Focus on Research

The Mitochondria and Complex II—Linchpin for Skin Aging and Photoaging

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Mitochondria (see box on page XX) are far more than the power plants of our cells. Few people realize that these tiny structures play a central role in our physiology in various contexts and maintain a complex, synergistic relationship with the nuclear genome. Molecular dermatologist Birch-Machin is among those who do.

And in studying the functions of mitochondria in human skin, Birch-Machin recently made an eye-opening discovery that is critically important to both mitochondrial and skin health in chronologic and photoaging. It concerns the unexpected protective role of complex II—the diminutive, unassuming member of the respiratory enzyme chain that completes conversion of the energy from glucose to ATP (see box on page XX). The implications are profound for understanding what can go wrong and why, and for developing therapeutic interventions to slow chronological aging, protect against photoaging, and reverse existing concerning changes.

Getting Hooked on Mitochondria—And Then the Skin
When Birch-Machin finished his PhD in molecular biology in 1986, he chose a post-doctoral fellowship with mitochondrial expert Professor Sir Doug Turnbull at the University of Newcastle in the U.K. Turnbull—now Director of the Wellcome Trust Centre for Mitochondrial Research there—was dedicated to uncovering the molecular mechanisms responsible for mitochondrial diseases. “I was very much impacted by the illnesses associated with mitochondrial dysfunction,” Birch-Machin recalls. “Children with these diseases died very early, and it was devastating.” He has been committed to understanding mitochondrial dysfunction ever since, with the ultimate goal of finding ways to correct it.

For the next decade Birch-Machin focused on pediatric mitochondrial myopathies, with assignments in the U.S., Canada, and then Paris. Along the way, he began to develop innovative techniques for gaining information about the mitochondrial genome. Then, while he was at the Hôpital Necker for Sick Children in Paris, the Department of Dermatology at the University of Newcastle expressed interest in having him apply his research techniques to mitochondria in human skin.

As he considered this, Birch-Machin began to think about the skin’s role of “communicating with the environment—and I had a kind of epiphany,” he recalls. “I knew from my mitochondrial research in muscle and brain that mitochondria play a role in chronologic aging, which involves declining metabolic energy and increasing oxidative stress. I also knew that mitochondria don’t repair very well,” he continues—“and it occurred to me that mitochondrial damage in the skin most likely increases measurably with sun exposure. I became really excited to study the role of mitochondria in skin aging, and also understand the effect that sun exposure has on this normal process. No one had explored
this before—and I wanted to do it.” Birch-Machin has focused on the skin ever since, expanding understanding in ways that will ultimately influence patient care.

**mtDNA Damage—Biomarker of Cumulative UV Radiation Exposure**

Specific properties of the mitochondrial genome had led Birch-Machin to hypothesize that mtDNA damage accurately reflects the cumulative impact of sun exposure. Mitochondrial genes lack the histone covers that are important protectors of nuclear genes, and their repair mechanisms are limited. The genome is also in close physical proximity to where superoxide generation—which occurs during UV exposure—takes place. So the mitochondrial genome is multiply vulnerable, and its limited repair capabilities allow damage to accumulate over time. The extent of this underlying damage, though, is not manifest. Because there are a great many nonclonal mitochondria within individual cells and multiple genomes within each mitochondrion (see box on page XX), there are usually enough molecules of functional mtDNA to compensate for those with mutations and maintain cell functions. But Birch-Machin suspected that assessing the extent of these silenced mutations should provide measurable evidence of UV-induced damage to mtDNA, and that the extent of damage would increase with increasing UV exposure—i.e., it would be a highly sensitive biomarker of UV exposure in human skin.

Previous studies had examined the frequency of just a single common deletion. But Birch-Machin believed that a complete picture was the only accurate way to begin. He and his team assessed 71 split-skin samples taken from body areas that were either unexposed, intermittently exposed, or highly sun exposed, and identified the full spectrum of mtDNA deletions in each skin sample. The number of deletions in the epidermis increased
significantly as UV exposure increased, but mtDNA in the dermis showed no effect. Next, they focused on a single, rarely reported 3895 bp mtDNA deletion in age-matched skin samples, again from body areas with large differences in sun exposure. The frequency of this rare deletion increased in line with increasing UV exposure—this time in both the epidermis and dermis. Exposing cultured human fibroblasts to a UVA+UVB light source confirmed this. The same rare mtDNA deletion appeared, then its presence increased in response to continued exposure.

Next, Birch-Machin and his team determined that the shorter UVR wavelengths (>320 nm) are primarily responsible for this mtDNA damage—the same part of the spectrum already implicated in both UVR-induced erythema and nuclear DNA damage in the skin. This time, dermal fibroblasts turned out to be far more sensitive to sun-induced mtDNA damage than keratinocytes, a discovery that holds “important implications for disease and photodamage mechanisms and for interventions,” Birch-Machin points out.

**Complex II—Newly Recognized Importance in Skin**

The four respiratory chain enzymes—complex I through complex IV—gradually transform the energy from glucose into ATP (see box on page XX). The byproducts of this process also make them the major generator of cellular oxidative stress. mtDNA vulnerability to this stress results in damage that in turn reduces the mitochondria’s ability to repair themselves, which increases the production of mutations and dysfunction, which further increases ROS production, which diminishes repair capabilities still further, producing additional mutations.... This vicious cycle is thought to underlie the mitochondria’s contribution to aging, cancer, neurodegeneration, and cell death in many tissues.
Birch-Machin and his group had already shown that UVA exposure increases both ROS production and mtDNA mutations in human skin. Now he wanted to begin exploring its role in this vicious cycle by identifying the most important sites of ROS production within the mitochondrial respiratory chain. Although he was not the first investigator to pose this question, he was among the very few to explore it in skin and the first to pursue it in human skin. Birch-Machin found this lack of attention to human skin quite surprising, “given that the skin is regularly exposed to the harmful UVA rays in sunlight.”

He and his team created multiple cultures of human keratinocytes (from the immortalized HaCaT cell line) and of fibroblasts (from neonatal foreskin). Each of the four respiratory chain enzymes can be inhibited by several chemical agents, and Birch-Machin modified the individual cultures by adding, separately, each of these inhibitors. Then he exposed these cultures to doses of UVA irradiation that are comparable to normal outdoor exposure. Representative cultures were left unexposed as controls. The expectation was that among the various UVA-exposed cultures, the missing enzyme associated with the largest drop in UVA-induced ROS production would—under normal circumstances—be the largest contributor to ROS production.

The results took them in a very different direction. Complex II stood out—but not for producing the lion’s share of ROS. Instead, it was unique for its ability to suppress ROS production, because inhibiting its activity increased ROS levels considerably.

Birch-Machin also engineered a comparison of young and aging tissues to see if the levels of these respiratory chain enzymes change with age. Shrinking telomeres are considered to be a biomarker of aging, so he and his team used cultures grown from two versions of human fetal lung fibroblasts. The youthful tissue cultures were grown from
fibroblasts engineered to overexpress the telomerase enzyme, which lengthens telomeres at the ends of nuclear genes. Because the unaltered—wild-type—cells had shorter telomeres, in relative terms they represented aging tissue. Assessing the individual levels of complexes I-IV in the young and aging fibroblast cultures highlighted complex II again. It was the only respiratory chain enzyme showing decreased activity in the aging lung fibroblasts. In line with this, recent data from a lab using mice to study aging skin in vivo had shown a decrease in complex II activity along with an increase in senescent cells in the skin as mice aged.

Birch-Machin realized for the first time that “the effect of complex II in human skin cells may be significantly more important than previously thought.” It would explain why the activity of this tiny enzyme activity is approximately two-fold greater in skin cells than in the liver, for example. Complex II also differs from the other three respiratory chain enzymes in a fundamental way. All of its subunits and assembling proteins are produced exclusively by the nuclear genome.

**Pursuing the Aging Connection**

Birch-Machin wasted no time in following up on this observation that “younger” fetal lung fibroblasts contained more complex II than the “older” ones did. “It is highly important to understand the aging process in skin,” he emphasizes. “Skin is the largest organ of the body, acting as a protective barrier to a spectrum of external insults that includes UV radiation, infection, toxicity, and mechanical stress,” he points out. A better understanding of the underlying biology will enable maintenance of skin health. And beyond this, “the skin is an organ that can be accessed and studied easily, and thus what is learned from this
research may have profound relevance and application to aging in other body tissues,” he adds.

To explore the role of complex II in skin aging, Birch-Machin and his team worked with foreskin tissue (sun-protected, eliminating any influence of UV exposure) from 27 males ranging from 6 to 72 years of age. They studied skin aging from two perspectives. Biological aging concerns the functional decline of the entire organism over time. Cellular senescence involves the transformation of proliferating cells to a state of irreversible growth arrest. On the positive side, senescence is an important tumor suppressive mechanism by preventing potentially malignant cells from undergoing replication, and senescent cells produce cytokines that aid wound healing. But senescent cells also have a number of significant negative effects—including ROS production and secretion of inflammatory cytokines—and thus are thought to be prominent in the aging process.

**Biological aging:** Birch-Machin and his team cultured fibroblasts and keratinocytes from each of these skin samples, then precisely calculated the activity level of complex II per unit of mitochondria in every culture. They also measured gene transcript expression and protein levels for complex II. Results were compared with complex IV, chosen as the control because—unlike complexes I and III—it is not directly linked to complex II within the electron transport chain. When the results were in, it became clear that complex II activity is pivotal to fibroblast function—but not to keratinocytes.

To begin with, complex II activity was substantially greater in fibroblasts than in keratinocytes, up to twice as high in cell cultures from younger donors. In addition, complex II activity decreased steadily with donor age—but only in fibroblasts. Because gene transcript expression and protein levels for complex II also declined with age in fibroblasts, it
appeared that in fibroblasts from this sun-protected area, the enzyme itself had not lost effectiveness with age but there was less and less of it. With the increasing loss of complex II’s protective actions, ROS-caused damage would exacerbate this diminishing activity. Complex IV showed no relation to age in either cell culture.

**Senescence:** When Birch-Machin and his team turned their attention to the relationship between senescence, complex II, and biological age in human skin, the ensuing results shed light on their observations with biological aging. Fibroblast cultures from 15 of the initial donors (ages 6-71 years) were separated into senescent and nonsenescent cell populations using the senescence biomarker lipofuscin, a wear-and-tear fluorescent pigment that is a remnant of the transformation to senescence. As donor age increased, the number of senescent fibroblasts increased while the activity level of complex II decreased—but only in senescent fibroblasts. (Complex IV activity level in senescent fibroblasts was unrelated to donor age.) The surprise was that purely nonsenescent fibroblast cultures showed no correlation between aging and complex II activity.

These unexpected results refocused the initial picture. The age-related decrease in complex II activity observed in the original fibroblast cultures did not actually reflect the entire fibroblast population, but was specifically a function of the senescent cell subset. Isolating the senescent fibroblasts for study emphasized that—in terms of mitochondrial complex II activity—senescent fibroblasts in the skin of older individuals are less efficient than those in younger individuals.

Human *in vivo* data will be required to confirm and flesh out the role of decreasing complex II activity in skin aging, and clarify whether this loss is a cause or a consequence of aging—or both, as the vicious cycle of aging would predict. Any of these scenarios,
including direct DNA damage initiated by increased ROS production, is likely to increase overall mitochondrial dysfunction. And the result is decreased tissue function.

Testing Interventions

Because antioxidant compounds neutralize ROS, thus reducing or eliminating oxidative damage, this is a logical area to explore for candidates to support skin health and prevent further damage in the face of ROS-inducing conditions. Birch-Machin has begun evaluating candidates from medicinal plants and plant extracts and from chemical molecules.

**Clitoria ternatea L:** Birch-Machin learned of *C. ternatea*—the butterfly pea plant—from Dr. Edward Okello, a neurology colleague who is also Executive Director of the Medicinal Plant Research Group. Indigenous to tropical Asia, it spread more widely across Asia and to Latin America and the East and West Indies, and has extensive traditional uses. One involves an herbal tea made from the colorful blue and white flower that is taken to protect the skin against age-related changes and sun-induced damage. Research on the plant’s biological activities have focused on the root, seeds, and leaf, but the flower was recently found to contain anthocyanins, which are known antioxidants.

Birch-Machin incubated human keratinocyte cultures with the flower water extract (CTW), then rinsed them before exposure to hydrogen peroxide or UV. There was significantly less cytotoxicity and mtDNA damage after CTW treatment than in nontreated cells. A phytochemical analysis of the flower identified major concentrations of polyacylated anthocyanins and flavonol glycosides, both with widely documented antioxidant actions. Birch-Machin points out that the flower extract’s significant ability to reduce UV-induced mtDNA damage indicates a potential for preventing oxidative stress arising from
mitochondrial dysfunction. Further research for therapeutic benefits is warranted. “We will also be studying additional Malaysian species, working in conjunction with Kew Gardens in London,” Birch-Machin says.

**Antioxidant molecules:** The first round of experiments compared the protective capability of two antioxidant molecules able to penetrate the mitochondria. **MitoQ** *(mitoquinone)—*a modified ubiquinone molecule developed by Mike Murphy, PhD, Program Leader of the MRC Mitochondrial Biology Unit, University of Cambridge, UK—is actively and exclusively attracted to mitochondria, and sufficiently small to penetrate the mitochondrial membrane. **Tiron** is a mitochondria-permeable and localized superoxide scavenger and antioxidant and also chelates metals (including iron and titanium). Human fibroblast cultures were incubated with one or the other, then exposed to physiologic doses of UVA or to hydrogen peroxide. Tiron completely prevented ROS-induced mtDNA damage across the board. MitoQ reduced damage by 17% and 32%, respectively. Birch-Machin suspected that tiron’s complete elimination of ROS production indicated a protective effect extending beyond the mitochondria, and further exploration also demonstrated complete protection of the nuclear genome from hydrogen peroxide-induced damage. MitoQ achieved 18%. Birch-Machin showed that it is completely independent of the Nrf2 signaling pathway, which is known to provide cellular protection against oxidative stress.

Tiron completely abrogated mitochondrial and nuclear DNA damage in human skin cells exposed to these stressors. “We postulated that these profound *in vitro* antioxidant effects are attributable to the combination of its antioxidant and metal-chelating properties,” Birch-Machin explains. “It targets not just the ROS, but also the increased free
intracellular metals that are released due to oxidative insults.” This points to the ideal therapeutic strategy of combining compounds with complementary capabilities.

**Conclusions**

It had first been speculated in the early 1970s that mitochondria play a key role in the aging process. Birch-Machin’s research in the skin “brings us one step closer to understanding how mitochondria may be contributing to this, with the hope of eventually targeting areas of the mitochondria in an attempt to counteract the signs of aging,” he states. This includes his search for a way to maintain ideal levels of complex II as an endogenous ROS suppressor, and his continuing work using tiron as a preventive.

He is also expanding his pursuit of mtDNA involvement in skin health and disease. He is looking at mtDNA in the context of treating psoriasis, in the relationship between oxidative stress, nutritional status, and skin aging, and in the science and use of sunscreens.

Birch-Machin is not mitochondria-centric though. “The fascination and beauty for me,” he explains, “is the way the two genomes communicate and affect each other. Those who focus on just one of them and ignore the other—it’s at their peril,” he adds. “Our perspective should be a healthy balance between the two, as they are equally important.”

**SIDEBAR 1**

**Mitochondria—The Tiny Organelles With the Huge Punch**

Back in the mists of evolutionary time, our mitochondria—the cellular organelles in our cytoplasm that produce 90% of the chemical energy that our cells need to survive, and
contribute substantially to other basic cellular functions—were independently dwelling purple photosynthetic bacteria. As eukaryotes evolved more than 1 billion years ago, these free-living bacteria became incorporated within them—the process of endosymbiosis—and developed a mutually dependent relationship. Their genomes, though, remained independent entities and mitochondria replication is independent of cell division. The human nuclear genome now contains 3 billion base pairs, with only about 2% actually coding for its 20,000 genes. The mitochondrial genome (mtDNA) evolved in the opposite direction, contracting down to a 16.5-kb circular structure containing just 16,569 base pairs and 37 genes (see illustration on page XX). Unlike nuclear DNA, mtDNA is a model of efficiency with no introns and no spacing between genes, so that almost all of it (~93%) represents a coding region. These dual-membrane mitochondria are rich in fats, proteins, and enzymes, but some of the proteins essential to their function—including the entire complex II respiratory enzyme (see box on page XX)—are produced by the cell, not by the mitochondria.

What this genome lacks in size, it makes up for in exceptional numbers. Excluding mature red blood cells, which are unique in their absence of mitochondria, the individual cellular presence of these organelles ranges from around 1,000 in cells with lower ATP needs to roughly 7,000 in individual human myocytes. Adding it all up, we contain roughly 500 trillion mitochondria, accounting for roughly 30% of our body weight. And each individual mitochondrion holds from 1 to 15 mtDNA molecules. Each cell’s population is not clonal, but include normal—ie, wild-type—and various mutation-altered states. The presence of wild-type genomes is potent. “There can actually be quite profound deficiencies and damage present, but the remaining wild-type genomes will complement the damaged
ones,“ Birch-Machin explains. “In some cases, just 10% of the mitochondrial genomes need to be normal to make up for the deficiencies of the other 90%.”

Although mitochondria are most well known as the cell’s powerhouse—transforming the energy from glucose into ATP—this is just part of what they do. Among other things, they participate in cellular differentiation, cell growth, the cell cycle, apoptosis, and steroid synthesis. They are platforms for intracellular signaling, regulators of innate immunity, modulators of stem cell activity, and are host to numerous biosynthetic and signaling processes that ultimately couple cellular metabolism to homeostatic regulatory mechanisms. Dysfunctional mitochondria are thus responsible for a number of human diseases and conditions. This past summer, mitochondrial research at the University of Southern California documented cross-regulation between the nuclear and mitochondrial genomes—noting that sometimes it’s the mitochondrial DNA in charge.

SIDEBAR 2

The Power of Complex II

Complex II is tiny—only four subunits in size—but particularly powerful because it is involved in two phases of the progression from glucose to ATP, not just one. Yet mitochondrial researchers have learned this only recently. They had paid scant attention to complex II because it is so much smaller than the other respiratory chain enzymes, which contain from 10 to 45 subunits. But its minimal size disguises immense impact—far greater than the other three complexes—because it is involved in the Krebs cycle as well as the electron transport chain. “Because it is involved in two major metabolic pathways,” Birch-Machin explains, “a small change in this enzyme has a profound effect.”
There are three steps in producing ATP, which begins with the release of energy from glucose. Then the Krebs—citric acid—cycle breaks this energy down and stores it in carrier molecules, enabled by complex II’s contribution of reduced ubiquinone. In the final stage of aerobic respiration—the electron transport stage—the respiratory chain enzymes complexes I, III, and IV engineer the production of ATP and enable it to leave the mitochondria. This involves electron transfers, proton gradients, and oxygen, producing reactive oxygen species (ROS) as byproducts. Complex II participates by—as Birch-Machin has discovered—minimizing ROS production and damage. Chronic oxidative stress has been linked to a myriad of pathologies.

**Suggested Readings**


