PNAS Plus Significance Statements

NAD^+ and SIRT3 control microtubule dynamics and reduce susceptibility to antimicrotubule agents

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Nicotinamide adenine dinucleotide (NAD^+) is an endogenous small molecule that has effects on diverse processes, including obesity, lifespan, and cancer. A major goal is to identify the NAD^+-regulated cellular pathways that may mediate these effects. In this study, we demonstrate that NAD^+ regulates the microtubule cytoskeleton. We find that these effects are mediated by the mitochondrial sirtuin-3. Our findings (pp. E2443–E2452) have implications for many clinically used chemotherapeutics that target microtubules, as we demonstrate that high NAD^+ levels can reduce sensitivity to these drugs. These results are also significant because they demonstrate for the first time that NAD^+, a molecule regulated by age, diet, and disease state, can influence basic microtubule functions.

Probing nuclear pore complex architecture with proximity-dependent biotinylation

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Proximity-dependent biotinylation (BioID) is a readily accessible method for identifying protein associations that occur in living cells. Fusion of a promiscuous biotin ligase to a bait protein for expression in live cells enables covalent biotin labeling, and thus identification, of proteins proximate to the bait. Here we used BioID to probe the organization of the nuclear pore complex, a large structure that regulates molecular transport between the nucleus and cytoplasm. These studies (pp. E2453–E2461) enhance our understanding of major subcomplexes within the nuclear pore complex and demonstrate the utility of BioID for studying the organization of large protein assemblies. Additionally, we have measured the labeling radius of BioID, thus enabling the rational application of this method and more meaningful data interpretation.

Single-cell nucleosome mapping reveals the molecular basis of gene expression heterogeneity

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Nucleosomes limit access to DNA, which antagonizes gene expression and prevents recruitment of transcription factors that cannot bind DNA wrapped around the histone octamer. Numerous studies using large cell populations determined that active genes promoters tend to be nucleosome-depleted. We developed (pp. E2462–E2471) a method to examine nucleosome positioning in single cells and revealed significant heterogeneity of nucleosome configurations within a population. In an inactive gene loaded with nucleosomes, a small subpopulation of nucleosome-depleted cells exists that were engaged in transcription. Single-cell mapping revealed that even in apparently nucleosome-free regions, some cells were occupied by nucleosomes. These data reveal an underlying complexity of nucleosome positioning and its role in regulating gene expression.

The endocannabinoid 2-AG controls skeletal muscle cell differentiation via CB1 receptor-dependent inhibition of K_v7 channels

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Although CB1 cannabinoid receptors control skeletal muscle insulin signaling, little is known of their role in muscle formation during differentiation from myoblasts to myotubes. The voltage-dependent K_v7 K^+ channels, which are tonically activated by the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2), instead activate myotube formation. We found (pp. E2472–E2481) that the levels of the endogenous CB1 agonist 2-arachidonoylglycerol are decreased during murine myoblast differentiation into myotubes, whereas CB1 expression is up-regulated. CB1 activation inhibits myotube formation. This effect is exerted by reducing PIP2 binding to $K_v7.4$, which represents the K_v7 subunit responsible for the pro-myogenic effects. Accordingly, CB1 activation inhibits $K_v7.4$-mediated currents in transfected CHO cells. The endocannabinoid system might thus play a role in skeletal muscle dystrophies.

11β-HSD1 is the major regulator of the tissue-specific effects of circulating glucocorticoid excess

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Glucocorticoids are widely prescribed for their anti-inflammatory properties but have Cushingoid side effects that contribute significantly to patient morbidity and mortality. Here (pp. E2482–E2491) we present data to demonstrate that the adverse side-effect profile associated with exogenous active glucocorticoid (GC) administration (including glucose intolerance, hyperinsulinemia, hypertension, hepatic steatosis, increased adiposity, and myopathy) is prevented by global deletion of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in mice. This study not only defines a significant shift in our understanding of the physiological and

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molecular mechanisms underpinning the adverse side effects associated with GC use but also raises the possibility of targeting 11β-HSD1 as a novel adjunctive therapy in the treatment of Cushing syndrome.

Manganese-enhanced magnetic resonance imaging reveals increased DOI-induced brain activity in a mouse model of schizophrenia

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Here, we model a positive symptom of schizophrenia, hallucination-like activity, in a mouse model of an environmental risk factor of schizophrenia, maternal immune activation (MIA). MIA offspring display an enhanced susceptibility to the hallucinogen 2,5-dimethoxy-4-iodoamphetamine (DOI) and demonstrate elevated DOI-induced brain activity as measured by induction of immediate early genes and manganese-enhanced MRI. High sensitivity to DOI in MIA offspring can be explained by an increased level of serotonin receptor 2A (5-HT2AR) that mediates the effect of DOI on the prefrontal cortex. Chronic treatment with the 5-HT2AR antagonist ketanserin reduces DOI-induction of head twitching in MIA offspring. Our data (pp. E2492–E2500) demonstrate that DOI-induced hallucination-like activity can be modeled in the MIA mouse model and suggest 5-HT2AR as a potential therapeutic target for schizophrenia.

Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2

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Recently it has been suggested that metformin, the most commonly used antidiabetic drug, might also possess general health-promoting properties. Elucidating metformin's mode of action will vastly increase its application range and will contribute to healthy aging. We reveal (pp. E2501–E2509) a signaling cascade in which metformin is able to extend lifespan by increasing the production of reactive oxygen species (ROS). This allowed us to further work at the crossroads of human disease and aging research, identifying a key molecule that is able to translate the ROS signal into a prolongevity cue: an antioxidant peroxiredoxin is also able to activate a lifespan-promoting signaling cascade, here described in detail. Continued research efforts in this field lead toward a targeted improvement of aging-related complications.