PNAS Plus Significance Statements

Characterization of the human ESC transcriptome by hybrid sequencing

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Isoform identification and discovery are an important goal for transcriptome analysis because the majority of human genes express multiple isoforms with context- and tissue-specific functions. Better annotation of isoforms will also benefit downstream analysis such as expression quantification. Current RNA-Seq methods based on short-read sequencing are not reliable for isoform discovery. In this study (pp. E4821–E4830) we developed a new method based on the combined analysis of short reads and long reads generated, respectively, by second- and third-generation sequencing and applied this method to obtain a comprehensive characterization of the transcriptome of the human embryonic stem cell. The results showed that large gain in sensitivity and specificity can be achieved with this strategy.

Evolutionary mix-and-match with MFS transporters II

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The Major Facilitator Superfamily (MFS), the largest family of secondary transport proteins, catalyzes transport of a wide range of substrates. Difficulty discerning underlying mechanistic principles is due to low sequence conservation. However, a common structural feature of MFS members, suggesting that they may have arisen by intragenic multiplication, is a repeat of four three-helix bundles organized in two pseudosymmetrical domains. An alignment of these triple-helix motifs in combinatorial fashion allows detection of functionally homologous positions. Thus, substrate and H^+-binding sites in distantly related symporters are located at the same relative positions (pp. E4831–E4838). The structural organization also suggests that an ordered kinetic mechanism similar to that determined for lactose permease may be operative in other MFS symporters.

ORAI1 calcium channel orchestrates skin homeostasis

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The epidermis of the skin is composed of keratinocytes that are organized in several layers. Basal cells divide and produce cells moving outwards the epidermis while undergoing the process of terminal differentiation, crucial for the barrier function of the skin. Calcium is an indispensible ion for differentiation, and calcium channels are of primary importance. Unexpectedly, we discovered (pp. E4839–E4848) that the Orai1 calcium channel is mainly expressed in the basal layer, functioning to negatively control differentiation. The Orai1 channel supplies calcium to sustain proliferation and, in particular, to drive migration of keratinocytes, both processes being the feature of basal keratinocytes.

Hierarchical recruitment of Plk4 and regulation of centriole biogenesis by two centrosomal scaffolds, Cep192 and Cep152

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Found in most eukaryotic cells, a centriole is a cylindrically shaped subcellular structure that plays an important role in various cellular processes, including mitotic spindle formation and chromosome segregation. Centriole duplication occurs only once per cell cycle, thus ensuring accurate control of centriole numbers to maintain genomic integrity. Although a growing body of evidence suggests that a Ser/Thr protein kinase, polo-like kinase 4 (Plk4), is a key regulator of centriole duplication, how Plk4 is recruited to centrosomes remains largely unknown. Here (pp. E4849–E4857) we showed that Plk4 dynamically localizes to distinct subcentrosomal regions by interacting with two hierarchically regulated scaffolds, Cep192 and Cep152. Highlighting the importance of these interactions, mutational disruption of either one of these interactions was sufficient to cripple Plk4-dependent centriole biogenesis.

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Transcription factor evolution in eukaryotes and the assembly of the regulatory toolkit in multicellular lineages

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Independent transitions to multicellularity in eukaryotes involved the evolution of complex transcriptional regulation toolkits to control cell differentiation. By using comparative genomics (pp. E4858–E4866), we show that plants and animals required richer transcriptional machineries compared with other eukaryotic multicellular lineages. We suggest this is due to their orchestrated embryonic development. Moreover, our analysis of transcription factor (TF) expression patterns during the development of animals reveal links between TF evolution, species ontogeny, and the phylotypic stage.

Suppression of WC-independent *frequency* transcription by RCO-1 is essential for *Neurospora* circadian clock

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Rhythmic clock gene transcription is essential for the functions of eukaryotic circadian clocks. In the *Neurospora* circadian oscillator, the WHITE COLLAR (WC) complex is responsible for rhythmic *frequency* (*frq*) transcription and was thought to be the only transcriptional activator for *frq*. Here, we show that *frq* can be constitutively transcribed in a WC-independent manner when the transcriptional corepressor *rco-1* is deleted. In *rco-1* mutants, high-level constitutive WC-independent *frq* transcription results in impaired WC activity and loss of circadian rhythmicity. Our results (pp. E4867–E4874) further indicate that RCO-1 acts together with the histone modifier SET-2 and the chromatin remodeling factor CHD-1 to regulate normal chromatin structure at the *frq* locus, which permits rhythmic *frq* transcription.

The *CentO* satellite confers translational and rotational phasing on cenH3 nucleosomes in rice centromeres

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Centromeres are sites on chromosomes that mediate attachment to microtubules for chromosome segregation and often comprise tandemly repeated "satellite" sequences. The function of these repeats is unclear because centromeres can be formed on single-copy DNA by the presence of nucleosomes containing a centromere-specific variant of histone H3 (cenH3). Rice has centromeres composed of both the 155-bp *CentO* satellite repeat and single-copy non-*CentO* sequences. This study (pp. E4875–E4883) shows that rice cenH3 nucleosomes are regularly spaced with 155-bp periodicity on *CentO* repeats, but not on non-*CentO* sequences. *CentO* repeats have an ∼10-bp periodicity in dinucleotide pattern and in nuclease cleavage that suggests that *CentO* has evolved to minimize its bending energy on cenH3 nucleosomes and that centromeric satellites evolve for stabilization of cenH3 nucleosomes.

A unique secreted adenovirus E3 protein binds to the leukocyte common antigen CD45 and modulates leukocyte functions

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Human adenoviruses encode Early region 3 (E3) proteins that manipulate the host immune response to establish an infection or to persist longer. To date, only a few E3 functions from a single adenovirus species (C) have been characterized, all of which act directly on infected cells. Here we describe a secreted E3 protein that is uniquely expressed by species D adenoviruses. This protein targets noninfected leukocytes using a cell surface phosphatase as a receptor. We provide evidence (pp. E4884–E4893) that this interaction suppresses leukocyte activation and effector functions, implying that species D adenoviruses can affect the host distant from the site of infection.

Tuberous sclerosis 1 (Tsc1)-dependent metabolic checkpoint controls development of dendritic cells

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A fundamental question in immunology is how the coordination of immune signals and metabolic programs regulates immune responses. The identification of metabolic pathways orchestrating the activation of lymphocytes and dendritic cells (DCs) has advanced our understanding of immune activation, but whether cell metabolism contributes to development of immune cells is unknown. Here (pp. E4894–E4903) we have genetically defined a crucial metabolic checkpoint for DC development that is mediated by the interplay between Tsc1-mTOR complex 1 signaling and Myc-dependent bioenergetic and biosynthetic programs. Dysregulation of this pathway impairs survival, proliferation, and functional differentiation of DCs, thereby highlighting the importance of metabolic programming of DC development.

Selective inhibitor of endosomal trafficking pathways exploited by multiple toxins and viruses

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Bacterial and viral infections are a significant public health burden. To corrupt normal host cellular functions, many bacterial toxins and all viruses must gain entry to host cells, a process that exploits the host's own cellular machinery. In this study (pp. E4904–E4912), we use high-throughput technologies to screen for chemical inhibitors of bacterial toxin and viral entry. We report the discovery of a small molecule that inhibits several viruses and bacterial toxins. In addition to the therapeutic potential, this compound represents a powerful probe for dissecting the mechanisms of mammalian membrane trafficking processes.

Lhx2 regulates a cortex-specific mechanism for barrel formation

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The somatosensory barrels are a unique feature of the rodent cortex. Each barrel represents a functional unit in which clustered innervation from an individual whisker connects with a ring of cortical neurons. This study (pp. E4913–E4921) reports that when a single transcription factor, LIM homeobox 2, is deleted specifically in the cortex, neither the barrel cores nor the cortical barrel walls are able to form, although a rudimentary functional mapping of the somatosensory innervation does occur. Understanding how barrels form will shed light on how functional neurocircuitry is assembled in its final stages, and this insight may be broadly applicable in the nervous system.

Intestinal HIF2α promotes tissue-iron accumulation in disorders of iron overload with anemia

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Several distinct congenital disorders can lead to tissue-iron overload with anemia. Tissue-iron accumulation is the major cause of mortality in these patients. Intestinal hypoxia-inducible factor-2α (HIF2α) and its downstream target gene divalent metal transporter-1 (DMT1) are essential for iron absorption during times of increased iron demand. However, the role of the intestinal HIF2α/DMT1 signaling axis in iron overload disorders has not been assessed. We demonstrate (pp. E4922–E4930) that HIF2α and DMT1 in the small intestine are highly activated early in mouse models of anemic iron overload and that disruption of their expression can prevent and improve tissue-iron accumulation in these disorders. These results demonstrate that HIF2α and DMT1 are ideal therapeutic targets in iron-overload disorders.

Long-period rhythmic synchronous firing in a scale-free network

Yuanyuan Mi, Xuhong Liao, Xuhui Huang, Lisheng Zhang, Weifeng Gu, Gang Hu, and Si Wu

Understanding the mechanisms of how neural systems process temporal information is at the core to elucidate brain functions, such as for speech recognition and music appreciation. The present study (pp. E4931–E4936) investigates a simple yet effective mechanism for a neural system to extract the rhythmic information of external inputs in the order of seconds. We propose that a large-size neural network with scale-free topology is like a repertoire, which consists of a large number of loops and chains with various sizes, and these loops and chains serve as substrates to learn the rhythms of external inputs.