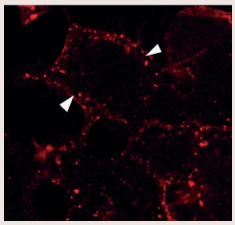
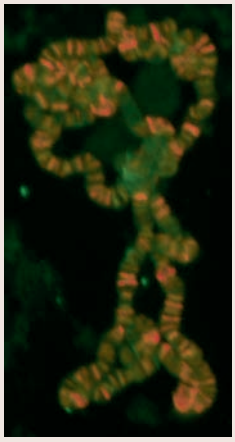
In this issue

by Jane Bradbury



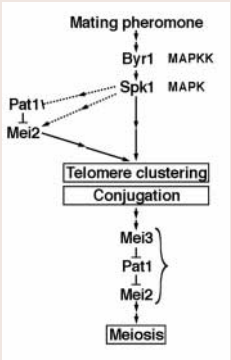
Not just a phospho-lipase

Phospholipase C-γ1 (PLC-γ1) is a critical signalling molecule in the regulation of cell proliferation. Its downstream effects depend both on its lipase activity – through which it generates inositol 1,4,5-trisphosphate and diacylglycerol – and on its SH2 and SH3 domains. Now, Pann-Ghill Suh and co-workers report that PLC-γ1 is also a guanine nucleotide exchange factor (GEF) that regulates the GTPase dynamin 1 and suggest that it is involved in the regulation of endocytosis (see p. 3785). Dynamin 1 drives clathrin-mediated endocytosis of numerous proteins, including growth factor receptors. In their paper, the authors show that PLC-γ1 functions as a dynamin 1 GEF in vitro and in vivo through a direct interaction between its SH3 domain and the GTPase. Overexpression of PLC-γ1 in PC12 cells enhances dynamin-1-dependent endocytosis of epidermal growth factor (EGF) receptor and as a result stimulates activation of the MAP kinase ERK, which is downstream of the EGF receptor. By contrast, downregulation of PLC-γ1 by siRNA reduces ERK activation. Suh and co-workers propose that PLC-γ1 functions as a key molecule in growth-factor-induced proliferation by regulating growth factor endocytosis.



DNA topology revealed

Changes in chromatin structure are thought to occur during the regulation of gene expression. However, relatively little is known about DNA topology in vivo because we lack suitable probes for its analysis. On p. 3797, Kuniharu Matsumoto and Susumu Hirose describe a technique that might remedy this gap in our knowledge, visualising transcription-coupled, unconstrained negative DNA supercoils for the first time in an interphase genome. Their technique relies on the ability of psoralen to intercalate into DNA. When exposed to UV light, psoralen crosslinks opposite DNA strands at a rate that depends on the degree of negative superhelicity in the DNA. Using biotinylated psoralen and fluorescent streptavidin, the authors visualised psoralen binding to *Drosophila* salivary gland DNA. Signals indicating unconstrained negative supercoils were visible on many but not all interbands and puffs, sites of active transcription in polytene chromosomes. Inhibition of transcription or nicking of chromatin DNA abolished psoralen crosslinking. Application of this technique to other interphase chromosomes should greatly increase our understanding of DNA topology in vivo.

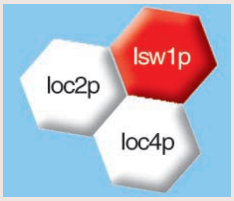


Meiosis mei way

In the fission yeast *Schizo-saccharomyces pombe*, meiosis usually occurs only in diploid zygotes. Upon nitrogen starvation, a mating pheromone signal is transduced through a MAP kinase cascade, and haploid cells with opposite mating types conjugate to form a diploid cell. Zygotic expression of Mei3 is then induced, which inactivates the Pat1 kinase, allowing activation of Mei2, which drives meiosis. On p. 3875, Yasushi Hiraoka and colleagues show that activation of Spk1, the pheromone-responsive MAP kinase, can drive haploid cells to undergo meiosis with normal chromosome behaviour. The authors achieve this through ectopic expression of a constitutively active form of the MAP kinase kinase Byr1 and show that, in this situation, induction of meiosis requires Mei2 but not Mei3. Furthermore, telomeres cluster at the spindle pole body to form a bouquet, as happens in normal meiosis, and the chromosomes separate normally. The authors conclude that Spk1 activation by constitutively active Byr1 can activate Mei2 sufficiently to drive meiosis in *S. pombe*, even in the absence of Mei3, and propose a new model for the genetic regulation of both meiosis and telomere clustering.

Strand bias in targeted gene repair

Point mutations can be repaired through targeted nucleotide exchange, in which the binding of an oligonucleotide specific for the target sequence leads to recruitment of cellular proteins and catalysis of nucleotide exchange. Transcription regulates targeted nucleotide exchange and, now, Erin Brachman and Eric Kmiec report that DNA replication also modulates this process in eukaryotes (see p. 3867). Previous studies indicated that transcriptionally active genes are more readily repaired by targeted nucleotide exchange than are non-expressed genes and that the untranscribed strand is corrected more efficiently. In prokaryotes, DNA replication also affects strand bias. To test whether this is also the case in eukaryotes, the authors constructed four SV40-genome-containing minichromosomes in which a mutant green fluorescent protein was incorporated on both sides of the SV40 origin of replication in both orientations. Using these constructs in COS-1 cells, the authors confirm the untranscribed strand repair bias and show that DNA replication increases this bias but only if the untranscribed strand is also the lagging strand in DNA synthesis.



Chromatin-remodelling machines

Remodelling of nucleosomes on DNA controls access of regulatory proteins to their target sequences. In this way, it endows chromatin with the plasticity required for regulation of transcription, DNA replication, recombination and repair. A variety of large, ATP-dependent multiprotein complexes carry out such remodelling, and these have been identified in yeast, flies and mammals. In Cell Science at a Glance, Anton Eberharter and Peter Becker survey the known remodelling machines, as well as the different 'nucleosomoid' structures that they generate (see p. 3707 + poster).

Development in press

The sound of silence

When an active eukaryotic gene is moved into a heterochromatic region of the genome, heterochromatin proteins 'spread' over the rearranged gene and silence it. One model for this spreading of silencing proposes that heterochromatin protein 1 (HP1) binds to a methylated histone near the gene and recruits Su(VAR)3-9, which methylates adjacent histones to form new HP1-binding sites. Reporting in *Development*, Danzer and Wallrath challenge this model by investigating HP1-mediated gene silencing in vivo in *Drosophila*. They describe a tethering system in which HP1 fused to the DNA-binding domain of the *E. coli lacI* repressor is expressed in *Drosophila* that carry *lac* operator repeats 1.9 and 3.7 kb upstream of two euchromatic reporter genes. Expression of the HP1 fusion protein silences both reporters and alters the euchromatin to a heterochromatin-like structure. Importantly, silencing of the nearby, but not the distant, reporter gene occurs in a *Su(var)3-9* mutant, which indicates that short-range silencing involves self-propagation of HP1 binding.

Danzer, J. R. and Wallrath, L. L. (2004). Mechanisms of HP1-mediated gene silencing in *Drosophila*. *Development* **131**, 3571-3580.