurement.
- **Sources:** [9] [10] [11]

C01: Intracellular and extracellular mechanics require different measurements

- **Category:** Component or external comparison
- **Organism context:** Mammalian cultured cells and jellyfish mesoglea
- **Supported inference:** Cytoplasmic poroelastic relaxation and osmotic-volume effects are intracellular component evidence; mesogleal rheology addresses extracellular material.
- **Not established:** Intracellular hydration as a demonstrated lasting cause of jellyfish repair.
- **Sources:** [4] [8]

C02: Membrane resealing and epithelial closure involve different signaling contexts

- **Category:** Component or external comparison
- **Organism context:** Mammalian cell membranes and Clytia epithelium
- **Supported inference:** Calcium/ATP-related membrane repair and extracellular ATP effects on epithelial closure are measured in their respective systems.
- **Not established:** An intracellular calcium or hydration response measured in the Clytia ATP experiment; a verified ATP pulse-and-washout memory test.
- **Sources:** [5] [6]

E01: Electrical conditions can reversibly redirect morphogenesis

- **Category:** Published experimental result
- **Organism context:** Freshwater Hydra spheroids
- **Supported inference:** Prolonged field exposure alters calcium activity and organizer/morphological state; withdrawal permits regrowth.
- **Not established:** Benefit of a brief naturalistic cue; calcium activity alone as a

- beneficial-state indicator; initial wound-sealing effect.
- **Sources:** [2]

E02: Electrode pacing controls movement during stimulation

- **Category:** Published experimental result
- **Organism context:** Marine Aurelia medusae
- **Supported inference:** Contractions and swimming/body dynamics respond under imposed stimulation.
- **Not established:** Post-off repair; natural ambient receiver field inferred from electrode voltage.
- **Sources:** [12] [13]

E03: Finite electrical treatment has later colony-level associations

- **Category:** Component or external comparison
- **Organism context:** Botryllus schlosseri colonial chordate
- **Supported inference:** Later normal budding and reported post-treatment RNA differences provide an external finite-treatment comparison.
- **Not established:** Jellyfish wound repair; precise first/final-session RNA time zero; molecular mediation; separation of all tank/sham alternatives.
- **Sources:** [23]

W01: Moving water is permissive for arm regeneration in a tested setup

- **Category:** Published experimental result
- **Organism context:** Aurelia ephyrae
- **Supported inference:** Regenerated outgrowth can contain canals/muscle/neurons and participate in pulsing; treatment responses vary.
- **Not established:** The causal contribution of loading versus feeding/transport/oxygen; an intracellular-water mechanism; brief exposure with withdrawal.
- **Sources:** [14] [15]

R01: Remodeling differs from replacement of a missing structure

- **Category:** Published experimental result
- **Organism context:** Aurelia symmetrization and Clytia medusae
- **Supported inference:** Mechanical reorganization and closure can be distinguished from organ outgrowth; hydroxyurea separates outcomes in Clytia.
- **Not established:** All forms of repair sharing one mechanism; drug effects being exclusively proliferation-specific.
- **Sources:** [16] [17]

R02: Tentacle regrowth can restore an assayed function

- **Category:** Published experimental result
- **Organism context:** Cladonema bulb-retaining tentacles
- **Supported inference:** Prey capture recovered to 55% at 48 h and 100% at 72 h in the reported n=36 time-point assays.
- **Not established:** Every sensory/neural function restored; exact origin of all repair-specific cells.
- **Sources:** [18]

R03: Sealing and tip regrowth differ by tentacle context

- **Category:** Preprint experimental result
- **Organism context:** Haliclystus preprint
- **Supported inference:** Both tested tentacle contexts seal while only secondary tips regrow within the observation window.
- **Not established:** Permanent or class-wide incapacity; proliferation alone causing the difference; peer-reviewed confirmation.
- **Sources:** [7]

R04: Rhopalial regrowth includes reported neural functions

- **Category:** Published experimental result
- **Organism context:** Tripedalia regeneration abstract
- **Supported inference:** The accessible abstract reports neural regrowth/pacemaking and lens-eye light responses.
- **Not established:** A learned state directing repair; unreviewed full-text details inferred from the abstract.
- **Sources:** [19]

R05: Life-cycle reversal is a separate biological endpoint

- **Category:** Published experimental result
- **Organism context:** Historical Turritopsis nutricula abstract
- **Supported inference:** Medusa-to-hyroid reversal is reported with the original published species name retained.
- **Not established:** Intracellular-water mechanism or indefinite survival of every animal.
- **Sources:** [30]

A01: WIC-associated gene functions contribute to blastema formation

- **Category:** Published experimental result
- **Organism context:** Aurelia rhopalium regeneration
- **Supported inference:** Authors report Sox2 and Otx5b knockdown outcomes 0/16 each; Wntless 4/16 versus 15/16 controls.
- **Not established:** Selective necessity of the entire WIC population; a separate environmental cue; gene-ID equivalence of every annotation candidate.
- **Sources:** [3]

A02: Early captured WIC proportions and counts have distinguishable maxima

- **Category:** Descriptive secondary analysis
- **Organism context:** Nine pooled Aurelia single-cell stages
- **Supported inference:** Maximum captured share is 38.341% at day0.5; maximum captured WIC count is 10767 at day1; share is 0.663% at day2.
- **Not established:** Absolute tissue abundance; independent animal replication; silently equating exact sampled maxima to broad phase wording.
- **Sources:** [3] [31] [33]

A03: Within-WIC RNA and all-cell RNA can have different timing

- **Category:** Descriptive secondary analysis
- **Organism context:** Aurelia raw-count RNA summaries
- **Supported inference:** The preselected 20-gene signature peaks at day0.5; Plg-annotated Aco20833 peaks within WICs at day0 and across all cells at day0.5.
- **Not established:** Validated pathway activity; certified symbol crosswalk; retained expression in the same cell.
- **Sources:** [31] [33]

A04: Late WIC estimates are sparse

- **Category:** Descriptive secondary analysis
- **Organism context:** Aurelia days4-7
- **Supported inference:** Captured WIC counts are 21/41/23/4; day7 Wntless-candidate detection 75% is 3 of 4 cells.
- **Not established:** Reliable late population activation or animal-level uncertainty calculated from those cells.
- **Sources:** [31]

I01: Cell trajectories and communication networks are inferred

- **Category:** Computational inference
- **Organism context:** Aurelia atlas and related cell-state analyses
- **Supported inference:** Expression-based ordering and changing cell identities inform candidate regenerative processes.
- **Not established:** Direct lineage tracing or repeated measurement of one cell; pseudotime as elapsed days.
- **Sources:** [3] [21]

I02: Mechanochemical and neural models generate hypotheses

- **Category:** Computational inference
- **Organism context:** Hydra and Aurelia modeling contexts
- **Supported inference:** Models complement measured activity and tissue dynamics; access limits of the Hydra polarity record are retained.
- **Not established:** Computational aftereffects being direct cellular-memory observations or repair outcomes.
- **Sources:** [22] [28] [29]

L01: Associative learning is not a measured learning-to-repair link

- **Category:** Published experimental result
- **Organism context:** Tripedalia behavior and isolated rhopalia
- **Supported inference:** Reported training changes behavior/neural responsiveness with a post-training test.
- **Not established:** Hours/days of specified stimulus-free retention; retained learning controlling rhopalian regeneration.
- **Sources:** [20] [19]

N01: Sound exposure can have later tissue-injury effects

- **Category:** Published experimental result
- **Organism context:** Two scyphozoan species under prolonged playback
- **Supported inference:** Later statocyst lesions establish a post-exposure tissue effect under tested high-level conditions.
- **Not established:** Brief noninjurious natural cue; beneficial repair; calibrated particle motion or heartbeat detection threshold.
- **Sources:** [24]

N02: Internal circuits and bioelectric source measurements do not calibrate a jellyfish heartbeat receiver

- **Category:** Component or external comparison
- **Organism context:** Aglantha circuits and marine/immersed electrical recordings
- **Supported inference:** The sources establish internal signaling or source/circuit measurements in their stated contexts.
- **Not established:** A defensible heartbeat-to-jellyfish distance or receiver dose; a subsequent repair effect.
- **Sources:** [25] [26] [27]

Q01: The complete separate-cue-to-retained-state-to-repair sequence remains open

- **Category:** Unresolved connection
- **Organism context:** Combined scoped evidence
- **Supported inference:** Each component can be defined and some are separately measured; post-injury regeneration is directly supported.
- **Not established:** All links demonstrated in one causal comparison; intracellular hydration as mediator; absence of literature as refutation.
- **Sources:** [1] [2] [3] [20] [23] [31]

5. Keep compartments and endpoints distinct

Observation	What it measures	What it does not establish
Medium osmolarity and Hydra cavity inflation	External conditions, lumen dynamics, tissue deformation [1]	Intracellular water organization
Mammalian cytoplasmic force relaxation	Intracellular mechanics under the tested conditions [4]	Jellyfish post-cue regenerative persistence
Calcium-sensitive fluorescence	Activity of the probe in the measured cells [2]	Hydration or a general measure of beneficial regeneration
Mesogleal rheology	Extracellular material mechanics [8]	Intracellular transport or a repair endpoint
RNA counts and captured cell labels	Transcript detection, normalized expression, and relative capture composition [31]	Absolute tissue abundance, protein activity, or same-cell persistence

Membrane resealing, tissue closure, remodeling/symmetrization, organ replacement, and life-cycle reversal remain separate outcome categories. Structural replacement plus the function actually assayed is stronger evidence than size alone, but no single assay demonstrates every aspect of recovery. [5] [16] [17] [18] [19] [30]

6. Source and identity audit

The uploaded synthesis contains 13 pages and matches the saved original PDF byte for byte. Its SHA-256 is ad945f3fcf6fa6c9d8575bf3e870e9e976306a13dcdccda5bcf6c1212a737933. The thirty original source numbers are retained; records 31–33 add the processed deposit, raw-archive metadata, and official supplements. Records 3 and 31–33 all concern the same Aurelia study, and record 15 is source data for record 14. They must not be counted as independent confirmations.

The new reference crosswalk preserves saved-evidence paths and access status. The Haliclystus record remains a preprint as checked on 18 September 2026. Tripedalia regeneration, Aglantha circuitry, and historical Turritopsis reversal are abstract-only here; the 2026 Hydra polarity record also includes accessible figure captions. Unseen full-text details are not assumed. [7] [19] [22] [25] [30]

For Aurelia, checksums verify the deposited files and official scientific supplement payloads. Cell totals match Table S2; raw RNA totals and detected-gene counts match every cell's metadata; 154/154 marker-detection fractions support correspondence of the chosen 16-cluster solution with Dataset S1. These checks establish computational consistency, not animal-level reproducibility. [31] [33]

7. Reproducibility index

All paths below are workspace-relative. External source links are in the references.

Material	Location
Original literature synthesis	exports/medusozoa-inputs-repair-research.md and its PDF
Original foundation packet	exports/medusozoa-regeneration-foundation-packet.md and its PDF
Saved primary sources and original register	research/sources/ and research/sources.json
Aurelia analysis, scripts, requirements, and tests	research/aurelia-timecourse/README.md
Aurelia sample/deposit/cluster audits	research/aurelia-timecourse/provenance/
Stage, gene-expression, and candidate-timing CSVs	research/aurelia-timecourse/results/
New manuscript, ledger, reference crosswalk, and input hashes	research/medusozoa-compiled-paper/

The Aurelia README gives sequential commands to retrieve the pinned public processed data, extract RNA counts, audit labels, calculate summaries, and run tests. It does not require sequencing-read alignment or execution of the authors' code. The compilation renderer reads those saved outputs directly; numerical manuscript tables are generated from the CSVs, not manually reconstructed from figures.

8. Specific open questions

The next information needed is narrow: an authoritative marker-to-gene-ID crosswalk, clarified phase wording, any independently measured single-cell pool/animal identities, the relationship of the archived day-14 run to the nine-stage atlas, and the location of the bulk expression matrix. A bounded sequencing-coverage sensitivity analysis could test the robustness of the early RNA pattern, but has not been performed.

The broader causal gap is separate: receiver-level natural exposure, verified cue cessation, a measured post-off internal variable, and its contribution to a clearly defined repair outcome in one comparison. No direct hydration or heartbeat variable is present in the Aurelia data.

This packet contains no proposed dosing or laboratory procedure, and initiates no author contact, new experiments, publication, or monitoring. It supports a working manuscript and future decisions about what evidence would materially change its conclusions.

References and source-access notes

1. [Mechanical oscillations orchestrate axial patterning through Wnt activation in Hydra](#) (2021-12-10; open full text; publication status: peer reviewed)
2. [Electric-Induced Reversal of Morphogenesis in Hydra](#) (2019-09-16; open full text; publication status: peer-reviewed; single-laboratory result, no independent replication located)
3. [Deciphering the ancestral mechanisms of neural regeneration through single-cell analyses of jellyfish rhopalium repair](#) (2026-05-20; open full text; publication status: peer-reviewed)
4. [The cytoplasm of living cells behaves as a poroelastic material](#) (2013-01-06; author manuscript open full text; publication status: peer reviewed)
5. [Repair of injured plasma membrane by rapid Ca²⁺-dependent endocytosis](#) (2008-03-10; open full text; publication status: peer reviewed)
6. [Epithelial wound healing in Clytia hemisphaerica provides insights into extracellular ATP signaling mechanisms and P2XR evolution](#) (2023-11-01; open full text; publication status: peer-reviewed)
7. [Regression of juvenile tentacles is driven by loss of cell proliferation in Haliclystus sanjuanensis, a cnidarian with limited metamorphosis](#) (2026-04-01; open full text; publication status: bioRxiv Version 1; not peer reviewed as of 2026-09-18)
8. [Micro- and Macrorheology of Jellyfish Extracellular Matrix](#) (2012-01-04; open full text; publication status: peer-reviewed)
9. [Mechanical characterization of regenerating Hydra tissue spheres](#) (2024-05-23; open full text; publication status: peer reviewed)
10. [Mechanical strain focusing at topological defect sites in regenerating Hydra](#) (2025-03-03; open full text; publication status: peer reviewed)
11. [Jellyfish shape as a mechanical balance](#) (2025-03-17; open full text; publication status: peer-reviewed; associated correction concerns acknowledgments)
12. [Low-power microelectronics embedded in live jellyfish enhance propulsion](#) (2020-01-29; open full text; publication status: peer-reviewed; later locomotion follow-ups exist, repair consequence not tested)
13. [Harnessing natural embodied intelligence for spontaneous jellyfish cyborgs](#) (2025-05-23; open full text; publication status: peer-reviewed 2025 follow-up; no repair endpoints)
14. [A conserved strategy for inducing appendage regeneration in moon jellyfish, Drosophila, and mice](#) (2021-12-07; open full text; publication status: peer reviewed)
15. [Figure 2 source data: Aurelia factor screen](#) (2021-12-07; open spreadsheet; publication status: published with peer-reviewed article)

16. [Self-repairing symmetry in jellyfish through mechanically driven reorganization](#) (2015-06-15; open full text; publication status: peer-reviewed)
17. [Pattern regulation in a regenerating jellyfish](#) (2020-09-07; open full text and source data; publication status: peer reviewed)
18. [Distinct stem-like cell populations facilitate functional regeneration of the Cladonema medusa tentacle](#) (2023-12-21; open full text and data; publication status: peer reviewed)
19. [Regeneration of the Rhopalium and the Rhopalial Nervous System in the Box Jellyfish Tripedalia cystophora](#) (2018-02-01; abstract via EPA HERO bibliographic record; publisher full text inaccessible; publication status: peer-reviewed)
20. [Associative learning in the box jellyfish Tripedalia cystophora](#) (2023-09-22; full text; publication status: peer-reviewed)
21. [Dynamic cell fate transitions during morphallactic regeneration](#) (2026-06-02; open full text; publication status: peer-reviewed)
22. [The dual nature of body-axis formation in Hydra regeneration: Polarity-morphology concurrency](#) (2026; primary abstract and figure captions; full article not reviewed; publication status: published journal research; experiment/model distinction retained)
23. [Electrical stimulation promotes longevity and regeneration in a colonial chordate](#) (2026-05-26; open full text; publication status: peer-reviewed; single-study comparison)
24. [Evidence of Cnidarians sensitivity to sound after exposure to low frequency noise underwater sources](#) (2016-12-21; open full text; publication status: single study in two scyphozoan species; no independent replication located in searches through 2026-09-18)
25. [Central Circuitry in the Jellyfish Aglantha digitale: I. The Relay System](#) (1995-11-01; abstract accessible; full text paywalled; publication status: older foundational circuit study; accessible page is abstract-only)
26. [Bioelectric Fields of Marine Organisms: Voltage and Frequency Contributions to Detectability by Electroreceptive Predators](#) (2013; open author-hosted PDF; publication status: component/source evidence only; receiver tests concern elasmobranch literature, not jellyfish)
27. [A novel method for noninvasive bioelectric measurement utilizing conductivity of seawater](#) (2021-03-29; open full text; publication status: component/source measurement only; not jellyfish evidence)
28. [Neuromechanical wave resonance in jellyfish swimming](#) (2021-03-08; open full text; publication status: peer-reviewed; experiment plus computational model)
29. [From single neurons to behavior in the jellyfish Aurelia aurita](#) (2019-12-23; open full text; publication status: model predictions must not be treated as direct stimulus-response measurements)
30. [Reversing the Life Cycle: Medusae Transforming into Polyps and Cell Transdifferentiation in Turritopsis nutricula \(Cnidaria, Hydrozoa\)](#) (1996; primary abstract only; publisher full text inaccessible in this pass; publication status: peer-reviewed; historical species name retained)
31. [Authors' processed-data/code Zenodo record 19466460 v3](#) (2026-04-08; open; publication status: published Zenodo record; version 3)
32. [PRJNA1224934 raw-archive metadata](#) (2025-02-17; public metadata; raw archive record; publication status: public archive metadata record)
33. [Official Europe PMC supplementary bundle for PMC13189125](#) (2026-05-20; official Europe PMC supplementary bundle; publication status: official supplementary bundle)