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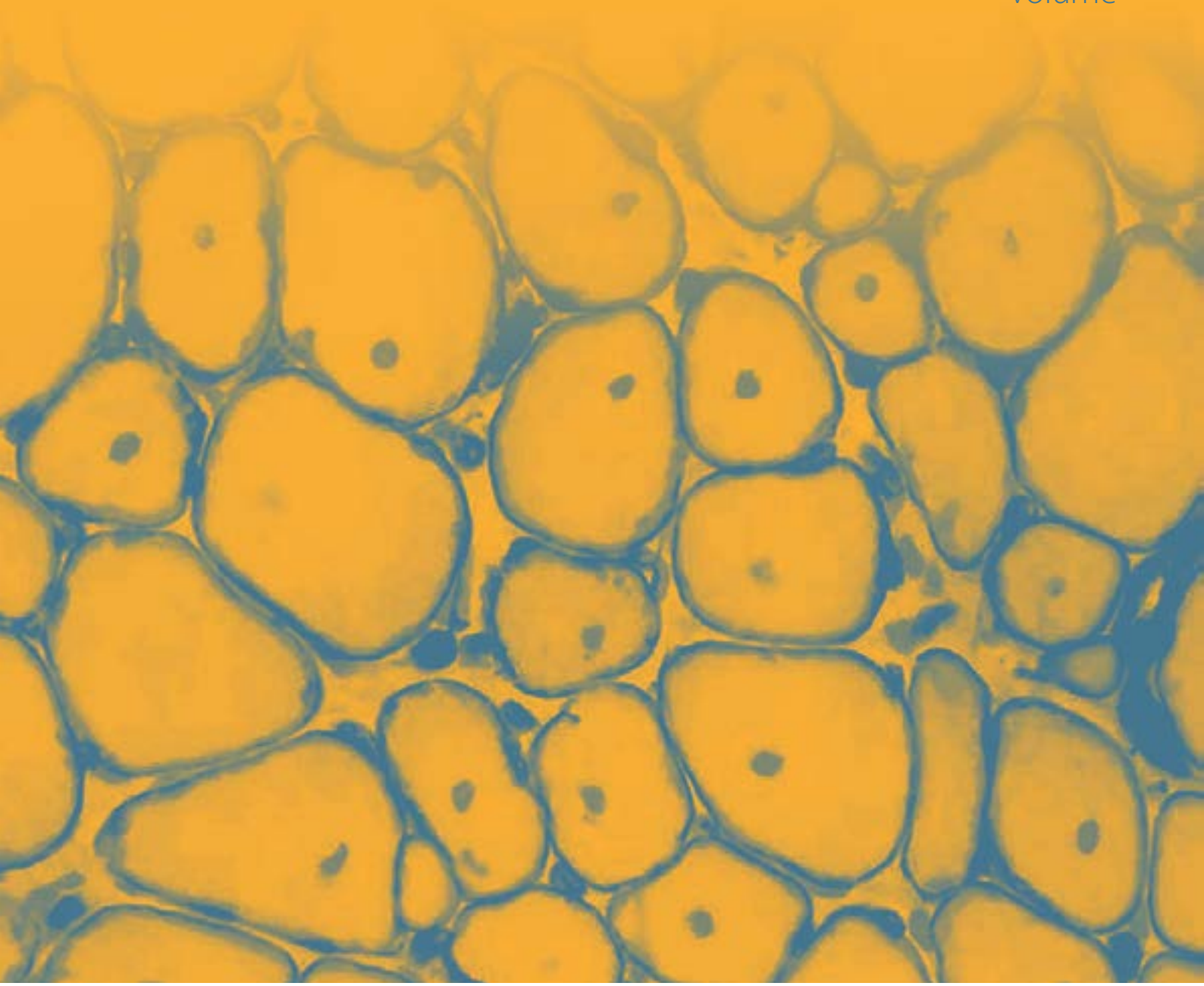

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Physiological Mini-Reviews is a scientific journal, publishing brief reviews on "hot" topics in Physiology. The scope is quite broad, going from "Molecular Physiology" to "Integrated Physiological Systems". As indicated by our title it is not our intention to publish exhaustive and complete reviews. We ask to the authors concise and updated descriptions of the "state of the art" in a specific topic. Innovative and thought-provoking ideas are welcome.

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"Physiological Mini-Reviews" will have a maximum of 3000 words, 50 references and 3 figures. Material will be addressed to scientific people in general but not restricted to specialist of the field. For citations in the text please refer to Instructions in our webpage. Final format will be given at the Editorial Office. Most contributions will be invited ones, but spontaneous presentations are welcome. Send your manuscript in Word format (.doc or .docx) to: pmr@safisiol.org.ar

Animal Models in Research

The present number presents three different animal models, revealing their principal characteristics and their importance as research tools.

These Mini Reviews were part of a satellite symposium organized by the Young Investigator Committee of the Argentinean Society of Physiology.

HYDRA AS AN ANIMAL MODEL SYSTEM IN PHYSIOLOGY

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ABSTRACT
Hydra has been used as a model in different topics in biology, including physiology. It pertains to the Phylum Cnidaria, an ancestral group of Metazoa that shares a common ancestor with Bilateria. Hydra provides an experimental framework to analyze mechanisms that regulate the homeostasis and, due to the high level of gene conservation, can be easily extrapolated to other animal groups. We use this model to analyze physiological and evolutionary aspects of communication systems activated by food, and by the peptide messengers, Allatotropin (AT) and Allatostatin-C (AST-C). Using immunohistochemistry, quantum dots, bioinformatic, and physiological assays, we show that these systems are present in Hydra regulating feeding behavior. We analyze the regulation of the extrusion of the hypostome (that contains the mouth), showing that, whereas AT mimics the effect of the food, AST-C acts as antagonist. We show that those effects depend on changes in the cytosolic Ca^{2+} concentration, revealing the specific signaling pathway activated. Our data support the ancestral functions and conservation of these signaling systems that control myoregulatory activities related with feeding.
Keywords: Hydra; myoregulation; model system; physiology.

RESUMEN
Hydra ha sido usada como modelo para el estudio de diferentes aspectos de la biología, incluyendo fisiología. Pertenece al phylum Cnidaria, grupo ancestral de metazoos que comparte un ancestro común con Bilateria. El modelo de Hydra representa un sistema de estudio útil para analizar el mantenimiento de la homeostasis que, debido al alto grado de conservación respecto de los metazoos, puede ser extrapolado a otros grupos animales. Analizamos en Hydra tanto aspectos fisiológicos, como evolutivos de sistemas de comunicación activados por el alimento y los péptidos allatotropina(AT) y allatostatina C (AST-C). Mediante análisis de datos inmunohistoquímicos, *quantum dots*, fisiológicos y de bioinformática demostramos que estos sistemas de comunicación están presentes en Hydra regulando el comportamiento alimentario. analizando la regulación de la extrusión del hipostoma (estructura que contiene la boca) demostramos que AT mimetiza el efecto del alimento, estimulando su extrusión, mientras que AST-C se comporta como antagosnista. Mostramos que estos efectos dependen de cambios en la concentración de Ca^{+2} citosólico, revelando sus vías de señalización especifica. Nuestros datos muestran las funciones y conservación de estos sistemas de comunicación, desde organismos ancestrales como Cnidarios.
Palabras clave: Hydra; mioregulación; sistema modelo; fisiología.

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Introduction

Hydra is a fresh-water member of the phylum Cnidaria, a basal group of metazoans. This phylum evolved 700 million years ago, and represents the first group of animals with a defined body axis and a nervous system [1]. Together with Ctenophora and Placozoa, it shares a common ancestor with Bilateria (http://tolweb.org/Animals/2374) (Figure 1D).

The anatomy of Hydra is simple (Figures 1A and B). It has a tubular body, with differentiated structures along their axis performing specific functions. The oral region is at the apical pole, and is responsible for the active feeding behavior. This region consists in a ring of tentacles (that participates in the prey capture) surrounding the hypostome, that contains the mouth. At the opposite pole (aboral) the body presents the basal disc, responsible for the adhesion to the substrate. The digestive system is incomplete, constituted by a gastrovascular cavity (GVC), in which the digestion takes place. This cavity opens at the oral pole in the mouth/anus area. The nervous system consists of a net of neurons (sensory-motor, ganglia, and mechanosensory) along the body column. This net shows a higher density at both extremities, and forms a nerve ring just around the tentacles. It provides robust neuro-muscular activities with sophisticated behaviors. The reproduction is mainly asexual. Indeed, when the food is abundant the body generates buds, that after an appropriated growth separate from their parents. In some conditions, the individuals can develop gonads to reproduce sexually.

The body wall consists of two myoepithelial layers separated by an extracellular matrix, called mesoglea (Figure 1C). The outer layer (ectoderm) consists of multifunctional myoepithelial cells. In the inner layer (endoderm) the myoepithelial cells also act as digestive cells, phagocytosing nutrients together with a population of gland cells that secrete proteases [2].

Myoepithelial cells may represent the most ancestral type of contractile cells, and exhibit the eumetazoan actin-myosin machinery [3]. They present contractile projections that are highly organized, running axially along the body in the ectoderm, and circumferentially in the endodermic layer. In this sense, contraction of ectodermal myoepithelial cells results in a decrease of the length of the body column, whereas the contraction of the cells in the endoderm produces the lengthening of these structures [4, 5]. These contractile cells are innervated, with synaptic structures that that resembles the neuromuscular junctions of the bilaterians [3].

Beyond myoepithelial and secretory cell populations, both layers also contain nerve cells, interstitial cells, and when appropriate, gametes. Moreover, Hydra sp. as a member of the phylum Cnidaria, present another particular and characteristic cell population, the nematocytes, which are a kind of mechano-sensory cells related with prey capture that constitute a synapomorphy of this phylum.

Finally, these cell lineages (i.e. myoepithelial and interstitial cells), behave as stem cells for a dozen of cell types [6].

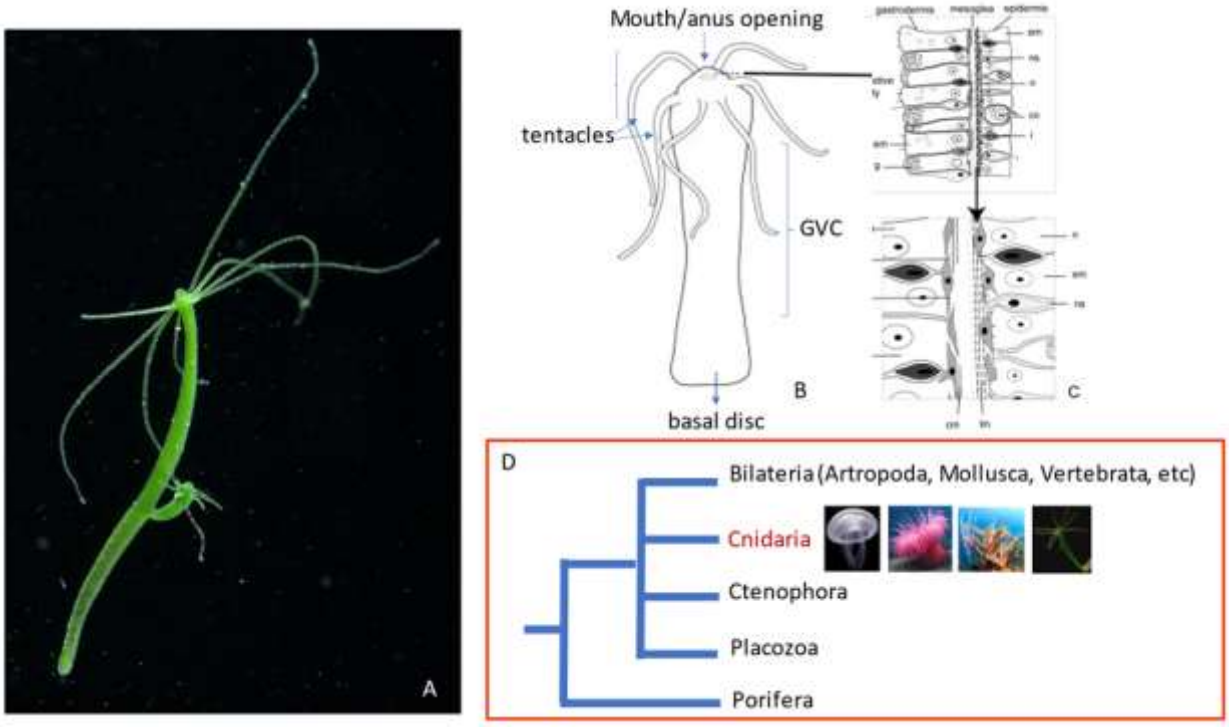

Figure 1. Anatomy of Hydra. A. General aspect of Hydra sp. B. Schematic representation of Hydra sp with the main regions of the body. GVC: gastrovascular cavity C. Cross sections of the hydroid body showing the two body layers (ectoderm or epidermis and endoderm or gastrodermis) and the mesoglea between them. The scheme also shows the different cells that form the body walls. cn: cnidocyte; eg: epithelial gland cell; ep: epithelial-muscular cell; g: mucous and enzymatic gland cell; i: interstitial cell; n: nerve cell and ns: neurosensory cell; cm: circular muscular layer formed by the contractile extensions of the nutritive muscle cells; lm: longitudinal muscle layer formed by the contractile extensions of the epithelial-muscular cells. (Modified of Alzugaray et al., 2013) D. Phylogenetical position of the Phylum Cnidaria showing its relationships with other groups of metazoans.

Hydra as an animal model system

Due to its great capacity of asexual reproduction and regeneration, Hydra colonies are easy to maintain in the laboratory. For many years different species were used as an animal model system to study different aspects of biology, including physiology, ecosystems, ecotoxicology, and developmental biology [reviewed in 7]. Regarding this last topic, Hydra is probably the most well-known cnidarian because of its low senescence rate, and its regenerative capabilities, being practically immortal. Indeed, Hydra polyps not only can regenerate any lost part of its body, but also, can reaggregate into an intact animal within a few days after being dissociated into single cells [8].

Hydra in physiological studies

Regarding physiology, since the late 19th century, Hydra became a model system because it provides an experimental framework to analyze mechanisms that regulate the maintenance of homeostasis that can be easily extrapolated to other animal groups. In this way, one of the most studied aspect has been its active feeding behavior, by which the prey is captured and ingested in a complex and coordinated fashion, when signals from their preys initiate this response.

Analyzing the feeding behavior in Hydra: effects of AT and AST-C peptides

Our laboratory has studied communication systems that involve the peptidic messengers Allatotropin (AT) and Allatostatin-C (AST-C). These signaling molecules were first described in insects by their function as regulators of the synthesis and secretion of juvenile hormones (JH) [9, 10]. They exert their functions by binding to membrane receptors of the rhodopsin subfamily of G protein-coupled receptors (GPCRs), that are considered orthologues of the orexin (Ox) and somatostatin (STT) receptors of Chordata, respectively [11, 12-15].

Both peptides have many other roles, being the regulation of muscle contraction one of the most well documented. Whereas AT has myo and cardiostimulatory effects, AST-C shows an inhibitory effect, acting as an antagonist of the myostimulatory activity of AT [16-18]. Although these peptides and their receptors were mainly studied in insects, the existence of AT and AST-C-like systems has been proposed in other phyla of invertebrates as Annelida, Mollusca, Platyhelminthes, and Cnidaria [19-21,13].

In our laboratory, we use the Hydra model to study not only the physiological aspects of this organism, but also to analyze communication systems based in AT and AST-C, in terms of the evolution in Metazoa. Using AT and AST-C peptides conjugated with nano-crystals (quantum dots), we showed that these peptides are recognized by different myoepithelial cell populations in Hydra sp., suggesting that Hydra cells can respond to these signaling molecules due to the existence of specific receptors for both peptides [13, 21].

Physiological assays showed that both, AT and AST-C act as myoregulators in Hydra, inducing different behaviors. The treatment with AT causes the extrusion of the hypostome, mimicking the effect of food during feeding behavior. Moreover, the treatment with an AT antiserum avoids hypostome extrusion induced by the presence of food [14, 21]. Regarding AST-C treatment, it induces a significant change in the length and shape of tentacles, mimicking those adopted during the capture of the prey. AST-C also causes a shortening of the body column, mainly by the shortening of the peduncle. Finally, the gastrovascular cavity (GVC) changes to a bottle like shape, resembling the appearance adopted after ingestion of the prey. When the maximum dose of this peptide was used, it triggered contractions which mimicked peristaltic waves [13]. Interestingly, this peptide did not show any relevant effect on the hypostome of starved hydroids, but prevented its extrusion when was applied on hydroids stimulated with food or AT [14]. Altogether these results suggest that AT and AST-C could participates in the regulation of the feeding behavior in Hydra.

Results obtained by the use of bioinformatic tools support those obtained by physiological assays. In spite of searches for AT and AST-C peptides in cnidarians genomes did not show any relevant sequences to be considered as orthologue of these neuropeptides, in silico searches for the receptors do show that Cnidaria genomes predict the existence of GPCRs that would be orthologues of the systems that are present in bilaterians. Regarding AST-C, we found proteins that show a high level of identity with the family of AST-C/Somatostatin receptors. Interestingly, the putative proteins from cnidarians show a high level of identity and similitude in the sequences considered as a signature of this family [13]. Proteins sharing homology with AT/Ox receptor also were predicted by cnidarian genomes [21,14,15]. Indeed, they have the E/DRWYAI motif, which is considered as a signature of this family of receptors [15].

Study of signaling pathways in Hydra: understanding the feeding behavior

Most of the studies performed in Hydra analyze pathways involved in the control of development and aging. However, basic physiological mechanisms such as muscle contractions, remains poorly understood.

An increase in the cytosolic calcium levels ([Ca2+]cyt) is needed to induce muscle contraction in any type of muscle cells. The pioneering studies of feeding behavior developed in Hydra sp., shows that EDTA (a Ca2+ chelator that acts in the extracellular medium) inhibits feeding, and also that the addition of Ca2+ reverses this effect [22, 23]. On the other hand, studies performed in muscles of jellyfish (Cnidaria), show that an increase in [Ca2+]cyt is due to both, the release of Ca2+ from intracellular stores, and its influx from the extracellular space [24, 25]. To understand the involvement of this ion in the extrusion of the hypostome in Hydra, we analyzed its behavior in the presence of different compounds that modify Ca2+ levels, and stimulates or inhibit the cellular Ca2+ machinery.

Hypostome extrusion induced by food and AT peptide depends on an increase of [Ca2+]cyt

Treatments of starved hydroids with thapsigargin (TG), a compound that inhibits the reuptake of Ca2+ into the sarco(endo)plasmic reticulum (ER) inducing an increment of its cytosolic levels, causes an increase in the length of the hypostome, similarly to the presence of both, food and AT. In agreement with these results, treatments with the intracellular Ca2+ chelator BAPTA/AM, prevent the hypostome extrusion induced by food or AT in a dose-dependent fashion. These facts suggest that an increase in the [Ca2+]cyt is required for hypostome activity, and that one of the ways involved is the release of Ca2+ from de ER [14, 26, 27]. Similar results were obtained in physiological assays using EDTA (a Ca2+ chelator) or nifedipine, a blocker of voltage-gated Ca2+ channels (VGCC), which prevent the influx from the extracellular media. In both assays the treatment avoided the hypostome extrusion. Altogether these results suggest that in Hydra, both mechanisms that increase [Ca2+] cyt (i.e. release ER, and extracellular influx throughout an L-Type VGCC) are relevant for hypostome extrusion [14]. Interestingly, EDTA did not completely prevent the response induced by food, suggesting that the influx of calcium is not the only way involved to cause the extrusion of the hypostome. The release of Ca2+ from the ER (the main intracellular source of Ca2+ in muscle cells) is mediated by two types of channels: Ryanodine receptors (RYR), which are primarily modulated by Ca2+, and inositol-3 phosphate receptors (IP3R) which are activated by different ligands that bind with cell membrane receptors which produce IP3. Using physiological experiments, we also tested the involvement of both ER Ca2+ receptor-channels in Hydra and found that treatments with Xetospongine-C (Xe-C) (an inhibitor of IP3R) prevents the extrusion of the hypostome induced by food or AT, while treatments of starved hydroids with caffeine (an agonist that causes the opening of the RyR) increase the hypostome length, mimicking the effect of the stimulators of the feeding behavior. Moreover, assays using inhibitory doses of ryanodine (Ry) (a vegetal compound that blocks the RyR) prevented hypostome extrusion in the presence of food but has no effect in hydroids treated with AT. Taking together, these results suggest that the two known ways to release Ca2+ from ER participate in the hypostome extrusion induced by food, but the IP3R is the one involved in the mechanism activated by AT [26, 27]. Using bioinformatic tools we found that H. vulgaris genome predicts a protein that fits all of the characteristics of an IP3R. Although experimental results suggest the existence of proteins reacting to Ry and caffeine, RyR orthologues were not found in this Phylum [26]. In agreement with our finding, other authors have also suggested that RyR would not be present in Cnidaria [28]. The existence of RyRs has been predicted in many other groups of invertebrates, as well as in the basal metazoan Trichoplax adhaerens (Placozoa) that lacks neurons and muscles, and it also is present in unicellular eukaryotes such as choanoflagellates, suggesting that RyR evolved early in evolution, but could have been lost in certain metazoan groups such as Cnidaria [28]. Regarding our results, it is possible that other kind of channel proteins could be acting as the Ry target in Cnidaria [26].

Hydra as a model to study GPCR and their signaling cascades

As we mentioned previously, AT acts by binding to a receptor of the GPCR superfamily. In spite that the receptor has been widely studied in insects, the signaling cascade is not completely understood. We use the Hydra model, and the extrusion of the hypostome, to analyzed the complete signaling pathway activated by AT peptide in vivo [27].

Taking into account that our previous results suggest the involvement of the IP3R in the mechanism activated by AT in Hydra, and due that IP3Rs open when they have bound the second messenger IP3, we analyzed this signaling cascade in Hydra. IP3 is produced by the cleavage of a membrane phospholipid, the PI2P by the enzyme phospholipase C (PLC). PLCs can be grouped into six subfamilies (i.e. β, γ, δ, ε, ζ, η), which are activated by different mechanisms, including GPCRs. In the lack of a subtype-selective PLC inhibitor, we used U73122, a compound that inhibits all PLC types. The treatment with this compound prevents the effect of AT peptide on hypostome extrusion. Altogether, the inhibition of PLC and IP3R, confirm the involvement of the IP3 pathway in the transduction of AT signaling.

In order to confirm the involvement of a GPCR in this signaling pathway, we treated hydroids with an inhibitor of agonist and antagonist binding to GPCRs (SCH-202676). The results showed that SCH completely inhibited hypostome extrusion induced by AT, providing supporting evidence on the involvement of this kind of receptor in the signal transduction of this peptide, and suggesting that ATR would be linked to a member of the Gαq subfamily (i.e. Gαq, Gα11, Gα14 or Gα15/16), which activates the β subfamily of PLCs [27]. Indeed, studies performed in Porifera and Cnidaria showed that genes codifying proteins involved in the inositol phospholipid signaling pathway, such as Gαq and PLCs (including the β subtype), would appeared early in the evolution of Metazoa, suggesting the ancestral origin in the earliest branching of animal phyla. [29].

Another possible pathway related to GPCRs, is the one that controls the levels of cAMP throughout the regulation of the activity of the enzyme adenylyl cyclase (AC). This enzyme converts ATP into cAMP. While Gαs subfamily of G protein stimulates AC, Gαi inhibits it. We also analyzed the involvement of this pathway in our experimental model. The results obtained in hydroids undergoing treatment with melittin, a compound that inhibits Gαs and stimulates Gαi activity, suggest that this pathway is not relevant for the hypostome extrusion in Hydra.

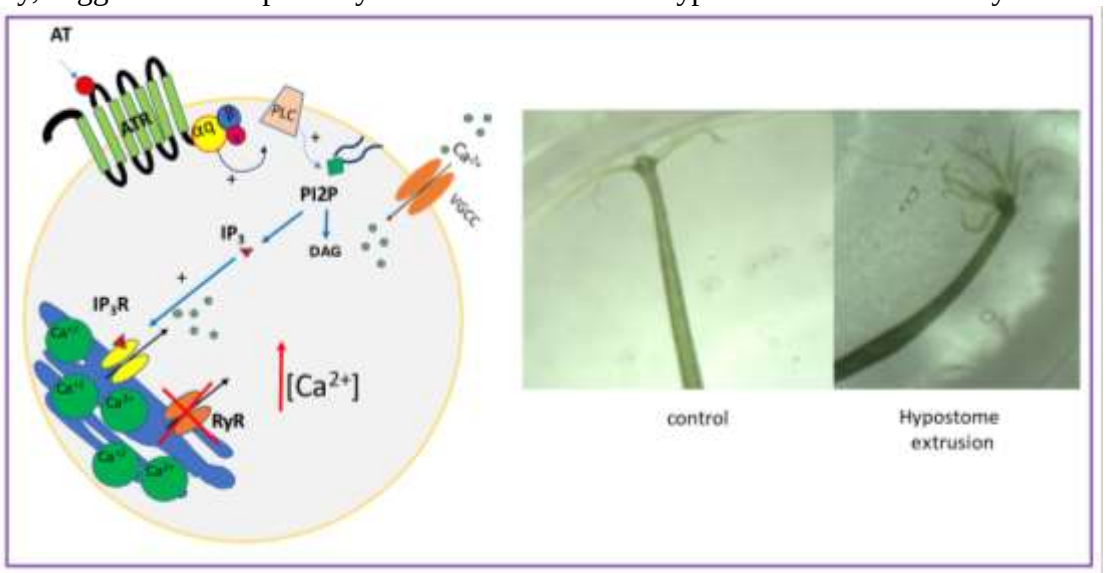

Figure 2. Signaling pathway of AT in Hydra sp activated during hypostome extrusion. Schematic representation of the signaling cascade in epithelia-muscle cells. VGCC: voltage-gated Ca2+ channels; ATR: Allatotropin receptor (GPCR); αβγ: subunits of the G protein; αq: subtype q of the G protein; PLC: phospholipase; PI2P: phosphatidyl inositol bi phosphate; DAG: diacyl-glycerol; IP3: inositol-tri-phosphate; IP3R: inositol-tri-phosphate receptor; RyR: ryanodine receptor.

Interestingly, the ATR signaling pathway proposed in our model resembles those ones activated by OxRs in vertebrates, which in fact are phylogenetically related [11, 14, 15] In spite of OxR signaling is complicated and versatile (for review see 30), both orexin receptors can be coupled to Gαq proteins, inducing PLC activity and Ca2+ release from ER. Even though Ox peptides regulate complex functions, they also act as myoregulators. In fact, it was shown that they modulate intestinal and cardiac muscle contraction [31, 32]. In fact, in intestinal smooth muscle, it was inhibited by the L-type VGCC blocker nifedipine, and by inhibitors of the IP3R [31]. This data supports the ancestral existence of the protein machinery involved, and the pathways that control myoregulatory activities related with feeding. Indeed, Hydra sp was the first species in which the conservation of molecules regulating cell differentiation and development across the biological evolution was demonstrated. Interestingly, some of these molecules show identical sequences between cnidarians and mammals. [33]. A trasncriptomic analysis showed that Hydra sp shares at least 6071 genes with humans, in contrast to Drosophila sp and Caenorhabditis elegans, which share only 5696 and 4571 genes, respectively, with humans [34]. Furthermore, when the genome of Hydra magnipapilata was available [3] it was confirmed that most of the gene families present in mammals, do have representatives in cnidarians.

Conclusions

For more than 250 years Hydra has been used as a model in different topics of biology. In addition to their high rate of reproduction and regeneration, this group of species also has the advantage of show a high level of gene conservation in comparison with more recent animal groups, that strengthens the validity of this model systems for studying processes shared by eumetazoans. In our laboratory, we use the Hydra model to study, not only the physiological aspects of this organism, but also to analyze processes in terms of the evolution of Metazoa.

References

[1] **Brusca C, Brusca GJ.** Invertebrates (*Sinauer Associates*, Sunderland, MA, 2003).

[2] **Lentz TL.** The Cell Biology of Hydra. *North Holland Publ. Co*., Amsterdam. 1966

[3] **Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T, Rattei T, Balasubramanian PG , Borman J, Busam D et al.** The dynamic genome of Hydra. *Nature* 2010; 464: 592-596.

[4] **Yum S, Takahashi T, Koizumi O, Ariura Y, Kobayakawa Y, Mohri S, Fujisawa T.** A Novel Neuropeptide, Hym-176, Induces Contraction of the Ectodermal Muscle in Hydra. *Biochem Biophys Res Commun* 1998; 248: 584-590.

[5] **Takahashi T, Hayakawa E, Koizumi O, Fujisawa T.** Neuropeptides and their functions in Hydra. *Acta Biol Hung* 2008; 59: 227-235.

[6] **Bode HB.** The interstitial cell lineage of hydra: a stem cell system that arose early in evolution. *J. Cell Sci.* 1996. 109: (Pt 6):1155-64.

[7] **Galliot B.** Hydra, a fruitful model system for 270 years. *Int. J. Dev. Biol*. 2012; 56: 411-423.

[8] **Galliot B & Schmid V.** Cnidarians as a Model System for Understanding Evolution and Regeneration. Int. J. Dev. Biol. 2002; 46: 39-48.

[9] **Kataoka H, Toschi A, Li JP, Carney RL, Schooley DA, Kramer SJ.** Identification of an allatotropin from adult Manduca sexta. *Science*1989; 243:1481–3.

[10] **Kramer SJ, Toschi A, Miller CA, Kataoka H, Quistad GB, Li JP, Carney RL, Schooley DA.** Identification of an allatostatin from the tobacco hornworm Manduca sexta. *Proceedings of the National Academy of Sciences*,1991; 88 (21) 9458-9462.

[11] **Horodyski FM, Verlinden H, Filkin N, Vandersmissen HP, Fleury C, Reynolds SE.** Isolation and functional characterization of an allatotropin receptor from Manduca sexta. *Insect Biochem Molec Biol* 2011; 41:804–14

[12] **Auerswald L, Birgül N, Gäde G, H J Kreienkamp HJ.** Structural, functional, and evolutionary characterization of novel members of the allatostatin receptor family from insects. *Biochem Biophys Res Commun* 2001; 282(4):904-9.

[13] **Alzugaray ME, Hernandez-Martínez S, Ronderos JR.** Somatostatin signaling system as an ancestral mechanism: Myoregulatory activity of an Allatostatin-C peptide in Hydra. *Peptides*, 2016; 82, 67–75.

[14] **Alzugaray ME, Ronderos JR.** Allatoregulatory-like systems and changes in cytosolic Ca2+ modulate feeding behavior in Hydra. *Gen. Comp. Endocrinol.,* 2018; 258, 70–78.

[15] **Alzugaray ME, Bruno MC, Villalobos Sambucaro MJ, Ronderos JR.** The evolutionary history of the Orexin/Allatotropin GPCR family: from Placozoa and Cnidaria to Vertebrata. *Sci. Rep*. 2019; 9, 10217.

[16] **Sterkel M, Riccillo FL, Ronderos JR.** Cardioacceleratory and myostimulatory activity of allatotropin in Triatoma infestans (Klüg). *Comp Biochem Physiol A* 2010; 155:371–377.

[17] **Villalobos-Sambucaro MJ, LorenzoFigueiras AN, Riccillo FL, Diambra LA, Noriega FG, Ronderos JR** () Allatotropin Modulates Myostimulatory and Cardioacceleratory Activities in Rhodnius prolixus (Stal). *PLoS ONE* 2015; 10(4): e0124131.

[18] **MJ Villalobos-Sambucaro, LA Diambra, FG Noriega, JR Ronderos.** Allatostatin-C antagonizes the synergistic myostimulatory effect of allatotropin and serotonin in Rhodnius prolixus (Stal). *General and comparative endocrinology* 2016; 233, 1-7.

[19] **Adami ML, Damborenea C, Ronderos JR.** Expression of a neuropeptide similar to allatotropin in free living Turbellaria (Platyhelminthes) *Tissue and Cell* 2011; 43 (6), 377-383

[20] **Adami ML, Damborenea C, Ronderos JR.** An allatotropin-like neuropeptide in Mesostoma ehrenbergii (Rhabdocoela, Platyhelminthes). *Zoomorphology* 2012; 131:1–9.

[21] **Alzugaray ME, Adami ML, Diambra AL, Hernandez-Martínez S, Damborenea C, Noriega FG, Ronderos JR.** Allatotropin: an ancestral myotropic neuropeptide involved in feeding. *PLoS One*, 2013; 8, e77520.

[22] **Lenhoff HM, Bovaird J.** Requirement of bound calcium for the action of surface chemoreceptors. *Science*. 1959;130(3387):1474-6.

[23] **Lenhoff HM, Heagy W.** Aquatic invertebrates: model systems for study of receptor activation and evolution of receptor proteins. *Annu Rev Pharmacol Toxicol*. 1977; 17:243-58.

[24] **Lin YC, Spencer AN.** Calcium currents from jellyfish striated muscle cells: preservation of phenotype, characterization of currents and channel localization. *J Exp Biol*. 2001; 204(Pt 21):3717-26.

[25] **Lin YC, Spencer AN**. Localization of intracellular calcium stores in the striated muscles of the jellyfish Polyorchis penicillatus: possible involvement in excitation-contraction coupling. *J Exp Biol.* 200; 204(Pt 21):3727-36.

[26] **Alzugaray ME, Gavazzi MV, Ronderos JR.** Calcium signaling in early divergence of Metazoa: Mechanisms involved in the control of muscle-like cells contraction in Hydra plagiodesmica (Dioni). *Can. J. Zool*. 2019; 824, 812–824.

[27] **Alzugaray ME, Gavazzi MV, Ronderos JR.** G protein-coupled receptor signal transduction and Ca2+ signaling pathways of the allatotropin/orexin system in Hydra. *Gen. Comp. Endocrinol*., 2021.; 300: 113637.

[28] **Mackrill JJ.** Ryanodine receptor calcium release channels: an evolutionary perspective. *Adv Exp Med Biol.* 2012;740:159-82.

[29] **Koyanagi M, Ono K, Suga H, Iwabe N, Miyata T.** Phospholipase C cDNAs from sponge and hydra: antiquity of genes involved in the inositol phospholipid signaling pathway. *FEBS Lett* 1998; 13;439(1-2):66-70.

[30] **Kukkonen JP.** Lipid signaling cascades of orexin/hypocretin receptors. *Biochimie*. 2014; 96:158-65.

[31] **Squecco R, Garella R, Luciani G, Fabio Francini F, Baccari MC.** Muscular effects of orexin A on the mouse duodenum: mechanical and electrophysiological studies. *J Physiol*. 2011; 589:5231-46.

[32] **Patel VH, Karteris E, Chen J, Kyrou I, Mattu HS, Dimitriadis GK, Rodrigo G, Antoniades C, Antonopoulos A, Tan BK et al.** Functional cardiac orexin receptors: role of orexin-B/orexin 2 receptor in myocardial protection. *Clin. Sci.* 2018; 132, 2547–2564.

[33] **Bodenmüller H, Schaller HC**. Conserved amino acid sequence of a neuropeptide, the head activator, from coelenterates to humans. *Nature*. 1981; 293(5833):579-80.

[34] **Wenger Y, Galliot B.** RNAseq versus genome-predicted transcriptomes: a large population of novel transcripts identified in an Illumina-454 Hydra transcriptome. *BMC Genomics*. 2013; 14:204.

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HOW SIMILAR ARE YOU TO THE FRUIT FLY? *DROSOPHILA MELANOGASTER* AS MODEL FOR STUDYING HUMAN DISEASES

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ABSTRACT

Drosophila melanogaster is a holometabolous insect with a short life cycle. Its genome is completely sequenced, and it is easy to grow in the laboratory. More than 120 years of history using *Drosophila* have allowed us to clarify the inheritance laws and to understand cellular and molecular mechanisms of mammalian development in this model. Approximately 75% of the genes responsible for several human diseases have homologs in the fruit fly. This work addresses some advantages of using this model organism for human pathophysiology studies. Neurodegenerative disorders, heart disease, and nephropathies can be modeled in transgenic organisms, affecting the homologous gene or expressing the human sequences. It is also possible to study multi-organ pathophysiological conditions such as aging, diabetes, and cancer. In addition, *Drosophila* has been used to explore substances of human consumption such as cocaine, methamphetamine, caffeine, alcohol, tobacco, and cannabinoids. Finally, the versatility of this organism and the knowledge of its genome have made it possible to undertake large-scale pharmacological studies to learn about the interactions between substances and genes, to find new drugs.

Keywords: *Drosophila*, diseases, drugs, modeling, pharmacogenomics

RESUMEN

Drosophila melanogaster es un insecto holometábolo que presenta un ciclo de vida corto; su genoma está completamente secuenciado y es muy fácil de criar en el laboratorio. Más de 120 años de historia utilizando a *Drosophila* han permitido clarificar las leyes de la herencia y entender algunos mecanismos celulares y moleculares del desarrollo del mamífero en este modelo. Aproximadamente el 75% de los genes responsables de ciertas patologías humanas tienen homólogos en la mosca de la fruta. Este trabajo aborda algunas de las ventajas de utilizar a *Drosophila melanogaster* como organismo modelo para estudiar la fisiopatología humana. Con el uso de organismos transgénicos que tienen afectado uno o más genes homólogos, o que expresan genes humanos, se pueden modelar diferentes enfermedades neurodegenerativas, cardiopatías y nefropatías. También es posible ahondar en el estudio de condiciones fisiopatológicas multiorgánicas como por ejemplo el envejecimiento, la diabetes y el cáncer. Además, se ha utilizado a *Drosophila* para estudiar sustancias de consumo humano tales como cocaína, metanfetamina, cafeína, alcohol, tabaco y cannabinoides. Finalmente, la versatilidad de este organismo y el conocimiento de su genoma han permitido abordar estudios farmacológicos a gran escala, para conocer las interacciones entre las sustancias y los genes, con el objetivo de diseñar nuevos fármacos.

Palabras claves: *Drosophila*, enfermedades, drogas, modelo, farmacogenómica

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Characteristics of the Fruit Fly

The fruit fly *Drosophila melanogaster* (Diptera: *Drosophilidae*) is a small holometabolous insect because its development consists of a complete metamorphosis through four stages: egg, larva, pupa, and adult. Adult individuals are about 3 mm long. Their bodies are brown with black stripes and their eyes are red. However, due to several genetic mutations, there are individuals with different eye and body colors. In nature, *Drosophila* feeds on fruit and other decaying items rich in sugar. The life cycle from oviposition to adults is relatively short and lasts about 10-12 days at 25°C. Females lay up to 100 eggs per day throughout their lives at room temperature. Adults live around 70 days or more, but both survival and fertility are modified according to temperature [1].

Looking to the past: remarkable experiments with the fruit fly

By 1901, scientists William Castle at Harvard University, Frank Lutz at Cold Spring Harbor, and Fernandus Payne at Indiana University were already using the fruit fly in their research. It was Castle's publications that sparked the interest of several laboratories in *Drosophila* as an organism for genetic and evolutionary studies [2].

In 1910, Thomas Hunt Morgan, an American biologist, identified for the first time a white-eyed mutant male. Through successive crosses, Morgan concluded that the inheritance of certain traits was sex-linked. Further experiments in his laboratory provided evidence supporting Walter Sutton and Theodor Boveri's chromosomal theory of inheritance. This theory highlights that the factors responsible for inheritance (genes) are found in chromosomes and that the behavior of chromosomes during meiosis could explain the laws of inheritance described by Gregor Mendel [2,3].

Morgan's discoveries through his research with the fruit fly allowed him to win the Nobel Prize in 1933. Five other Nobel prizes were awarded to scientists who developed their experiments with *Drosophila melanogaster*: Hermann Joseph Muller (1946), Edward B Lewis, Christiane Nüsslein-Volhard, Eric F Wieschaus (1995), Richard Axel (2004), Jules A Hoffmann (2011) Jeffrey C Hall, Michael Rosbash and Michael W Young (2017). They elucidated the laws of inheritance, the generation of mutations, aspects of embryogenesis, the development of the olfactory system, the activation of the immune system, and the molecular mechanisms that control circadian rhythms, *i.e.*, the internal biological clocks of organisms, through assays in *Drosophila* [4]. Thus, in addition to becoming the exemplary model for genetic studies, *Drosophila melanogaster* became important for studies on pathophysiology in multiple areas of biomedical sciences.

Why *Drosophila* is a useful model in biology?

Drosophila melanogaster can be grown in the laboratory, easily. It is possible to raise hundreds of individuals in vials, occupying a small space and with a low maintenance cost. Their diet can be sustained by simple sources of carbohydrates (cornmeal) and protein (yeast extract). The food must be changed regularly every 10-14 days at 25°C or every 5 weeks at 18°C. The life cycle is short, so it is possible to work with successive generations in a shorter time compared to mammals [4].

The genome, transcriptome, and proteome of *Drosophila melanogaster* are characterized [5–7]. Sixty percent of its genome shows homology to the human, but unlike the human genome, it is less redundant, *i.e.*, it has fewer duplicated genes. In addition, about 75% of the genes responsible for human diseases have homologs in the fruit fly [8].

The possibility of modifying the genome and generating transgenic organisms has made it feasible to mutate fly genes and reproduce symptoms of human diseases such as Alzheimer's, Parkinson's, Friedrich's ataxia, heart disease, metabolism, among others. It has also allowed the insertion of fluorescent proteins as markers and has made it possible to regulate gene expression over time or in response to stimuli [4,9]. On the other hand, several public repositories provide numerous lines to be used by different laboratories in the world. Figure 1, shows the general aspect of a wild type fly (Top panel) and the recognizing of structures and molecular mediators mediating transgenic approaches inserting reporter system (Bottom panel).

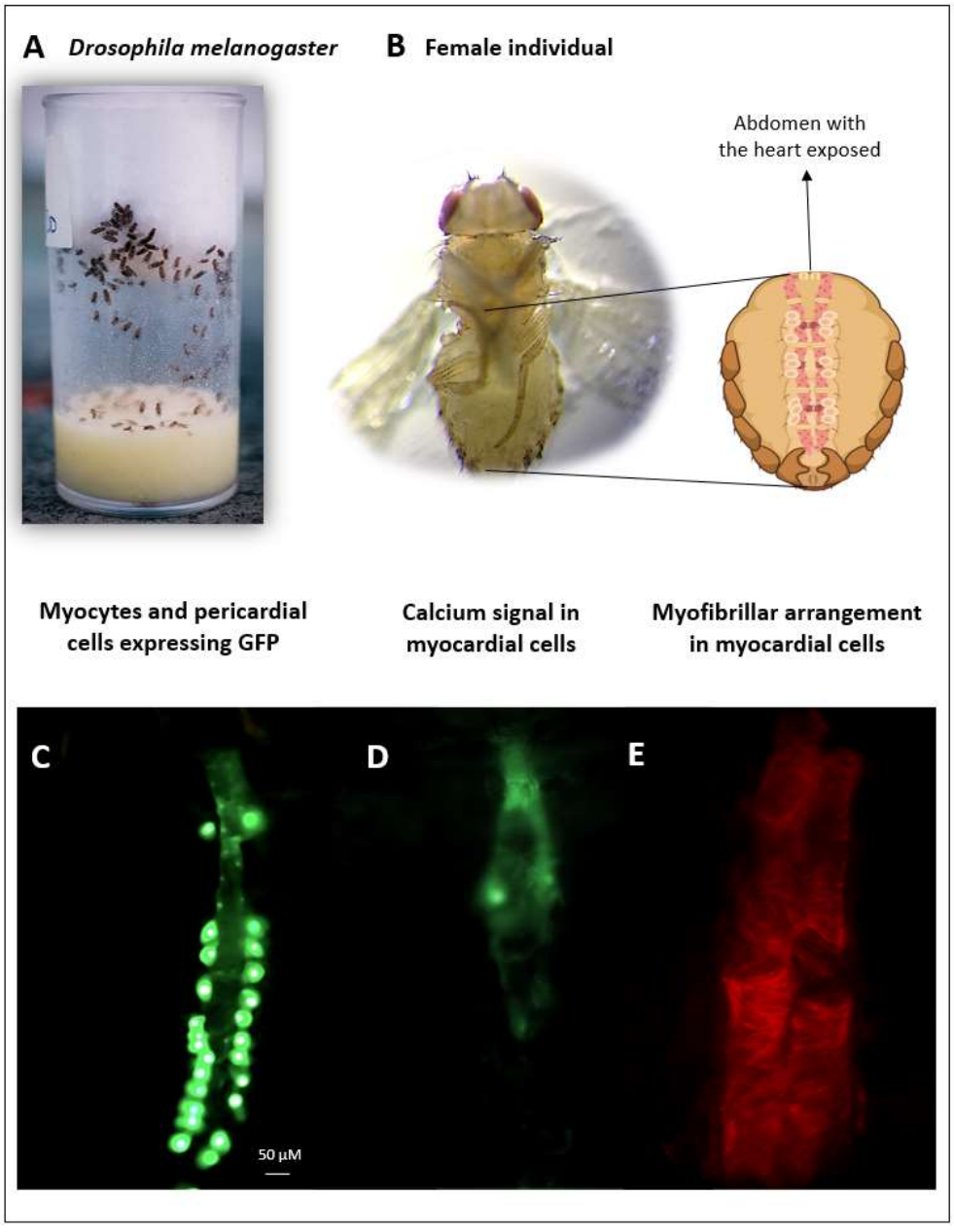

Figure 1. A. Adults individuals of wild type *Drosophila melanogaster* growing into a vial. **B.** Aspect of a female individual and scheme of an opened abdomen to visualize the heart. C-D. Two transgenic approaches to recognize structures and intracellular mediators: **C.** Cardiomyocytes and pericardial cells expressing a green fluorescent protein (GFP) under the driver *Hand C*. **D.** Cardiac-specific expression of Ca^{2+} sensing fluorescent protein used to assess intracellular Ca^{2+} transients in the cardiomyocytes. **E.** Myofibrillar arrangement of the cardiac fibers identified with phalloidin (magnification 40X).

Drosophila and diseases

Almost all human organ systems have a counterpart in the fruit fly [8]. Below is shown how the major organ systems of *Drosophila* have contributed to understanding human physiological processes and pathologies. Figure 2 represents the correspondence between the most important system in humans and flies.

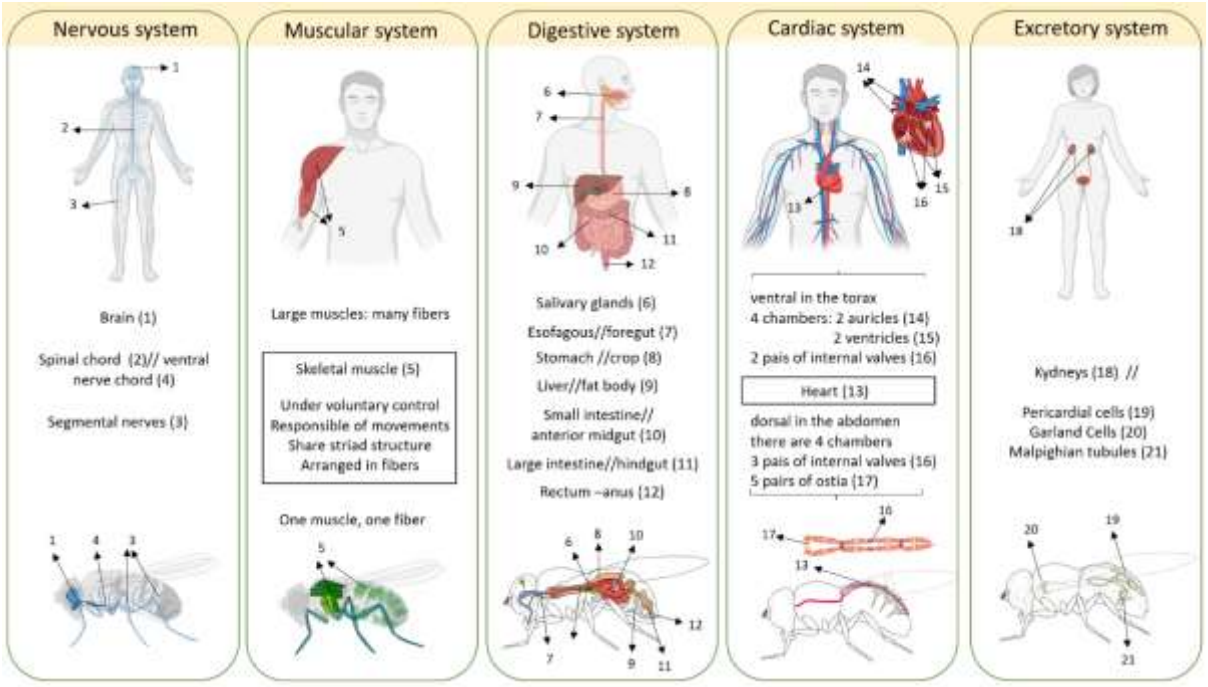

Figure 2. Comparison of the human and *Drosophila melanogaster* organ system of interest in biomedical research. Each organ is numbered and indicated in the correspondent scheme.

Nervous system

The central nervous system of *Drosophila* consists of a bilateral and symmetrical brain, composed of neurons and glial cells [9]. From there, neuronal extensions extend to the rest of the body. Many human neurological disorders can be modeled in the fruit fly. One of them is Parkinson's disease (PD) which is characterized by progressive deterioration of motor functions. Inherited forms of PD are caused by mutations in genes encoding key proteins in the development of the pathophysiology of the disease. Most of the genes implicated in familial forms of PD have at least one homolog in the fly, such as LRRK2, parkin, PINK1, and DJ-1 and the pathways regulated by these genes are also conserved in this organism [10]. Several key neuropathological and clinical features of PD are reproduced in this model because flies can perform complex motor behaviors such as walking, climbing, and flying.

Alzheimer's disease is a progressive neurological disorder that results in the irreversible loss of neurons in the cortex and hippocampus. The formation of amyloid plaques and neurofibrillary aggregates are characteristic events. Transgenic flies that model this pathology overexpress the gene coding for presenilin, a component of the alpha-secretase complex which induces an intracellular calcium deficit and is related to one of the early events of Alzheimer's pathology. Other models are based on the expression of the human gene tau, which allows the analysis of myofibrillar aggregates detected in this pathology. In addition, there are models of co-expression of the beta-amyloid precursor protein and human tau [11].

Another pathology that has been reproduced in fruit flies is Friedreich's Ataxia, an autosomal recessive disease. The mutation conducts to loss of function of the gene encoding the frataxin protein [12]. Patients present a neurodegenerative disorder that causes motor problems and in a subgroup of individuals, hypertrophic cardiomyopathy [13]. Moreover, it is possible to model epilepsy, a neurological disorder characterized by sudden recurrent episodes of sensory and motor disturbances caused by abnormal electrical activity in the brain. Some types of epilepsy are caused by genetic factors such as mutations in genes that code for ion channels. There are models of epilepsy in *Drosophila* with mutations in *eag* and *sh* genes. Their products are components of potassium channels and mutant flies show neuronal hyperexcitability and seizures [14].

The scheme of neurodegenerative disorders that can be modeled in *Drosophila* also includes amyotrophic lateral sclerosis, spinocerebellar ataxia, and Huntington's disease [9]. On the other hand, as in humans, sleep disorders have a major impact on the physiological functions of *Drosophila* and might even lead to death. Since many of these disorders have a genetic component, it is possible to study the molecular mechanisms associated with these alterations by modifying the homologous of the human gene in the fruit fly.

Cardiac system

The adult *Drosophila* heart is a longitudinal tube extending along the abdomen in the dorsal region. It consists of four chambers arranged in series and connected by valves. The entry of hemolymph into the chambers occurs through the ostia. Hemolymph is the intracellular fluid that provides nutrients and hormones to the internal organs of the fly. Unlike mammals, the respiratory system is composed of tracheas and provides gaseous exchange independently of hemolymph circulation. This characteristic allows flies to live for days with a severely damaged heart and allows drastic interventions to be made in the circulatory system with less impact on the rest of the body's tissues and organs [15]. Genes encoding for contractile proteins such as channels, pumps, and other proteins present in cardiomyocytes are conserved between *Drosophila* and mammals [16].

Drosophila can present heart failure and, as in humans, it can be distinguished between dilated and hypertrophic cardiomyopathies. Cardiomyopathies secondary to other types of disorders can be detected in fruit flies. For example, mutants for the presenilin gene, associated with early-onset Alzheimer's disease, present dilated cardiomyopathy. Changes in the expression levels of this gene increase age-associated arrhythmias, mitochondrial and myofibrillar degeneration [17]. Regarding the channelopathies, KCNQ potassium channels have been studied during aging and it has been demonstrated that they play a protective role. Mutations of the KCNQ1 channel, lead to prolonged QT syndrome causing cardiac arrhythmias [18]. In models of diabetes, flies subjected to a diet enriched with sugar were observed to develop cardiomyopathies associated with insulin resistance and type 2 diabetes. Increased arrhythmias and impaired cardiac function modulated by insulin and MAPK pathways were observed [19].

In addition, flies have been sent to the space to examine the effects of microgravity on cardiac structure and function to understand the changes that astronauts might undergo in space missions. Exposure to microgravity resulted in structural and functional cardiac remodeling, reduced output along with decreased expression of genes encoding for sarcomere and extracellular matrix elements in flies [20].

Digestive system

The *Drosophila* and mammalian intestine are of endothelial origin. Both of them include a monolayer of cuboidal epithelial cells called enterocytes, as well as stem cells. Mammalian and *Drosophila* intestinal cells (ISCs) share intracellular mechanisms that control intestinal regeneration. Due to the physiological similarities between the *Drosophila melanogaster* and

vertebrate gut, the midgut epithelium of the fruit fly has been used to study the contribution of signaling pathways (*i.e.*, EGFR, Notch, Hedgehog, and Wg/Wnt) to the renewal of stem cells (Stem cells ISCs) [21]. Also, *Drosophila* constitutes a model organism for high-throughput studies on gut microbiota-host interactions. The intestinal mucosa must distinguish between commensal, mutualistic, and pathogenic bacteria. The imbalance between components of the microbiota can disrupt intestinal homeostasis and lead to disorders [22].

Excretory system

In humans, podocytes, specialized cells in the glomeruli of the kidneys, are responsible for filtration. Analogous cells in the fruit fly are nephrocytes, present in the thorax, named Garland cells, and in the abdomen, named pericardial cells. It has been possible to model conditions such as diabetic nephropathies and monogenic forms of nephrotic syndromes in *Drosophila* [23].

Pathologies with multiorgan impact

Aging

Aging in all organisms can be described as the deterioration of various functions over time until death occurrence. In *Drosophila*, the ability to feed oneself, as well as mobility, resistance to stress, and fertility, are reduced over time. Sleep disturbances are common. Neurological functions such as learning and memory are impaired. Old flies frequently exhibit heart failure [24]. Aging reduces heart rate and alters intracellular calcium handling. Thus, aging affects multiple organ systems simultaneously.

Obesity

Obesity increases the risk of diabetes, metabolic syndrome, cardiovascular disease, and cancer. Therefore, uncovering the complications associated with obesity is challenging. *Drosophila* has been used to model obesity induced by a diet rich in sugars and fatty acids. In *Drosophila*, the organ that stores carbohydrates and lipids are the fat body, analogous to human adipose tissue. Obese individuals accumulate triglycerides in the fat body. The fat body also performs liver-like functions. Flies with high sugar intake, like humans, exhibit hyperglycemia, insulin resistance, and cardiomyopathies. Obesity in fruit flies can also be genetically induced to analyze the consequences of obesity without altering nutrient-sensitive signaling mechanisms [25].

Diabetes

Diabetes is a chronic metabolic disease caused by deficiency or loss of insulin. *Drosophila* produces seven insulin-like peptides secreted by insulin-secreting cells in the brain. These peptides participate in signaling pathways similar to those in mammals. It is possible to model type I and type II diabetes in *Drosophila.* As in mammals, high-sugar diet leads to insulin resistance. This is accompanied by decreased weight and increased glucose in the hemolymph and fat body in larvae as in adults [27].

Cancer

Cancer is a disease based on deregulation in the activity of oncogenes and/or loss of function of tumor suppressor genes, often induced by environmental factors that increase the risk of developing the disease. Most of the signaling pathways linked to cancer development are present in *Drosophila*. Cell polarity mediators and pathways such as Salvador-Warts-Hippo (SWH), RAS/RAF/ERK, PI3K/TOR, JNK, and cMyc, deregulated in cancer, can be also altered in the fruit fly [27].

A summary of human diseases modelized in *Drosophila* is shown in figure 3.

Drosophila and drugs

Drug addiction is a disorder characterized by excessive use of one drug, to the point of compulsive drug seeking and use. This can lead to dependence on the substance, which includes physical symptoms such as tolerance and withdrawal. Although addiction is considered a human phenomenon, animal models provide insight into the molecular mechanisms underlying addictions. Flies exhibit complex behaviors such as associative learning, sensor-motor integration, and social behaviors [28]. This has allowed to used them in the study of the effects induced by various substances of abuse.

Alcohol

Ethanol is a known substance to fruit flies because it is found in nature as a product of sugar fermentation. In the laboratory, studies focus on the locomotor response to acute ethanol exposure, tolerance to the compound, and preference behaviors that model aspects of addiction. For example, male flies rejected by previously mated females showed a preference for consuming ethanol-supplemented food. Interestingly, although virgin males showed a preference for ethanol-supplemented food, this was not greater than that manifested by rejected males. The researchers demonstrated that sexual deprivation would create a deficit of a specific peptide (neuropeptide F, NPF) that would increase reward-seeking behavior, such as ethanol consumption [29].

Caffeine

Caffeine has been used in experiments in which sleep deprivation has been induced in *Drosophila*. Utilizing this strategy, it was possible to compare insomniac individuals treated or not with compounds such as γ-aminobutyric acid (GABA) and 5-hydroxytryptophan (5-HTP), both proposed as candidates in the search for drugs that can solve sleep problems. On the other hand, in our laboratory, we observed that the application of caffeine in the hemolymph of *Drosophila* has direct effects on the heart modifying the heart rate and the force of contraction [30].

Cocaine

Studies on the action of cocaine focused on behavior and neurophysiology in *Drosophila* showed similar results that human responses to this substance. Flies that consumed cocaine had altered dopamine levels causing increased adult mortality and defects in the formation of female reproductive cells (gametes). Other defects observed in the formation of gametes in these flies were attributed to the dysregulation of another neurotransmitter, serotonin [31].

Amphetamines

Amphetamine and methamphetamine are used clinically to treat attention deficit hyperactivity disorder and narcolepsy, among other conditions. Long-term use of these compounds creates the risk of addiction. Side effects of methamphetamine in humans, like increased oxidative stress, heart failure, neurotoxicity have been reported, among others. In *Drosophila*, methamphetamine causes toxic effects like in humans. It was observed that exposure of flies to this substance, increased locomotor activity and induced anorexia. It has also been identified that these substances affect the set of several genes (genome) and proteins (transcriptome) [31].

Cannabis

Mammals and other vertebrates respond to phytocannabinoids. These compounds are present in the flowers of the *Cannabis sativa*, known as marijuana. Phytocannabinoids are similar in structure and/or function to components of the vertebrate endocannabinoid system. This system regulates many functions such as pain response, nutritional status, and overall balance of the body. Fruit flies possess endocannabinoids in their hemolymph although the most studied cannabinoid receptors in mammals, CB1 and CB2, are not present in insects [32]. However, other receptors mediate responses to cannabinoids in mammals, such as the transient receptor potential channels (TRPs) and peroxisome proliferator-activated receptors (PPARs). Numerous actions of cannabinoids cannot be explained by binding to CB1 and CB2 receptors. Thus, the interest on alternative pathways mediated by other receptors, is increasing. Studies from our laboratory have shown that the behavior and cardiac function of healthy flies exposed to phytocannabinoids, were affected. Chronic treatment with a strain rich in the cannabinoid tetrahydrocannabinol (THC) initially increased arrhythmias. However, longer exposure to phytocannabinoids induced increased contractility [33].

Nicotine

Nicotine has been shown to influence the development and survival of *Drosophila melanogaster*. The gene encoding for the nicotinic cholinergic receptor is present in *Drosophila melanogaster* and it has been shown that nicotine increases heart rate in larvae, which have the particularity that their heart is not innervated. The cardiac muscle of *Drosophila melanogaster* also presents receptors for glutamatergic neurons and beta-adrenergic receptors that connect with the nervous system, on which the mechanisms of action of nicotine are known in more detail. Studies of our group have shown that flies chronically exposed to tobacco presented an incremented heart rate and alterations in the dynamics of the calcium transient in the heart. Mutant organisms for the alpha 1 and 7 subunits of nicotinic receptors allowed us to elucidate the relative contribution to the observed effects of tobacco and nicotine [34].

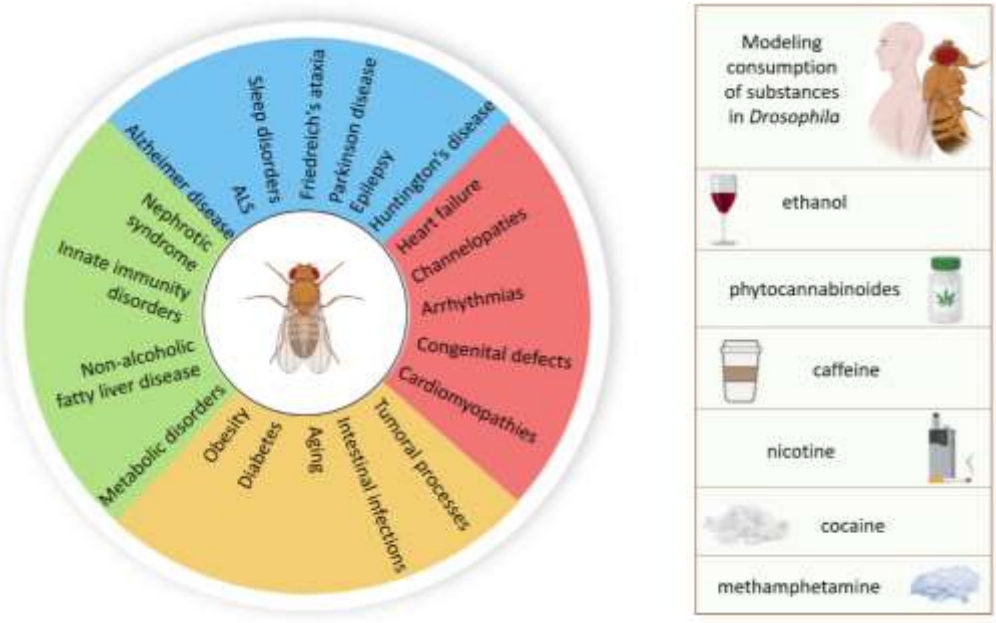

Figure 3. Left: Different diseases can be modelized in the fruit fly. Right: list of substances of human consumption studied in *Drosophila*.

Pharmagenomics

Finally, *Drosophila melanogaster* is a useful organism for high-throughput pharmacological studies, accelerating the discovery of new compounds for therapeutic purposes [35]. Pharmacogenomics analyzes the response of the genome to drugs. This discipline allows to determine which drugs and doses are adequate for patients, according to their genetic polymorphisms. High-throughput studies in flies, whose genome is completely sequenced, conduct to the identification of genes responsible for different responses to substances. Thus, it might be possible to find new drugs for subsequent trials.

Although many human pathologies have been mentioned that can be investigated in *Drosophila*, this model has limitations like other animal models. Oher pathologies of different etiology than genetic origin cannot be easily modeled or the results cannot be safely extrapolated to humans. Disease modeling reproduces some but not all symptoms. Complex polygenic diseases are more difficult to address. However, the versatility of the model after more than 100 years of research history, turns it into a possibility to be explored through studies in new areas of biomedical disciplines.

References

[1] **Perveen FK.** Introduction to *Drosophila*. In Perveen FK ed. *Drosophila melanogaster* - Model for Recent Advances in Genetics and Therapeutics. London, *Intech Publisher Headquarters*; 2018; doi: 10.5772/67731.

[2] **Carlson EA.** How fruit flies came to launch the chromosome theory of heredity. *Mutation Research*. 2013; 753(1):1–6.

[3] **Frezza G, Capocci M.** Thomas Hunt Morgan and the invisible gene: the right tool for the job. *History and Philosophy of the Life Sciences.* 2018; 40(2):31.

[4] **Allocca M, Zola S, Bellosta P**. The Fruit Fly, *Drosophila melanogaster*: The Making of a Model (Part I). In Perveen FK ed. *Drosophila melanogaster* - Model for Recent Advances in Genetics and Therapeutics. *London, Intech Publisher Headquarters*; 2018; doi: 10.5772/intechopen.72832.

[5] **Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Sherer SE, Li PW, Hoskins RA, Galle RF et al.** The genome sequence of *Drosophila melanogaster*. *Science*. 2000; 287(5461):2185–95.

[6] **Zeitouni B, Sénatore S, Séverac D, Aknin C, Sémériva M, Perrin L.** Signalling Pathways Involved in Adult Heart Formation Revealed by Gene Expression Profiling in *Drosophila*. *PLOS Genetics*. 2007; 3(10): e174.

[7] **Cammarato A, Ahrens CH, Alayari NN, Qeli E, Rucker J, Reedy MC, Zmasek CM, Gucek M, Cole RN, Van Eyk JE et al.** A Mighty Small Heart: The Cardiac Proteome of Adult *Drosophila melanogaster*. *PLOS ONE*. 2001; 6(4):e18497.

[8] **Ugur B, Chen K, Bellen HJ.** *Drosophila* tools and assays for the study of human diseases. *Disease Models & Mechanisms.* 2016; 9(3):235.

[9] **Allocca M, Zola S, Bellosta P.** The Fruit Fly, *Drosophila melanogaster*: Modeling of Human Diseases (Part II). In Perveen FK ed. *Drosophila melanogaster* - Model for Recent Advances in Genetics and Therapeutics. London, Intech Publisher Headquarters; 2018; DOI: 10.5772/intechopen.73199.

[10] **Whitworth AJ.** *Drosophila* Models of Parkinson's Disease. *Advances in Genetics.* 2011; 73(C):1–50.

[11] **Jeon Y, Lee JH, Choi B, Won S-Y, Cho KS**. Genetic Dissection of Alzheimer's Disease Using *Drosophila* Models. *International Journal of Molecular Sciences*. 2020; 21(3):884.

[12] **Monnier V, Llorens JV, Navarro JA.** Impact of *Drosophila* Models in the Study and Treatment of Friedreich's Ataxia. *International Journal of Molecular Sciences*. 2018; 19(7):1989.

[13] **Calap-Quintana P, Navarro JA, González-Fernández J, Martínez-Sebastián MJ, Moltó MD, Llorens JV.** *Drosophila melanogaster* Models of Friedreich's Ataxia. *BioMed Research International*. 2018; 5065190.

[14] **Broughton SJ, Kitamoto T, Greenspan RJ.** Excitatory and Inhibitory Switches for Courtship in the Brain of *Drosophila melanogaster*. *Current Biology*. 2004; 14(7):538–47.

[15] **Rotstein B, Paululat A.** On the Morphology of the *Drosophila* Heart. *Journal of Cardiovascular Development and Disease* 2016; 3(2):15.

[16] **Lin N, Badie N, Yu L, Abraham D, Cheng H, Bursac N, Rockman HA, Wolf MJ**. A method to measure myocardial calcium handling in adult *Drosophila*. *Circulation research*. 2011; 108(11):1306.

[17] **Li A, Zhou C, Moore J, Zhang P, Tsai T-H, Lee H-C, Romano DM, McKee ML, Schoenfeld DA, Serra MJ et al.** Changes in the Expression of the Alzheimer's Disease-Associated Presenilin Gene in *Drosophila* Heart Leads to Cardiac Dysfunction. *Current Alzheimer research*. 2011; 8(3):313.

[18] **Ocorr K, Reeves NL, Wessells RJ, Fink M, Chen H-SV, Akasaka T, Yasuda S, Metzger JM, Giles W, Posanoy JW et al**. KCNQ potassium channel mutations cause cardiac arrhythmias in *Drosophila* that mimic the effects of aging. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(10):3943.

[19] **Na J, Musselman LP, Pendse J, Baranski TJ, Bodmer R, Ocorr K, Ocorr K, Cagan R**. A *Drosophila* Model of High Sugar Diet-Induced Cardiomyopathy. *PLOS Genetics.* 2013; 9(1):e1003175.

[20] **Walls S, Diop S, Birse R, Elmen L, Gan Z, Kalvakuri S, Pineda S, Reddy C, Taylor E, Trinh B et al.** Prolonged Exposure to Microgravity Reduces Cardiac Contractility and Initiates Remodeling in *Drosophila*. *Cell Reports*. 2020; 33(10):108445.

[21] **Apidianakis Y, Rahme LG**. *Drosophila melanogaster* as a model for human intestinal infection and pathology. *Disease Models & Mechanisms*. 2011; 4(1):21–30.

[22] **Trinder M, Daisley BA, Dube JS, Reid G.** *Drosophila melanogaster* as a High-Throughput Model for Host–Microbiota Interactions. *Frontiers in Microbiology.* 2017; 8:751.

[23] **Helmstädter M, Huber TB, Hermle T.** Using the *Drosophila* Nephrocyte to Model Podocyte Function and Disease. *Frontiers in Pediatrics*. 2017; 5:1.

[24] **Piper MDW, Partridge L.** *Drosophila* as a model for ageing. Biochimica et Biophysica Acta (BBA) - *Molecular Basis of Disease*. 2018; 1864(9):2707–17.

[25] **Musselman LP, Fink JL, Baranski TJ.** Similar effects of high-fructose and high-glucose feeding in a *Drosophila* model of obesity and diabetes. *PLoS ONE*. 2019; 14(5).

[26] **Graham P, Pick L.** *Drosophila* as a Model for Diabetes and Diseases of Insulin Resistance. *Current Topics in Developmental Biology*. 2017; 121:397–419.

[27] **Mirzoyan Z, Sollazzo M, Allocca M, Valenza AM, Grifoni D, Bellosta P.** *Drosophila melanogaster*: A Model Organism to Study Cancer. *Frontiers in Genetics*. 2019; 10:51.

[28] **Greenspan RJ, Ferveur J-F**. Courtship in Drosophila. *Annu Rev Genet*. 2000; 34:205-232.

[29] **Shohat-Ophir G, Kaun KR, Azanchi R, Heberlein U.** Sexual deprivation increases ethanol intake in *Drosophila*. *Science.* 2012; 335(6074):1351–5.

[30] **Santalla M, Portiansky L, Ferrero PV.** *Drosophila melanogaster*, an Emerging Animal Model for the Study of Human Cardiac Diseases. *Argentine Journal of Cardiol*. 2016; (84)5.

[31] **Highfill CA, Baker BM, Stevens SD, Anholt RRH, Mackay TFC**. Genetics of cocaine and methamphetamine consumption and preference in *Drosophila melanogaster*. *PLOS Genetics*. 2019; 15(5):e1007834.

[32] **Khaliullina H, Bilgin M, Sampaio JL, Shevchenko A, Eaton S.** Endocannabinoids are conserved inhibitors of the Hedgehog pathway. *Proceedings of the National Academy of Sciences*. 2015; 12(11):3415–20.

[33] **Gómez IM, Rodríguez MA, Santalla M, Kassis G, Colman Lerner JE, Aranda JO, Sedán D, Andrinolo D, Valverde CA, Ferrero P.** Inhalation of marijuana affects *Drosophila* heart function. *Biology Open*. 2019; 8(8): bio044081.

[34] **Santalla M, Pagola L, Gómez I, Balcazar D, Valverde CA, Ferrero P.** Smoking flies: testing the effect of tobacco cigarettes on heart function of *Drosophila melanogaster*. *Biology Open*. 2021; 10(2).

[35] **Fernández-Hernández I, Scheenaard E, Pollarolo G, Gonzalez C.** The translational relevance of *Drosophila* in drug discovery. *EMBO reports*. 2016; 17(4):471–2.

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A LOOK INTO A WORMY FRIEND: THE MODEL OF *CAENORHABDITIS ELEGANS*

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ABSTRACT
This mini-review aims to be a glimpse into the widely used model *Caenorhabditis elegans,* a free-living nematode found in nature. As the information around this worm is vast and it cannot be summarized in these few lines we will try to introduce its main features, the interest to work with it, and how to begin the journey to incorporate it into your laboratory.
Keywords: Caenorhabditis elegans; nematode; approach model; physiology.

RESUMEN
Esta mini revisión pretende ser un vistazo al modelo ampliamente utilizado *Caenorhabditis elegans*, un nematodo de vida libre que se encuentra en la naturaleza. Como la información en torno a este gusano es vasta y no se puede resumir en estas pocas líneas, intentaremos introducir sus principales características, el interés por trabajar con él y cómo iniciar el viaje para incorporarlo a tu propio laboratorio.
Palabras clave: Caenorhabditis elegans; nematodo; modelo de abordaje; fisiología.

Introduction
It was Sydney Brenner, in 1965, who saw the potentiality of using nematodes as models for the future of molecular biology, especially for the study of development and the nervous system (Brenner, 1988; Brenner, 2003). Nematodes were already an interesting target as they were seen as simple eukaryotic organisms, with a manageable size and number of cells, and Brenner saw *Caenorhabditis briggsae* as an ideal system (Corsi et al., 2015). Later, he switched to *C. elegans* because the strain grew better than the *briggsae* isolate; which was for the better as *C. elegans* proved to be an even better model than what Brenner could have predicted. *C. elegans* is so far the only hermaphroditic free-living species in which external application of dsRNAs inactivates gene expression, which proved to be a great tool that rocketed genetic studies to the sky. Second, its syncytial germ line makes for easy transgenesis, as injected DNA recombines and forms an additional chromosome that is frequently passed onto later generations. Third, chemical mutagenesis proved to be efficient due to a balance between toxicity and mutagenic effect (Félix, 2008). Finally, in 1969 John Sulston successfully froze *C. elegans*, which meant that strains could be stored in labs for several years. From then, the *C. elegans* community started to grow rapidly and nowadays there are over a thousand laboratories registered worldwide inwww.wormbase.org, with over 1700 research articles published each year for the last 5 years (Corsi et al., 2015).

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A worm's life

C. elegans is a free-living nematode found worldwide growing in rich soil or compost with decomposing plant material, which provides bacterial food. In nature, *C. elegans* colonizes food sources and goes through four larval stages (L1, L2, L3, and L4) after hatching. When food starts to scarce or the population crowds, individuals can shift during L1 stage to an alternative developmental path, delaying its molt in a pre-dauer stage (L2d) and afterward enters a nonfeeding diapause stage called "Dauer". This stage is crucial for the nematode population as it can endure long periods without feeding, changing its metabolic pathways and stress resistance. It also changes its behavior as it moves faster and stands on its tail (called nictation), making these larvae more susceptible to be carried to another niche, where upon reaching a food source it can escape the dauer stage and reassume the development at L4 stage(Golden & Riddle, 1984). The alternation between life-cycles in the wilderness, coupled with environmental changes through seasons, gives a boom-and-bust behavior to the *C. elegans* population (Frézal & Félix, 2015).

In the laboratory, the boom-and-bust cycle is lost as worms are grown with plenty of food and are transferred to new plates once food has been depleted or the population starts to crowd. The worms are grown between 15 and 25°C and go through the four larval stages, reaching fertile adulthood in three days at 22°C (Fig. 1). Its size, ranging from 0.25mm at the first larval stage to 1mm at the adult stage, means that worms are usually observed in Petri dishes with solid agar under dissecting microscope as they move, eat, develop and lay eggs. Compound or confocal microscopes are used for much finer resolutions and address observation at the cellular level. As *C. elegans* is transparent, many of its structures can be directly seen. Moreover, its constant number of cells (956 cells) meant working with individual cells was easy to achieve and even individual cells and subcellular details can be visualized. Details can be enhanced using fluorescent proteins as tags, allowing the study of developmental processes, easy screen for mutants, isolating cells, and characterizing protein interactions in vivo (Boulin et al., 2006; Chalfie et al., 1994; Feinberg et al., 2008; Husson et al., 2013; Kerr & Schafer, 2006). One of the main goals of Brenner was to identify individual neurons and their synaptic connections. Thanks to electron microscopy and new technology tools, something not found in any other organism was achieved, mapping the neuronal connections for the 302 neurons found in the hermaphrodite, making it the first connectome. This was also recently achieved for the male worms (White, 1986; Cook et al., 2019; Jarrell et al., 2012).

The male population is scarce (0.1-0.2%) and rises under unfavorable conditions, increasing offspring and generating different genetic compositions. Therefore, hermaphrodites reproduce mainly by self-fertilization (often referred to as "selfing") and not by mating with other hermaphrodites; as Sydney Brenner said "the animals are driven to homozygosity" (Brenner, 1974), making mutant strains isogenic. Selfing reduces the effort needed to find a mutant and simplifies maintaining stocks, as a single animal can give rise to an entire population (Corsi et al., 2015).

C. elegans was the protagonist of another research milestone, as it was the first eukaryotic multicellular organism to have its genome fully sequenced, unlocking new interesting findings and genetic tools (C. elegans Sequencing Consortium, 1998).

The discovery of RNA interference in 1998 was achieved by injecting double-stranded RNA into worms (Fire et al., 1998). Afterward, other techniques were developed as soaking worms in dsRNA and feeding worms bacteria engineered to produce dsRNA also could induce a robust RNAi response (Tabara et al., 1998; Timmons and Fire, 1998). Feeding proved to be an easy and effective method that is commonly used in genetic studies and two libraries of RNAi bacteria

strains were developed in mass silencing experiments, the library of Ahringer lab and the Vidal lab (Kamath et al., 2003; Rual et al., 2004).

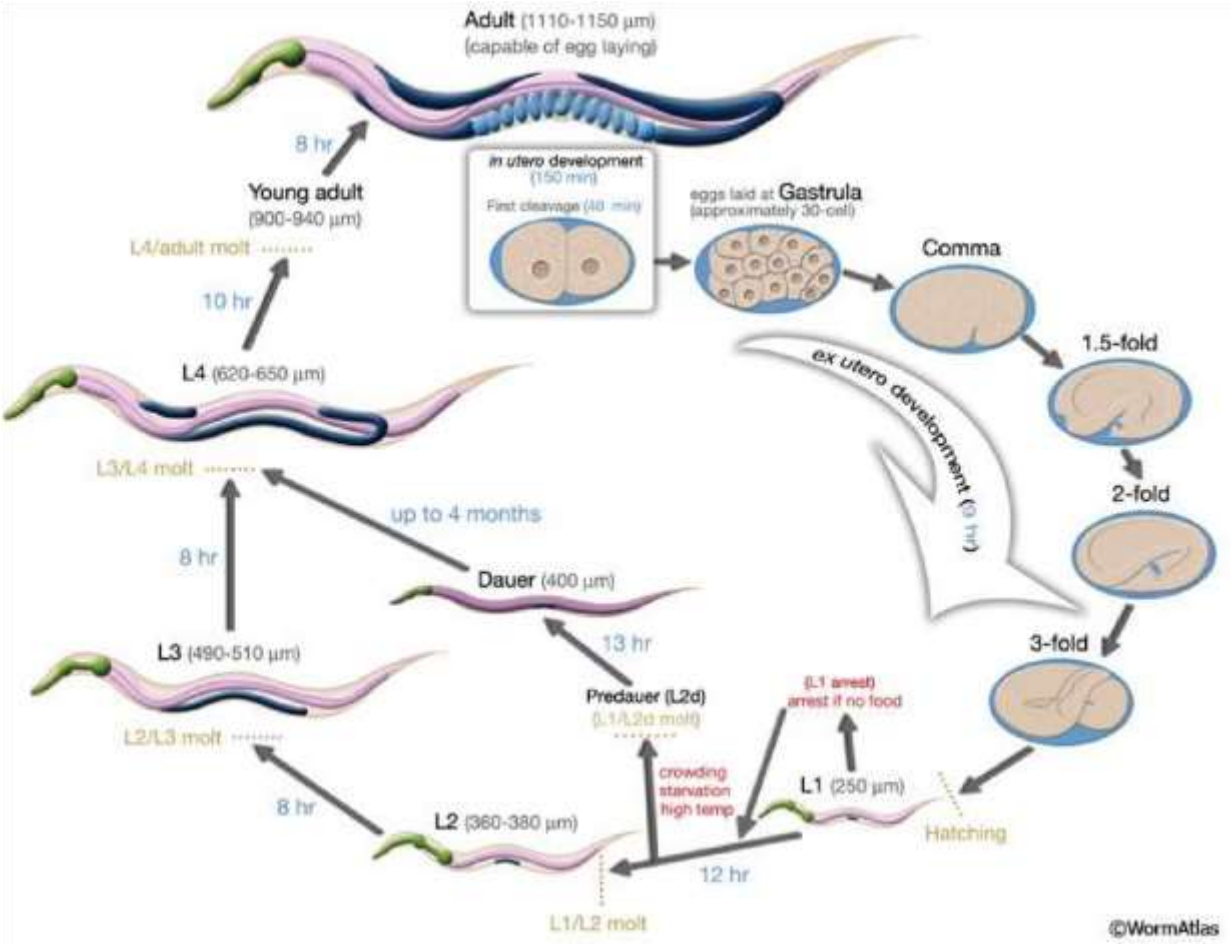

Figure 1. The life cycle of C. elegans at 22°C. 0 min is fertilization. Numbers in blue along the arrows indicate the length of time the animal spends at a certain stage. The length of the animal at each stage is marked next to the stage name in micrometers (µm).

The many fields of *C. elegans* (*C. elegans* community)

Although the *C. elegans* research started with neurology and development, many other fields have expanded with the help of this nematode. For example, we have parasitology, evolution, and ecology, cell biology, biochemistry and metabolism, aging, stress, model of human diseases, drug discovery, and behavior. The first critics of the model were that its behavior was simplistic for a neurobiological study, that it only knew how to move forward and backward. But *C. elegans* proofed them wrong, as it has many sensorial features and new are being discovered.

Although work in *C. elegans* covers many different fields, with lots of laboratories across the globe, it is still a friendly and close community. With International Worm Meetings every two years a good flow of homebrew methods, research data, and exchange of strains is guaranteed. There are also region-specific meetings in Latin American, Europe, India, Asia-Pacific (https://wormmeetings.weebly.com/). This welcoming and open spirit is also seen in the early beginnings of *C. elegans* work, with the "Worm breeders Gazzette" being published in 1975, whose intention was to publish the different methods that were being developed for the work in

C. elegans, months before the publication was finished; making them rapidly available to other labs (The et al., 2009).

The worm in the web

With the advancement of technology, not only new techniques were available to study the biology of *C. elegans*, but new ways of registering and reaching data rose with the coming of the World Wide Web; and this worm was also caught in it. Many platforms were developed to spread the information or make available specific tools to analyze the work done in *C. elegans*, but they can be classified into two types, "portals" and "knowledge environments", where the first organizes links relevant to the domain of interest while the second offers direct information and services. The main and most used sites are the Wormbook, the Wormbase, the Wormatlas, the Caenorhabditis Genetics Center (CGC), and the "Worm breeders Gazzette" previously mentioned, but there are many others. (Lee, 2005)

Wormbook is an open-access collection of original, peer-reviewed chapters that provides basic information about the biology of *C. elegans* and other nematodes. It also has WormMethods, with a collection of protocols, and WormHistory, which is a compilation of personal perspectives, lectures, journals, and ideas. (http://www.wormbook.org/index.html)

WormBase is an international consortium of biologists and computer scientists providing the research community with accurate, current, and accessible encyclopedic information concerning the genetics, genomics, and biology of *C. elegans* and related nematodes. It also provides useful bioinformatics tools for genetic analysis and browsing, providing easy to access curated information about anatomy, genes, papers, phenotypes proteins, mutant strains, etc. Moreover, it presents a register of the *C. elegans* lab community, making it easier to contact other researchers. Founded in 2000, the WormBase Consortium is led by Paul Sternberg (CalTech), Matt Berriman (The Wellcome Trust Sanger Institute), Kevin Howe (EBI), and Lincoln Stein (The Ontario Institute for Cancer Research). WormBase is a founding member of the Alliance of Genome Resources Project. (https://wormbase.org)

WormAtlas is a database featuring behavioral and structural anatomy of *C. elegans*, linking to handbooks, images, and movies, slideable anatomical cuts of the worm, a map of the neuronal wiring, and more available tools to visualize and comprehend the biology of the model. It is a great source of visual content and information. (https://wormatlas.org/)

From the University of Minnesota – USA, the Caenorhabditis Genetics Center (CGC) gathers the nomenclature, strain collection, purchase and distribution, and the genetic map construction. Here mutant strains of *C. elegans*, wild isolates, and bacterial strains for feeding can be purchased for a fee. The C. elegans Gene Knockout Consortium has created a knockout of approximately 21000 individual genes in the *C. elegans* genome, all available for purchase and study. (https://cbs.umn.edu/cgc/home)

There are many more cites that address specific fields of research, different projects, tools, and resources that are not presented here but can be easily accessed by the ones mentioned.

How to start your own *C. elegans* laboratory

All the methods, techniques, and recipes needed to start working in *C. elegans* are available at the Wormbook site, in the section "Worm Methods", Maintenance of *C. elegans* (Stiernagle, 2006). Nevertheless, from a protocol to the lab bench there will always be a distance to jump, and working with this model comes with the day-to-day struggles of a biologic organism. Here we will summarize the basic infrastructure and supplies needed, adding a few suggestions for beginners. To start a laboratory in *C. elegans*, you will need:

- An incubator capable of reaching temperatures between 15-25°C. The usual temperature for growing is 20°C, as the worms grow comfortably and reaches adulthood in three days. Although worms can be maintained at room temperature, an incubator gives a controlled environment suited for many experiments. Apart from this, having an incubator at 15°C is useful for medium-term storage, as worms grow slower and food lasts longer; this also gives flexibility to accelerate or slow the worms cycle, for example, to obtain a certain stage of development for an experiment.
- Stereoscopic microscope for checking plates, seeing how the worms are growing, and detecting any contamination. Every stage of development can be seen with an approximate magnification of 20X and easy observation analysis can be done to detect deficiencies in movement, morphology, behavior, and development.
- Petri dishes and NGM media. 60mm Petri dishes are the most commonly used but bigger ones can be used if larger populations are required; 30mm Petri dishes are frequently used in experiments that use expensive reagents and are easier for working with smaller populations like is the case of RNAi experiments. NGM is the basic solid media for growing worms, easy to make, with a few extra saline sterile buffers and cholesterol that has to be added after the media is autoclaved. After plates are poured they can be dried under a hood cabin, which reduces humidity in the plates diminishing the possibility of contamination. Plates can be stored either at room temperature or at 4°C and NGM media can be stored after sterilization but not after adding the buffers, as it cannot be reheated.
- Food. OP50 bacteria is the most widely used *E. coli* strain for feeding the worms and it has a mutation that limits its growth, which facilitates the observation under the microscope. Bacteria can be grown in LB media with antibiotics at 37°C and then put a drop in each plate, letting it dry afterward (Fig. 2). This will leave a spot of bacteria (preferably in the center of the plates) and clear agar around it, facilitating the visualization of the worms. Apart from OP50, there are many other strains used for food, but it is important to read how it affects worm growth and to be consistent and use the same bacteria through experiments.

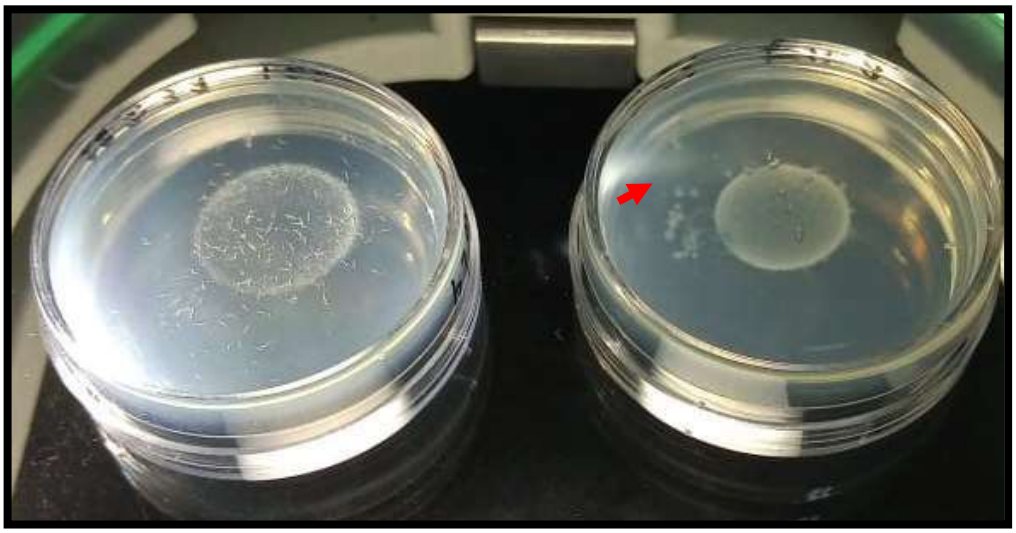

Figure 2. Two plates with NGM and a spot of OP50 bacteria (red arrow). In the left one, adults canbe seen in plain sight mainly in the spot of food, but there is no adult on the right one.

- Extensive workspace is not a must, but certain protocols require sterile environments like under a Bunsen burner or biosecurity hood cabin. Glass alcohol burners are a practical option to use next to the microscope.
- Ultrafreezer at -80° or liquid Nitrogen. As each generation of worms passes, mutations could be accumulated. It is important to remember that these worms are hermaphrodites with self-fertilization. This would affect the original strain genome and, in consequence, the results of experiments and observations carried. To avoid this, it is important to store any strain received, which can be done by collecting early larval stages and freezing them with the proper Freezing Buffer.
- Spatula and worm picker. Transferring worms from one plate to another is a crucial dayto-day practice for maintaining and expanding the worm population. There are two simple methods to achieve this, Chunking and Picking. Chunking is done from a plate, usually a starved or nearly starved plate with crowded worms, cutting a small square of agar using a sterilized small spatula and placing it in a fresh plate; this method allows moving bulk of worms from different stages and getting a decent population in a few days. The other method consists of moving one worm at a time using the worm-picker, a thin flattened platinum wire attached to a Pasteur pipette; touching a worm and pinking it up, and then moving it to a new plate and allowing it to leave the pick.

On a side note, contaminated plates are the main issue when maintaining *C. elegans* strains. Measurements to work properly, on a clean bench with sterile supplies, must be taken. Nevertheless, fungus and other bacteria will grow on plates eventually. However, they can be saved if contamination is not excessive. Worms that are not close to the contamination can be picked and placed in new plates. Contamination can be chunked out and removed from the plate with a spatula. As a last resource, plates can be bleached (See bleaching protocol in the WormBase site) killing contamination and all worm stages except for the eggs that can then be placed in a fresh plate; this can be also used as a method to synchronize the population (Stiernagle, 2014).

Concluding remarks

The work in *C. elegans* is an ever-expanding world with many different lines of research, new techniques being developed, and novel discoveries; with still many questions to be answered, despite the abundance of investigation done and achieved. Nevertheless, *C. elegans* remains to be humble and easy to approach model, with cheap maintenance, many resources available and flexible techniques that can be adapted to each researcher´s need.

References

Altun ZF and Hall DH 2009. Introduction. In WormAtlas. doi:10.3908/wormatlas.1.1

Boulin T, Etchberger JF & Hobert O. (2006). Reporter gene fusions. *WormBook : The Online Review of C. Elegans Biology*, 1–23. https://doi.org/10.1895/wormbook.1.106.1

Brenner S. The genetics of Caenorhabditis elegans. Genetics. 1974 May;77(1):71-94. doi: 10.1093/genetics/77.1.71. PMID: 4366476; PMCID: PMC1213120.

Brenner S. 1988 *Foreword. The Nematode Caenorhabditis elegans,* edited by W. B. Wood, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Brenner S. (2003). Nature's gift to science (Nobel lecture). *ChemBioChem*, *4*(8), 683–687. https://doi.org/10.1002/cbic.200300625

C. elegans Sequencing Consortium, 1998 Genome sequence of the nematode *C. elegans*: a platform for investigating biology. Science 282: 2012-2018.

Chalfie M, Inouye S, Tsuji Frederick I, Tu Y, Euskirchen G, Ward William W & Prasher Douglas C (1994). Expression of the gene and fluorescence characteristics of the recombinant protein Green fluorescent protein as a marker for gene expression. *Fed. Eur. Biochem. Letters*, *341*(5148), 277–280.

Cook SJ, Jarrell TA, Brittin CA, Wang Y, Bloniarz AE, Yakovlev MA, Nguyen KCQ, Tang LTH, Bayer EA, Duerr JS, Bülow HE et al. (2019). Whole-animal connectomes of both Caenorhabditis elegans sexes. *Nature*, *571*(7763), 63–71. https://doi.org/10.1038/s41586-019-1352-7

Corsi AK, Wightman B & Chalfie M. (2015). A Transparent Window into Biology: A Primer on Caenorhabditis elegans. *Genetics*, *200*(2), 387–407. https://doi.org/10.1534/genetics.115.176099

Feinberg EH, VanHoven MK, Bendesky A, Wang G, Fetter RD, Shen K & Bargmann CI. (2008). GFP Reconstitution Across Synaptic Partners (GRASP) Defines Cell Contacts and Synapses in Living Nervous Systems. *Neuron*, *57*(3), 353–363. https://doi.org/10.1016/j.neuron.2007.11.030

Félix MA. (2008). RNA interference in nematodes and the chance that favored Sydney Brenner. *Journal of Biology*, *7*(9), 5–9. https://doi.org/10.1186/jbiol97

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE & Mello CC. (1998). 35888. *Nature*, *391*(February), 806–811. https://www.nature.com/articles/35888.pdf

Frézal L & Félix MA. (2015). C. elegans outside the Petri dish. *ELife*, *4*, 1–14. https://doi.org/10.7554/eLife.05849

Golden JW & Riddle DL. (1984). The Caenorhabditis elegans dauer larva: Developmental effects of pheromone, food, and temperature. *Developmental Biology*, *102*(2), 368–378. https://doi.org/10.1016/0012-1606(84)90201-X

Husson SJ, Gottschalk A & Leifer AM. (2013). Optogenetic manipulation of neural activity in C. elegans: From synapse to circuits and behaviour. *Biology of the Cell*, *105*(6), 235–250. https://doi.org/10.1111/boc.201200069

Jarrell TA, Wang Y, Bloniarz AE, Brittin CA, Xu M, Thomson JN, Albertson DG, Hall DH & Emmons SW. (2012). The connectome of a decision-making neural network. *Science*, *337*(6093), 437–444. https://doi.org/10.1126/science.1221762

Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le Bot N, Moreno S, Sohrmann M et al. (2003). Systematic functional analysis of the Caenorhabditis elegans genome using RNAi. *Nature*, *421*(6920), 231–237. https://doi.org/10.1038/nature01278

Kerr RA & Schafer WR. (2006). Intracellular Ca2+ imaging in C. elegans. *Methods in Molecular Biology (Clifton, N.J.)*, *351*(3), 253–264.

Lee R. (2005). Web resources for C. elegans studies. *WormBook : The Online Review of C. Elegans Biology*, 1–16. https://doi.org/10.1895/wormbook.1.48.1

Rual JF, Ceron J, Koreth J, Hao T, Nicot AS, Hirozane-Kishikawa T, Vandenhaute J, Orkin SH, Hill DE, van den Heuvel S et al. (2004). Toward improving Caenorhabditis elegans phenome mapping with an ORFeome-based RNAi library. *Genome Research*, *14*(10 B), 2162–2168. https://doi.org/10.1101/gr.2505604

Stiernagle, T. (2006). Maintenance of C. elegans. *WormBook : The Online Review of C. Elegans Biology*, *1999*, 1–11. https://doi.org/10.1895/wormbook.1.101.1

Stiernagle, T. (2014). Maintenance of Caenorhabditis elegans. *International Journal of Biomedical and Health Sciences*, *10*(2), 55–67. http://www.asopah.org

Tabara H, Grishok A & Mello CC. (1998). RNAi in C. elegans: Soaking in the Genome sequence. *Science*, *282*(5388), 430–431. https://doi.org/10.1126/science.282.5388.430

The C, Generation N & Nelson G. (2009). THE WORM BREEDER'S GAZETTE Vol 18-1. *The Worm Breeder'S Gazette*, *18*(1).

Timmons L, Fire A. Specific interference by ingested dsRNA. Nature. 1998 Oct 29;395(6705):854. doi: 10.1038/27579. PMID: 9804418.

White JG, Southgate E, Thomson JN and Brenner S. 1986 The structure of the nervous system of *Caenorhabditis elegans*. Philos. Trans. *R. Soc. Lond. B Biol. Sci.* 314: 1-340.

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