PNAS Plus Significance Statements

MPE-seq, a new method for the genome-wide analysis of chromatin structure

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The structure of chromatin is critical for processes such as transcription, DNA replication, and DNA repair. The most commonly used techniques for analyzing chromatin structure involve the use of enzymes such as micrococcal nuclease (MNase) and DNase I. These enzymes, however, have distinct characteristics that can at some times be an advantage but in other situations be a drawback. Here we describe methidiumpropyl-EDTA sequencing (MPE-seq), a method in which we use the chemical MPE-Fe(II) for the genome-wide analysis of chromatin structure. MPE-Fe(II) cleaves chromatin with minimal DNA sequence bias. Moreover, MPE-seq reveals noncanonical chromatin structures in active promoter regions that are not seen with standard MNase-seq conditions. MPE-seq provides insights into chromatin structure that complement the information gained from MNase-seq. (See pp. E3457–E3465.)

An eIF2α-binding motif in protein phosphatase 1 subunit GADD34 and its viral orthologs is required to promote dephosphorylation of eIF2α

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Phosphorylation of eukaryotic translation initiation factor 2α (eIF2α) is the principal mechanism cells use to regulate translation initiation. Specific kinases phosphorylate eIF2α to inhibit protein synthesis under stress conditions; however, eIF2α dephosphorylation is catalyzed by general protein phosphatase 1 (PP1). In mammalian cells, specific *trans*-acting targeting proteins, growth arrest and DNA damage-inducible protein 34 (GADD34) and constitutive repressor of eIF2α phosphorylation (CReP), bind to PP1 and promote dephosphorylation of eIF2α. We show that GADD34 directly binds to eIF2α, and we identify and demonstrate the function of an eIF2α-binding motif that is shared among GADD34, CReP, and several viral proteins. Thus, these cellular and viral PP1-targeting proteins bind independently to PP1 and to eIF2α to form a trimeric complex and promote the specific dephosphorylation of eIF2α to maintain cellular protein synthesis. (See pp. E3466–E3475.)

DNA polymerase from temperate phage Bam35 is endowed with processive polymerization and abasic sites translesion synthesis capacity

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Functional classification of DNA polymerases (DNAPs) usually divides them into replicative faithful replicases and error-prone enzymes devoted to DNA repair and DNA damage tolerance through translesion synthesis (TLS). When we analyzed the biochemical properties of phage Bam35 replicative DNAP, we found it to be a highly faithful DNAP that can couple strand displacement to processive DNA synthesis, suitable for rolling circle amplification of plasmidic DNA. Interestingly, it is also endowed with intrinsic TLS capacity opposite abasic sites and processive primer extension beyond the lesion. These features configure a versatile enzyme for accurate maintenance of viral genomic information over generations and, besides, to deal with DNA lesions, which suggest a possible application of Bam35 DNAP for the amplification of damaged or ancient DNA. (See pp. E3476–E3484.)

Structural analysis of a class III preQ$_1$ riboswitch reveals an aptamer distant from a ribosome-binding site regulated by fast dynamics

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Riboswitches are RNA molecules found mostly in bacteria that control genes by sensing cellular levels of metabolites, such as the simple organic compound preQ$_1$. The diversity of riboswitches and their potential as novel antibiotic targets continue to elicit interest in these regulatory sequences. Here we present the crystal structure of a newly discovered bacterial preQ$_1$-III riboswitch that senses preQ$_1$ using an unusual, two-part architecture. A complementary analysis of flexibility and dynamics showed that recognition of preQ$_1$ induces riboswitch compaction, while concomitantly enhancing formation of a distant double-helix possessing a regulatory signal that zips and unzips rapidly, producing gene "off" and "on" states. These observations expand our knowledge of riboswitch construction and suggest a broader role for dynamics than previously recognized. (See pp. E3485–E3494.)

DNA damage during the G0/G1 phase triggers RNA-templated, Cockayne syndrome B-dependent homologous recombination

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Unrepaired DNA strand breaks at transcriptionally active sites are expected to be more deleterious than elsewhere in the genome because the integrity of the coding regions is likely to be compromised. The commonly recognized homologous recombination (HR) process occurs in the G2/M phase and depends on the presence of sister chromatids as a donor template. Our data demonstrate a Cockayne syndrome protein B- and RNA-dependent mechanism of transcription-associated HR in the G0/G1 phase and offer insight into double strand break repair at sites of active transcription. The data suggest that a deficiency in this repair mechanism might explain why neurodegeneration as well as tumorigenesis may be associated with seemingly stable, terminally differentiated (G0) cell populations. (See pp. E3495–E3504.)

Vimentin filament precursors exchange subunits in an ATP-dependent manner

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Although vimentin intermediate filaments (VIFs) are the most stable cytoskeletal component in motile cells, VIFs undergo dramatic reorganization during cell spreading, cell division, and motility. Here, we studied the first step of IF assembly using the vimentin^{Y117L} mutant, which forms oligomers called unit-length filaments (ULFs) but cannot assemble into mature VIFs. We discovered that ULFs, unlike VIFs, are extremely dynamic and rapidly exchange subunits with the soluble vimentin pool. Surprisingly, this process requires ATP but seems independent of the vimentin phosphorylation events previously shown to trigger filament disassembly. We believe that

dynamic exchange of subunits could play a role in the regulation of ULF assembly and maintenance of a soluble vimentin pool during the reorganization of the filament network. (See pp. E3505–E3514.)

Miniaturized mitogenome of the parasitic plant *Viscum scurruloideum* is extremely divergent and dynamic and has lost all *nad* genes

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The mitochondrial genomes of flowering plants are characterized by an extreme and often perplexing diversity in size, organization, and mutation rate, but their primary genetic function, in respiration, is extremely well conserved. Here we present the mitochondrial genome of an aerobic parasitic plant, the mistletoe *Viscum scurruloideum*. This genome is miniaturized, shows clear signs of rapid and degenerative evolution, and lacks all genes for complex I of the respiratory electron-transfer chain. To our knowledge, this is the first report of the loss of this key respiratory complex in any multicellular eukaryote. The *Viscum* mitochondrial genome has taken a unique overall tack in evolution that, to some extent, likely reflects the progression of a specialized parasitic lifestyle. (See pp. E3515–E3524.)

Environmental CO_2 inhibits *Caenorhabditis elegans* egg-laying by modulating olfactory neurons and evokes widespread changes in neural activity

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Carbon dioxide (CO_2) gradients are ubiquitous, but fluctuations in CO_2 provide an important cue shaping animal behavior. This paradox suggests that CO_2 provides contextual information that is integrated with other inputs. Here, we show that *Caenorhabditis elegans* CO_2-sensing circuits are much more sophisticated than assumed hitherto. A surprisingly large number of neurons, including nociceptors, gustatory neurons, and olfactory neurons, respond to CO_2 in vivo. Glia also exhibit large Ca^{2+} responses to CO_2. Worms therefore may couple detection of CO_2 and other cues at the earliest stages of sensory processing. Besides avoiding CO_2, *C. elegans* stops laying eggs at high CO_2. Inhibition of oviposition involves sustained activation of the AWC olfactory neurons by CO_2 and enduring inhibition of neurons innervating the egg-laying muscles. (See pp. E3525–E3534.)

Genome-wide binding and mechanistic analyses of Smchd1-mediated epigenetic regulation

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Structural maintenance of chromosomes flexible hinge domain containing 1 (Smchd1) is a protein that plays an important role in maintaining gene silencing in many biological circumstances, including facioscapulohumeral muscular dystrophy; however, how it brings about gene silencing is unknown. Understanding the molecular mechanism by which Smchd1 contributes to stable transcriptional silencing is critical to appreciate how it functions in normal biology and when it is mutated in facioscapulohumeral muscular dystrophy. This study reveals, for the first time to our knowledge, where Smchd1 binds genome-wide, its hitherto unappreciated functional interaction with chromatin organizer CCCTC-binding factor in gene regulation, and which part of the protein is required for chromatin binding. These data lead to a new model of Smchd1 function, where it directly binds DNA to mediate 3D chromatin architecture. (See pp. E3535–E3544.)

Genetic and epigenetic architecture of sex-biased expression in the jewel wasps *Nasonia vitripennis* and *girault*i

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This paper provides a comprehensive analysis of sex differential gene expression in haplodiploid jewel wasps. Between two closely related species, 75% of genes display differential expression, despite males having half the genetic complement of females, with no sex chromosomes. These differences are not directly mediated by sex-specific methylation because almost no sex differences in methylation were observed. Genes with sex-specific expression show low frequency of methylation. However, the majority of female-biased genes are methylated (in both sexes), whereas male-biased ones are mostly nonmethylated in either sex. We conclude that female-biased genes are more likely to be recruited from conserved methylated genes over evolutionary time, whereas most male-biased genes are from genes after recent duplication events that are not methylated. (See pp. E3545–E3554.)

Genetic architecture of natural variation in *Drosophila melanogaster* aggressive behavior

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Aggressive behavior is evolutionarily conserved and genetically complex, but the genetic basis of natural variation in aggression is largely unknown. We performed genome-wide association analyses using the inbred, sequenced lines of the *Drosophila* Genetic Reference Panel (DGRP) and an advanced intercross population derived from the most and least aggressive DGRP lines. These analyses identified largely nonoverlapping genes that mapped onto a genetic interaction network inferred from an analysis of pairwise epistasis in the DGRP. We functionally validated candidate genes and genetic interactions. Epistasis for aggressive behavior causes cryptic genetic variation in the DGRP that is revealed by changing allele frequencies. This observation may apply to other fitness traits and species, with implications for evolution, applied breeding, and human genetics. (See pp. E3555–E3563.)

Platelet microparticles are internalized in neutrophils via the concerted activity of 12-lipoxygenase and secreted phospholipase A_2-IIA

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On activation, blood platelets package components from their cytoplasm into microparticles (MPs), tiny vesicles released by cytoplasmic membrane budding and shedding. Given that MPs can impact other cellular lineages on internalization, we aimed to decipher the mechanisms promoting MP internalization by cellular recipients. We modeled MP internalization by neutrophils and identified a predominant lipid, 12(S)-hydroxyeicosatetranoic acid, as a mediator critical for the promotion of MP internalization. MPs were found inside neutrophils from individuals with rheumatoid arthritis, and their presence in neutrophils in the joints of mice treated with arthritogenic serum is dependent on the expression of enzymes implicated in the generation of 12(S)-hydroxyeicosatetranoic acid. These findings reveal a unique molecular mechanism implicated in MP internalization relevant to inflammatory processes. (See pp. E3564–E3573.)

Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health

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Klebsiella pneumoniae is rapidly becoming untreatable using last-line antibiotics. It is especially problematic in hospitals, where it causes a range of acute infections. To approach controlling such a bacterium, we first must define what it is and how it varies genetically. Here we have determined the DNA sequence of *K. pneumoniae* isolates from around the world and present a detailed analysis of these data. We show that there is a wide spectrum of diversity, including variation within shared sequences and gain and loss of whole genes. Using this detailed blueprint, we show that there is an unrecognized association between the possession of specific gene profiles associated with virulence and antibiotic resistance and the differing disease outcomes seen for *K. pneumoniae*. (See pp. E3574–E3581.)

Enhanced memory consolidation in mice lacking the circadian modulators Sharp1 and -2 caused by elevated Igf2 signaling in the cortex

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SHARP1 and SHARP2 transcription factors are modulators of the sleep/wake homeostasis. Sleep is thought to be important for efficient memory consolidation by gradual stabilization of hippocampus-dependent memory traces in stable cortical modules. Here, SHARP1 and SHARP2 single and double null mutant mice were investigated in cognitive processing. SHARP1 and SHARP2 double null mutants show enhanced cortex-dependent remote fear memory formation, although hippocampus-dependent recent fear memory formation is not changed. Molecular analyses revealed that insulin-related growth factor 2 (IGF2)/MAPK signaling is elevated in the cortex of double mutants and that IGF2 overexpression in the anterior cingulate cortex is sufficient to enhance fear memory consolidation. Our analyses provide evidence that the control of sleep and memory consolidation may share common molecular mechanisms. (See pp. E3582–E3589.)

Activity-dependent synaptic GRIP1 accumulation drives synaptic scaling up in response to action potential blockade

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Brain circuits need plasticity mechanisms that stabilize activity to function properly. Although such homeostatic plasticity mechanisms have been widely described in a number of brain circuits, little is known about the molecular pathways that mediate them. Here, we show that an important form of homeostatic synaptic plasticity at excitatory synapses, synaptic scaling, relies on the activity-dependent accumulation of the glutamate receptor-binding protein glutamate receptor-interacting protein-1 (GRIP1) at synaptic sites. Our data show that GRIP1 is recruited to synapses under conditions of hypoactivity and, through a direct interaction with AMPA-type glutamate receptors, in turn, recruits more AMPA receptors to the synapse. These findings generate molecular insight into the mechanisms that adjust excitatory synaptic strength in response to perturbations in firing. (See pp. E3590–E3599.)

N-linked glycosylation of protease-activated receptor-1 at extracellular loop 2 regulates G-protein signaling bias

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G-protein-coupled receptors (GPCRs) are the largest class of mammalian signaling receptors and mediate vast physiological responses. The capacity to modulate GPCR signaling therapeutically is important for treatment of various diseases, and discovering new aspects of receptor signaling is critical for drug development. Protease-activated receptor-1 (PAR1) is GPCR for thrombin. Similar to other GPCRs, PAR1 is promiscuous and couples to multiple heterotrimeric G-protein subtypes in the same cell. How a single GPCR can couple to multiple G-protein subtypes concurrently has remained an enigma. We demonstrate that N-linked glycosylation of PAR1 regulates G-protein coupling specificity and differentially controls cellular responses. Thus, the status of GPCR glycosylation is a critical determinant for specifying coupling to distinct G-protein subtypes. (See pp. E3600–E3608.)

Allosteric interactions between agonists and antagonists within the adenosine A_{2A} receptor-dopamine D_2 receptor heterotetramer

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G protein-coupled receptors (GPCRs) constitute the largest plasma membrane protein family involved in cell signaling. GPCR homodimers are predominant species, and GPCR heteromers likely are constituted by heteromers of homodimers. The adenosine A_{2A} receptor (A_{2A}R)-dopamine D_2 receptor (D_2R) heteromer is a target for the nonselective adenosine receptor antagonist caffeine. This study uncovers allosteric modulations of A_{2A}R antagonists that mimic those of A_{2A}R agonists, challenging the traditional view of antagonists as inactive ligands. These allosteric modulations disappear when agonist and antagonist are coadministered, however. A model is proposed that considers A_{2A}R-D_2R heteromers as heterotetramers, constituted by A_{2A}R and D_2R homodimers. The model predicted that high concentrations of A_{2A}R antagonists would behave as A_{2A}R agonists and decrease D_2R function in the brain. (See pp. E3609–E3618.)

A structural, functional, and computational analysis suggests pore flexibility as the base for the poor selectivity of CNG channels

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Cyclic nucleotide-gated (CNG) channels underlie sensory transduction in photoreceptors and olfactory epithelium and share a high degree of homology with K^+ channels. However, these channels conduct Na^+ and K^+ differently: although K^+ channels discriminate with high accuracy Na^+ from K^+, CNG channels do not discriminate among different cations. By combining electrophysiology, molecular dynamics simulations, and X-ray crystallography we found that the pore region exhibits a dynamic structure. We show that (*i*) the selectivity filter can adapt to large and small ions with a different geometry and (*ii*) the pore diameter critically depends on the ion within. We conclude that the pore of CNG channels is highly flexible and that this flexibility is at the basis of their poor ionic selectivity. (See pp. E3619–E3628.)