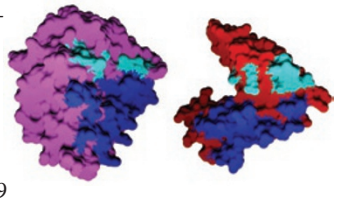
On complement with complementarity



Found on B cells and follicular dendritic cells, complement receptor 2 (CR2) binds the degradation products of complement C3 (iC3b, C3b, C3dg or C3d) covalently 'tagged' to an antigenic surface. At the same time, CR2 is associated with CD19 and CD81 on B cells, and this complex leads to sensitization of B cells to antigens. Therefore, understanding the interaction between CR2 and C3d is of great importance both for adaptive and innate immunity. An X-ray crystal structure of C3d bound to the CR2 SCR domains (CR2(SCR1-2)) had been determined previously; however, the interactions seen in that structure were in conflict with several biochemical studies. Now, van den Elsen and Isenman report a new crystal structure of the C3d–CR2(SCR1-2) complex. In this structure, the interaction interface between C3d and CR2(SCR1-2) is formed through shape complementarity, as the V-shaped SCR domains interact with the concave surface of C3d. Interestingly, none of the interactions in the previous model were seen in this new structure, whose interface is corroborated by previous biochemical data as well as mutational analysis revealed by the authors. Additionally, the new structural model reveals how other SCR1 and SCR2 residues contribute to binding. The insights from this structure will allow researchers to target this interaction and thus either up- or downregulate B cell reactivity as desired. (*Science* **332**, 608–611, 2011) *SM*

Pooling resources to fight oncogenes

Increased chromosomal instability is detectable early in oncogenesis as a consequence of DNA double-strand break (DSB) formation. One event that can contribute to DSB formation, particularly at fragile sites, is replicative stress, which occurs upon oncogene activation. Oncogenes are also known to aberrantly switch on the Rb-E2F cell-proliferation pathway. Bester *et al.* have now modified poorly proliferating primary cells to express the human papillomavirus-16 oncogenes *E6* and *E7* (proteins referred as E6/E7 here). Upon activation, replication rate was strongly suppressed, and movement of the two forks from the same origin became uncoupled, indicating that replication processivity was affected. To compensate, the E6/E7-expressing cells activated a number of usually dormant origins. Fragile sites experienced instability earlier than other regions of the genome, and, consistent with this, SNPs near these sites were among the first to undergo loss of heterozygosity. One factor that controls the dynamics of replication is the available nucleotide pool. When E6/E7 are present, the amount of all of the dNTPs (and rNTPs, to a lesser extent) is reduced, meaning cells proliferate with an insufficient nucleotide pool to support normal replication. This alteration contributes to the observed instability, as supplementing oncogene-expressing cells with exogenous nucleosides suppressed the above phenotypes and limited cellular transformation in soft agar. Another oncogenic condition that activates the Rb-E2F pathway, cyclin E overexpression, showed similar effects on nucleotide pool size. Although some proliferation factors regulate genes involved in nucleotide biosynthesis, oncogene activation of Rb-E2F did not induce many of these genes. c-Myc is known to regulate nucleotide biosynthesis, and it also restored dNTP pools along with replication rate and genomic stability, when overexpressed in the oncogene-expressing cells. This study thus establishes dysregulated nucleotide biosynthesis as the basis for the genomic instability arising from oncogene-induced activation of the Rb-E2F pathway. (*Cell* **145**, 435–446, 2011) *AKE*

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Unraveling G4

Single-stranded DNA containing guanine tracts can form a very stable structure called G-quadruplex (G4) *in vitro*. Sequences with the potential to form G4 are abundant in the genome across organisms, and some sites are conserved, suggesting G4 might have a functional role. In contrast, G4 would pose an obstacle for DNA replication and hence require the action of DNA helicases such as Pif1, which can efficiently unwind G4 *in vitro*. Paeschke, Capra and Zakian now use a genome-wide approach to identify Pif1-binding sites in *Saccharomyces cerevisiae*. The authors used a catalytically dead Pif1 mutant for ChIP-on-chip analysis, increasing the sensitivity of the method; the identified sites were also validated with wild-type Pif1. The analyses excluded rDNA and telomeric regions, which contain repetitive sequences and where Pif1 is already known to act. The data reveal over a thousand Pif1-binding sites, with a subset (about 10%) containing G4 motifs. In cells deficient in Pif1, these G4 regions caused the replication fork to slow down and increased the frequency of chromosomal breaks and homologous recombination. Interestingly, binding of Pif1 to the G4 motifs peaked at late S phase, after these regions have been replicated. The authors propose that the replication fork can bypass G4 structures but progresses at a slower pace; resolving the G4 structures prior to chromosomal condensation and mitosis is important to avoid breaks and genomic instability. This work provides strong evidence for the need to unwind G4 structures during DNA replication (and hence that G4 structures do form *in vivo*). The data also indicate that Pif1 has functions other than unwinding G4, as the majority of the sites identified do not contain G4 motifs but include highly transcribed genes and sites for mitotic and meiotic double-strand breaks. (*Cell* **145**, 13–14, 2011) *IC*

Stressing out with Hog1

Responding to stress is obviously crucial to survival across organisms, and in *Saccharomyces cerevisiae*, osmotic stress triggers signaling through the mitogen-activated protein kinase (MAPK) Hog1. Upon stimulation, Hog1 translocates to the nucleus, activating a transcriptional stress response program. This pathway is involved in a transient response—the so-called adaptation response—and Peter and colleagues have now tested whether this response has the same underlying cell-to-cell variability previously observed for cells undergoing sustained MAPK signaling. To test this, the authors generated reporters driven by defined promoters involved in the transcriptional leg of the Hog1 response. At low osmotic stress, reporters had background fluorescence, but at higher salt concentrations, all cells expressed the reporters tested. However, at intermediate concentrations, cells showed a bimodal distribution, with some cells expressing reporter and others not. Using dual-reporter expression, the authors were able to show that this was due to intrinsic variability in the cell, rather than variation due to extrinsic factors. Hog1 nuclear translocation did not show the same variability, indicating that a downstream event was responsible for bimodality. Indeed, by examining cells with impaired chromatin-remodeling complexes, the authors implicated INO80, SAGA and RSC in this bimodal gene expression, as well as the transcription factors Sko1 and Hot1. How these activities are integrated at the promoter, leading to varying levels of expression, as well as whether this variability has an adaptive role under stress, are exciting questions for future exploration. (*Science*, **332**, 732–735, 2011) *SL*