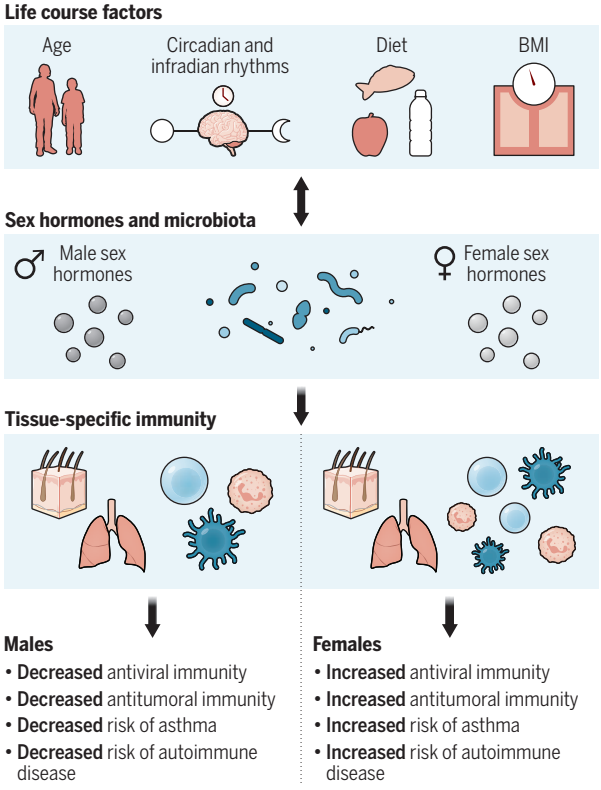
actions in tissue immunity. Recent shifts in dietary habits, including high-fat "Western" diets and trends to vegetarian, vegan, and ketogenic (high fat) diets, will also affect immune and physiological functions. Indeed, in humans, a ketogenic diet modulated adaptive immune cell pathways, whereas a vegan diet up-regulated pathways associated with antiviral immunity (*11*).

A key environmental factor regulating sex differences is age. Androgens are elevated in males and females during perinatal development and later in males during onset of adolescence (*2*). By contrast, in humans, concentrations of estrogen and progesterone fluctuate throughout life span in both sexes, with circulating levels of both hormones changing over critical points in the female life span during puberty, pregnancy, and menopause. In a mouse model of autoimmune colitis, cell-specific deletion of estrogen receptor-α (ERα) reduced pathogenic T cells and increased T regulatory cells (T_{regs}) in female mice, thereby ameliorating disease pathology (*12*). However, how estrogen signaling affects other barrier sites such as the lungs or skin remains undefined. This is important because, for example, asthma is more common in boys than girls, but then more prevalent in women compared to men, and severity tends to fluctuate with critical phases of hormonal change (*13*). Therefore, investigating how estrogen regulates immune function, particularly in T cells and type 2 immunity, and factoring in microbiota, age, and the developmental milestones associated with hormonal shifts, would help clarify precisely how sex hormones influence tissue pathology.

It is vital that studies factor biological sex as an essential immunological variable into experimental design, interpretation, and reporting in both human and animal studies. Inclusion of both sexes in all experimental settings is now stipulated by major funding agencies internationally. It may also be valuable to plan human immunological studies that include transgender individuals taking sex hormones to better understand the impact of artificial hormone replacement therapy on immune function. Such studies will help define relationships between sex hormone signaling, microbiota, and immune pathways across the life course, while also factoring in environmental variables such as age, circadian and infradian rhythms, BMI, and diet (see the figure). ■

Layers of immunoregulation

The tone and strength of immune responses in different tissues are calibrated by multilayered factors. Central to this immunoregulation is cell-cell and cell-protein cross-talk. Sex hormones and the microbiota are important modulators of tissue immunity, and their effects are influenced by age, diet, circadian and infradian rhythms, and body mass index (BMI). This can result in sex differences in tissue immunity. For example, T cell immunity is increased in females compared to males, which reduces viral infections and cancers in females but also increases the risk of developing inflammatory diseases such as asthma and autoimmunity compared to males.



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MICROBIOLOGY

The nitroplast: A nitrogen-fixing organelle

A bacterial endosymbiont of marine algae evolved to an organelle

By **Ramon Massana**

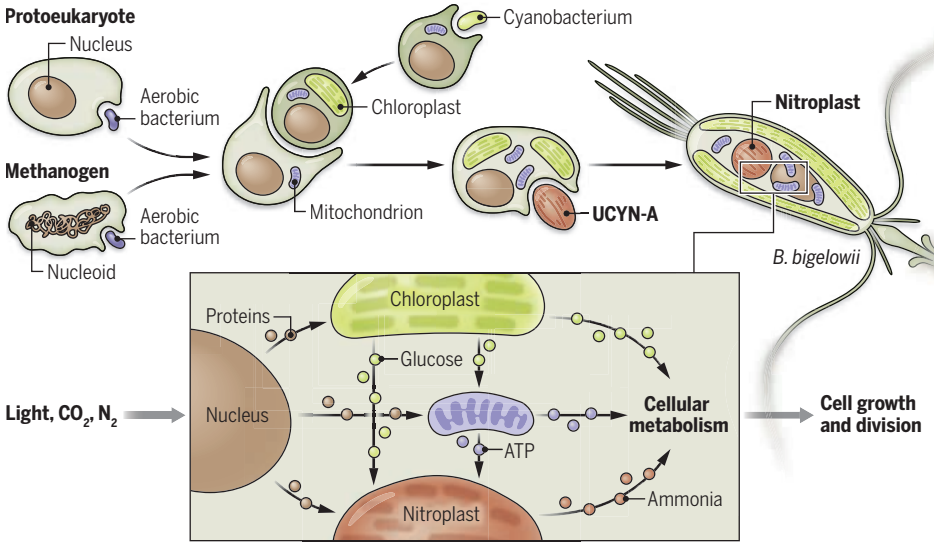
Eukaryotic cells are notably complex—for example, they have various organelles, which are membrane-bound structures with specific functions. Two of these organelles, mitochondria and chloroplasts, which function in respiration and photosynthesis, evolved from the integration of endosymbiotic bacteria to the eukaryotic cell (*1*). In marine systems, some nitrogen-fixing bacteria are endosymbionts of microalgae, such as *Candidatus* Atelocyanobacterium thalassa (UCYN-A), a cyanobacterial symbiont of the unicellular algae *Braarudosphaera bigelowii* (*2*). On page 217 of this issue, Coale *et al.* (*3*) report a close integration of the endosymbiont into the architecture and function of the host cell, which is a characteristic of organelles. These findings show that UCYN-A has evolved from a symbiont to a eukaryotic organelle for nitrogen fixation—the nitroplast—thereby expanding a function that was thought to be exclusively carried out by prokaryotic cells to eukaryotes.

Biological nitrogen fixation, which reduces atmospheric dinitrogen gas (N_2) into reactive ammonia (NH_3), is central in the nitrogen biogeochemical cycle as the only path to incorporate the abundant dinitrogen gas into biomass. This process represents a main driver of fertilization for aquatic and terrestrial systems and is continuously studied to increase crop yields in agriculture (*4*). To directly benefit from the resulting ammonia, many photosynthetic organisms, from terrestrial plants to microalgae, incorporate nitrogen-fixing symbionts (*5*). This is the case for *B. bigelowii* and relatives (belonging to the algal class Prymnesiophyceae) that carry the nitrogen-fixing UCYN-A cyanobacteria. The UCYN-A symbiont lacks the genes for the oxygen-evolving photosystem II and carbon fixation, which suggests that it is unable to perform oxygenic photosynthesis and is in-

Institut de Ciències del Mar (CSIC), Barcelona, Catalonia, Spain. Email: ramonm@icm.csic.es

Evolution and function of the nitroplast

Multiple organelles in eukaryotic cells, including mitochondria, chloroplasts, and nitroplasts, evolved from the integration of endosymbiotic bacteria. In *Braarudosphaera bigelowii*, the chloroplast fixes inorganic carbon to produce glucose, which feeds the respiratory chain in mitochondria that produces adenosine triphosphate (ATP), which in turn fuels nitrogen fixation in the nitroplast. Glucose, ammonia, and ATP generated by the organelles, together with externally incorporated compounds (phosphorous, mineral nutrients, and vitamins), are the building blocks for cell metabolism, resulting in cell growth and division.



GRAPHIC: N. BURGESS/*SCIENCE*

volved in a tight partnership with the host, providing it with fixed nitrogen and receiving fixed carbon in return (*6*). This symbiosis is now known to be very stable, to be widespread in sunlit coastal and oceanic waters, and to play a crucial role in the nitrogen biogeochemical cycle (*2*). However, challenges in obtaining stable cultures of *B. bigelowii* and UCYN-A have limited studies on this symbiosis.

Coale *et al.* successfully grew *B. bigelowii* in culture, which enabled them to further probe its interactions with UCYN-A. Tridimensional subcellular images taken with soft x-ray tomography were used to follow the development of the nucleus, mitochondria, chloroplast, and UCYN-A during the cell cycle. The data revealed a coordinated line of events for the replication and fission of these four components that shows that UCYN-A is as integrated within the eukaryotic cell architecture as the other three organelles. These findings also suggest that UCYN-A division is tightly controlled by the host and that the symbionts are transmitted to daughter cells during cell division. Furthermore, proteomics and comparative genomics analyses showed that UCYN-A contains many proteins that are imported from the eukaryotic host. These proteins are encoded in the host nucleus, translated in the host cytoplasm, signaled for transport to the nitroplast, and complement metabolic pathways that appear to be incomplete in the UCYN-A genome, such as those involved in the synthesis of some amino acids, nucleotides, or cofactors. The synchronized division and the import of essential eukaryotic proteins indicate that UCYN-A has evolved beyond endosymbiosis (*7*) and that it can instead be considered a eukaryotic organelle under the full control of the host. The organelle is called the nitroplast, taking the name proposed years ago for analogous systems (*8*) and denoting its role in nitrogen fixation and its cyanobacterial origin (by analogy to plastids, which are also derived from cyanobacteria).

Distinguishing an endosymbiont from an organelle can be challenging (*9*), and each reported endosymbiosis may appear at a different stage of a putative endosymbiont-organelle continuum. Nevertheless, the deep cellular integration of UCYN-A into the host and its severe genetic dependency support the view that the nitroplast of *B. bigelowii* can be added to the short list of endosymbiosis-derived organelles. The evolutionary history of the nitroplast is analogous to that of mitochondria and chloroplasts, including gene loss, coordinated division, and subjugation to the host. Besides the mitochondrion, chloroplast, and nitroplast, there are only a few additional cases of endosymbiosis-derived organelles (*10*), such as the chromatophore of the amoeba *Paulinella*. Additionally, the spheroid bodies of freshwater diatoms, which resemble UCYN-A in many ways, may represent another independently evolving nitroplast (*11*). Nonetheless, it is still intriguing that so few endosymbiosis-derived organelles are known, which emphasizes how difficult it is to achieve this transition (*12*).

The transitions from endosymbionts to the various organelles happened independently at different times of eukaryotic evolution, and this influences their taxonomic coverage. Mitochondria acquisition (thought to have occurred around 2 billion years ago) predates the origin of the eukaryotic cell, and these organelles are found throughout the eukaryotic tree of life, with some cases of secondary loss or modification. The primary endosymbiosis that originated the chloroplast also occurred in ancient times (likely around 1.5 billion years ago) in the supergroup Archaeplastida. Chloroplasts were later transferred to other eukaryotic supergroups by secondary or tertiary endosymbiosis. The establishment of the nitroplast is more recent—about 100 million years ago (*13*)—and this may explain why this organelle is taxonomically constrained to prymnesiophytes. Even within this narrow host range, this system has coevolved, revealing a remarkable relationship between organelle size and host size in related species (*14*). Given enough time, the nitroplast might be transferred to other lineages through secondary endosymbiosis, securing nitrogen supply to distant eukaryotes.

The study from Coale *et al.* shows that a renowned endosymbiont is actually the nitroplast organelle—an optimal adaptation of the microalgae to thrive in nitrogen-limited waters. Like in photosynthesis, a prokaryotic innovation that was incorporated by endosymbiosis into the eukaryotic cell and is now considered a eukaryotic function, these new data support the claim that nitrogen fixation is no longer an exclusive prokaryotic function and that eukaryotes can fix molecular nitrogen through the nitroplast. The nitroplast represents a textbook case of a eukaryotic organelle that complements the energy, carbon, and nitrogen needs of the algal host (see the figure) and is another example of how ecology is the theater where evolution takes place. ■

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