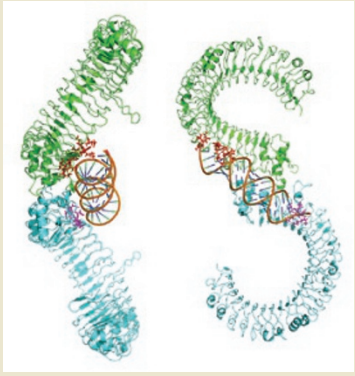
siRNA activates TLR3



Several small interfering RNA (siRNA)-based drugs are already showing promise in clinical trials, including one for macular degeneration that inhibits angiogenesis purportedly by targeting vascular endothelial growth factor (VEGF). But now, Kleinman and colleagues cast doubt on the exquisite target specificity often claimed for the approach by showing that siRNA suppression of angiogenesis can be sequence independent. In these researchers' hands, six siRNAs unrelated to VEGF inhibited choroidal neovascularization when injected into the vitreous humor of mice. Finding that fluorescently labeled siRNAs did not enter cells, they focused on surface receptors and found evidence that Toll-like receptor 3 (TLR3) is involved in the effect: suppression of angiogenesis did not occur in mouse mutants lacking TLR3, and soluble TLR3 abrogated the response. In addition, siRNAs identical to a drug targeting *VEGFA* failed to do so in TLR3 mutants, but did so in mutants of the supposed target gene *VEGFA*. The researchers carried out structural and genetic studies and found that the TLR3 response required a minimum of 21 nucleotides, and that polymorphisms of the human *TLR3* gene responded differently to the molecules. The work raises interesting possibilities for patient selection with siRNA therapeutics, but also some troubling possibilities for nonspecific effects of siRNA, particularly when administered systemically. (*Nature* **452**, 591–597, 2008) *LD*

Parkinson's strikes transplanted neurons

Small-scale clinical trials of fetal midbrain neurons for Parkinson's disease have had mixed outcomes, with some transplant recipients gaining improved motor skills and others showing little effect or developing dyskinesias. Three analyses of postmortem brain tissue from long-term transplant recipients shed new light on the fate of the grafted cells. In the most notable finding, two of the studies (by Li *et al.* and Kordower *et al.*, on three subjects 11–16 years post-transplant) detected characteristic Parkinson's histopathology in some of the grafted cells, including α-synuclein- and ubiquitin-positive Lewy bodies. How these relatively young cells acquired a disease phenotype generally associated with advanced age could not be determined, but future investigation of this process, which may depend on a prion-like mechanism, could provide insight into the normal progression of Parkinson's disease. Interestingly, the third study, by Mendez *et al.* (on three subjects 9–14 years post-transplant and two subjects 3–4 years post-transplant), found no evidence of Parkinson's pathology in the grafts. Although the disease spread to some grafted neurons in two of the studies, large numbers of these cells remained healthy more than a decade after transplantation, supporting the potential of cell therapy for Parkinson's disease. (*Nat. Med.* **14**, 501–503, 504–506, 507–509, 2008) *KA*

Written by Kathy Aschheim, Laura DeFrancesco, Peter Hare & Jan-Willem Theunissen

Mast cell activators as adjuvants

Mast cells are important in the initiation of not only the innate but also the adaptive immune response. McLachlan *et al.* demonstrate that several well-known mast cell activators, including the small-molecule compound 45/80 (c48/80), can be used as vaccine adjuvants to boost the antigen-specific serum immunoglobulin G (IgG) response in a mast cell– and dendritic cell–dependent manner. Whereas protective antigen (PA) from *Bacillus anthracis* with c48/80 induced significant PA-specific IgG titers in wild-type mice, these responses were attenuated in mast cell–deficient $Kit^{W}/Kit^{W\text{-}v}$ mice. Furthermore, transient depletion of the dendritic cell population in mice that received PA in combination with c48/80 resulted in failure to mount an antibody response; c48/80 was also a potent mucosal and nasal adjuvant. Administration of c48/80 with PA into the nose induced antigen-specific $CD4^{+}$ T cells and increased levels of PA-specific IgG, but it only modestly affected $CD8^{+}$ T cell responses. Finally, in combination with the B5R poxvirus protein, c48/80 effectively vaccinated mice against vaccinia virus. (*Nat. Med.* **14**, 536–541, 2008) *JWT*

Expanding the pluripotency network

Early investigations of pluripotency in embryonic stem (ES) cells identified a small number of transcription factors, such as Oct 4, Sox2 and Nanog, that have essential roles in establishing or maintaining the pluripotent state. More recently, interest has shifted to system-wide approaches for describing the molecular networks that underpin pluripotency. Extending previous work that used microarray-based chromatin immunoprecipitation (ChIP-Chip) to identify the target genes of Oct4, Sox2 and Nanog, Kim *et al.* carried out a ChIP study in mouse ES cells to identify the target genes of nine pluripotency-associated transcription factors: Oct4, Sox2, Nanog, Klf4, Myc, Dax1, Nac1, Zfp281 and Rex1. More than one-third (6,632) of promoters in the mouse genome were occupied by at least one of the nine transcription factors. Half of these were bound by one of the factors and the rest by multiple factors, with >100 promoters bound by seven or more. The degree of occupancy correlated with gene expression: promoters bound by more than four factors were generally transcriptionally active, and those bound by a single factor were inactive. The authors also characterize new transcriptional regulatory circuits among the nine factors that include autoregulation, feed-forward regulation and interconnectivity. (*Cell*, doi:10.1016/j.cell.2008.02.039) *KA*

Amplification-free DNA resequencing

Effective high-throughput single-molecule sequencing has long been sought to replace bulk sequencing strategies that require PCR-mediated amplification. Amplification introduces errors and bias in template representation, and necessitates onerous and costly DNA manipulation. Now, Harris *et al.* describe the prototype of a technology that may contribute to making the lofty goal of a $1,000 human genome sequence a reality. Their approach, which requires a reference sequence to piece together ~23-nucleotide reads, involves anchoring poly(dT) oligos to glass coverslips and then using these to first capture poly(dA)-tailed single-stranded templates (in this study, <200 nucleotides long) and then prime template-directed extension with fluorescently labeled nucleotides. Nucleotide incorporation is detected by fluorescence imaging. 224 sequencing cycles, each analyzing >280,000 DNA molecules simultaneously, enabled the authors to resequence the ~7.2 kbp M13 bacteriophage genome to an average depth of >150× with complete coverage and efficient detection of single-nucleotide mutations. The ability to sequence the same DNA strand twice *in situ* further reduces error rates. (*Science* **320**, 106–109, 2008) *PH*