DNA REPAIR

A perfect symphony

The carefully orchestrated recruitment over time of a large number of repair and checkpoint proteins to the site of DNA damage was studied by Rodney Rothstein and colleagues in live yeast cells. By visualizing fluorescently tagged checkpoint and repair proteins in a series of mutant strains, they identified which proteins were recruited in response to DNA double-strand breaks (DSBs) or replication stress, and dissected the order of their recruitment.

The MRX complex (which comprises Mre11, Rad50 and Xrs2) is the first to be detected at a DSB site, which confirms its status as the DSB sensor. The Tel1 kinase is recruited next, followed by replication protein A (RPA), which recognizes and protects single-stranded DNA ends. In turn, RPA directs the recruitment of several checkpoint proteins including the Rad24–Rfc2–5 clamp loader, the Ddc1–Mec3–Rad17 clamp, the Mec1–Ddc2 kinase, the Rad9 mediator and the Rad53 kinase.

The actual DNA-repair proteins — the homologous recombination (HR) machinery — are recruited last, and only in the S and G2 phases. Their recruitment depends on Rad52 and coincides with the disappearance of MRX from the site of repair. Sae2 seems to facilitate this transition from the damage-recognition phase to the repair phase, as the disassembly of MRX and the recruitment of recombination proteins are both delayed in an *sae2* mutant strain.

Only a subset of the proteins that are recruited to DSBs also assemble at stalled replication forks, as MRX and Rad52 (and, therefore, all other recombination proteins) are lacking. However, when replication forks collapse, MRX and Rad52 are recruited, which, presumably, is triggered by exposed DNA ends.

These new insights raise an intriguing question: why do some proteins (MRX, RPA and others) bind to the DNA-repair site irrespective of the cell-cycle phase, whereas the recruitment of others (the HR machinery) is restricted to the S and G2 phases?

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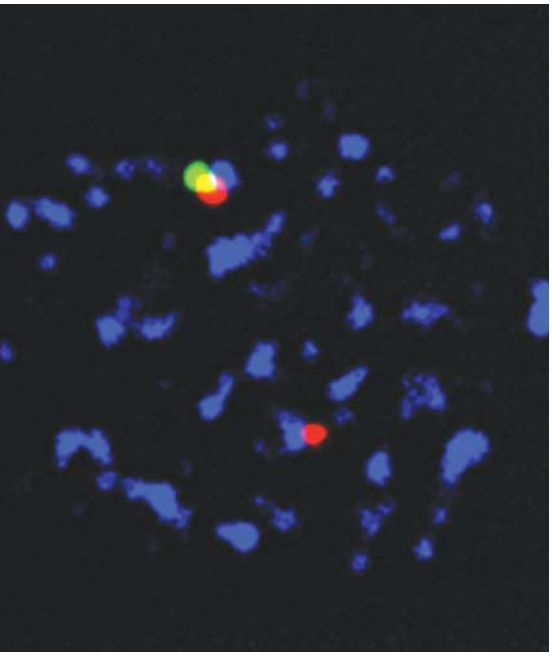
References and links

ORIGINAL RESEARCH PAPER Lisby, M. *et al.* Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell* **118**, 699–713 (2004)

NUCLEAR ORGANIZATION

Migrant workers



Single optical section through a mouse erythroid cell showing the colocalization of an actively expressed *β-globin* allele (green), an actively expressed *Eraf* allele (red) and an RNAP-II transcription factory (blue). Image kindly provided by Peter Fraser,The Babraham Institute, Cambridge, UK.

For most of us, a productive day's work involves a journey to the office and now, according to a recent study, it seems as if our genes face a similar commute. Reporting in *Nature Genetics*, Peter Fraser and colleagues show that, when actively expressed, spatially distant genes travel to sites in the nucleus that are enriched in the factors required for their transcription.

The authors examined the subnuclear location of *Hbb-b1* (a mouse *β-globin* gene) and that of a panel of genes, which included the erythroid-specific gene *Eraf* and several other active loci. All the genes were located on mouse chromosome 7 and were separated from *β-globin* by 25–40Mb.

First, Osborne *et al.* used double-label, three-dimensional RNA fluorescence *in situ* hybridization (3D-RNA FISH) to compare the positions of the transcribed *β-globin* locus with each of the distal chromosome-7 genes in the nucleus of erythroid cells. Despite the large distance between their chromosomal locations, the actively transcribing *β-globin* gene colocalized with the other active genes at a high frequency. The authors then carried out RNA immuno-FISH using intron-specific probes combined with antibodies that were directed against RNA polymerase II (RNAP II). These studies confirmed that most of the actively transcribing genes were associated with nuclear foci that are enriched in RNAP II — so called 'RNAP-II transcription factories' — and that two transcribed genes often congregated in the same factory.

Biochemical analysis by chromosome conformation capture (3C) showed that *β-globin* interacted with the *Eraf* locus in erythroid cells, but not in brain cells (where both these genes are silent), confirming that this association was transcription dependent.

RNAP-II transcription factories have been well described in HeLa cells but, until now, it was not known if these nuclear domains accumulated at individual loci or if they were shared by many genes to facilitate transcription. The authors reasoned that transcription signals would be unlikely to coincide if each gene assembled its own transcription factory. Also, they observed only 100–300 RNAP-II foci per mouse-erythroid-cell nucleus — much fewer than the estimated number of expressed genes. This study indicates that several genes travel to the same RNAP-II factory to share the limited polymerase molecules. Genes are rapidly turned on and off and so it will be interesting to identify the efficient transport systems that are used by these important nuclear commuters.

Shannon Amoils

References and links

ORIGINAL RESEARCH PAPER Osborne, C. S. *et al.* Active genes dynamically colocalize to shared sites of ongoing transcription. *Nature Genet.* **36**, 1065–1071 (2004)