Chapter 11

New Technologies for Monitoring Marine Mammal Health

Annalaura Mancia
University of Ferrara, Ferrara, Italy

INTRODUCTION

Traditionally, biology was a descriptive discipline, and that is how the biology of marine mammals has also been for a long time. In his work *Historia Animalium*, Aristotle was the first to make many pertinent observations about dolphins, including the fact that they would bear their young alive, suckle them, breathe air, and communicate by underwater sounds. From his descriptions and writings, we know that in the 4th century BC there were dolphins in the Mediterranean and porpoises in the Black Sea. Accurate natural history observation on the biology of marine mammals expanded throughout the time associated to morphologic descriptions in the pre-1900s and followed by description of behavior and distribution during the times of hunting and whaling activities. Studies of life history patterns, habitat use, and behavior in captivity or in nature started only in the second half of the last century, slowly combining aspects of mammalogy, ethology, ecology, conservation, evolutionary biology, and, finally, molecular biology.

Molecular biology is the field of biology that studies the composition, structure, and interactions of the most important macromolecules for each living organism, such as nucleic acids (DNA and RNA) and proteins that carry out the biologic processes essential for each cell's functions and maintenance. The DNA contains the genes that determine how the individual organism will be. The therapeutic control of a medical condition is generated from the basic concept that genes make proteins that catalyze a biochemical reaction and control the phenotype of the organism. Thus, the understanding of DNA and the identification of gene and protein defects responsible for a specific disease is very important in maintaining the health of an organism.

The method of dissecting biologic systems into their constituent parts, known as reductionist method, has been very useful in explaining the chemical basis of

numerous living processes and has been largely responsible for the amazing progress seen in biology during the past five decades or so. However, biologic systems are extremely complex and have emergent properties that cannot be explained, or even predicted, by studying a gene at the time. New experimental techniques for investigating the unique complexity of biologic systems that results from the diversity of interactions and regulatory networks were, all of a sudden, a necessity.

Recent developments in high-throughput nanotechnologies and bioinformatics have enabled the examination of biologic systems in remarkable detail, providing the data that molecular biologists need to simulate the behavior of complex biologic networks and systems. We can now monitor thousands of molecules simultaneously and generate real-time pictures of any biologic system in any condition. The opportunity to assess the expression of hundreds to thousands of genes, proteins, or metabolites simultaneously has been made possible by the development of the "omics" technologies.

THE OMICS TECHNOLOGIES

Omics technologies aim at understanding a complex system, considered a whole. Therefore, in a specific biologic sample, the universal detection of genes is called *genomics*, of the totality of the mRNA is called *transcriptomics*, the study of the entire set of peptides and proteins is called *proteomics*, and that of all the intermediate products of metabolism is called *metabolomics*. Taking advantages of the new technologies, many other applications can be added to the list of these major omics technologies, generated by the necessity of deepening information on a specific mechanism or/and at a specific level.

The integration of all the omics technologies is characterized by the generation of an enormous amount of data that can be interpreted only by the involvement of many scientific disciplines (e.g., biology, computer science, engineering, bioinformatics, physics) (Chen et al., 2010). The result aims at deciphering systems in their complexity, at predicting how they can change over time and conditions, and at proposing solutions to health and environmental issues (Fig. 11.1).

Genomics

Genomics is an area that concerns the sequencing and analysis of an organism's genome. The cost of genome sequencing has gone down drastically from the 3 billion dollars used for the first human genome sequencing in the beginning of this century to about 3 thousand dollars in the year 2016 (and probably the half of that by the end of 2017), resulting in the establishment of the size and sequence of the genome of over a thousand species of organisms. Such knowledge of genome sequence has been useful in many ways. For example, we know that the minimum number of genes to sustain life by a bacterium is only 260. It also led to the surprising understanding that humans carry only 20,000 genes, against the previous guess that humans may have up to 100,000
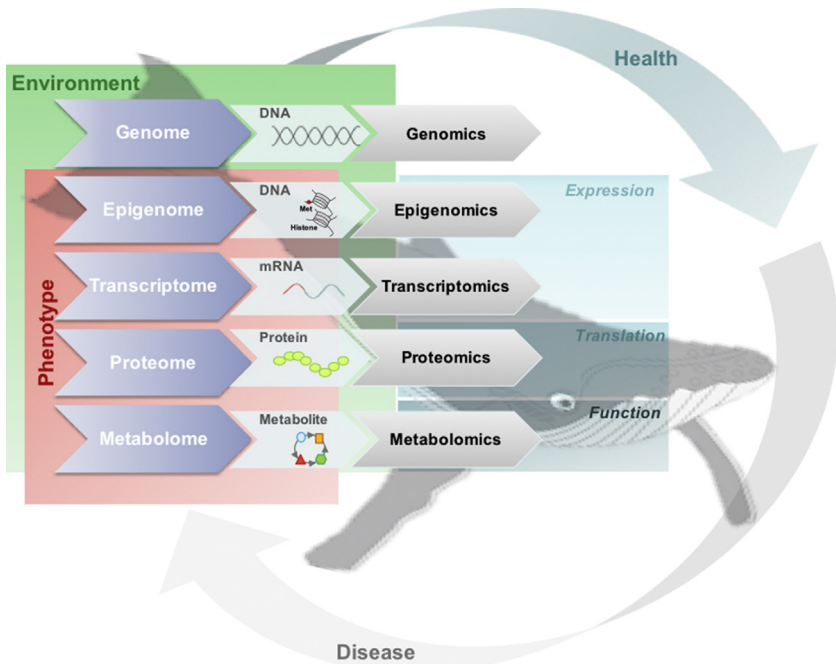
FIGURE 11.1 **Outline of the omics sciences describing the complexity of systems.** The omics profile of each individual is characterized by its biologic molecules that translate into the structure, function, and dynamics of the organism. Genome, epigenome, transcriptome, proteome, and metabolome are linked to the phenotype; they can change over time and conditions, in relation to health and environmental issues.

genes. The sequencing of the entire DNA of an organism is called a whole genome sequence (WGS). A WGS project involves the sequencing of DNA, the assembly of that sequence to create a representation of the original chromosome, and the annotation and analysis of that representation, with an emphasis on significance and function. It aims at the collective characterization of coding and noncoding DNA regions, their structure, function, and evolution. It reveals the complete DNA makeup of an organism, enabling the better understanding of the variations both within and between species, allowing an accurate differentiation between organisms. Comparisons of genome sequences from different individuals have led to the establishment of copy number variation with over 10 million single-nucleotide polymorphisms (SNP). SNPs can provide a genetic fingerprint for use in identity testing and are found to be involved in the etiology of many diseases. Several studies have demonstrated the diagnostic utility of WGS, for example, in mutation detection (Lupski et al., 2010; Herdewyn et al., 2012; Bae et al., 2014) or in the identification of clinically relevant variants in ~40% of pediatric populations with autism (Weedon et al., 2014) and ~60% of those with intellectual disability (Gilissen et al., 2014).

Transcriptomics

Transcriptomics is the study of the transcriptome: the complete set of transcripts in a cell and their quantity in a specific developmental stage or physiologic condition. Transcriptomics studies RNA in any of its forms: mRNA, rRNA, tRNA, and other noncoding RNA produced in one or a population of cells. The term can be applied to the total set of transcripts in a given organism, or to the specific subset in a particular tissue. Currently, there are two key techniques used for transcriptomic analysis: microarrays, which quantify a set of predetermined sequences, and RNA sequencing, which uses high-throughput sequencing to capture all sequences.

Differently from the genome, the transcriptome can vary with external environmental conditions. Generally transcriptomic studies are referred to expression profiling studies, examining the expression level of mRNAs in a given cell population. Transcriptome analysis allows us to understand the expression of a genome at the transcription level, which provides information on gene structure, regulation of gene expression, gene product functions, and genome dynamics. Understanding the transcriptome is essential for interpreting the functional elements of the genome and revealing the molecular constituents of cells and tissues, and also for understanding development and disease. Transcriptome analysis can be carried out at the resolution of single cells, a powerful strategy to connect gene expression networks, cell lineage, and phenotype of individual cells and to study complex disease such as cancer, as well as other biologic phenomena such as tissue regeneration, embryonic development, and immune response (Liu and Trapnell, 2016; Kanter and Kalisky, 2015). Comparison of transcriptomes allows the analysis of interspecies differences (Shay et al., 2013), the identification of genes that are differentially expressed in distinct cell populations, across tissues and individuals (Mele et al., 2015), in response to an injury (Khan et al., 2017) or to different treatments (Datta et al., 2016). Transcriptome sequencing can evaluate absolute transcript levels of sequenced and unsequenced organisms, detect novel transcripts and isoforms, and reveal sequence variations and splice variants. Affordable and fast, it is the most informative assay to start with, offering an overview of the expressed genes to guide subsequent analyses (carried through proteomics, metabolomics, and other methods).

The combination of genomics and transcriptomics is called functional genomics. Functional genomic studies aim at deciphering the connection between phenotypes and genotypes and have brought a revolution in the fields of medicine and modern biology.

Proteomics

Proteomics is the study of the proteome, the totality of the proteins in a cell, tissue, or organism and their identity, their biochemical properties, and functional roles. Differently from genomics and transcriptomics, propelled by advancement in sequencing technologies, proteomics has been driven by advances in

mass spectrometry (MS) and other techniques that allow the analysis of a large number of protein samples at low cost (e.g., 2D gel electrophoresis). Proteomics is the study of how protein quantities, modifications, and structures change during development and in response to internal or external stimuli. The proteome of an organism is much larger and complex than its genome. Contrarily from the genome, the proteome changes constantly: DNA in organisms is essentially constant throughout their lives, while the kinds and amounts of proteins that are synthesized at any instant are subject to much variation. Proteins are continuously made, modified, and eliminated; many genes encode for more than one version of a protein, and a protein can be modified differently in response to cellular stimuli. The field of proteomics investigates which proteins are expressed at what stages in an organism's life and exactly how and why these proteins are expressed. Protein controls the structure and function of a cell by facilitating all biochemical reactions. Proteomics contributes to improve biomarker translation to modern medicine (Rifai et al., 2006; Veenstra, 2007). Biomarkers are measurable characteristics that reflect physiologic, pharmacologic, or disease processes and can be used to screen an individual for diagnostic and therapeutic purposes. Proteomic-based approaches for biomarker investigation can be employed in different aspects of medicine, to better understand pathways affected in a disease, to identify individuals at a high risk of developing the disease, and to identify individuals who are most likely to respond to specific treatment (Guest et al., 2013). The application goes beyond medicine: biomarkers may, in fact, be the best approach to identify an early response to contaminants (Broeg et al., 2005) and are very sensitive for identifying an organism's stress, although it is not always clear the origin of the stress, and there are often multiple stressors present (Smit et al., 2009). Biomarker responses can be measured in organisms collected from or deployed in field sites to integrate the effects of chemical and nonchemical stressors, reducing the need for complex laboratory exposure scenarios.

The integration of proteomics with genomics and transcriptomics is called proteogenomics. Posttranscription regulation, protein half-life, and posttranslational modification are examples of what defines the proteome dynamics and cannot be deduced from data of functional genomics; consequently, proteomics is a crucial, corresponding methodology to both transcriptomics and genomics.

Metabolomics

Metabolomics refers to the systematic identification and quantification of the metabolome of a biologic system (cell, tissue, organ, biologic fluid, or organism) at a specific point in time. Metabolome refers to low molecular weight organic molecules, intermediates, and products of metabolism, such as hormones, other signaling molecules, and secondary metabolites: like the transcriptome and the proteome, the metabolome is dynamic. Metabolomics is a powerful approach because metabolites and their concentrations directly reflect the underlying

biochemical activity and state of the cell, thus representing best the molecular phenotype. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are the techniques most often used for metabolome profiling.

One of the applications of metabolic profiling studies is in the toxicology field. Metabolomics can detect the physiologic changes caused by toxic insult of a chemical (or a mixture of chemicals) especially of urine or blood plasma samples. The observed changes can be often related to specific lesion in liver or kidney.

A wider application of metabolomics is in the characterization of the interactions of organisms with their environment (environmental metabolomics) (Samuelsson and Larsson, 2008; Viant, 2008). A study can involve individuals to populations, and it can be related to the traditional fields of ecophysiology and ecology, and from an instantaneous effect to those over evolutionary time scales, enabling studies of genetic adaptation (Bundy et al., 2009). Metabolomics has a number of advantages over the other omics approaches. The metabolome is the final product of gene transcription, and therefore, changes in the metabolome are amplified relative to changes in the transcriptome and the proteome (Urbanczyk-Wochniak et al., 2003). The metabolome contains the smallest domain (made up of approximately 5000 metabolites vs. 100,000 proteins in the proteome and 20,000 expressed genes in the genome), but it also contains the most diverse biologic molecules, which can make it more physically and chemically complex than the other "omes."

Epigenomics

Different from the omics technologies listed so far describing the "ome" representing the totality of the principal macromolecules presents in all living organisms, epigenomic studies the modifications of the DNA that do not change its sequence, while changing the way the DNA sequence gives its instructions. The epigenome is made up of chemical compounds and proteins that can attach to DNA and can turn genes on or off, controlling the production of proteins in precise cells. These modifications are sometimes passed on from cell to cell as cells divide (mitosis) and can be passed down from one generation to the next (meiosis); hence, they are heritable. The epigenome can also change throughout a person's lifetime. Adjustments to next-generation sequencing protocols to enrich for the DNA regions carrying modifications (e.g., bisulfite treatment or antibody-based methods) are applied to the study of epigenetic processes. One type of modification is called DNA methylation, where methyl groups attached to the bases of the DNA molecule turn genes on or off. A second type is called histone modification, where chemical tags are attached to the histones (DNA-wrapping proteins that enable the DNA to be wound up into chromosomes), giving information about regions in the DNA that should be used or ignored.

Usually, the modifications occur as a natural process of development and tissue differentiation, but they can be altered in response to environmental

exposures or disease. Epidemiological evidence increasingly suggests that early life exposures to dietary and environmental exposures can have a profound effect on the epigenome, resulting in birth defects and diseases developed later in life (Dolinoy et al., 2007; Jirtle and Skinner, 2007). The normal role in development and differentiation of epigenetic regulation can be misdirected, leading to a number of diseases including cancer (Jones and Baylin, 2007; Robertson, 2005). The epigenetic alterations are more readily reversible than genetic events, offering potential for the development of therapies (Reamon-Buettner and Borlak, 2007).

Toxicogenomics

Toxicogenomics is the application of genomic technologies (genome sequence analysis, gene expression profiling, proteomics, metabolomics) to study the adverse effects of environmental stressors and toxicants on the organisms and the environment health. The application of transcriptomics, proteomics, and metabolomics enables the study of adverse effects of xenobiotic substances in relation to structure and activity of the genome to understand the role of gene–environment interactions in disease (Heijne et al., 2005). This area promises to have a large impact on many other scientific and medical disciplines, as scientists can now generate complete descriptions of how components of biologic systems work together in response to various stresses, drugs, or toxicants (Gomase and Tagore, 2008). Due to the rapid advent in genomics technologies and attention to ecologic risk assessment, the term "ecotoxicogenomics" has recently emerged to describe integration of omics technologies (i.e., transcriptomics, proteomics, metabolomics, and epigenomics) into ecotoxicologic fields. Ecotoxicogenomics is defined as study of an entire set of genes or proteins expression in nontarget organisms that is important in responses to environmental toxicant exposures, offering benefit in ecologic risk assessment (Kim et al., 2015). While the availability of genomic information about nonmodel organisms is expanding, the application of ecotoxicogenomics to a variety of organisms becomes a powerful tool for evaluating the effects of chemicals on the entire ecosystems (Iguchi et al., 2006; Watanabe and Iguchi, 2003).

OMICS OF THE MARINE MAMMAL WORLD

Many marine mammal species and populations, considered to be most vulnerable to human activities, are endangered or threatened, or designated as depleted worldwide (Endangered Species Act; International Union for Conservation of Nature Species Programme). Marine mammals cannot escape the legacy of the global decline of the ocean health due to anthropogenic impacts such as overfishing, coastal habitat destruction, deep sea mining, oil and gas exploration, release of chemical contaminants, and pollutants from industrial applications (Desforges et al., 2016; Lane et al., 2015; Schwacke et al., 2012; Van Bressem

et al., 2009). Noise can also critically impact marine mammal behavior and fitness (Blair et al., 2016; Ellison et al., 2012; Peng et al., 2015). Moreover, marine mammals are also constantly exposed to natural factors; biotoxins from harmful algae can produce mass mortalities that have devastating effects on population dynamics both in the short and long term (Van Dolah, 2000). Among the consequences, there is an increase in reports of diseases, such as metabolic disorder, opportunistic infections, and population changes in growth, reproduction, and survival (Di Guardo et al., 2011; Gulland and Hall, 2007). Current understanding of the long-term effects of these factors, alone and in combination, has been limited by the lack of comprehensive methodologies and by the protected status of the animals.

The potential of the application of omics technologies to marine environmental science and especially in environmental risk assessment is recognized by many researchers and official organizations (Bozinovic and Oleksiak, 2011; Kim et al., 2015; Kumar and Denslow, 2017; Veldhoen et al., 2012). The application of the omics technologies holds promises toward a significant progress in the understanding of marine mammal health and physiology challenged by a marine environment subject to continuous changes.

Marine Mammal Genome Projects

The pregenomic era was characterized by the Human Genome Project (HGP), an international research effort to determine the sequence of the human genome and to identify the genes that it contains. The first draft was released in 2001 (Lander et al., 2001), and the project was finally completed in 2003, 2.5 years ahead of time, and also significantly under budget, thanks to the advancement in sequencing technology and reduced cost. By the time the HGP began in the late 1990s, the highest estimates put the number of human protein-coding genes at 100,000, and since then the number has continued to shrink. However, at present, the final number of true protein-coding genes in the reference genome lies between 19,000 and 20,000, accounting for 1.5% of the 3 billion base pairs representing the entire genome. Of course, this information led to many unanswered questions that characterize what is presently called "the postgenomic era." The focus is on the discovery and explanation of all the functional elements encoded within the genome sequence, and the comparison of related genomes has emerged as a powerful instrument for genome interpretation. In an effort seeking the identification of functional elements that are conserved across mammals, a project funded by the National Human Genome Research Institute, the bottlenose dolphin has been chosen as one of 24 animals whose genome has been sequenced as part of the comparative genomic annotation (Lindblad-Toh et al., 2011). The first marine mammal genome to be sequenced was sequenced at 2× coverage, with its first version publicly available in 2010. In a 2× coverage, each genomic base is represented in roughly two sequence reads ("2×" redundancy), leaving many gaps in the final sequence due to

statistical fluctuations in read placement, biases in preparative libraries, and difficulties leading to a low-quality assembly. The low coverage has significant effects on the subsequent analyses: the absence of a protein-coding gene, or a disruption of its open reading frame, may represent a deficiency of the assembly or may represent a real evolutionary gene loss. Moreover, low depth can introduce sequence errors that can be propagated, leading to wrong conclusions of a study (Green, 2007). However, a low-redundancy genome, such as the 2× dolphin genome sequenced, is useful to obtain biologic information: partial sequences of most genes and other evolutionarily conserved segments average estimates of mutation rates, as well as a comprehensive assessment of interspersed repeat content including the identification of lineage-specific families. A comparison of about 10,000 protein-coding sequences from the bottlenose dolphin genome with nine other amniotes genomes documented rates of synonymous substitution in the dolphin lineage that were significantly lower than other mammals and equivalent to that of humans and elephants. The dolphin lineage exhibited evidence of positive selection of multiple genes associated with the nervous system, metabolic processes, and glycemic regulation, and others possibly linked to cetacean specializations such as deep diving, blubber, and fat storage. In addition, the dolphin lineage showed a significant increase in selection on genes expressed in the mitochondrion in comparison with other mammalian genomes (McGowen et al., 2012). A deeper analysis using 11,838 high-quality orthologous gene alignments selected from the dolphin and four other terrestrial mammalian genomes identified genes that had undergone positive selection that are significantly enriched in the categories of lipid transport and localization, ATPase activity, sense perception of sound, and muscle contraction, all areas that are potentially related to cetacean adaptations (Nery et al., 2013; Sun et al., 2013). The dolphin genome has been used to obtain information on the independent evolution of echolocation in bats and cetaceans (Parker et al., 2013). More information has been obtained, increasing the coverage of the bottlenose dolphin genome; the analysis revealed that parallel substitutions are widespread in marine mammals, but also that while convergent phenotypic evolution can result from convergent molecular evolution, in the evolution process are more often used different molecular pathways to reach the same phenotypic outcome (Foote et al., 2015; Zhou et al., 2015). Among the whales, a comparative genomic analysis identified the expansion in the whale lineage of gene families associated with stress-responsive proteins and anaerobic metabolism, whereas gene families related to body hair and sensory receptors were contracted (Yim et al., 2014). Further insights into the genomic basis of aquatic adaptations in marine mammals that can be linked to their physiology and health may rely on functional or genomic analyses of noncoding regions, which will be soon achieved thanks to the latest release of novel genomes, first among all the bottlenose dolphin genome with 114.5× coverage. From the current annotation report, the bottlenose dolphin genome seems to contain about 17,000 protein-coding genes in 2.1 billion base pairs. The current coverage is

about 95% versus the 86% of the previously implemented version. The advancement in sequencing technologies, together with the reduced cost, has made possible the sequencing of genomes of several other marine mammals in the last few years. To date, the genomes of several marine mammal species have been annotated and released by the National Center for Biotechnology Information (NCBI) Eukaryotic Genome Annotation Pipeline: five belonging to the order of Cetartiodactyla (*Balaenoptera acutorostrata scammoni*, minke whale; *Lipotes vexillifer*, Yangtze River dolphin; *Orcinus orca*, killer whale; *Physeter catodon*, sperm whale; *Tursiops truncatus*, bottlenose dolphin), three belonging to the order of Carnivora (*Leptonychotes weddellii*, Weddell seal; *Ursus maritimus*, polar bear; *Odobenus rosmarus divergens*, Pacific walrus), and one belonging to the order of Sirenia (*Trichechus manatus latirostris*, Florida manatee).

Omics Findings for Marine Mammal Health

Cetacea (Order: Cetartiodactyla)

Transcriptomics in marine mammal science is unquestionably the omics approach more represented in the literature. The reasons are several, the first being the cost of the experimental procedure: it is extremely less expensive to sequence only the small coding fraction of the genome instead of the whole with best coverage and resolution. Moreover, transcriptomic analysis does not necessarily need a reference genome. Therefore, the transcriptomic approach has been in use for many years now, refined by the advancement in sequencing technologies. Transcriptomic analyses aid discovery of novel gene functions and the connection of molecular and physiologic responses to a large scale of stimuli (ecologic, anthropogenic). The principal methods used are the gene expression microarrays and the more recent RNA-seq. With gene expression microarrays, thousands of genes are analyzed simultaneously in any given sample, thus obtaining a lot of information about the physiologic systems and the impact of environmental challenges. In 2007, the first microarray for marine mammal studies was developed. It was a species-specific cDNA microarray containing 1395 unigenes selected from targeted cloning and T and B cells cDNA libraries features (Mancia et al., 2007). The first microarray was useful for stress response and immune function studies in wild dolphins (Mancia et al., 2008, 2010). The blood transcriptome reveled that (1) the dolphin immune system mechanisms have high similarity to those of humans and other terrestrial mammals; (2) the immune system of the dolphins, resident inhabitants of coastal locations of temperate waters worldwide, reflects the environmental condition in which they live; and (3) the sampling method impact on downstream analysis underlines the need for species-specific baseline data. The same microarray was applied to screen a different tissue in a study focused on the study of the vitamin D3 pathway on cell cultures from dolphin skin; the analysis showed the importance of nonclassic functions of vitamin D3, such as its role in innate immunity, similar to what has been demonstrated in other mammals (Ellis et al., 2009).

In 2014, a much more comprehensive system, a species-specific oligo microarray, containing 24,418 unigene sequences from cDNA libraries of seven different tissues from bottlenose dolphin was generated (Mancia et al., 2014). The microarray was used to screen wild animal blood transcriptomes and was effective in the differentiation of populations of dolphins inhabiting different geographic locations and by the effects of environmental contaminants on dolphin health (Mancia et al., 2014, 2015). Dolphins inhabiting the coastal waters of Georgia in the United Statees, known to be heavily contaminated by Aroclor 1268 (Kucklick et al., 2011), an uncommon polychlorinated (PCB) mixture, displayed variation in expression of genes involved in xenobiotic metabolism, development/differentiation, and oncogenic pathways (Mancia et al., 2015). The same microarray was also applied to evaluate the skin tissue as a source of information, giving the minimal disruption inferred from the sampling methods (e.g., dart biopsy and/or stranding events). The skin was useful to evaluate the activation of an immune response to the exposure of contaminant of emerging concerns, such as bisphenol A and perfluorooctanoic acid (Lunardi et al., 2016) and also to establish baseline health parameters for investigations on contaminant exposure or health status. Gene expression was greatly impacted by season, with one-third of all the genes on the array varying between winter and summer, highlighting the need for creating a baseline for natural variability for a better investigation of the effects of a stressor (Van Dolah et al., 2015).

Despite its ability to interrogate the expression of thousands of genes, the microarray can still lack information, due to the incorrect abundance of some of the transcripts. The most recent RNA sequencing (RNA-seq) method, in which the cDNA made from the RNA sample is directly sequenced through high-throughput DNA sequencing, provides a less biased evaluation of the transcriptome. RNA-seq analyses are very sensitive and offer the advantages of detecting all the unique sequences and of quantifying levels of RNAs expressed at a very low level. Most importantly, the reduced cost of RNA-seq provides an efficient approach to generate sequences for functional genomics analyses in a nonmodel organism with unsequenced genomes using a de novo assembly procedure (Gui et al., 2013). Gui et al. (2013) characterized the leucocyte transcriptome of the Indo-Pacific humpbacked dolphin, now an endangered species because of the dramatic decline in population size of the past decades. The dataset provides a substantial genomic-level resource for the endangered species, while the identification of genetic markers and genes involved in immune system response and adaptive evolution can be useful in understanding the molecular mechanisms of various pathways in cetaceans.

Proteomic analyses have been demonstrated to support and validate studies on marine mammal health. One of the first proteomic studies on dolphins was a screening of the dolphin skin proteome by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), to compare the proteomic profile of skin tissue samples with that of skin-derived cultures. Results demonstrate that samples share distinct similarities sustaining the validity of the use of epidermal cell lines

for the study of the interaction occurring between dolphins and the environment (Yu et al., 2005). Another example was a study investigating a dolphin condition similar to human metabolic syndrome using serum lipid profiles between two groups of dolphins, where high or low insulin were analyzed. The study found differences in phospholipid fatty acids in the two groups, which may play a role in the susceptibility to or development of an insulin-resistant-like state: the proteomic analysis of dolphin serum showed correlation of changes in the fatty acids with an insulin-sensitizing phenotype (Sobolesky et al., 2016).

An approach to direct, noninvasive, health assessments of wild marine mammals used metabolomics to profile exhaled breath metabolites, providing a first library of volatile and nonvolatile compounds in cetacean exhaled breath. Dolphin breath contains a large variety of low-abundance metabolites, many of which are common with those found in human breath and considered indicative of human health status. Providing a link between dolphin breath and certain health conditions or exposures, the monitoring of exhaled breath metabolome content provides useful diagnostic information that can be used by veterinary personnel and conservation managers in their decision-making processes (Aksenov et al., 2014; Zamuruyev et al., 2016).

Pinnipedia (Order: Carnivora)

Diseases of wild marine mammals can be difficult to diagnose because of their protected status and their unknown life history. A study using tools relying on cross-hybridization between closely related species presented the blood transcriptome of the California sea lion (*Zalophus californianus*) as a diagnostic classifier for rapid diagnosis and treatment of infection, disease, intoxication, or other causes of compromised health status in stranded animals (Mancia et al., 2012). The results achieved with this work indicate that cross-species microarray technologies, using a selected gene set for microarray design and analysis could be of use to wildlife managers and veterinarians in handling species with little genomic data. The California sea lion, a protected marine mammal inhabiting the western coast of North America, is a good sentinel species for coastal habitats, and the most common cause of stranding is reported to be domoic acid toxicosis, caused by the ingestion of domoic acid, a potent neurotoxin produced by diatom species of the genus *Pseudo-nizschia*. Human domoic acid intoxication cases present with abnormal behaviors, even seizures and epilepsy linked to hippocampal lesions, sharing the neuropathology profile observed in the California sea lions exposed to domoic acid, in particular in the recently discussed link between hippocampal lesions and epilepsy (Buckmaster et al., 2014; Ramsdell and Gulland, 2014). The blood transcriptomic analysis showed enrichment of pathways consistent with that neuropathology profile, confirmed by the analysis of the proteome of cerebrospinal fluid from intoxicated animals (Neely et al., 2015). Proteomic analysis of blood indicated that several apolipoprotein E charge forms decreased in domoic acid–intoxicated sea lions and may be important in the progression of domoic acid toxicosis (Neely et al., 2015).

The transcriptomic and the proteomic approaches taken, combined with machine learning approaches, performed as a robust and accurate tool to diagnose domoic acid toxicosis, demonstrating promise for future studies combining additional variables in multidimensional space to create robust classifiers (Mancia et al., 2012; Neely et al., 2015).

Omics studies on California sea lion (family: Otaridae) were the first attempt to combine a global-scale analysis to the health status of marine mammals belonging to the order of Carnivora, mostly related to the possibility of screening a high number of unhealthy animals. In fact, data analysis was guided and/or supported by evaluation obtained by clinical observations and health parameters measurements carried out on the high number of unhealthy and ill cases that seasonally were hospitalized and rehabilitated at the Marine Mammal Center, in Sausalito, CA. Lately, the attention seems to be focused on different species, pinnipeds belonging to the family of the Phocidae, and to the understanding of the mechanisms activated by environmental chemical or biologic stress. Transcriptomics was used to evaluate brain tissues from stranded harbor seals (*Phoca vitulina*) to understand the cause of death. The study identified pathways involved in innate and adaptive immunity in samples with a phocine herpesvirus (PhV-1) brain infection and found a strong upregulation of fatty acid metabolic genes in animals that did not die from viral infection. Although the cause of the dysregulation of fatty acid metabolism in the brains of these harbor seals is unknown, it may be correlated to exposure to toxins or nutrient depletion (Rosales and Vega Thurber, 2016). A de novo assembly of the blubber transcriptome in ringed seal (*Pusa hispida*) was used to identify molecular indicators of PCBs exposure. Transcript levels for gene targets were found to be correlated with increasing levels of blubber PCBs, linking an energy metabolism imbalance to the toxicity of the chemicals (Brown et al., 2017). Transcriptome analysis of muscle tissue of another member of the family Phocidae, the northern elephant seal (*Mirounga angustirostris*), provides a resource for a deeper investigation of the alteration of molecular pathways triggered by environmental stress, including modifications of metabolic and immune signaling as well as muscle tissue maintenance (Khudyakov et al., 2015a). An example comes from a study of the cellular responses to hypothalamic-pituitary-adrenal axis stimulation by measuring corticosteroid hormones, metabolites, and muscle gene expression before, during, and following administration of exogenous adrenocorticotropic hormone (ACTH); results suggests a compensatory, tissue-sparing mechanism used to maintain cortisol and aldosterone sensitivity while avoiding deleterious long-term consequences of stress (Khudyakov et al., 2015b). Reference sequences specific to elephant seals were also obtained from the blubber tissue and used to profile the transcriptomic response to hypothalamic-pituitary-adrenal stress axis activation and to identify tissue-specific molecular markers of stress in the pinniped fasting-adapted species (Khudyakov et al., 2017).

Sirenia (Superorder: Afroheria)

The completion of the genome draft of the Florida manatee (*Trichechus manatus latirostris*) in 2012 made immunogenetic exploration of the Sirenia order more feasible. The Florida manatee is one of the most endangered marine mammals in American coastal waters, continuously facing demographic challenges due to anthropogenic activities and stochastic factors. Brevetoxicosis and papillomavirus are disease-associated agents that have been described in their populations (Bossart et al., 2002; Walsh et al., 2015), in addition to several parasites including trematodes, nematodes, and coccidians (Bando et al., 2014). Discovering the genetic details of their immune system is an important step in the development of manatee-specific tools for monitoring health. The genomic scaffolds of the Florida manatee were used to characterize the organization and expression of the immunoglobulin heavy chain locus. The potential segmental diversity and constant region isotypic diversity described may be related to the mechanisms of defense against infectious disease in their environment.

New Technologies: Challenge and Progress

The number of advantages using the new omics technologies constantly increase, but there are also limitations and challenges that need to be considered. With the completion of the human genome, the relationship between one or more genes to a specific disease and the importance of the interaction with complex environmental factors became clear, making the understanding of the disease process more challenging. Moreover, knowledge of physiologic processes such as SNPs, epigenetic modifications, and posttranslational modifications increased the complexity of host–disease interactions. Combining the study of multiple genes, proteins, and metabolites, the multiomics approach proved to be the correct one to understand the global picture of how each disease affects its host. Each omics study provides information between disease and nondisease, describing alterations of genes (transcriptomics), proteins (proteomics), or metabolites (metabolomics). Thanks to the advent of the bioinformatics with the development of computational and statistical techniques, these large datasets can be combined to offer an integrated view of how an organism may react to a disease, leading to potential biomarkers that can be used in the future for therapeutic applications.

However, technical difficulties, expensive instruments, cost versus benefit uncertainty, and clinical stringency make the use of omics still limited. In marine mammal studies, these limitations are associated with limited sample availability and genetic heterogeneity. Beside these restrictions, the massive amount of data generated from each omics study with often small sample size complicates the data interpretation and restricts the clinical value of the results. While using experimental replication and validation methods as well as reference samples and baseline data will improve confidence in the reliability of the results, there are parameters that need to be well evaluated. The small sample size used in the experimental setting caused by the protected status of marine mammals and by the cost of the

techniques/sample can be a source of false discovery. Solutions to maximize the value of an omics study rely on repeated observations over long period of times, the examination of multiple tissues or body fluids correlated to the disease, the use of more than one technique to validate results, and the integration of the results with the biology or pathology of disease. However, different types of high-throughput technologies, each simultaneously collecting a large set of molecular data, used to collect information on the molecular components of biologic systems need to be integrated and analyzed. Taken together, the complexity of biologic systems, the technological limits, the large number of biologic variables, and the relatively low number of biologic samples make data integration of multilayer datasets one of the most relevant problems computational scientists are addressing nowadays.

LINKING MARINE MAMMALS TO HUMANS: NEW RESOURCES FOR HEALTH

Mammals have returned to the water in at least seven separate lineages: Cetacea, Sirenia, Desmostylia (extinct order), Pinnipedia, *Ursus maritimus* (polar bear), *Enhydra lutris* (sea otter), and *Thalassocnus* spp. (aquatic sloths, extinct genus). Some of these lineages have retained most of their terrestrial form while spending most of their time in the water, while others have changed their morphology dramatically to spend the entire time in the water. Clades of extant marine mammals seem to have originated at two discrete times. Cetacea and Sirenia originated during the early Eocene (50 million years ago, MYA), a time of high productivity in aquatic environments, with warm, broad, shallow seas and abundant resources to exploit, whereas Pinnipedia originated during the Oligocene (35 MYA), when productivity of the oceans, like today, was more concentrated around areas of upwelling. Both Cetacea and Sirenia were fully aquatic by the end of the Eocene, while Pinnipedia are semiaquatic animals (Gingerich et al., 1983; Lipps and Mitchell, 1976; Uhen, 2007). Each of these evolutionary histories is different from the others. Despite the finding that these adaptations evolved in mosaic patterns, they all have aquatic characteristics in common, and different morphologic solutions to aquatic conditions were achieved separately in each clade. The transition from terrestrial animals to fully aquatic animals took about 12 MY, and eventually, they diversified into the species we know today: the genus *Tursiops*, which bottlenose dolphins belong to, first appeared in the fossil record about 5 MYA.

While some mammals were evolving in the oceans, others, on land, evolved into the first primates, humans ancestors. The human lineage split from the modern chimpanzees and bonobos around 7 MYA, with the early hominins (human-like primates) that were our direct ancestors. Current estimates of the similarity between the DNA sequences of both the human and chimpanzee genome range between 95% and 99% (Varki and Altheide, 2005).

The Earth is old and so is life: while the Earth formed 4.5 billion years ago, the oldest known fossils are around 3.5 billion years old. In this time scale, the evolution of marine mammals and primates are relatively recent events. Many

studies have compared primates and cetaceans in the perspective to elucidate the social evolution of highly intellectual mammals in terrestrial and aquatic environments. Despite a deep evolutionary divergence, adaptation to physically dissimilar environments, and very different neuroanatomic organization, some primates and cetaceans show striking convergence in social behavior, artificial language comprehension, and self-recognition ability (Marino, 2002; Yamagiwa and Karczmarski, 2014). Thanks to next-generation sequencing and the availability of the genomes, we can now correlate the knowledge on the observations made on the ecology, social relationships, behavior, in the molecular mechanisms operated by genes, proteins, and metabolites. Using the current genomic available data, we can look briefly at the identity, at transcripts and protein level, between humans and marine mammals. In Table 11.1, coding regions of the Cetacea, the bottlenose dolphin (*Tursiops truncatus*), the minke whale (*Balaenoptera acutorostrata scammoni*), the killer whale (*Orcinus orca*), the sperm whale, (*Physeter catodon*), and the Yangtze River dolphin (*Lipotes vexillifer*) are compared to those of humans and to those of the closest relatives in the Cetartiodactyla order. Coding regions of the Pinnipeds, the Weddell seal (*Leptonychotes weddellii*), and the walrus (*Odobenus rosmarus divergens*) are compared to those of humans and to those of the closest relatives in the Carnivora order. Coding regions of the Florida manatee (*Trichechus manatus latirostris*) are compared to those of humans and to those of the closest relatives in the Afrotheria superorder. As expected, the similarity is higher between closely related species within the same order and superorder. But the results from alignment of sequences of human and marine mammals is still very high, at least in the coding regions analyzed. Unquestionably, more of the genome needs to be understood and analyzed in both humans and marine mammals, but the similarity that we can already observe is an important instrument for basic research and translational science. Marine mammals are used as sentinels for ocean and human health (Bossart, 2011), a choice driven by the characteristics shared with the human species (e.g., mammals with long life spans, long-term coastal residents, feed at a high trophic level). Marine sentinels allow the characterization and management of potentially negative impacts linked to the environment degradation that can affect animal and human health associated with the oceans. Marine mammals have also developed unique adaptations, some of which are species-specific, to live their entire life, or most of it, in the aquatic environment (e.g., deep long dives, swimming, thermoregulation, echolocations). These fascinating differences with land mammals can sometimes be turned into powerful tools to understand and treat complex pathologic processes in human medicine.

Applications of Modern Genomics Techniques: Metagenomics for Zoonoses

Marine mammals are top predators that are essential for the health and function of the oceans, too often affected by various factors that can be detrimental

TABLE 11.1 Transcript and Protein Alignment Within Marine Mammal Species of the Order of Cetartiodactyla (Infraorder: Cetacea), Carnivora (Clade: Pinnipedia), and Sirenia (Superorder: Afrotheria) and Between Marine Mammals and *Homo sapiens*

Cetacea

Tursiops truncatus

	Transcript		Protein	
	% identity	% coverage	% identity	% coverage
Homo sapiens	89.52	82.84	76.30	78.73
Cetartiodactyla	91.99	93.52	79.73	81.75

Balaenoptera acutorostrata scammoni

	Transcript		Protein	
	% identity	% coverage	% identity	% coverage
Homo sapiens			77.51	80.51
Cetartiodactyla	91.47	95.27	80.06	84.37

Lipotes vexillifer

	Transcript		Protein	
	% identity	% coverage	% identity	% coverage
Homo sapiens	89.09	82.86	77.34	81.03
Cetartiodactyla	91.32	96.21	79.93	85.50

Orcinus orca

	Transcript		Protein	
	% identity	% coverage	% identity	% coverage
Homo sapiens	89.39	84.93	76.97	83.03
Cetartiodactyla	91.88	96.98	80.17	87.05

Physeter catodon

	Transcript		Protein	
	% identity	% coverage	% identity	% coverage
Homo sapiens			77.61	78.67
Cetartiodactyla	91.47	91.94	80.28	83.02

Pinnepedia

Leptonychotes weddellii

	Transcript		Protein	
	% identity	% coverage	% identity	% coverage
Homo sapiens	89.18	76.02	77.85	75.98
Carnivora	92.67	89.45	78.05	80.65

Odobenus rosmarus divergens

	Transcript		Protein	
	% identity	% coverage	% identity	% coverage
Homo sapiens	89.43	83.41	77.00	83.59
Carnivora	94.12	97.64	81.06	89.19

Sirenia

Trichechus manatus latirostris

	Transcript		Protein	
	% identity	% coverage	% identity	% coverage
Homo sapiens	89.18	82.89	75.96	82.24
Afrotheria	90.64	96.85	78.42	88.42
Cetartiodactyla			77.29	84.75

Sets of transcripts and proteins were retrieved from Entrez, aligned to the genome by Splign (transcripts) or ProSplign (proteins) and passed to Gnomon, NCBI's gene prediction software. The sequences used for the alignments are reads from RefSeq or from the GenBank database. The number in the report is the sum of multiple GenBank queries. The query of GenBank should retrieve the list of organisms with txid91561 for the Cetartiodactyla, txid311790 for the Afrotheria, and txid33554.

to their populations. Forty-four percent of stranded marine mammals die from unknown causes (Gulland and Hall, 2007). Disease is a major cause of marine mammal population decline, and the role of the microbiome in disease has generated considerable interest. The etiology of stranding events still remains poorly characterized, but high-throughput sequencing technology can identify and yield new insights into the virome and microbiome for disease identification and surveillance. The microbiome acts strongly and significantly in maintaining host health with a vital role in disease manifestation and immune system function. Members of the microbial community can directly influence the progression of a disease and modulate the host's immune system regulation and response, making the host's microbial partners essential to immune system function (Maynard et al., 2012).

A promising approach for pathogen identification in stranded marine mammals is the use of metagenomics, the characterization of the collective genome of microorganisms isolated from an organism using high-throughput sequencing technologies. Metagenomic studies do not require prior information about the disease agents and allow detailed comparisons of health and disease, identifying new insights into the virome and microbiome of wildlife. Metagenomic studies of marine mammals focused on the viral and microbial community of many tissues and body niches have displayed a big diversity of the microbiota according to the organ type and may be used as a baseline survey for comparison with samples from stranded animals during unexplained disease outbreaks (Godoy-Vitorino et al., 2017). A viral metagenomic study to investigate potential viral pathogens associated with a mortality event of captive California sea lions identified a novel species-specific anellovirus (ZcAV) (Ng et al., 2009). A similar study on lung samples of the Pacific harbor seals (*Phoca vitulina richardsii*) revealed another novel seal anellovirus (SealAV), which clusters phylogenetically with anelloviruses from California sea lions and domestic cats (Ng et al., 2011). The description of ZcAV and SealAV in the lungs of pinnipeds suggests that anellovirus infections may be common and play a role in marine mammal health and disease. A different metagenomic study used next-generation sequencing to get a comprehensive view of the fecal viral populations from wild and temporarily captive California sea lions, reporting previously uncharacterized viruses, including astroviruses, picornaviruses, bocaviruses, and sapoviruses (Li et al., 2011). A metagenomic survey of viromes from feces of the Subantarctic fur seal (*Arctocephalus tropicalis*) and south American fur seals (*Arctocephalus australis*) used next-generation sequencing to explore the viral diversity of southern hemisphere marine mammals (Kluge et al., 2016). A study of the bacterial communities in hundreds of samples from different body sites in healthy dolphins and sea lions showed a highly diverse bacterial taxonomic composition, which varies according to body site and host species (Bik et al., 2016). These findings provide species-specific databases that can be compared to later virome surveys and microbiota to detect alterations associated with changes in marine mammal health or population size.

Metagenomic studies in cetaceans have also indicated how the cetacean microbiome is affected by human-related bacteria (Godoy-Vitorino et al., 2017). This is particularly relevant since many human infections have a zoonotic, i.e., wild or domestic animal, origin. The rise in zoonotic diseases is driven by a complex interplay of environmental, ecologic, and epidemiologic factors. Therefore, the identification of pathogens in marine mammals may also moderate disease outbreaks and prevent zoonotic transmission (Delwart, 2012). Currently, there are about 15 known zoonotic marine mammal pathogens (Waltzek et al., 2012). The bacterial pathogen that causes tuberculosis, *Mycobacterium tuberculosis*, was introduced to the Americas via pinnipeds (Bos et al., 2014). The influenza A virus, a global human threat, is present in cetaceans and pinnipeds and has been shown to be transmitted from seals to humans (Geraci et al., 1982; Reperant et al., 2009; Webster et al., 1981). Most recent emerging diseases have been associated with host switches, including severe acute respiratory syndrome coronavirus, H5N1 avian influenza, Hendra virus, Nipah virus, and acquired immunodeficiency syndrome (Woolhouse and Gowtage-Sequeria, 2005). The risk of being injured or acquiring zoonotic diseases is highest in marine mammal researchers, rehabilitators, trainers, veterinarians, and volunteers, but there also numerous recreational activities now permitting contact with these animals. Subsistence hunters such as whalers and sealers, as well as human rescuers during marine mammal stranding events, are at risk of disease acquisition through their direct physical contact with infected marine mammals or through the ingestion of marine mammal food products (Hunt et al., 2008; McLaughlin, 2004; Webster et al., 1981). Luckily, so far the majority of zoonotic marine mammal diseases have resulted in localized skin infections in man that resolved spontaneously or with appropriate medical therapy. However, other marine mammal zoonoses, if left untreated, could induce life-threatening systemic diseases that could pose public health risks.

As the number of zoonotic diseases rises, the identification of pathogens in marine mammals has become an indicator of environmental health. The list comprises bacterial, viral, and fungal infections producing seal finger, brucellosis, leptospirosis, mycobacteriosis, mycoplasmosis, influenza, lobomycosis, and blastomycosis (Waltzek et al., 2012). As the closest oceanic relatives of humans, marine mammals are sentinel species for both human and ocean health, and they are long-lived, top predators, inhabiting the same inshore ecosystems utilized by man (Bossart, 2011).

Applications of Marine Mammal Unique Adaptations: Marine Biomedicine

Medical research has necessitated the integration of the omics and computational biology data to diagnose, interpret, and prognosticate human disorders, even with the current more comprehensive knowledge of the human physiology and molecular mechanisms of diseases. Omics technologies, including

genomics, transcriptomics, proteomics, metabolomics, and epigenomics have transformed human medical research in the last decade, but there are still unresolved underlying mechanisms in human disease. The exploitation of marine mammals' unique adaptations through omics technologies can give valuable insights into uncertain human biomedical conditions.

An example is the astonishing wound healing mechanisms observed in dolphins. In humans, the healing of tissue wounds is often associated with infection and results in scars. Zasloff, a professor of immunology at the Georgetown University, observed the clinical course of the recovery of two dolphins showing shark's bites about 30 cm in length and 3 cm in depth (including the blubber layer and the underlying muscle). He reported that, during the first day postinjury, blubber from surrounding tissues had already migrated over the open wound surface. On the second day, newly generated tissues were described, which would gradually fill the wound from its base, restoring the original volume (Zasloff, 2011). The wound healed completely in 4 weeks and without any infections, which is remarkable considering the analogies between their immune system and that of terrestrial mammals (Beineke et al., 2010; Mancia et al., 2007). Zasloff suggested a role of the components of the dolphin blubber during the healing process. Stem cells present in the blubber could have a role in this remarkable wound healing process. The composition of dolphin's blubber is in fact different from other marine mammals: the isovaleric acid is higher in concentration, being 2%–5% of total fatty acid in bottlenose dolphin, while there is no detection in several species of whales (Koopman et al., 2003). The isovaleric acid accumulates in the blubber and does not get burned for fuel during times of starvation; moreover, it seems to have antimicrobial activity (Hayashida-Soiza et al., 2008). A multilayered omics approach describing transcripts, proteins, and metabolites present in the blubber after a serious injury may unravel the mechanisms involved in the healing process and in the protection from infection. Advances in the comprehension of the mechanisms controlling the healing process could lead to the improvement in the control of wound healing in humans and terrestrial mammals, leading to new therapies: regenerative medicine is an emerging field, with numerous open trials in the veterinary and human fields.

Another example is given by the recent discoveries of the unique dolphin metabolism that can aid research for human metabolic syndrome and diabetes. People affected by metabolic syndrome can develop not only type II diabetes but also cardiovascular disease and the possibilities to have strokes. Like humans, dolphin can develop metabolic syndrome, characterized by elevated insulin, glucose, triglycerides, and ferritin (Venn-Watson et al., 2011, 2015). Fatty liver disease has been found both in wild dolphins and dolphins under human care, suggesting that dolphins are susceptible to metabolic disorders. A study compared a wild population of dolphins to one kept under human care, which have higher annual survival and lower mortality rates. A deep study into these animals' diets as a possible risk factor for longer life and metabolic

disease highlighted the potential benefits of C17:0, the margaric or heptadeca-noic acid, a saturated fatty acid also present in bovine milk fat. High levels of C17:0 on erythrocyte membranes or plasma phospholipids have been identified as protective factors against development of metabolic syndrome, type 2 diabe-tes, and associated inflammation (Venn-Watson et al., 2015). In a parallel study supported by proteomic analysis, differences in serum lipid profiles between two groups of dolphins, with high or low insulin, found differences in phospho-lipid fatty acids. Shifting the dolphin diet to fish rich in odd chain saturated fatty acids, such as C17:0, resulted in increased serum levels of the insulin-sensitiz-ing hormone adiponectin and serum sphingosines consistent with an insulin-sensitizing phenotype (Sobolesky et al., 2016). A better understanding of the networks activated by the mechanisms regulating adiponectin in dolphins could aid research of metabolic syndrome and diabetes affecting human populations.

Each year, millions of individuals die or become ill because of conditions or diseases that reduce the oxygen supply to hypoxia-sensitive tissues such as the brain. Hypoxia is also involved in and is the cause of many neuronal disorders in humans, for example, Alzheimer disease (Peers et al., 2007), Parkinson dis-ease (Speer et al., 2013), and cerebral ischemia (stroke). Acute metabolic insults like stroke have an especially devastating impact, which is mostly impossible to repair. By contrast, brains of diving mammals tolerate extended periods of systemic hypoxia without damage. There are several behavioral, anatomic, and physiologic adaptations that are associated with the remarkable dive capacity of many marine mammals (Davis, 2014) that may be partly explained on the genetic level by specific substitutions within the coding sequences (Foote et al., 2015) or by selective gene duplications and losses (Yim et al., 2014). Different studies used transcriptional analysis of the brain of the hooded seal (*Cystophora cristata*) and showed differential regulation of specific genes that may have a central role in the protection of the diving brain. In large parts, the seal brain responds to the hypoxic challenge in a similar way as the brain of other mam-mals, which includes the upregulation of typical stress proteins like cytokines and immediate early genes. However, the genes involved in the energy metabo-lism seem to be a specific response of the seal brain to hypoxia. These genes may also be suitable drug targets for human neuronal disorders associated with hypoxia (Fabrizius et al., 2016; Hoff et al., 2017).

CONCLUDING REMARKS

A new era of discoveries in the marine mammal field began in 2011, with the completion of the first marine mammal genome, 8 years after the first whole-genome assembly of the human genome. Since then, the bottlenose dolphin genome has been greatly implemented thanks to supplement of new high-throughput sequences data and to the newer high-coverage version of the genome. To date, genomes from nine species of marine mammals have been sequenced, annotated, and are publicly available at the NCBI database. Genome-guided

transcriptome assembly as well as de novo transcriptome assembly are providing novel insights into the mechanisms underlying the variety and diversity of marine mammals, revealing novel genes, functions, and connections of molecular and physiologic mechanisms related to their adaptations, health, and disease.

But the study of the genome and of the information that is encoded within the full DNA sequence of an organism will not unlock the code of life. The genotype is not the only thing responsible for the final physical makeup of the organism. The study of the epigenome with the genome-wide mapping of DNA methylation, histone modifications, nucleosome positioning, and three-dimensional architecture and the integration of genome and epigenome, of the RNA information from coding (mRNA) and noncoding RNA (epigenetic-related RNAs, such as micro RNA, short interfering RNA, piwi-interacting RNA, and long noncoding RNA) are necessary to understand how environment and genetic inclinations can intertwine in the complexity of cell biology. The network and pathways observed can in predictive models identify potential risks and propose solutions, making the new technologies relevant as prognostic and diagnostic tools.

The exploitation and combination of the new advanced omics techniques and computational methods will finally allow the discovery of a constantly changing environment thanks to the understanding of the interaction between marine mammals, humans, and the oceans.

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