Targeted gene trapping in mouse ES cells

Roland Friedel et al. report a method allowing efficient generation of targeted gene knockouts by homologous recombination in mouse ES cells. Current gene targeting techniques use vectors with a strong promoter to drive expression of a selectable antibiotic resistance marker, but such methods usually yield low homologous recombination frequencies because nonhomologous insertions (false positives) also result in antibiotic resistance. Friedel et al. improved upon the previously developed strategy of targeted trapping, in which promoterless gene trap vectors depend on the endogenous promoter of the targeted gene for expression of the antibiotic resistance marker. The authors achieved targeting frequencies as high as 90%, purportedly higher than any other approach reported to date. Although targeted genes must be expressed in ES cells at sufficient levels to confer antibiotic resistance, the authors found a threshold level of expression, and they estimate that about half of all genes are expressed above this level. — P.D.

“Gene targeting using a promoterless gene trap vector (“targeted trapping”) is an efficient method to mutate a large fraction of genes” by Roland H. Friedel, Andrew Plump, Xiaowei Lu, Kerri Spilker, Christine Jolicoeur, Karen Wong, Tadmir R. Venkatesh, Avraham Yaron, Mary Hynes, Bin Chen, Aimi Okada, Susan K. McConnell, Helen Rayburn, and Marc Tessier-Lavigne (see pages 13188–13193)

Two DNA repair genes associated with lung carcinogenesis

Christine Hollander et al. demonstrate that the XPC and Gadd45a genes are involved in the initiation and progression of lung cancer in mice, respectively. Chromosome 1p and 3p deletions are among the most frequent genetic changes associated with lung cancer, although no definitive tumor suppressor genes have been identified in these regions. XPC, located on chromosome 3, and Gadd45a, on chromosome 1, are both involved in DNA repair and have been previously associated with increased cancer risk. The authors report that 100% of XPC−/− mice developed spontaneous lung tumors in 16–17 months, primarily classified as benign adenomas, whereas Gadd45a−/− mice exhibited no increased incidence of lung tumors. However, in XPC−/− Gadd45a−/− double-mutant strains, >60% of the mice developed at least one malignant carcinoma in addition to multiple adenomas. Hollander et al. note that because XPC−/− mice have compromised DNA repair but show late onset of tumors and rarely develop malignancies, these mice may provide a useful animal model to study carcinogen-induced tumorigenesis. — N.Z.

“Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis” by M. Christine Hollander, Robyn T. Philburn, Andrew D. Patterson, Susana Velasco-Miguel, Errol C. Friedberg, R. Ilona Linnoila, and Albert J. Fornace, Jr. (see pages 13200–13205)

Lymphocyte attenuator binding site identified

Timothy Cheung et al. report the location of the binding site of the receptor herpesvirus entry mediator (HVEM) for B and T lymphocyte attenuator (BTLA), on the receptor’s cysteine-rich domain-1 (CRD1). HVEM, which is expressed by T lymphocytes, acts as a molecular switch to modulate T cell activation. The binding site for BTLA, which inhibits T cells activation, was previously unknown. A viral ligand for HVEM, herpes simplex virus-1 envelope glycoprotein D
Positively (blue) and negatively (red) charged residues of HVEM: K64 mutation causes loss of BTLA binding.

“Evolutionarily divergent herpesviruses modulate T cell activation by targeting the herpesvirus entry mediator cosignaling pathway” by Timothy C. Cheung, Ian R. Humphreys, Karen G. Potter, Paula S. Norris, Heather M. Shumway, Bonnie R. Tran, Ginelle Patterson, Rochelle Jean-Jacques, Miri Yoon, Patricia G. Spear, Kenneth M. Murphy, Nell S. Lurain, Christopher A. Benedict, and Carl F. Ware (see pages 13218–13223)

IMMUNOLOGY

Class switching linked to B cell division regulator

According to James Rush et al., antibody class switch recombination (CSR) by B cells relies on the cell division-regulated expression of cytidine deaminase (AID). Previous research has shown that cell division is linked to CSR, but the molecular mechanism underlying this division dependence is not well understood. Rush et al. examined the molecular events associated with CSR in activated B cells isolated from different divisions. The frequency of switching at the protein and DNA levels increased with successive cell divisions and was not dependent on culture duration. Germ-line transcript levels in division-sorted cells did not correlate well with the frequency of switching. However, AID expression increased with successive cell divisions in a time-independent manner and correlated with the frequency of CSR. B cells constitutively expressing AID experienced an almost 2-fold increase in the frequency of switched cells compared with controls. The authors suggest that the division-linked regulation of AID expression is a primary mechanistic control of CSR. — F.A.

Expression of activation-induced cytidine deaminase is regulated by cell division, providing a mechanistic basis for division-linked class switch recombination” by James S. Rush, Man Liu, Valerie H. Odegard, Shyam Unniraman, and David G. Schatz (see pages 13242–13247)

NEUROSCIENCE

Neuroanatomy of emotion and asthma

Melissa Rosenkranz et al. report that activity in the anterior cingulate cortex (ACC) and insula regions of the brain, in response to asthma-related emotional stimuli, is associated with inflammation and airflow obstruction in asthma. Chronic diseases characterized by dysregulation of inflammation, such as asthma, are known to be susceptible to modulation by stress and emotion. The authors sought to identify a specific neural circuitry through which cognitive and emotional factors interact with physiological events to influence asthma symptom severity. Functional MRI was used to evaluate brain activity in six individuals with asthma in response to asthma-relevant and non-relevant emotional stimuli. The subjects were told particular speech words, such as “wheeze” (asthma-related), “loneliness” (non-asthma-related negative), and “curtains” (neutral), after induced bronchoconstriction with methacholine or inhaled allergen. The ACC and insula areas of the subjects’ brains showed more activity with asthma-related words compared with other words. The results suggest that these brain regions are hyperresponsive to disease-specific emotional signals and that this might contribute to the dysregulation of the peripheral inflammatory process. — R.N.

Neural circuitry underlying the interaction between emotion and asthma symptom exacerbation” by Melissa A. Rosenkranz, William W. Busse, Tom Johnstone, Cheri A. Swenson, Gina M. Crisafi, Maryjo M. Jackson, Jos A. Bosch, John F. Sheridan, and Richard J. Davidson (see pages 13319–13324)