mostly heart and muscle diseases, as well as cancer. This is not too surprising, as the pathogenesis of these disorders is often characterized by the impairment of certain mechanical processes (e.g. altered contractility or abnormal motility). A key question, however, is how these LIM proteins directly contribute. Since their role as cellular mechanosensors is hypothesized to maintain mechanical homeostasis in cells and tissues, it begs the question of whether impaired mechanosensing underlies their role in these diseases.

Where can I find out more?

Hunter, C.S., and Rhodes, S.J. (2005). LIM-homeodomain genes in mammalian development and human disease. Mol. Biol. Rep. *32*, 67–77.
Ibar, C., Kirichenko, E., Keepers, B., Enners, E., Fleisch, K., and Irvine, K.D. (2018). Tension-dependent regulation of mammalian Hippo signaling through LIMD1. J. Cell Sci. *131*, jcs214700.
Koch, B.J., Ryan, J.F., and Baxevanis, A.D. (2012). The diversification of the LIM superclass at the base of the metazoa increased subcellular complexity and promoted multicellular specialization. PLoS One *7*, e33261.
Matthews, J.M., Lester, K., Joseph, S., and Curtis, D.J. (2013). LIM-domain-only proteins in cancer. Nat. Rev. Cancer *13*, 111–122.
Sala, S., and Ampe, C. (2018). An emerging link between LIM domain proteins and nuclear receptors. Cell Mol. Life Sci. *75*, 1959–1971.
Sala, S., and Oakes, P.W. (2021). Stress fiber strain recognition by the LIM protein testin is cryptic and mediated by RhoA. Mol. Biol. Cell *32*, 1758–1771.
Sang, M., Ma, L., Sang, M., Zhou, X., Gao, W., and Geng, C. (2014). LIM-domain-only proteins: multifunctional nuclear transcription coregulators that interact with diverse proteins. Mol. Biol. Rep. *41*, 1067–1073.
Smith, M.A., Blankman, E., Gardel, M.L., Luettjohann, L., Clare, M., and Beckerle, M.C. (2011). A zyxin-mediated mechanism for actin stress fiber maintenance and repair. Dev. Cell *19*, 365–376.
Smith, M., Hoffman, L., and Beckerle, M. (2014). LIM proteins in actin cytoskeleton mechanoresponse. Trends Cell Biol. *24*, 575–583.
Sun, X., Phua, D.Y.Z., Axiotakis, L., Smith, M.A., Blankman, E., Gong, R., Cail, R.C., Espinosa de Los Reyes, S., Beckerle, M.C., Waterman, C.M., and Alushin, G.M. (2020). Mechanosensing through direct binding of tensed F-actin by LIM domains. Dev. Cell *55*, 468–482.e7.
Wang, F., Zhao, J., Zhang, M., Yang, J., and Zeng, G. (2021). Genome-wide analysis of the mouse LIM gene family reveals its roles in regulating pathological cardiac hypertrophy. FEBS Lett. *595*, 2271–2289.
Winkelman, J.D., Anderson, C.A., Suarez, C., Kovar, D.R., and Gardel, M.L. (2020). Evolutionarily diverse LIM domain-containing proteins bind stressed actin filaments through a conserved mechanism. Proc. Natl. Acad. Sci. USA *117*, 25532–25542.
Yoshigi, M., Hoffman, L.M., Jensen, C.C., Yost, H.J., and Beckerle, M.C. (2005). Mechanical force mobilizes zyxin from focal adhesions to actin filaments and regulates cytoskeletal reinforcement. J. Cell Biol. *171*, 209–215.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Department of Cell and Molecular Physiology, Loyola University Chicago, Stritch School of Medicine, Maywood, IL 60153, USA.
*E-mail: poakes@luc.edu

Quick guide

Siamese fighting fish

Steven J. Portugal

What is a Siamese fighting fish? Siamese fighting fish (*Betta splendens*; Figure 1) belong to the Anabantoidei group, native to the freshwaters of southeast Asia. Often living in oxygen-poor water, they are small (*c*. 7 cm) carnivorous fish that have a long history with humans, with their domestication dating back at least 1,000 years. Today Siamese fighting fish remain one of the most popular species worldwide in the tropical fish aquarium trade, and decades of domestic breeding have created many elaborate colors and fin shapes (Figure 2). Wild male Siamese fighting fish are typically not as brightly colored or long-finned as the selectively bred domestic breeds, with the most common colours being dark red and blue. Females are typically smaller than males, with shorter less-elaborate fins and more subdued coloration. Siamese fighting fish thrive in shallow water with dense vegetation. This vegetation provides cover from predators while also supporting the invertebrate communities upon which they feed. It is likely the predominance of paddy fields in Southeast Asia contributed to our long history with this species, as Siamese fighting fish thrive in the conditions under which rice is grown. An unusual feature of all members of the Anabantoidei is the presence of an air-breathing labyrinth organ, akin to a lung, that allows them to obtain oxygen by taking gulps of air at the surface of the water. Siamese fighting fish are facultative air breathers, and if access to the water surface is restricted, they will drown. Their Latin name, *Betta splendens*, indicates what has made this species so famous around the world: *bettah* in Malay refers to an ancient warrior tribe. Male Siamese fighting fish are extremely territorial and engage in a series of aggressive displays when in combat with another male. These displays are intense and result in a substantial increase in metabolic rate, and unusually, can in some instances involve fights to the death of one opponent. It is this interaction between physiology and behavior, and the reliability of their aggressive responses, that has made Siamese fighting fish such a popular study species.

What happens when they fight? One of the most frequent displays of aggression in male Siamese fighting fish is the flaring of the opercula (Figure 2), whereby the fish spread out their gill covers. This flaring creates an illusion of greater size, while also being an honest signal about the condition of the male. While flaring the opercula, ventilation of the gills is reduced, akin to the fish holding its breath. The duration of opercular flare displays largely determines who wins an aggressive encounter, with the victor being the individual who flared for the longest. If Siamese fighting fish are kept in water with extremely low oxygen concentrations, opercular flaring is significantly reduced, suggesting a link between the duration of breath holding and body condition. Oxygen demands increase significantly during these energetic displays, yet the fish are unable to extract any more oxygen from the water than they can when they are at rest, due to their reduced gill surface area. This means the fish can only meet their increased oxygen requirements during combat via more air-breathing from the surface of the water. To make matters more complicated for the fish, they are unable to increase oxygen uptake per breath — they can't just take deeper breaths — thus the only solution is to take more breaths at the surface, at each visit. This creates a problem when two fish are dueling underwater; how do you ensure enough trips to the surface, without being attacked by your rival? Siamese fighting fish have evolved an unusual system of stereotyped synchronous surface breathing, whereby one of the fish will lead the other to the surface and will take a breath of air.

Why is their behavior and physiology so extreme? Their extreme behavior is linked to their reproduction. Male

Figure 1. Male Siamese fighting fish flaring their opercula.
Photo: Huy Lam.

Siamese fighting fish undertake all parental care duties and the female departs immediately after spawning, being chased away by the male. Males build an elaborate bubble nest at the surface of the water that can contain over 5,000 bubbles, each one individually created by the male. The male displays under his nest to a potential female in a similar fashion to how they interact with other males, with opercular flaring and vigorous body movements and fin extensions. Females frequently eavesdrop on two males dueling and will select the winner of the fight to breed with. When the female is ready to spawn, the male wraps himself around her in an embrace, prompting the female to release the eggs. These eggs are then collected by the male in his mouth and placed in the bubble nest. While the eggs develop, the male does not eat, as he guards the eggs and fans the bubble nest to ensure an adequate supply of oxygen. If any eggs or fry fall from the nest, the male collects them in his mouth and delicately places them back within the bubbles. The bubble nests can be quite delicate, and calm waters are required for breeding. It is likely that the paucity of suitable breeding sites, coupled with a relatively short lifespan, is what drives male Siamese fighting fish to be so aggressive.

What is our history with the species? Siamese fighting fish have played an important cultural role in many countries throughout Southeast Asia, and they are the national aquatic animal of Thailand. Originally, Siamese fighting fish were bred for their fighting tendencies with people gambling on the outcome of these duals, much like cockfighting. Around 1830, King Rama III of Siam (Thailand) presented Danish zoologist Theodore Cantor with a specimen, and the first Siamese fighting fish arrived to Europe in 1874 in France. While selective breeding in Asia had focused on heightening the aggression of the species, in Europe the emphasis was more on beauty for ornamental purposes. By the 1920s the first formal breeds were described, with at least 70 domesticated breeds now recognized today. In the wild, *Betta splendens* is now listed as vulnerable, due to interbreeding with domesticated fish which have been released into the wild, coupled with pollution and habitat destruction.

***What is left to learn from* Betta splendens?** While remaining a popular model species in ethology, in more recent times Siamese fighting fish have become notable for their hormonal responses to aquatic pollutants. Agricultural toxins and estrogen, much of which is inadvertently released into sewers through the urine of women taking birth control pills, are influencing the behavior of male Siamese fighting fish. These pollutants appear to dampen the male's aggressive territorial responses, negatively alter how they display to females and increase the likelihood of fathers consuming their own eggs. Siamese fighting fish are becoming a problematic invasive species in South America, Australia and the United States. Their ability to survive in low-oxygen water makes them able to outcompete other species, and in Australia, they are posing a threat to native fish and invertebrates.

Where can I find out more?

Alton, L.A., Portugal, S.J., and White, C.R. (2013). Balancing the competing requirements and display-behavior during male-male interactions in Siamese fighting fish, *Betta splendens*. Comp. Biochem. Physiol. *164*, 363–367.
Castro, N., Ros, A.F.H., Becker, K., and Oliveira, R.F. (2006). Metabolic costs of aggressive behavior in the Siamese fighting fish, *Betta splendens*. Aggressive Behav. *32*, 474–480.
Hammer, P.H., Michelle, N., Simoes, S., Needham, E.W., Wilson, D.N. Barton, M.A., and Lonza, D. (2019). Establishment of Siamese fighting fish on the Adelaide River floodplain: the first serious invasive fish in the Northern Territory, Australia. Biol. Invas. *21*, 2269–2279.
Jaroensutasinee, M., and Jaroensutasinee, K. (2001). Bubble nest habitat characteristics of wild Siamese fighting fish, *Betta splendens*. J. Fish. Biol. *58*, 1311–1319.
Kang, C.K., and Lee, T.H. (2010). The pharyngeal organ in the buccal cavity of the male Siamese fighting fish, *Betta splendens*, supplies mucus for building bubble nests. Zool. Sci. *27*, 861–866.

Figure 2. Siamese fighting fish breeds.
Male Siamese fighting fish flaring behaviour (top), and a number of different breeds and colours.

Kramer, D.L., and Graham, J.B. (1976). Synchronous air-breathing, a social component of respiration in fishes. Copeia *1976*, 689–697.
Meliska, C.J., Meliska, J.A., and Peeke, H.V. (1980). Threat displays and combat aggression in *Betta splendens* following visual exposure to conspecifics and one-way mirrors. Behav. Neural. Biol. *28*, 473–486.
Rüber, L., Britz, R., and Zardoya, R. (2006). Molecular phylogenetics and evolutionary diversification of labyrinth fishes (Perciformes: Anabantoidei). Sys. Biol. *55*, 374–397.
Tate, M., McGoran, R.E., White, C.R., and Portugal, S.J. (2017). Life in a bubble: the role of the labyrinth organ in determining territory, mating and aggressive behaviours in Anabantoid fish. J. Fish. Biol. *91*, 723–749.
Verbeek, P., Iwamoto, T., and Murakami, N. (2008). Variable stress-responsiveness in wild type and domesticated fighting fish. Physiol. Behav. *93*, 83–88.

DECLARATION OF INTERESTS

The author declares no competing interests.

Department of Biological Sciences, School of Life and Environmental Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK.
E-mail: Steve.Portugal@rhul.ac.uk

Primer

Riboswitches

Hubert Salvail[1]
and Ronald R. Breaker[1,2,3,]*

Riboswitches are structured noncoding RNA domains that are typically found embedded in messenger RNAs, where they sense specific target molecules or elemental ions and regulate gene expression. These RNAs thus serve as genetic switches that can activate or repress gene expression in response to changing levels of their target ligand. To many observers, riboswitches might seem like rare oddities that are not as sophisticated as, or competitive with, the various protein factors that perform these same roles. However, as the number of experimentally validated riboswitch classes increases, and their true biochemical sophistication is recognized, it is becoming clearer that many species from all three domains of life entrust RNAs to make important chemical sensing and gene control decisions without the necessary participation of protein factors.

To date, more than 55 riboswitch classes have been experimentally validated, and the ligands they sense comprise a diverse list of biologically relevant elemental ions and fundamental metabolites that are commonly derived from RNA nucleotides or their precursors. This notable bias in the ligand specificities strongly suggests that the most common riboswitches of today's organisms are descendants from ancient versions that first evolved in the 'RNA World', which is a proposed era of life when chemical transformations and molecular sensing were carried out predominantly by enzymes and receptors made of RNA.

Thus, by more closely examining the functions, structures, and mechanisms of riboswitches, it becomes possible to look back in time to understand more deeply how organisms of the RNA World survived, and even thrived, without the help of protein factors that have come to dominate these functions in modern organisms. This can reveal how a relatively simple nucleic acid polymer with only four common nucleotide components can