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The origins of multicellularity: a multi-taxon genome initiative

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The emergence of multicellular organisms from single-celled ancestors – which occurred several times, independently in different branches of the eukaryotic tree – is one of the most profound evolutionary transitions in the history of life. These events not only radically changed the course of life on Earth but also created new challenges, including the need for cooperation and communication between cells, and the division of labor among different cell types. However, the genetic changes that accompanied the several origins of multicellularity remain elusive. Recently, the National Human Genome Research Institute (NHGRI) endorsed a multi-taxon genome-sequencing initiative that aims to gain insights into how multicellularity first evolved. This initiative (which we have termed UNICORN) will generate extensive genomic data from some of the closest extant unicellular relatives of both animals and fungi. Here, we introduce this initiative and the biological questions that underpin it, summarize the rationale guiding the choice of organisms and discuss the anticipated benefits to the broader scientific community.

The origins of multicellular organisms: animals and fungi as a case study

The emergence of multicellular organisms is one of the most important and enigmatic evolutionary leaps in the history of life on Earth. In contrast to other major evolutionary transitions, multicellularity seems to have arisen multiple times independently in eukaryotes: in fungi, animals, slime molds, charophyte algae (and their descendants, the land plants), and certain other green, red and brown algae. However, despite the obvious importance of the transition from unicellular organisms to multicellular ones, little is known about how this pivotal event occurred in different eukaryotic lineages. New, comprehensive molecular and genomic data are required to improve our current understanding of the origins of multicellularity. In this context, the eukaryotic supergroup known as Opisthokonta provides an important evolutionary window. The opisthokonts include two of the most noticeable multicellular eukaryotic kingdoms – Metazoa (animals) and Fungi – the origins of which have been intensely debated for years [1]. The opisthokonts also include several unicellular lineages, all of which share a single-celled common ancestor with animals and fungi (Figure 1). By comparing the gene content of multicellular animals and fungi with that of their unicellular opisthokont relatives, it should be possible to improve our understanding of how multicellularity evolved in these two main groups of multicellular organisms. This is the specific aim of UNICORN (unicellular opisthokont research initiative), which we describe here. Knowledge gained from this initiative will also provide a basis for elucidating the degree to which the emergence of multicellularity in other groups followed similar or independent evolutionary trajectories.

What do we know about the genetic basis of multicellularity?

The evolution of multicellularity not only opened the door to a diverse range of evolutionary novelties but also posed new challenges to the organisms that acquired this trait. Genes involved in cell–cell communication, cell adhesion and cell differentiation probably arose before, or concomitant with, the origins of multicellularity. On the basis of recently obtained molecular data, the emerging hypothesis of how animals and fungi originated posits that their ancestors probably had a substantial part of the genetic toolkit needed to construct a multicellular body plan [2–5]. For example, most of the key regulatory genes and signaling molecules deployed during development of multicellular animals are widely conserved across Metazoa. Even the sponges, members of one of the earliest branching and morphologically simplest animal phyla, have recently been shown to express genes that encode components of at least six of the seven main animal cell signaling pathways and to have representatives of all animal-specific gene families involved in cell adhesion [4]. Similarly, Cnidaria, which includes organisms such as sea anemones and corals and which (similar to sponges) is another diploblastic phylum branching deeply within Metazoa, has a small

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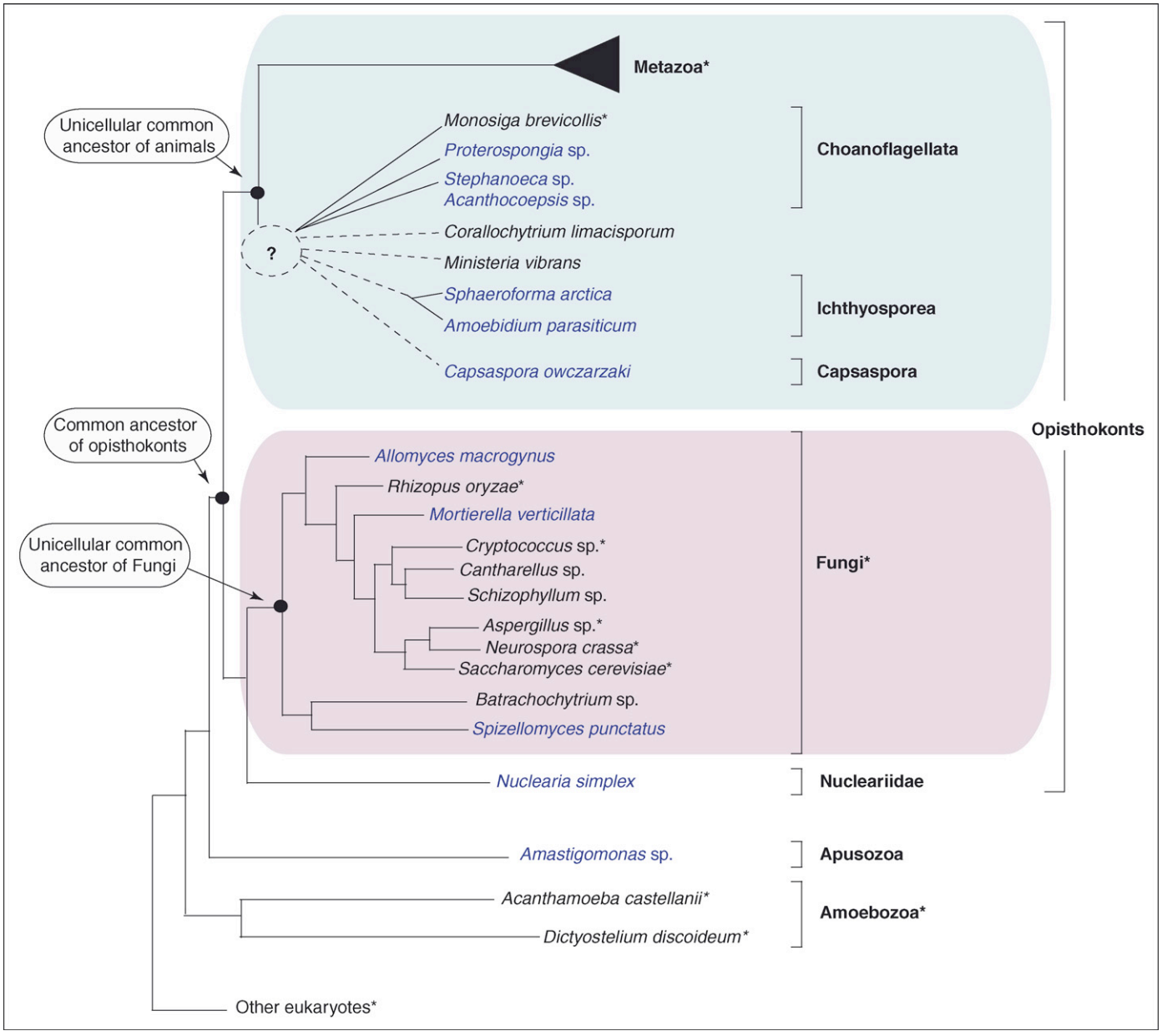

Figure 1. Phylogenetic tree of opisthokonts based on molecular data. The tree incorporates unpublished information available to the authors, in addition to published data [20,24]. Species for which genome sequencing is to be carried out under the auspices of UNICORN are labeled in blue. Asterisks indicate species for which genome sequences are either already available or slated for completion. Unicellular opisthokonts known to be more closely related to animals than to fungi are depicted as an unresolved polytomy (a node with more than two descendant branches). Some studies based on large concatenated data sets of nuclear-DNA-encoded [16] or mitochondrial-DNA-encoded [17] proteins have robustly identified choanoflagellates as the sister group to animals; however, in these analyses, taxon sampling for this part of the tree was limited (e.g. *Capsaspora, Corallochytrium* and *Ministeria* were not included). The sister-group relationship of Choanoflagellata and Metazoa has been challenged in other studies that include these previously unsampled taxa [20,24]; however, these analyses are based on data sets that comprise relatively few genes. Additional data, such as those expected from UNICORN, undoubtedly will help to resolve the branching order at the base of the opisthokont lineage.

set of Hox genes that control early axial patterning [6,7]. Finally, many structural features of cell and tissue organization – for example, desmosomes, other cell–cell junctions and extracellular matrix proteins – are distributed ubiquitously throughout Metazoa [5].

Surprisingly, numerous genes associated with multicellularity are also present in the closest known relatives of animals, the choanoflagellates (a group of unicellular and colonial flagellated protozoans). Pilot expressed-sequence tag (EST) studies in *Monosiga brevicollis* have revealed the first known non-animal homologs of animal genes involved in cell signaling and cell adhesion, such as protein tyrosine kinases, C-type lectins and cadherins [8]. Because the functions of these genes have been studied exclusively in multicellular animals and their cell-line derivatives, important questions are what roles these signaling and adhesion gene homologs have in the unicellular context of choanoflagellate biology, and what this information might tell us about the functions of these genes before the origin of animals. For example, in animals, protein tyrosine kinases respond to extracellular growth factors to mediate signaling between neighboring cells, and they are required for regulating proper development and cell proliferation. In choanoflagellates, protein tyrosine kinase function is also required for regulating cell proliferation, although what triggers the activity of

these enzymes is unknown. Interestingly, transfer of choanoflagellates from a nutrient-depleted environment to one that is nutrient rich results in a rapid change in the profile of tyrosine-phosphorylated proteins. Therefore, it is possible that protein tyrosine kinases in choanoflagellates respond directly to specific environmental cues (e.g. nutrient availability) or that they detect signals released from other cells in response to environmental change [8]. This observation raises the possibility that the function of many of the choanoflagellate homologs of animal genes involved in cell signaling and adhesion is to enable choanoflagellates to interpret and respond to their physical and biological environment. Such proteins might function specifically in activities such as predation (which requires recognition and binding of particular bacterial species), quorum sensing and sex (unknown so far from choanoflagellates, but probable given its widespread distribution throughout eukaryotes [2,8]).

Many metazoan gene families, however, have not yet been detected in their closest single-celled relatives [i.e. choanoflagellates, ichthyosporeans (a group of single-celled parasites [1,9]), and another class, of which *Capsaspora* is the sole member at present; Figure 1]. For example, although homeobox genes are present in animals, fungi and green plants, certain classes of these genes emerged during animal evolution. So far, the ANTP (Antennapedia) and PRD (paired) classes of homeobox genes have been detected only in multicellular animal genomes. These classes include the Hox, Dlx (distal-less), En (engrailed), Emx (empty spiracles), Msx (muscle segment) and Otx (orthodenticle) gene families. This is also true for many other transcription factors [e.g. T-box and Pax (paired box) families] and intercellular signaling molecules [e.g. Wnt proteins, fibroblast growth factors, ephrins, transforming growth factor-β, hedgehog and delta]. These findings raise the question of whether homologs, or perhaps progenitors, of these regulatory and structural proteins (or protein domains) could be present in the unicellular relatives of animals. It is certainly conceivable that some of the key gene families mediating animal development had already appeared and/or diversified in the unicellular ancestors of animals (reviewed in Ref. [2]). Alternatively, it is probable that some of these proteins are metazoan specific, some being new inventions and others being assembled by rearrangement of protein-coding gene modules in the ancestors of animals. One example of such rearrangements is the hedgehog gene family (which encodes key signaling proteins), for which a homolog of one region of the encoded proteins has been found in a choanoflagellate [10].

Considering fungi raises additional questions. For example, most of the genes associated with multicellularity that are ubiquitous in animals seem to be absent from many fungal lineages. This situation is particularly evident in budding and fission yeasts, which have markedly fewer nuclear genes than other fungi, probably owing to their unicellular life style [11]. However, some ascomycetes have numerous developmental genes (estimated at ~100 [12]) that are involved in building the multicellular structures (i.e. the fruiting bodies) of these fungi. Again, some of these multicellularity-associated gene families might be fungi specific, whereas others might have already diversified in unicellular common ancestors of Fungi.

The aim of the National Human Genome Research Institute (NHGRI)-endorsed initiative outlined here is to test hypotheses about the evolutionary emergence of multicellularity, for all metazoan-specific and fungi-specific gene families, through genome comparisons among diverse metazoans and fungi and their protistan relatives. It should be stressed that, for such an exploration, genome sequences, rather than ESTs, are required, for two reasons: first, because genes expressed at a low level are generally not represented in EST libraries, precluding determination of the overall genetic complexity of the organisms under study; and, second, because the number and types of transcripts being expressed are greatly influenced by the life-cycle phase of the organism. The genomic data gathered by this initiative will surely lead us to a better understanding of the genetic composition of the unicellular common ancestor of both animals and fungi and, ultimately, to a determination of the putative metazoan and fungal 'genetic starter kits' for multicellularity. However, progress in achieving these goals can only be made if we know which specific unicellular lineages are most closely related to animals and fungi.

The unicellular opisthokonts: extant relatives of animals and fungi

Molecular phylogenies have shown convincingly that Metazoa and Fungi are derived from a common ancestor [13–16]. Recent molecular studies have demonstrated that Opisthokonta, the taxonomic supergroup that contains animals and fungi, also includes several unicellular lineages such as the nucleariids (Nucleariidae), the choanoflagellates (Choanoflagellata; also known as Choanoflagellida), the ichthyosporeans (Ichthyosporea; also known as Mesomycetozoea [9]), *Capsaspora*, *Corallochytrium* and *Ministeria* [1,15–23] (Figure 1). However, the precise phylogenetic relationships within the opisthokonts have not been resolved completely. For example, the phylogenetic positions of *Corallochytrium* and *Capsaspora* have weak statistical support and need to be tested by generating additional data. Nevertheless, most other groups are strongly supported, even with the data that have been gathered so far. For example, it is clear that nucleariids constitute the unicellular lineage that is most closely related to fungi, whereas the remaining unicellular opisthokonts (i.e. choanoflagellates, ichthyosporeans, *Ministeria, Corallochytrium* and *Capsaspora*) are decidedly more closely related to animals than to fungi [17–20,24] (B. Franz Lang, unpublished). It should be emphasized that, at present, these single-celled opisthokonts are the only known eukaryotic microorganisms with genomes that have the potential to inform our understanding of the origin(s) of multicellularity in these two eukaryotic clades.

Of all of the opisthokont lineages, Metazoa is the only one for which genomic and EST data are available for a wide diversity of species, thus enabling comprehensive comparative analyses within this clade. For Metazoa, several complete genome sequences are available for bilaterian animals, and genome-sequencing projects for

Table 1. List of UNICORN taxa

Species	Taxonomic classification	Culture	Other features	Coverage
Proterospongia sp. or similar species	Choanoflagellata	Monoxenic	Colonial species	Draft (5×)
Stephanoeca sp. or similar species	Choanoflagellata	Mixed bacteria	Loricate species, complex exoskeleton	Low (2×)
Capsaspora owczarzaki	Choanozoan relative	Axenic	Medical relevance (schistosomiasis), known genome size	Draft (5×)
Sphaeroforma arctica	Ichthyosporea	Axenic	Multicellular-like microcolonies	Draft (5×)
Amoebidium parasiticum	Ichthyosporea	Axenic	Multicellular-like microcolonies	Low (2×)
Nuclearia simplex	Nucleariidae	Monoxenic	Fish pathogen	Draft (5×)
Spizellomyces punctatus	Chytridiomycota (Fungi)	Axenic	Used for molecular studies of mitochondrial tRNA editing	Draft (5×)
Allomyces macrogynus	Chytridiomycota (Fungi)	Axenic	Best-developed chytrid model	Low (2×)
Mortierella verticillata	Zygomycota (Fungi)	Axenic	Medical relevance (zygomycosis)	Low (2×)
Amastigomonas sp.	Apusozoa	Monoxenic	Opisthokont outgroup	Draft (5×)

a sponge and two cnidarians are nearly complete. By contrast, only one genome-sequencing project for a single-celled opisthokont relative of metazoans (the choanoflagellate *M. brevicollis*) is underway, and several key basal fungal lineages remain unsampled at a genomic level. To fill these gaps in a systematic manner, ten single-celled organisms have been selected for comprehensive genome sequencing, as described in the following section.

Rationale for selection of organisms targeted for genome sequencing

Species selection for this initiative was based on a combination of phylogenetic relevance, biological knowledge, availability, culturability and technical feasibility. In this section, we discuss the rationale for selection of the particular organisms, the genomes of which will be sequenced in this initiative (Table 1).

To gain insight into the origins of multicellularity in animals, genomic data for a range of taxa near the base of the metazoan branch are needed, in addition to data for unicellular opisthokonts more broadly. The key candidate species are found in two diverse protistan taxa, the choanoflagellates and the ichthyosporeans, plus three rather enigmatic genera, *Capsaspora, Corallochytrium* and *Ministeria*. This genome-sequencing initiative will, therefore, target choanoflagellates, ichthyosporeans and *Capsaspora*.

The choanoflagellates are mostly unicellular, although several colony-forming species are also known. Their possible relevance to animal origins was first noted 140 years ago, when Henry James-Clark observed structural similarity between some choanoflagellates and the feeding cells of sponges [25]. It should be noted, however, that there is considerable structural diversity within the choanoflagellates, with some forms producing a complex silicate 'lorica' around the cell, others having a rigid theca, and still others existing as naked cells [26]. The genome of one naked choanoflagellate, *M. brevicollis,* is being sequenced at the Joint Genome Institute (JGI). UNICORN will extend this sampling to encompass more of the diversity within this clade, notably through selection of a colonial choanoflagellate such as *Proterospongia* sp. and a loricate choanoflagellate such as a species of *Stephanoeca* or *Acanthocoepsis*.

The other metazoan relatives, *Capsaspora owczarzaki* and the ichthyosporeans, also occupy pivotal phylogenetic positions close to the base of the metazoan branch (Figure 1), so study of these should prove highly informative. Furthermore, EST data are already available for *C. owczarzaki* and two ichthyosporean species, *Sphaeroforma arctica* and *Amoebidium parasiticum* (see the Taxonomically Broad EST Database; http://amoebidia.bcm.umontreal.ca/pepdb); these data should assist in the interpretation and annotation of genome sequences. More importantly, for all three of these species, in contrast to the experience with most unicellular opisthokonts, we and other investigators have established axenic (bacteria free) cultures that grow well. Notably, the selected candidates all have additional biological features that point in favor of their inclusion in this initiative. For example, *Capsaspora* has relevance to human health, because its host, the snail *Biomphalaria glabrata*, is also the intermediate host of the digenean flatworm *Schistosoma*

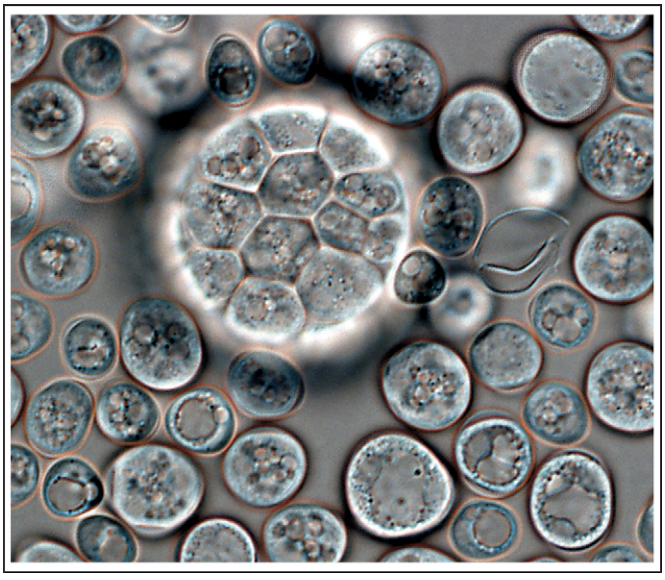

Figure 2. Differential interference contrast (DIC) micrograph of *Sphaeroforma arctica*. The micrograph illustrates the formation of pseudo-multicellular colonies. Under optimal conditions, individual microcolonies of this species develop spheres containing multiple (up to 30) individual cells, which eventually are released. The sphere shown is ~30 μm in diameter. *Amoebidium parasiticum* forms similar structures. Studying both of these species is likely to yield insights into the transition from unicellularity to multicellularity.

mansoni, the causative agent of widespread schistosomiasis in humans. *C. owczarzaki* not only parasitizes the intermediate host of *S. mansoni* but also attacks and kills the sporocysts of the flatworm living inside the snail [27]. Thus, because the genome sequences of a parasite (*S. mansoni*) and one host (*Homo sapiens*) have been completed, and because the genome of the second host (*B. glabrata*) is being sequenced, the *Capsaspora* genome sequence should offer important insights into genes implicated in this complex host–parasite–vector system. Moreover, the genome of *Capsaspora* has recently been determined to be of a reasonable size (~22–25 Mb) for complete sequencing [24]. In addition, both *S. arctica* and *A. parasiticum* show 'multicellular-like' characteristics (i.e. under optimal conditions, they form pseudomulticellular colonies; Figure 2) that promise to yield further insights into the transition from unicellular organisms to multicellular ones. Although *Corallochytrium* and *Ministeria* are also relevant to the questions we pose, our knowledge of their biology and genetic composition is minimal, and both are difficult to culture *en masse*.

Multicellularity evolved independently in animals and fungi, and a different unicellular opisthokont species, *Nuclearia*, is the outgroup to this transition in Fungi. The phylogenetic position of *Nuclearia* as the sister group of Fungi is so crucial to an understanding of the origin(s) of multicellularity in the fungal lineage that we included this organism in the initiative despite the difficulties involved in culturing *Nuclearia*. In addition to examining the genome of *Nuclearia*, it is also necessary to increase the genomic sampling within Fungi more broadly if we are to discern which genes and proteins arose at the base of this clade. In particular, our knowledge of the biology and of the genomes of chytrids and zygomycetes is marginal compared with what we know about other fungi. Therefore, UNICORN aims to obtain genomic data from two chytrids, *Spizellomyces punctatus* and *Allomyces macrogynus*, and from the zygomycete *Mortierella verticillata*. These taxa all have the advantage of growing in the absence of bacteria. Moreover, chytrids are the only fungi that produce flagellated spores; the zygomycetes, in turn, include many pathogens and are, therefore, relevant from a medical perspective.

We chose the taxa mentioned here because of their potential to illuminate the unicellular-to-multicellular transitions that took place independently in the lineages leading to animals and to fungi. It is conceivable that there is a genetic or cellular predisposition to multicellularity in the opisthokont lineage as a whole. To investigate this possibility, and to delve further into the unique nature of opisthokonts, it will be necessary to compare the genomes within this group to the genome of an organism that represents an outgroup to the entire opisthokont clade. For this reason, we have chosen to sequence the genome of *Amastigomonas* sp. or another species of the apusomonads (small gliding flagellates), which are thought to branch as an outgroup to the opisthokonts (Figure 1).

The proposed genome-sequencing projects within this initiative have been prioritized by considering both their scientific importance and the ease of providing clean nuclear DNA. Thus, we aim for a moderate coverage level (5×) for the genomes of six organisms, while providing lower – but still useful coverage (2×) – for the remaining genomes (Table 1). However, it should be noted that these initial genome-sequencing targets might be reassessed after the genome size of each organism has been obtained (or confirmed) through pilot sequencing.

Concluding remarks

The main goal of UNICORN is to fill a major gap in current genomic data so that we can gain insights into the origin(s) of multicellularity. The initiative will provide the first comprehensive data sets with which to carry out meaningful comparative genomic analyses of animals, fungi and their specific unicellular relatives.

The comprehensive and coherent genomic data that will be generated in this initiative will provide a unique resource for fundamental life sciences research, including parasitology, comparative genomics, pathogenomics, macromolecular modeling, molecular evolution, gene discovery, and development of new experimental model systems (see the supplementary material online). A more detailed description of the genome-sequencing proposal endorsed and funded by the NHGRI can be found on the agency's website (http://www.genome.gov/10002154). The Broad Institute (http://www.broad.mit.edu), the genome center to which this initiative is assigned, is expected to begin pilot sequencing within the next few months. Interested members of the scientific community are invited to participate in analysis and annotation of the data as they become available.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tig.2007.01.005.

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