Proposal to Sequence the Genomes of the Spiny Dogfish Shark (*Squalus acanthias*) and the Little Skate (*Raja erinacea*)

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Introduction

Chondrichthyes fishes appeared approximately 450 million years ago and today are the oldest existing jawed vertebrates. Elasmobranchs (sharks, rays and skates) comprise most chondrichthyan organisms. They exhibit fundamental vertebrate characteristics, including a developmental neural crest, jaws and teeth, and an adaptive immune system. They are also the oldest existing vertebrates with a closed, pressurized circulatory system and related signaling molecules and receptors such as platelet-derived growth factor and adenosine receptors. These characteristics have been exploited experimentally to promote significant understanding about human physiology, immunology, genomics, stem cell and cancer biology, pharmacology, toxicology and neurobiology. Among elasmobranchs, the spiny dogfish shark (*Squalus acanthias*) and little skate (*Raja erinacea*) have particular experimental advantages and are the most widely used as biomedical models. Genomic sequences from these two organisms will greatly facilitate studies in molecular evolution and comparative genomics and provide important molecular insights into basic human biology and disease.

Specific biological rationales for the utility of new sequence data: informing human biology.

Elasmobranchs exhibit primitive but powerful processes for dealing with salt and water homeostasis, cell volume regulation, and environmental and internal osmotic sensing. The mechanisms by which humans regulate intra- and extracellular small molecule concentrations are fundamentally the same as those utilized by elasmobranchs. However, the major transporting tissues of sharks and skates are easier to study than the mammalian kidney, liver, intestine or eye, because elasmobranch tissues are simpler in organization, more compartmentalized and dedicated to fewer functions. Genomic information applied to existing data in these physiological systems will allow the testing of molecular hypotheses and understanding of regulatory mechanisms that are directly applicable to human biology.

The value of elasmobranch models can be seen in studies of the rectal (salt) gland of the spiny dogfish shark. This organ does not exist in mammals, but provides unique opportunities to study critical physiological functions that are more dispersed and less experimentally accessible in other model animals. For example, the spiny dogfish shark rectal gland is uniquely composed entirely of a single cell type of transporting epithelium and has the highest density of cystic fibrosis transmembrane conductance regulator (CFTR) of any tissue from any organism (Hanrahan et al., 1996; Lehrich et al., 1998). CFTR is a cAMP-regulated, phosphorylation-sensitive chloride channel for which mutations in function or cellular localization lead to cystic fibrosis in humans. A comparison of the properties of the cloned shark and human CFTR has provided insights into structural domains related to functional differences in the normal and mutant proteins. Genomic sequence will provide other important information about the regulation and function of this protein.

Genomic sequence will also provide critical information for many other research areas in which elasmobranch models are used. These include elucidation of the organization and functional significance of several gene families critical in immunology. Work with elasmobranchs showed that immunoglobulin genes are clustered in a manner unlike that of birds, reptiles, amphibians and mammals (Hinds and Litman, 1986) whereas major histocompatibility complex (MHC) genes appear be more mammal-like than those of bony fishes. Considerable effort has also been devoted to examination of genes related to adaptive immunity in the clearnose skate (*Raja egeanteria*), as well as some work on the closely related little skate. Genomic sequence data from elasmobranchs will be necessary to better understand immunologically important multigene families, generation of molecular diversity and regulation of expression of the molecules involved in adaptive immunity (Ohta et al., 2000).

Other active research fields that will benefit from spiny dogfish shark and little skate genomic sequences include neurobiology using the skate retina, another distinctively simplified tissue, and the central nervous system. Shark brains are unusually resistant to hypoxia and also exhibit a unique blood-brain barrier defined by glia rather than endothelial junctions, and a choroid plexus that is extraordinarily accessible experimentally. These models lend themselves to less complicated studies in physiology, development, immunology, neurobiology and toxicology, and will benefit greatly from the availability of sequence data, as was demonstrated for the zebrafish model organism. See Tables I and II for summaries of current biomedical research with *S. acanthias* and *R. erinacea* from investigators worldwide.

Improving human health and understanding human disease

The experimental accessibility of elasmobranchs and their evolutionary distance from humans has promoted many unique insights into conserved functional domains of genes associated with human liver biology, multidrug resistance, cystic fibrosis and transport of ions, urea and xenobiotics (Aller et al., 1999; Ballatori and Villalobos, 2002; Cai et al., 2003; Cai et al., 2001; Gagnon et al., 2002; Marshall et al., 1991; Morgan et al., 2003a; Smith and Wright, 1999). The little skate is a particularly valuable model for liver toxicity and xenobiotic transport studies. For example, studies of the multidrug resistance-associated protein, MRP2, in skate show up to 76% identity to the orthologous human protein in several transmembrane domains known to function in substrate recognition and transport (Cai et al., 2003). Compared to the human ortholog, the skate MRP2 exhibited similar substrate specificity, transport function and expression patterns (Cai et al., 2003). Importantly, little skate primary hepatocytes retain hepatobiliary polarity for at least 8 hours and possibly up to several days in culture, offering significant advantages over mammalian hepatocyte cultures for studying the function of multidrug resistance-associated ATP-binding cassette (ABC) genes (Ballatori and Villalobos, 2002).

Mount Desert Island Biological Laboratory (MDIBL) provides valuable experience and support for toxicological studies using the spiny dogfish shark and little skate through its Center for Membrane Toxicity Studies (CMTS) supported by the National Institute of Environmental Health Sciences (NIEHS). The CMTS was established in 1985 as a Marine and Freshwater Biomedical Sciences (MFBS) Center, one of only four such Centers in the US. Annually, this center convenes internationally recognized investigators to study cellular and molecular mechanisms of toxicity of environmental pollutants using elasmobranch and other aquatic models. The CMTS is under the direction of Dr. James Boyer, Ensign Professor of Medicine and Director of the Yale University Liver Center. Additional collaborative research projects involving elasmobranch models at MDIBL include the Center for Comparative Functional Genomics, under the direction of Dr. John Forrest, Professor of Medicine, Yale University, and the NIEHS-supported development of a publicly available resource, the Comparative Toxicogenomics Database (CTD; Mattingly et al., 2003; Mattingly et al., 2004). Major goals of CTD include annotating genes and proteins of biomedical significance and promoting comparative studies of these genes and proteins across evolutionarily diverse organisms with a particular emphasis on aquatic species. It is the intent that these comparisons will lead to better understanding of molecular evolution, the significance of conserved sequences and the genetic basis of variable susceptibility to disease and toxicity. Advisory boards for these MDIBL initiatives include world renowned scientists with an impressive range of expertise such as Sidney Brenner and Frank Ruddle, pioneers in molecular and comparative genetics.

The striking sequence and functional similarity of many elasmobranch proteins to their human orthologs further supports the use of these organisms as experimental and genomic models to study human disease. In addition to the unique physiological opportunities afforded by the rectal gland of the spiny dogfish shark, molecular analysis of CFTR has also demonstrated that this organism is an important model for cystic fibrosis research. The spiny dogfish shark CFTR protein is 72% identical to the human ortholog and comparison of the human and shark CFTR sequences revealed conservation of five cyclic AMP-dependent kinase phosphorylation sites and three residues that, when mutated in the human protein, are associated with cystic fibrosis (Marshall et al., 1991). The ongoing work using spiny dogfish shark and little skate to understand disease states, including those associated with genetically abnormal CFTR and anionic transport proteins, will greatly benefit from genomic sequences of the spiny dogfish shark and little skate. These sequences will provide opportunities to study coding and regulatory regions of genes important to human health and evaluate potential nucleic acid target sites of a broad range of environmental and clinical chemicals.

Elasmobranchs have received much publicity for providing insights into cancer biology. Although much of the media reports claiming that sharks do not get cancer are scientifically unsubstantiated, the spiny dogfish shark

and other elasmobranchs are sources of antibiotics and angiogenesis inhibitors that may be promising cancer treatments. Genomic analysis will lead to a better understanding about how these compounds are synthesized and interact within the organism and may have important implications on the development of therapeutic treatments. Genomic sequences from spiny dogfish shark and little skate will also contribute to our understanding of substrate specificity and regulation of several anionic and xenobiotic transport proteins of the ABC protein superfamily shown to be involved in drug-resistant cancers and other drug-resistance phenomena.

Providing additional surrogate systems for human experimentation

Elasmobranchs have interesting properties regarding aging, cancer, protooncogene and telomerase expression, stem cells, and immortalization. Complete genomic data from the spiny dogfish shark and little skate will provide a systematic framework to address many basic questions of human medicine through rigorous experimental and comparative sequence studies. The age of a spiny dogfish shark can be estimated by an examination of cartilage disposition, and these animals may live as long as 100 years, continually increasing body size if food and space are unrestricted. Organs similarly increase in size, and several lines of evidence suggest that sharks and skates possess stem cells of considerable potential well into adulthood. For instance, a region of continual renal regeneration has been identified in the little skate, with new tubules being formed continually through adulthood. With a solid genomic foundation, these animals will represent a unique experimental model for examining the basic assumptions regarding normal and abnormal cell proliferation and aging. Spiny dogfish sharks are large and hardy enough to withstand removal of tissue samples, generation of proliferating cell cultures in vitro, genetic manipulation in vitro and transplantation of the altered cells back into the original donor animal. This would allow experimental cell-transfer approaches with animals caught from the wild similar to those achieved with inbred models.

Another research area that may expand rapidly with genomic data is the use of the skate embryo in developmental biology. Egg-laying females with stored sperm can be identified by palpation, and can be maintained in tanks for many months while predictably producing eggs in pairs at intervals of about seven days. Embryonic development from the earliest stages can be held in stasis simply by storing the embryos at refrigerator temperatures. Hatching requires about 6 months at 15^{o}C, and newly hatched animals average 4 inches long. Development is slow enough for removal and *in vitro* culture of embryonic cells and transplantation of altered cells back into an embryo. With genomic information, skate embryos would be amenable to transgenics, morpholino knock-downs and *in situ* hybridization. Parthenogenesis in sharks has also been reported and provides even larger potential for genomics-based studies. The spiny dogfish shark and little skate could be exploited as models of vertebrate development offering new perspectives for comparative studies with the more traditional zebrafish, Xenopus and mammalian developmental models.

Informing the human sequence and providing a better connection between the sequences of human and non-human organisms

Sequencing of the human genome catalyzed a new way of thinking in biology. It promised to facilitate a better understanding of disease-associated genes; however, our understanding of the sequence is limited by our ability to retrieve meaning from it (Aparicio et al., 2002; Miller and Kumar, 2001). The human genome and other vertebrate sequencing projects have underscored the importance of comparative studies to reveal conserved sequences that are functionally significant (Pennacchio and Rubin, 2003; Thomas and Touchman, 2002). Comparative analysis of the mouse and human genomes helped to identify orthologous genes and intraspecies polymorphisms, reveal the extent of sequence similarity and gene order between the species and provide insights about genomic evolution (Thomas et al., 2003; Waterston et al., 2002). However, in many cases, mammalian sequences may have limited comparative value for differentiating between conserved regions that are functionally significant and those that simply lack divergence time. For regions of genomes that evolve more slowly, the need for evolutionarily distant organisms may be particularly important. Convincing support for the value of comparative studies using evolutionarily divergent organisms for gene prediction was demonstrated by the pufferfish (*Fugu rubripes*) genome which led to the discovery of nearly 1000 human genes not previously described in the public domain (Aparicio et al., 2002).

Despite the significance of non-coding sequences in regulating biomedically significant genes, our ability to identify and predict the function of these DNA regions is limited (Pennacchio and Rubin, 2003). Similar to gene

and protein function predictions, comparative analysis of evolutionarily diverse organisms may also help identify important non-coding regions (Chiu et al., 2002; Dubchak et al., 2000; Thomas et al., 2003). This approach is supported by a recent study of HoxA and HoxA-like clusters in human, horn shark and zebrafish (Chiu et al., 2002). Extensive conservation of non-coding sequence motifs were found between the human and shark sequences, whereas zebrafish sequences exhibited significant loss of conservation (Chiu et al., 2002). Different rates of molecular evolution and gene duplication underscore the importance of genomic sequences from an evolutionary range of organisms for comparative sequence analyses.

The genomic sequences of the spiny dogfish shark and little skate will provide unique opportunities to study coding and non-coding, regulatory regions that have been conserved throughout the evolution of jawed vertebrates. Coupled with the experimental advantages of these organisms, predictions of conserved sequences from comparative genomic analyses will allow biologists to design targeted functional and regulatory studies. This approach will significantly enhance the study of genes associated with diseases in humans using these organisms.

Each new vertebrate genomic sequence is expected to benefit the annotation of existing sequences. Whereas annotation of mammalian and teleost genomes will be important for informing the shark and skate genomic sequences; elasmobranch sequences may play an important role in refining predictions of conserved coding and non-coding regions in the human sequence.

Expanding understanding of evolutionary processes

Despite the increase in vertebrate genomes that are being sequenced, there are still portions of evolutionary history without genomic representation (Thomas and Touchman, 2002). Recent additions of *Xenopus tropicalis, Gallus gallus* and *Canis familiaris* to the sequencing pipeline will provide important complementary perspectives for tetrapods and *Danio rerio* and Fugu sequences should have a similar impact on teleosts. By contrast, chondrichthyes, which include elasmobranchs, are the only major line of gnathostomes, or jawed vertebrates, for which there are no genomic sequences.

Chondrichthyan fishes are considered the monophyletic sister group to all other living gnathostomes (Schwartz and Maddock, 2002; see Figure 1). They diverged from the line leading to actinopterygian and sarcopterygian fishes over 400 million years ago. It is estimated that most living elasmobranch families, which include the spiny dogfish shark and little skate, diverged by the late Jurassic period or approximately 155 million years ago (Schwartz and Maddock, 2002). Among jawed vertebrates, elasmobranchs are the most evolutionarily distant from humans. The spiny dogfish shark and little skate sequences will close a significant gap in genomic data and for the first time, enable comparative genomic studies to be conducted within and across both gnathostome lines. Comparative studies will also promote a better understanding of molecular evolution and the connection between genomic sequence and morphological and physiological specialization. Understanding the genomic basis of ancient circulatory systems, immunological response and mechanisms of transport and chemical detoxification will provide important insights into human evolution and environmental adaptation.

Strategic Issues

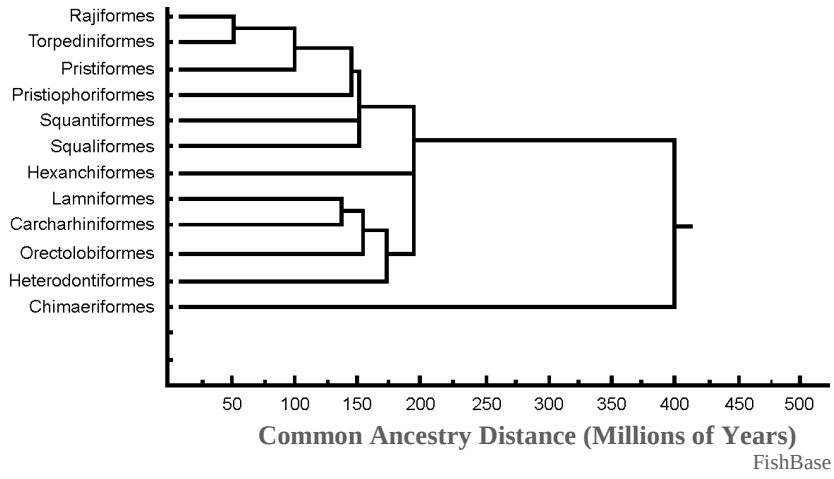
The suitability of the organism for experimentation

The spiny dogfish shark and little skate live in ocean temperatures of 5-10^{o}C. These cold-water organisms exhibit much reduced rates of metabolism, ion transport and oxygen consumption compared with many animal models. This results in increased stability of cells, tissues and cellular macromolecules, including nucleic acids, and more accessible quantitative physiological measurements, such as membrane transport rates. Furthermore, these animals are sufficiently large to provide plentiful amounts of material for cell culture or biochemistry studies. Use of the dogfish shark rectal gland as a source of Na/K-ATPase was a critical part of the Nobel (1997) prize-winning work of Dr. JC Skou on this enzyme. These animals can be obtained and maintained in the laboratory year-round. It has been suggested that the dogfish shark may be the most abundant of all shark species. Adults are 2 to 4 feet long and can weigh up to 20 pounds. Developing shark embryos at various stages can be removed from captured pregnant females and maintained while developing in saltwater tanks. The potential use of skate embryos in developmental biology has been described above. Specific monoclonal antibodies and antisera, cell cultures and reagents for cellular-molecular biological analysis are available within the research community.

A recent program implemented at MDIBL has established transfection and cell culture technology for spiny dogfish shark and little skate (Parton et al., 2004). Once sufficient genomic information is available, *in vitro* approaches can be used to conduct targeted studies to evaluate functionality of proteins and regulatory regions in the spiny dogfish shark and little skate genomes. Proliferating cultures have been initiated from the spiny dogfish shark rectal gland, spiral valve, eye, liver, brain, early embryo (several early stages) and kidney and skate liver and embryo (multiple somite stages). Multipassage culture of telomerase-positive cells has been achieved with spiny dogfish shark eye, brain, kidney and embryo and little skate embryo and liver, and it is expected that these lines will proliferate indefinitely. Cell lines are available to any researcher requesting them from MDIBL. These cultures can be DNA-transfected by electroporation or lipofection and will express plasmid genes under control of the CMV early promoter/enhancer. Cell cultures in combination with genomic information would allow physical mapping and construction of radiation hybrid panels.

The organism's state of readiness for sequencing and resources available to complement the sequence

The haploid chromosome number is 49 for little skate and 30 for spiny dogfish shark. The haploid genome sizes of the little skate and spiny dogfish shark are 3.5 pg/nucleus and 6.5 pg/nucleus, respectively (Schwartz and Maddock, 2002; Stingo and Rocco, 2001). An extensive biological literature exists on these organisms; a search of BIOSIS Previews (1969-September 2003) revealed 1,318 for *Squalus acanthias*, and 470 for *Raja erinacea*. A PubMed search identified more than 3000 references for shark and more than 700 references for skate. About one third of the shark references were devoted to *Squalus acanthias*. The remaining references described a variety of species including the spotted dogfish shark (order Carcharhiniformes: *Scyliorhinus canicula, ~ 270 references),*nurse shark (Order Orectolobiformes: *Ginglymostoma cirratum*; ~100 references) and the horn shark (Order Heterodontiformes: *Heterodontus francisci*; ~20 references). Despite their common names, the spiny and spotted dogfish sharks are quite distant evolutionarily, as are the nurse and horn sharks. The Squaliformes, which contain *Squalus acanthias,* and Rajiformes, which contain *Raja erinacea,* shared a common ancestor with the other orders 200 million years ago. Squaliformes and Rajiformes shared a common ancestor 150 million years ago. Heterodontiformes shared a common ancestor with Orectolobiformes and Carcharhiniformes 175 million years ago. Orectolobiformes and Carcharhiniformes shared a common ancestor 150 million years ago and Charcharhiniformes appeared about 135 million years ago. The most cited skate in PubMed is the little skate, with about four times the number of citations than the clearnose skate. Genomic DNA or cDNA sequences submitted to GenBank total 94 for *Squalus acanthias*, including the $Na^+/K^+/2Cl^-$ cotransporter, K channel; CFTR; natriuretic peptide receptor; Gi-α-2 subunit of G-protein; Sgk-1 and sgk-2 serine-threonine protein kinases.



Expressed sequence tags (ESTs) for *Squalus acanthias* and *Raja erinacea* presently are being generated at MDIBL. In a pilot analysis of 172 expressed sequence tags from spiny dogfish shark rectal gland, 39% were novel genes or untranslated regions. Thus far, the expressed sequence tag program for *Squalus acanthias* rectal gland has produced 1,358 ESTs in 920 contigs. Among these sequences were ESTs similar to HoxA10, zinc finger protein 235 and transcription factors NERF-1a and 1b, sox11, znf6. From little skate, the program has produced 552 ESTs in 323 contigs. BLAST analysis of skate liver ESTs revealed sequences similar to Bmp2-inducible kinase, complement C3 precursor, hepatic nuclear factor 4, zinc finger protein C3HC4 and translation elongation factor 2 and a number of liver-related genes. The results of these pilot EST projects are being submitted to the EST database (dbEST) at the National Center for Biotechnology Information (NCBI). They are also available through an MDIBL collaboration on the Hollings Marine Laboratory website (http://www.marinegenomics.org). Efforts are also directed at sequencing ESTs from other organs including shark brain, heart and kidney.

A recent project at MDIBL produced normalized cDNA libraries representing 10 different tissues from both shark and skate. From each of these libraries at least 5,000 clones are being sequenced from the 5' end. These will be compared against sequences in GenBank, and annotated for inclusion in dbEST. EST sequencing will increase to 50,000 clones from each normalized library as time and funding permit.

A DNA microarray expression unit also is currently under development at MDIBL. Alignment of EST sequences from a sampling of shark and skate genes (F1ATPase, glutamate decarboxylase, urea transporter; myelin basic protein, Pax2 and CFTR) showed that the skate and shark orthologs were 70 to 99% identical at the nucleotide level. This suggests that it may be possible to use skate microarrays for analysis of shark gene expression, and to identify and PCR-clone segments of shark genes from orthologous skate sequences. BAC libraries exist for horn shark and clearnose skate, and a nurse shark library is under construction. Sequence identity between the two skate species looks quite good (C. Amemiya) and similarly can be explored further. The close relatedness of the two species makes it likely that information obtained from the little skate will be of nearly direct use for laboratories using the clearnose skate.

Bioinformatics. The initial assembly and preliminary annotation of the shark and skate genomic sequences will be performed at the Washington University Sequencing Center. In order to facilitate public access to the sequence data, we plan to leverage the uniquely appropriate resources at MDIBL. In addition to being an international center for elasmobranch researchers, researchers at MDIBL are developing the Comparative Toxicogenomics Database (CTD). CTD will provide the necessary infrastructure to integrate these genomic sequences into a curated database dedicated to comparative studies and provide users with the ability to query, retrieve and download data. We will explore several open-source software packages to provide additional capabilities for viewing and comparing genomic sequences such as the Generic Genome Browser and ZPicture (Ovcharenko et al., 2004). CTD is being developed in collaboration with investigators at MDIBL, three other MFBS Centers (Oregon State University, University of Miami, and University of Wisconsin-Milwaukee) and investigators from other research institutions. Collaborations are in place for a community-based curation effort of data in CTD, particularly with respect to aquatic organisms. This initiative will contribute to the annotation of the shark and skate genomic sequences once they are publicly available. Genomic sequences will also be provided on genome browser websites, such as NCBI, ENSEMBL, and UCSC.

The bioinformatics group at MDIBL has close working collaborations with Dr. Monte Westerfield at the Zebrafish Information Network (ZFIN) and Dr. Janan Eppig at the Mouse Genome Informatics (MGI) Databases. Drs. Westerfield and Eppig have significant experience integrating genomic sequences with curated information in their databases. In addition to providing support for this sequencing proposal they have offered to provide guidance to the MDIBL bioinformatics staff with integrating shark and skate genomic sequences with curated data in CTD and to integrate their databases with CTD to promote the exchange of comparative data (indicated in letters of support).

Community Support for Genomic Sequencing of *S. acanthias* and *R. erinacea*.

MDIBL received letters supporting the genomic sequencing of *Squalus acanthias* and *Raja erinacea* from 61 distinguished scientists from national and international institutions. The authors of these letters strongly expressed their belief that genomic sequences from these organisms will add significant value to many important areas of biomedical research and our understanding of the human genome and molecular evolution. A list of these individuals is provided in Table III and copies of the letters will be provided upon request.

The letters come from investigators in North America, Europe and Asia. They include scientists and physicians using the spiny dogfish shark and little skate in biomedical research (eg., Dawson, Cutting, Skou, Haller, Kinne, Zeidel). They also include those using other shark or skate species, particularly in immunology (eg., Litman, Marchalonis, Hueter, Luer Hazon), who feel that the spiny dogfish shark and little skate are the most valuable choices for the scientific community as a whole. Also in the list are scientists involved in genome projects with other organisms who see the value of data from spiny dogfish shark and little skate for comparative genomics (eg., Eppig, Iwama, Lindblad-Toh, Postlethwait, Robinson, Samollow, Shima, Zon, Westerfield). In addition, there are investigators who are currently pursuing questions in areas of comparative genomics or evolution that will find the spiny dogfish shark and little skate genome data valuable (eg., Wagner, Ruddle, Stubbs, Ovcharenko, Kultz, Hahn).

Sequencing strategy and cost

Spiny Dogfish shark (*S. acanthias)*

The large genome size of sharks poses unique challenges not previously encountered with other animal genome sequencing projects. It will be necessary to investigate novel approaches to achieve genomic reduction for this organism; a strategy that has been successful in sequencing the plant, *Zea mays*. Strategies developed to facilitate a representation of the shark genome may also serve as a model for sequencing other large and/or repetitive genomes. Methods used to resolve the shark genome first must sufficiently represent genic regions, and secondly present them in context to the overall structure and organization of the genome. A physical map along with paired end sequences from mapped clones will be crucial to providing a framework for the method used. An extensive EST project will provide a reference to evaluate the effectiveness of a method for identifying genic regions. Furthermore, EST and mapped BAC end sequences will be vital for linking partial gene assemblages, so that entire genes maybe finished. Sequencing the shark genome offers the potential to explore alternative strategies that might be both cost-efficient and meet the objectives of a large-scale sequencing project.

Little skate (*R. erinacea*)

The sequencing of most genomes usually proceeds via a whole-genome shotgun (WGS) approach in its initial phase but in later stages relies on paired sequence reads from large-insert clones for long-range continuity. Our strategy for sequencing the genome of the little skate genome will utilize a map-assisted WGS approach. This will consist of a BAC-based physical mapping component targeted at 15x physical clone coverage, ~6-fold WGS component consisting of both small and large insert clones and an automated finishing component. The physical map, along with paired end sequences from fosmids and the mapped BAC clones, will provide a framework by which the genome sequence can be accurately assembled, and ordered and oriented on the chromosomes. In addition, we in collaboration with others will confirm the anchoring of supercontigs to the genome using FISH with fosmids from major supercontigs. We expect that the cost of generating a physical map and 6X sequence coverage would be approximately $39M.

Proposed whole genome shotgun sequencing of the *R. erinacea* genome.

Description	Quantity	Coverage
4 kb plasmids	37M	(6x)
40 kb fosmids	2.5M	(.3x)
BAC end reads	0.1M	(0.02X)
BAC fingerprints	0.3M	(15X physical coverage)

Two BAC libraries (10x and 5x) will be constructed using DNA from the same animal as the WGS sequencing effort. Fosmid libraries will also be constructed. All libraries will be available for distribution. From the BAC library a map will be created by standard fingerprinting methods, which will result in a fifteen-fold clone coverage of the genome (Marra et al., 1997). End sequences from 225,000 BACs will assist in the assembly of the whole genome shotgun and anchor the assemblies to the physical map. The genome sequences will be assembled with *Pcap* and *Arachne2*, and the higher quality assembly will be released via our websites (Huang et al., 2003; Jaffe et al., 2003). To improve the overall quality of the 6x assembly, one round of automated finishing will be performed. The program Autofinish will select sequencing reactions for closing gaps and improving low quality areas (Gordon et al., 2001). For the typical genome, a 50-60% reduction in contig number will be realized from a single round of Autofinish (unpublished data).

Genome annotation

Analysis and annotation of the little skate or spiny dogfish shark will be accomplished in a manner similar to our work on the *C. elegans* genome. We will work with the shark and skate community to ensure that the resulting annotation is consistent with the requirements of their respective research communities. A preliminary gene set will

be built using software packages, such as *Genwise* (EBI) or *FGENESH* (Softberry) based on protein similarity. This gene set will then be corrected based on EST and cDNA sequences and then released to the research community for further annotation and curation. The final gene set will be corrected using a modification of Eannot.pl, a tool developed at the WUGSC for the analysis of human chromosomes 2 and 4 (manuscript in preparation). If both the little skate and spiny dogfish shark genomes are sequenced, then a comparative *Twinscan* approach will also be employed (Korf et al., 2003).

Data availability

Shotgun reads will be downloaded daily to the NCBI Trace Archive. All pertinent sequence information from the little skate and spiny dogfish shark will be displayed on genome browser websites, such as NCBI, ENSEMBL, and UCSC as well as MDIBL's Comparative Toxicogenomics Database.

Additional consideration

The heterozygosity of the skate or shark is unknown, but needs to be investigated before a WGS project ensues. High polymorphic rates in other WGS organisms have posed significant problems in assembling sequences and creating BAC maps. To address the question of heterozygosity of the shark and skate genomes the WUGSC will initiate heterozygosity analyses of several animals of both fish provided by MDIBL.

Figure 1. Phylogeny adapted from NCBI (Belle et al., 2002).

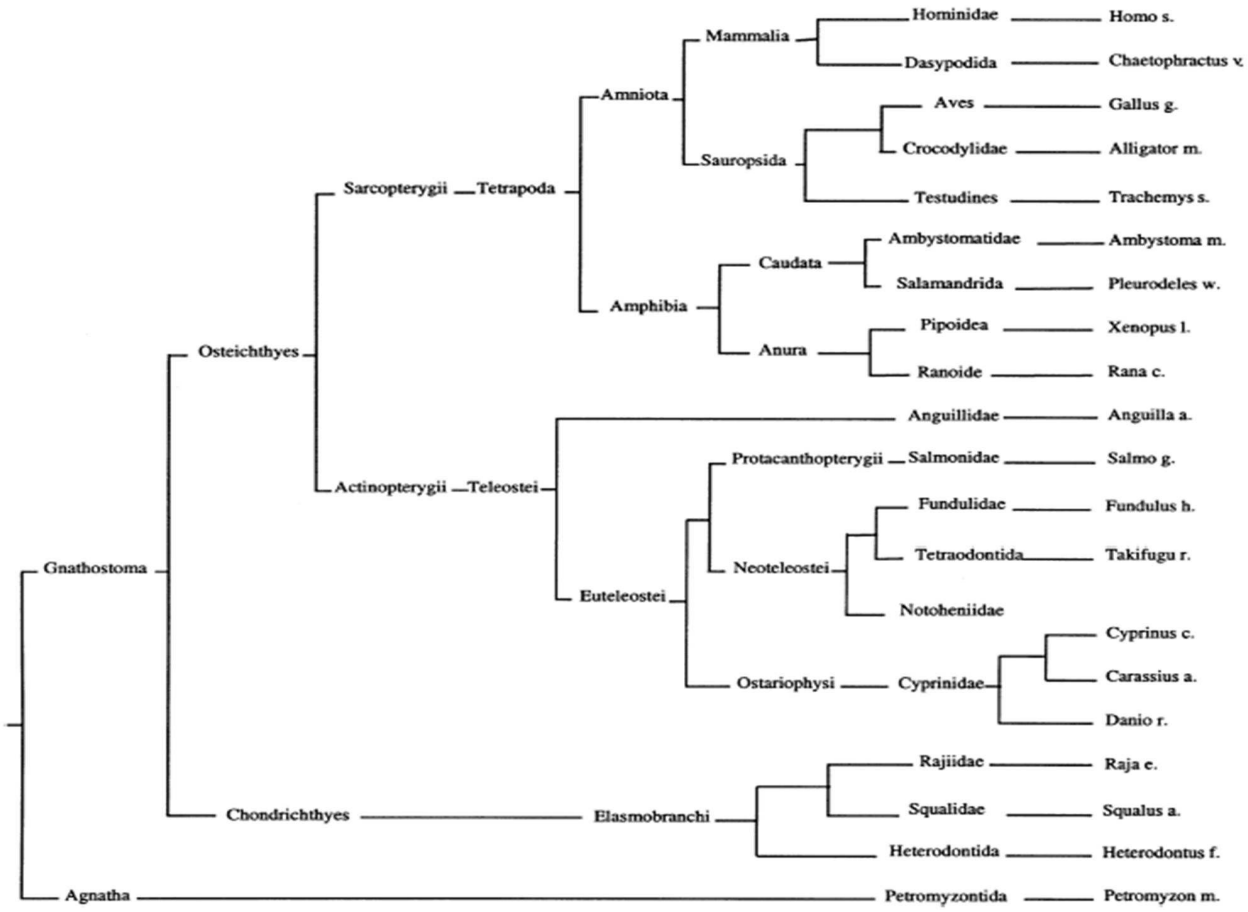


Table I. Summary of current biomedical research with *S. acanthias* from investigators worldwide.

Tissue	Research Focus	Recent Citations (examples)
Rectal gland	CFTR	Hanrahan et al., 1996; Lehrich et al., 1998
	Transport regulation	Aller et al., 1999; Waldegger et al., 1998; Yang et al., 2002
	Na-K-Cl cotransporter	Darman and Forbush, 2002; Flemmer et al., 2002; Gagnon et al., 2004; Gagnon et al., 2003
	Na-K ATPase	Cornelius and Mahmmoud, 2003; Mahmmoud et al., 2003; Skou, 1998
	Contractility	Evans and Piermarini, 2001
	Ultrastructure/cytoskeleton	Silva and Epstein, 2002; Valentich et al., 1996
	Xenobiotic transport	Karnaky et al., 2003; Miller et al., 2002a; Miller et al., 2002b
	Heavy metal toxicity	Ballatori and Villalobos, 2002; Ke et al., 2002; Kinne-Saffran and Kinne, 2001
	Salinity sensing	Fellner and Parker, 2002
Brain/CNS	Blood-brain barrier	Miller et al., 2002a
	Xenobiotic transport	Villalobos et al., 2002
	Glia	Kalman and Gould, 2001
Kidney	Regeneration	Elger et al., 2003; Hentschel et al., 1998
	Ion/urea transport	Fines et al., 2001; Gagnon et al., 2002; Smith and Wright, 1999
	Glucose transport	Kipp et al., 1997
	Salinity sensing	Hentschel et al., 1998; Nearing et al., 2002
Liver	Lipid metabolism	Duggan et al., 2002
	Antibiotics	Rao et al., 2000
Heart/vascular	Transport regulation	Woo and Morad, 2001
	Vascular regulation	Agnisola et al., 2003; Evans and Piermarini, 2001
	Angiogenesis	Bhargava et al., 2001; Gingras et al., 2003
Gill	Transport regulation	Evans and Gunderson, 1999; Wilson et al., 2002; Wilson et al., 1997
Other	Functional genomics	Ballatori et al., 2003; Hong et al., 1996; Mattingly et al., 2003; Mattingly et al., 2004
	Cell Culture	Parton et al., 2004
	Toxicology	Betka and Callard, 1999
	Protein Biochemistry	Schuurmans Stekhoven et al., 2003
	Embryology/Endocrinology	Koob and Callard, 1999; Ringholm et al., 2003

Table II. Summary of current biomedical research with *R. erinacea* from investigators worldwide.

Tissue	Research Focus	Recent Citations (examples)
Liver	Xenobiotic transport	Cai et al., 2003; Rebbeor et al., 2000
	Anion transport	Ballatori et al., 2000; Cai et al., 2001; Cai et al., 2002; Seward et al., 2003
	ATP receptor	Dranoff et al., 2000
Eye	Neurotransmitter receptor	Chappell et al., 2002; Kreitzer et al., 2003; Malchow and Andersen, 2001
	Heavy metal neurotoxicity	Redenti and Chappell, 2003; Rosenstein and Chappell, 2003
Brain	Glia	Kalman and Gould, 2001
Kidney	Regeneration	Elger et al., 2003
	Glucose Transport	Kipp et al., 1997
	Urea Transport	Morgan et al., 2003b
Gill	Ion transport	Choe and Evans, 2003
Blood	Cell volume regulation	Goldstein et al., 2003; Guizouarn et al., 2003
Embryo	Endocrinology	Koob and Callard, 1999
Other	Functional genomics	Ballatori et al., 2003; Mattingly et al., 2003; Mattingly et al., 2004
	Toxicology	Ballatori and Villalobos, 2002; Grosell et al., 2003; Runnegar et al., 1999
	Cell Culture	Parton et al., 2004
	Immunology	Anderson et al., 1995; Widholm et al., 1999

Table III. Individuals submitting letters of support for the genomic sequencing of *Squalus acanthias* and *Raja erinacea*.

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