Capsule

Black mamba venom peptides target acid-sensing ion channels to abolish pain

Polypeptide toxins have played a central part in understanding physiological and physiopathological functions of ion channels. In the field of pain, they led to important advances in basic research and even to clinical applications. Acid-sensing ion channels (ASICs) are generally considered principal players in the pain pathway, including in humans. A snake toxin activating peripheral ASICs in nociceptive neurons has been recently shown to evoke pain. Diochot et al. show that a new class of three-finger peptides from another snake, the black mamba, is able to abolish pain through inhibition of ASICs expressed either in central or peripheral neurons. These peptides, called mambalgins, are not toxic in mice but show a potent analgesic effect upon central and peripheral injection that can be as strong as morphine. This effect is, however, resistant to naloxone, and mambalgins cause much less tolerance than morphine and no respiratory distress. Pharmacological inhibition by mambalgins combined with the use of knockdown and knockout animals indicates that blockade of heteromeric channels made of ASIC1a and ASIC2a subunits in central neurons and of ASIC1b-containing channels in nociceptors is involved in the analgesic effect of mambalgins. These findings identify new potential therapeutic targets for pain and introduce natural peptides that block them to produce a potent analgesia.

Nature 2012; 490: 552

Eitan Israeli

Capsule

Melanomas resist T cell therapy through inflammation-induced reversible dedifferentiation

Adoptive cell transfer therapies (ACTs) with cytotoxic T cells that target melanocytic antigens can achieve remissions in patients with metastatic melanomas, but tumors frequently relapse. Hypotheses explaining the acquired resistance to ACTs include the selection of antigen-deficient tumor cell variants and the induction of T cell tolerance. However, the lack of appropriate experimental melanoma models has so far impeded clear insights into the underlying mechanisms. Landsberg et al. establish an effective ACT protocol in a genetically engineered mouse melanoma model that recapitulates tumor regression, remission and relapse as seen in patients. They report the unexpected observation that melanomas acquire ACT resistance through an inflammation-induced reversible loss of melanocytic antigens. In serial transplantation experiments, melanoma cells switch between a differentiated and a dedifferentiated phenotype in response to T cell-driven inflammatory stimuli. The authors identified the pro-inflammatory cytokine tumor necrosis factor-alpha (TNFα) as a crucial factor that directly caused reversible dedifferentiation of mouse and human melanoma cells. Tumor cells exposed to TNFα were poorly recognized by T cells specific for melanocytic antigens, whereas recognition by T cells specific for non-melanocytic antigens was unaffected or even increased. Our results demonstrate that the phenotypic plasticity of melanoma cells in an inflammatory microenvironment contributes to tumor relapse after initially successful T cell immunotherapy. On the basis of our work, they propose that future ACT protocols should simultaneously target melanocytic and non-melanocytic antigens to ensure broad recognition of both differentiated and dedifferentiated melanoma cells, and include strategies to sustain T cell effector functions by blocking immune-inhibitory mechanisms in the tumor microenvironment.

Nature 2012; doi:10.1038/nature11538

Eitan Israeli

Capsule

A vaccine strategy that protects against genital herpes by establishing local memory T cells

Most successful existing vaccines rely on neutralizing antibodies, which may not require specific anatomical localization of B cells. However, efficacious vaccines that rely on T cells for protection have been difficult to develop, as robust systemic memory T cell responses do not necessarily correlate with host protection. In peripheral sites, tissue-resident memory T cells provide superior protection compared to circulating memory T cells. Shin et al. describe a simple and non-inflammatory vaccine strategy that enables the establishment of a protective memory T cell pool within peripheral tissue. The female genital tract, which is a portal of entry for sexually transmitted infections, is an immunologically restrictive tissue that prevents entry of activated T cells in the absence of inflammation or infection. To overcome this obstacle, the authors developed a vaccine strategy that they term "prime and pull" to establish local tissue-resident memory T cells at a site of potential viral exposure. This approach relies on two steps: conventional parenteral vaccination to elicit systemic T cell responses (prime), followed by recruitment of activated T cells by means of topical chemokine application to the restrictive genital tract (pull), where such T cells establish a long-term niche and mediate protective immunity. In mice, prime and pull protocol reduces the spread of infectious herpes simplex virus 2 into the sensory neurons and prevents development of clinical disease. These results reveal a promising vaccination strategy against herpes simplex virus 2, and potentially against other sexually transmitted infections such as human immunodeficiency virus.

Nature 2012; 491: 463

Eitan Israeli

Capsule

A FOXO3-IRF7 gene regulatory circuit limits inflammatory sequelae of antiviral responses

Antiviral responses must be tightly regulated to defend rapidly against infection while minimizing inflammatory damage. Type 1 interferons (IFN-I) are crucial mediators of antiviral responses and their transcription is regulated by a variety of transcription factors; principal among these is the family of interferon regulatory factors (IRFs). The IRF gene regulatory networks are complex and contain multiple feedback loops. The tools of systems biology are well suited to elucidate the complex interactions that give rise to precise coordination of the interferon response. Litval et al. have used an unbiased systems approach to predict that a member of the forkhead family of transcription factors, FOXO3, is a negative regulator of a subset of antiviral genes. This prediction was validated using macrophages isolated from Foxo3-null mice. Genome-wide location analysis combined with gene deletion studies identified the Irf7 gene as a critical target of FOXO3. FOXO3 was identified as a negative regulator of Irf7 transcription and they have further demonstrated that FOXO3, IRF7 and IFN-I form a coherent feed-forward regulatory circuit. These data suggest that the FOXO3-IRF7 regulatory circuit represents a novel mechanism for establishing the requisite set points in the interferon pathway that balances the beneficial effects and deleterious sequelae of the antiviral response.

Nature 2012; 490: 421

Eitan Israeli

Capsule

MR1 presents microbial vitamin B metabolites to MAIT cells

Antigen-presenting molecules, encoded by the major histocompatibility complex (MHC) and CD1 family, bind peptide- and lipid-based antigens, respectively, for recognition by T cells. Mucosal-associated invariant T (MAIT) cells are an abundant population of innate-like T cells in humans that are activated by an antigen(s) bound to the MHC class I-like molecule MR1. Although the identity of MR1-restricted antigen(s) is unknown, it is present in numerous bacteria and yeast. Kjer-Nielsen and co-workers show that the structure and chemistry within the antigen-binding cleft of MR1 is distinct from the MHC and CD1 families. MR1 is ideally suited to bind ligands originating from vitamin metabolites. The structure of MR1 in complex with 6-formyl pterin, a folic acid (vitamin B9) metabolite, shows the pterin ring sequestered within MR1. Furthermore, the authors characterize related MR1-restricted vitamin derivatives, originating from the bacterial riboflavin (vitamin B2) biosynthetic pathway, which specifically and potently activate MAIT cells. Accordingly, they show that metabolites of vitamin B represent a class of antigen that are presented by MR1 for MAIT-cell immunosurveillance. As many vitamin biosynthetic pathways are unique to bacteria and yeast, these data suggest that MAIT cells use these metabolites to detect microbial infection.

Nature 2012; doi:10.1038/nature11605

Eitan Israeli

Capsule

Phosphorylation of NLRC4 is critical for inflammasome activation

NLRC4 is a cytosolic member of the NOD-like receptor family that is expressed in innate immune cells. It senses indirectly bacterial flagellin and type III secretion systems, and responds by assembling an inflammasome complex that promotes caspase-1 activation and pyroptosis. Qu et al. use knock-in mice expressing NLRC4 with a carboxy-terminal 3×Flag tag to identify phosphorylation of NLRC4 on a single, evolutionarily conserved residue, Ser 533, following infection of macrophages with *Salmonella enterica* serovar Typhimurium (also known as *Salmonella typhimurium*). Western blotting with a NLRC4 phospho-Ser 533 antibody confirmed that this post-translational modification occurs only in the presence of stimuli known to engage NLRC4 and not the related protein NLRP3 or AIM2. Nlrc4−/− macrophages reconstituted with NLRC4 mutant S533A, unlike those reconstituted with wild-type NLRC4, did not activate caspase-1 and pyroptosis in response to *S. typhimurium*, indicating that S533 phosphorylation is critical for NLRC4 inflammasome function. Conversely, phosphomimetic NLRC4 S533D caused rapid macrophage pyroptosis without infection. Biochemical purification of the NLRC4-phosphorylating activity and a screen of kinase inhibitors identified PRKCD (PKCδ) as a candidate NLRC4 kinase. Recombinant PKCδ phosphorylated NLRC4 S533 in vitro, immunodepletion of PKCδ from macrophage lysates blocked NLRC4 S533 phosphorylation in vitro, and Prkcd−/− macrophages exhibited greatly attenuated caspase-1 activation and IL-1β secretion specifically in response to *S. typhimurium*. Phosphorylation-defective NLRC4 S533A failed to recruit procaspase-1 and did not assemble inflammasome specks during *S. typhimurium* infection, so phosphorylation of NLRC4 S533 probably drives conformational changes necessary for NLRC4 inflammasome activity and host innate immunity.

Nature 2012; 490: 539

Eitan Israeli

Capsule

Genetic basis for Intellectual and neurological disabilities

Intellectual and neurological disabilities can arise from diverse developmental aberrations. Novarino and group have now determined the genetic basis for one such disorder for a small group of patients. Exome sequencing led to identification of mutations in a kinase *BCKDK* (branched chain ketoacid dehydrogenase kinase) that regulates metabolism of branched-chain amino acids such as valine, leucine, and isoleucine. Mice with homozygous mutations in the *BCKDK* gene showed developmental and neurological abnormalities resembling those in certain mouse autism models. Analysis of transport mechanisms responsible for carrying amino acids across the blood-brain barrier revealed competition between the branched-chain amino acids and large neutral amino acids. Nutritional supplementation with extra branched-chain amino acids in the diet of mice carrying homozygous mutations in the *BCKDK* gene normalized their phenotype.

Science 2012; 338: 394

Eitan Israeli

Capsule

Clonal allelic predetermination of immunoglobulin-κ rearrangement

Although most genes are expressed biallelically, a number of key genomic sites – including immune and olfactory receptor regions – are controlled monoallelically in a stochastic manner, with some cells expressing the maternal allele and others the paternal allele in the target tissue. Very little is known about how this phenomenon is regulated and programmed during development. Using mouse immunoglobulin-κ (Igκ) as a model system, Farago and colleagues demonstrate that although individual hematopoietic stem cells are characterized by allelic plasticity, early lymphoid lineage cells become committed to the choice of a single allele, and this decision is then stably maintained in a clonal manner that predetermines monoallelic rearrangement in B cells. This is accompanied at the molecular level by underlying allelic changes in asynchronous replication timing patterns at the κ locus. These experiments may serve to define a new concept of stem cell plasticity.

Nature 2012; 490: 561

Eitan Israeli

"It is a truism that almost any sect, cult, or religion will legislate its creed into law if it acquires the political power to do so"

Robert A. Heinlein (1907-1988), American science fiction writer and one of the most influential and controversial authors of the genre in his time

Capsule

Mutations in the gene *CIB2* contribute to Usher syndrome and non-syndromic deafness

Individuals with the hereditary disorder Usher syndrome suffer from hearing loss. Associated genetic mutations impair function of the inner ear, where sensory cells fail to convert sound waves into electrical signals. Riazuddin et al. have determined that mutations in the gene *CIB2* contribute to Usher syndrome and non-syndromic deafness. *CIB2* encodes calcium and integrin binding protein 2, which is widely expressed in human and mouse tissue. In the mouse inner ear, the protein localizes to the tips of stereocilia of inner ear cells. When deflected by sound waves, ion channels in these hairlike projections open, triggering a mechanoelectrical signaling cascade. *CIB2* interacts with whirlin, a protein that organizes molecular complexes that maintain stereocilia structure and growth. Suppression of *CIB2* expression in zebrafish disrupted responses to acoustic stimuli and caused abnormal balance during movement. Overexpression of *CIB2* in cultured cells decreased the release of calcium from intracellular stores. *CIB2* may help to maintain intracellular calcium homeostasis in inner ear cells by sequestering calcium and influencing the release of stored calcium during mechanoelectrical signal transduction.

Nat Genet 2012; 44: 10.1038/ng.2426

Eitan Israeli

Capsule

Targeting VEGF-B as a novel treatment for insulin resistance and type 2 diabetes

The prevalence of type 2 diabetes is rapidly increasing, with severe socioeconomic impacts. Excess lipid deposition in peripheral tissues impairs insulin sensitivity and glucose uptake, and has been proposed to contribute to the pathology of type 2 diabetes. However, there are few treatment options that directly target ectopic lipid accumulation. Recently it was found that vascular endothelial growth factor B (VEGF-B) controls endothelial uptake and transport of fatty acids in heart and skeletal muscle. Hagberg el al. show that decreased VEGF-B signaling in rodent models of type 2 diabetes restores insulin sensitivity and improves glucose tolerance. Genetic deletion of Vegfb in diabetic db/db mice prevented ectopic lipid deposition, increased muscle glucose uptake and maintained normoglycemia. Pharmacological inhibition of VEGF-B signaling by antibody administration to db/db mice enhanced glucose tolerance, preserved pancreatic islet architecture, improved β cell function and ameliorated dyslipidemia, key elements of type 2 diabetes and the metabolic syndrome. The potential use of VEGF-B neutralization in type 2 diabetes was further elucidated in rats fed a high fat diet, in which it normalized insulin sensitivity and increased glucose uptake in skeletal muscle and heart. Their results demonstrate that the vascular endothelium can function as an efficient barrier to excess muscle lipid uptake even under conditions of severe obesity and type 2 diabetes, and that this barrier can be maintained by inhibition of VEGF-B signaling. The authors propose VEGF-B antagonism as a novel pharmacological approach for type 2 diabetes, targeting the lipid transport properties of the endothelium to improve muscle insulin sensitivity and glucose disposal.

Nature 2012; 490: 426

Eitan Israeli

"What you really value is what you miss, not what you have"

Jorge Luis Borges (1899-1986), Argentinian short-story writer, essayist, poet and translator

Capsule

Structure of the agonist-bound neurotensin receptor

Neurotensin (NTS) is a 13-amino-acid peptide that functions as both a neurotransmitter and a hormone through the activation of the neurotensin receptor NTSR1, a G-protein-coupled receptor (GPCR). In the brain, NTS modulates the activity of dopaminergic systems, opioid-independent analgesia, and the inhibition of food intake; in the gut, NTS regulates a range of digestive processes. White and collaborators present the structure at 2.8 Å resolution of Rattus norvegicus NTSR1 in an active-like state, bound to NTS8-13, the carboxy-terminal portion of NTS responsible for agonist-induced activation of the receptor. The peptide agonist binds to NTSR1 in an extended conformation nearly perpendicular to the membrane plane, with the C terminus oriented towards the receptor core. These findings provide, to our knowledge, the first insight into the binding mode of a peptide agonist to a GPCR and may support the development of non-peptide ligands that could be useful in the treatment of neurological disorders, cancer and obesity.

Nature 2012; doi:10.1038/nature11558

Eitan Israeli

Capsule

Generation of functional thyroid from embryonic stem cells

The primary function of the thyroid gland is to metabolize iodide by synthesizing thyroid hormones, which are critical regulators of growth, development and metabolism in almost all tissues. So far, research on thyroid morphogenesis has been missing an efficient stem cell model system that allows for the in vitro recapitulation of the molecular and morphogenic events regulating thyroid follicular-cell differentiation and subsequent assembly into functional thyroid follicles. Antonica et al. report that a transient overexpression of the transcription factors NKX2-1 and PAX8 is sufficient to direct mouse embryonic stem cell differentiation into thyroid follicular cells that organize into three-dimensional follicular structures when treated with thyrotropin. These in vitro-derived follicles showed appreciable iodide organification activity. Importantly, when grafted in vivo into athyroid mice, these follicles rescued thyroid hormone plasma levels and promoted subsequent symptomatic recovery. Thus, mouse embryonic stem cells can be induced to differentiate into thyroid follicular cells in vitro and generate functional thyroid tissue.

Nature 2012; doi:10.1038/nature11525

Eitan Israeli

"No man, for any considerable period, can wear one face to himself and another to the multitude, without finally getting bewildered as to which may be true"

Nathaniel Hawthorne (1804-1864), American writer whose many works featuring moral allegories with a Puritan inspiration and deep psychological complexity, exemplified by his most famous work *The Scarlet Letter*

Capsule

Androgenetic haploid embryonic stem cells produce live transgenic mice

Haploids and double haploids are important resources for studying recessive traits and have large impacts on crop breeding, but natural haploids are rare in animals. Mammalian haploids are restricted to germline cells and are occasionally found in tumors with massive chromosome loss. Recent success in establishing haploid embryonic stem (ES) cells in medaka fish and mice raised the possibility of using engineered mammalian haploid cells in genetic studies. However, the availability and functional characterization of mammalian haploid ES cells are still limited. Li and co-scientists show that mouse androgenetic haploid ES (ahES) cell lines can be established by transferring sperm into an enucleated oocyte. The ahES cells maintain haploidy and stable growth over 30 passages, express pluripotent markers, possess the ability to differentiate into all three germ layers in vitro and in vivo, and contribute to germlines of chimeras when injected into blastocysts. Although epigenetically distinct from sperm cells, the ahES cells can produce viable and fertile progenies after intracytoplasmic injection into mature oocytes. The oocyte-injection procedure can also produce viable transgenic mice from genetically engineered ahES cells. These findings show the developmental pluripotency of androgenentic haploids and provide a new tool to quickly produce genetic models for recessive traits. They may also shed new light on assisted reproduction.

Nature 2012; 490: 407

Eitan Israeli

"It takes a great deal of courage to stand up to your enemies, but even more to stand up to your friends"

J.K. Rowling (b. 1965), British novelist, best known for the *Harry Potter* fantasy books, the best-selling book series in history. She has led a 'rags to riches' life story, in which she progressed from living on social security to multi-millionaire status within five years, and is a notable philanthropist

Capsule

An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background

People with pale skin, red hair, freckles and an inability to tan – the 'red hair/fair skin' phenotype – are at highest risk of developing melanoma, compared to all other pigmentation types. Genetically, this phenotype is frequently the product of inactivating polymorphisms in the melanocortin 1 receptor (*MC1R*) gene. *MC1R* encodes a cyclic AMP-stimulating G-protein-coupled receptor that controls pigment production. Minimal receptor activity, as in red hair/fair skin polymorphisms, produces the red/yellow pheomelanin pigment, whereas increasing *MC1R* activity stimulates the production of black/brown eumelanin. Pheomelanin has weak shielding capacity against ultraviolet radiation relative to eumelanin, and has been shown to amplify ultraviolet-A-induced reactive oxygen species. Several observations, however, complicate the assumption that melanoma risk is completely ultraviolet radiation dependent. For example, unlike non-melanoma skin cancers, melanoma is not restricted to sun-exposed skin and ultraviolet radiation signature mutations are infrequently oncogenic drivers. Although linkage of melanoma risk to ultraviolet radiation exposure is beyond doubt, ultraviolet-radiation-independent events are likely to have a significant role. Mitra et al. have introduced a conditional, melanocyte-targeted allele of the most common melanoma oncoprotein, BRAFV600E, into mice carrying an inactivating mutation in the *MC1R* gene (these mice have a phenotype analogous to red hair/fair skin humans). The authors observed a high incidence of invasive melanomas without providing additional gene aberrations or ultraviolet radiation exposure. To investigate the mechanism of ultraviolet radiation-independent carcinogenesis, they introduced an albino allele, which ablates all pigment production on the Mc1re/e background. Selective absence of pheomelanin synthesis was protective against melanoma development. In addition, normal Mc1re/e mouse skin was found to have significantly greater oxidative DNA and lipid damage than albino-Mc1re/e mouse skin. These data suggest that the pheomelanin pigment pathway produces ultraviolet radiation-independent carcinogenic contributions to melanomagenesis by a mechanism of oxidative damage. Although protection from ultraviolet radiation remains important, additional strategies may be required for optimal melanoma prevention.

Nature 2012; 491: 449

Eitan Israeli

"Most people think that shadows follow, precede, or surround beings or objects. The truth is that they also surround words, ideas, desires, deeds, impulses and memories"

Elie Wiesel (b. 1928), Romanian-born Jewish-American writer, professor, political activist, Nobel laureate, and Holocaust survivor

Capsule

A vaccine strategy that protects against genital herpes by establishing local memory T cells

Most successful existing vaccines rely on neutralizing antibodies, which may not require specific anatomical localization of B cells. However, efficacious vaccines that rely on T cells for protection have been difficult to develop, as robust systemic memory T cell responses do not necessarily correlate with host protection. In peripheral sites, tissue-resident memory T cells provide superior protection compared to circulating memory T cells. Shin et al. describe a simple and non-inflammatory vaccine strategy that enables the establishment of a protective memory T cell pool within peripheral tissue. The female genital tract, which is a portal of entry for sexually transmitted infections, is an immunologically restrictive tissue that prevents entry of activated T cells in the absence of inflammation or infection. To overcome this obstacle, the authors developed a vaccine strategy that they term "prime and pull" to establish local tissue-resident memory T cells at a site of potential viral exposure. This approach relies on two steps: conventional parenteral vaccination to elicit systemic T cell responses (prime), followed by recruitment of activated T cells by means of topical chemokine application to the restrictive genital tract (pull), where such T cells establish a long-term niche and mediate protective immunity. In mice, prime and pull protocol reduces the spread of infectious herpes simplex virus 2 into the sensory neurons and prevents development of clinical disease. These results reveal a promising vaccination strategy against herpes simplex virus 2, and potentially against other sexually transmitted infections such as human immunodeficiency virus.

Nature 2012; 491: 463

Eitan Israeli

Capsule

Host microbe interactions have shaped the genetic architecture of inflammatory bowel disease

Crohn's disease and ulcerative colitis, the two common forms of inflammatory bowel disease (IBD), affect over 2.5 million people of European ancestry with rising prevalence in other populations. Genome-wide association studies and subsequent meta-analyses of these two diseases as separate phenotypes have implicated previously unsuspected mechanisms, such as autophagy, in their pathogenesis and showed that some IBD loci are shared with other inflammatory diseases. Jostins and team expand on the knowledge of relevant pathways by undertaking a meta-analysis of Crohn's disease and ulcerative colitis genome-wide association scans, followed by extensive validation of significant findings, with a combined total of more than 75,000 cases and controls. The authors identified 71 new associations, for a total of 163 IBD loci, that meet genome-wide significance thresholds. Most loci contribute to both phenotypes, and both directional (consistently favoring one allele over the course of human history) and balancing (favoring the retention of both alleles within populations) selection effects are evident. Many IBD loci are also implicated in other immune-mediated disorders, most notably with ankylosing spondylitis and psoriasis. They also observed considerable overlap between susceptibility loci for IBD and mycobacterial infection. Gene co-expression network analysis emphasizes this relationship, with pathways shared between host responses to mycobacteria and those predisposing to IBD.

Nature 2012; 491: 119

Eitan Israeli