PNAS Plus Significance Statements

Impact of adolescent marijuana use on intelligence: Results from two longitudinal twin studies

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Marijuana is the most commonly used recreational drug in the United States. Some studies suggest that marijuana use in adolescence is linked to declines in intellectual functioning. Because of the infeasibility of studying this phenomenon experimentally, it is unclear whether the association can be causally attributed to marijuana use itself or is instead the result of confounding factors. We approach this issue quasiexperimentally using longitudinal samples of adolescent twins. Among twin pairs discordant for marijuana use, we assessed intelligence quotient (IQ) score changes while adjusting for the effects of genetic influences and other factors shared by members of the same twin pair. Results suggest that familial confounds underlie the association between adolescent marijuana use and declining IQ scores. (See pp. E500–E508.)

Nicastrin functions to sterically hinder γ-secretase–substrate interactions driven by substrate transmembrane domain

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γ-Secretase is a conserved and ubiquitous intramembrane-cleaving protease (I-CLiP). Its normal function is required for proper notch signaling, and its processing of amyloid precursor protein is implicated in Alzheimer's disease. Although γ-secretase has over 90 reported substrates, little is known about the mechanism by which γ-secretase recruits its substrates while at the same time distinguishing substrate from nonsubstrate within the protein-crowded environment of cellular membranes. In contrast to previous studies, our data demonstrate that substrate transmembrane domain drives its interaction with γ-secretase. We find that the γ-secretase component nicastrin acts to sterically block substrates with large ectodomains from interacting with γ-secretase, providing the mechanism by which γ-secretase selectively recruits ectodomain-shed substrates while also preventing cleavage of nonsubstrates. (See pp. E509–E518.)

Structure of NDP-forming Acetyl-CoA synthetase ACD1 reveals a large rearrangement for phosphoryl transfer

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Acyl-CoA thioesters are key substrates for energy conversion. Related ATP/GTP-producing synthetases form a large superfamily with members in all kingdoms of life. In contrast to their general importance, the underlying reaction mechanism of these enzymes is still not understood in all steps. Here, we describe various structures of a nucleoside diphosphate-forming acetyl–CoA synthetase from an evolutionary very old archaeon. A large conformational rearrangement within the enzyme is observed. The structures reveal a partial unwinding and reorientation by 120° of a phosphohistidine-containing segment. This conformational rearrangement couples the acyl-CoA binding site with the nucleoside diphosphate binding site. The presented structures prove a long-standing hypothesis and provide insight into the determinants for substrate selectivity. (See pp. E519–E528.)

Intrinsic regulation of FIC-domain AMP-transferases by oligomerization and automodification

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FIC-domain enzymes are found in all kingdoms of life and catalyze posttranslational modifications of various target proteins to modulate their function. Because the vast majority of Fic proteins are expressed in an inhibited form, their physiological importance has escaped attention for a long time. This article reveals an autonomous mechanism of inhibition relief for class III Fic proteins, which hinges on autoadenylylation of an inhibitory helix. Because the process occurs *in cis*, the Fic enzyme constitutes a molecular timer that operates independent of enzyme concentration. Furthermore, we show that Fic-mediated adenylylation of DNA gyrase leads to bacterial growth arrest. Thus, the time-dependent inactivation of DNA gyrase may serve as a switch to bacterial dormancy under starvation or other stress conditions. (See pp. E529–E537.)

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SIRT6 deacetylates PKM2 to suppress its nuclear localization and oncogenic functions

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SIRT6 (sirtuin 6) is a member of the highly conserved sirtuin family of NAD^+-dependent deacetylases. SIRT6 regulates diverse cellular processes including tumorigenesis. However, the role of SIRT6 deacetylase activity in its tumor-suppressor functions is not well understood. Here we report that SIRT6 deacetylates nuclear PKM2 (pyruvate kinase M2). PKM2 is a glycolytic enzyme with nonmetabolic nuclear oncogenic functions. SIRT6-mediated deacetylation results in PKM2 nuclear export in an exportin 4-dependent manner. As a result of SIRT6-mediated deacetylation, PKM2 nuclear protein kinase and transcriptional coactivator functions are abolished. Thus SIRT6 suppresses PKM2-dependent cell proliferation and tumorigenesis. Taken together, our findings demonstrate the pivotal role of deacetylase activity in SIRT6 tumor-suppressor functions and delineate a mechanism of PKM2 nuclear export. (See pp. E538–E547.)

Transcription factor 7-like 1 is involved in hypothalamo–pituitary axis development in mice and humans

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The relevance of transcription factor 7-like 1 (TCF7L1) during hypothalamo–pituitary (HP) axis development remains unknown. Using mouse genetics, we show that TCF7L1 acts as a transcriptional repressor to regulate the expression of the hypothalamic signals involved in pituitary formation. In addition, we screened a cohort of human patients with forebrain and/or pituitary defects and report two independent missense variants, p.R92P and p.R400Q, in human TCF7L1. Functional studies in vitro and rescue experiments in zebrafish mutants deficient for *tcf7l1a* and *tcf7l1b* show that the p.R92P and p.R400Q variants exhibit reduced repressing activity compared with wild-type TCF7L1. In summary, we identify TCF7L1 as a determinant for the establishment of HP axis development and as a potential candidate gene to be mutated in congenital hypopituitarism. (See pp. E548–E557.)

Utilization of a photoactivatable antigen system to examine B-cell probing termination and the B-cell receptor sorting mechanisms during B-cell activation

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B-cell receptor (BCR) and antigen engagement induces several responses resulting in B-cell activation. However, it has been difficult to study these responses due to their dynamic nature. To solve this problem, a photoactivatable antigen, caged 4-hydroxy-3-nitrophenyl acetyl (caged-NP), was developed. B cells contacting caged-NP exhibited probing behaviors that are cell intrinsic with strict dependence on F-actin remodeling. B-cell probing behaviors were terminated within 4 s after the photoactivation of caged-NP. The termination of B-cell probing was concomitant with the accumulation response of the BCRs into the BCR microclusters. The analysis of temporally segregated single molecule images demonstrated that antigen binding induced trapping of BCRs into the BCR microclusters is a fundamental mechanism for B cells to acquire antigens. (See pp. E558–E567.)

T-cell–intrinsic Tif1α/Trim24 regulates IL-1R expression on T_H2 cells and T_H2 cell-mediated airway allergy

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The increasing number of patients presenting with severe asthma throughout the world present a clear unmet medical need. This study identified putative transcriptional regulators in T-helper 2 (T_H2) cells with the aim of identifying previously unidentified targets to inhibit T_H2-mediated allergy. Genetic deletion of *Trim24* (tripartite motif-containing 24) in T cells showed that Trim24 was essential for T_H2-mediated allergy. Transcriptional analysis showed that *Trim24* was required for many of the pathogenic properties of T_H2 cells and that IL-1–regulated signaling is compromised in $Trim24^{-/-}$ cells. In vivo, in vitro, and in silico approaches identified a previously overlooked role for Trim24 in T_H2-mediated allergy and validate a combined approach to interrogate transcriptional datasets to identify new therapeutic targets to prevent allergy and asthma. (See pp. E568–E576.)

Subtype-specific addiction of the activated B-cell subset of diffuse large B-cell lymphoma to FOXP1

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We demonstrate that forkhead box P1 (FOXP1) is a central transcriptional regulator of the most aggressive activated B cell (ABC) subtype of diffuse large B-cell lymphoma (DLBCL), the most prevalent non-Hodgkin's lymphoma worldwide. We used a variety of methods to identify and functionally confirm FOXP1 target genes in DLBCL cell lines and primary clinical isolates. We found that FOXP1 target genes are sufficient to segregate ABC-DLBCL from the more indolent germinal center B-cell (GCB)-DLBCL subtype as well as to identify both hallmark and previously unidentified pathways underlying DLBCL pathology. Our findings extend the role of FOXP1 from a prognostic indicator of unknown mechanism to a driver of ABC-DLBCL neoplasia. (See pp. E577–E586.)

Sensing of latent EBV infection through exosomal transfer of 5′pppRNA

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Increasing evidence suggests that the exosomal messenger pathway warns neighboring cells against cellular stress and infection. Recent studies have shown that viruses and cancer cells exploit exosomes to transmit functional RNAs. Our studies reveal that a viral small RNA signal for innate immunity Epstein–Barr virus (EBV)-EBER1 is produced by latent EBV-infected B cells and recognized by noninfected dendritic cells activating an

inflammatory response. We detected high amounts of EBV-EBER1 transcripts and EBV-microRNAs in inflamed skin lesions of autoimmune patients that are infiltrated with dendritic cells. Importantly, we found virtually no EBV-DNA present in these tissues, suggesting that continuous cell–cell EBER1 transmission via exosomes occurs in humans. We propose that innate sensing of latent EBV in predisposed individuals may be more harmful than previously thought. (See pp. E587–E596.)

SutA is a bacterial transcription factor expressed during slow growth in *Pseudomonas aeruginosa*

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Pathogens that are dormant or growing slowly play important roles in chronic infections, but studying how cells adapt to these conditions is difficult experimentally. This work demonstrates that time-selective analysis of cellular protein synthesis, using bioorthogonal noncanonical amino acid tagging (BONCAT), can provide the sensitivity needed to identify important factors in slow-growth physiology. We identified in *Pseudomonas aeruginosa*, a previously uncharacterized transcriptional regulator that is expressed preferentially under slow-growth conditions, binds RNA polymerase, and has widespread effects on gene expression. This factor is one of several proteins of unknown function identified in our proteomic analysis, and our results suggest that further characterization of fundamental cellular processes under these conditions will shed light on important and understudied realms of biology. (See pp. E597–E605.)

Stimulus-induced visual cortical networks are recapitulated by spontaneous local and interareal synchronization

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The greatest proportion of brain activity is endogenously generated. The brain's endogenous activity is highly structured and affects sensory coding, behavior, and perception. The observation of structured endogenous activity across spatial scales suggests that it plays a role in the maintenance and formation of brain networks. The correlation of spontaneous functional MRI signals has demonstrated the existence of multiple intrinsic networks, previously observed during controlled cognitive paradigms. The prevalence and reliability of intrinsic networks have generated intense interest in the functional relevance and electrophysiological basis of interareal correlations. Using multisite recordings from areas V1 and V4 of awake monkeys, we show that endogenously generated activity, both during stimulation and passive fixation, exhibits a similar pattern of local and interareal rhythmic synchronization. (See pp. E606–E615.)

Brain responses in humans reveal ideal observer-like sensitivity to complex acoustic patterns

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We reveal the temporal dynamics and underlying neural sources of the process by which the brain discovers complex temporal patterns in rapidly unfolding sound sequences. We demonstrate that the auditory system, supported by a network of auditory cortical, hippocampal, and frontal sources, continually scans the environment, efficiently represents complex stimulus statistics, and rapidly (close to the bounds implied by an ideal observer model) responds to emergence of regular patterns, even when these are not behaviorally relevant. Neuronal activity correlated with the predictability of ongoing auditory input, both in terms of deterministic structure and the entropy of random sequences, providing clear neurophysiological evidence of the brain's capacity to automatically encode high-order statistics in sensory input. (See pp. E616–E625.)

GR SUMOylation and formation of an SUMO-SMRT/NCoR1-HDAC3 repressing complex is mandatory for GC-induced IR nGRE-mediated transrepression

Guoqiang Hua, Laetitia Paulen, and Pierre Chambon

Glucocorticoids (GCs), acting through binding to the GC receptor (GR), are peripheral effectors of circadian and stress-related homeostatic functions fundamental for survival throughout vertebrate life span. They are widely used to combat inflammatory and allergic disorders, and their therapeutic effects have been mainly ascribed to their capacity to suppress the production of proinflammatory cytokines. The present study unveils, at the molecular level, the mechanisms that underlie the GC-induced GR direct transrepression function mediated by the evolutionary conserved inverted repeated negative response element. This knowledge paves the way to the elucidation of the functions of the GR at the submolecular levels and to the future educated design and screening of drugs, which could be devoid of undesirable debilitating effects on prolonged GC therapy. (See pp. E626–E634.)

Glucocorticoid-induced tethered transrepression requires SUMOylation of GR and formation of a SUMO-SMRT/NCoR1-HDAC3 repressing complex

Guoqiang Hua, Krishna Priya Ganti, and Pierre Chambon

The antiinflammatory property of natural glucocorticoids (GCs) was demonstrated more than 60 years ago. Since then, synthetic GCs have been widely used to combat inflammatory and allergic disorders. However, multiple severe undesirable side effects associated with long-term GC treatments, as well as induction of glucocorticoid resistance associated with such treatments, limit their therapeutic usefulness. In the present study, we unveiled the molecular mechanism underlying the GC-induced GC receptor (GR)-mediated tethered indirect transrepression. This knowledge paves the way to the future educated design and screening of drugs, collectively named selective GR agonists, which would exhibit the major therapeutically beneficial properties of GCs, but would be devoid of undesirable debilitating effects upon prolonged GC therapy. (See pp. E635–E643.)

Subunit stoichiometry and arrangement in a heteromeric glutamate-gated chloride channel

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Cys-loop receptors (CLRs) are transmembrane ion channels activated by neurotransmitters to mediate chemoelectric excitation or inhibition throughout the nervous system. Hence, CLRs play a key role in our day-to-day life, from coordination of motions to cognition. Impairment of CLRs' activity leads to various pathophysiological conditions. The CLR studied here is a glutamate-gated chloride-selective receptor (GluClR). GluClRs are unique to invertebrates, yet they are pharmacologically important because they serve as targets for ivermectin, an anthelmintic drug used to treat humans suffering from filarial diseases. This study provides better understanding of the subunit arrangement and stoichiometry of Glu-binding sites in GluClRs. (See pp. E644–E653.)

Assessment of fight outcome is needed to activate socially driven transcriptional changes in the zebrafish brain

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Within social groups, there are animals of different social status that express different behavioral profiles that are paralleled by different patterns of gene expression in the brain. However, social status is not fixed, but rather depends on social interactions; hence, group living animals must be able to switch between different status-dependent behavior and brain gene expression profiles. Here we show for the first time, to our knowledge, that what triggers a genomic response to a social interaction in zebrafish is the subjects' assessment of the interaction rather than a fixed response to a releaser cue in the environment. The occurrence of fighting assessment in zebrafish suggests that a cognitive ability classically considered complex is also present in a simple-minded vertebrate. (See pp. E654–E661.)