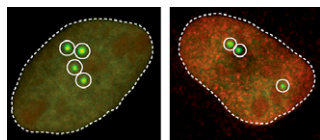


## If it ain't broke, don't fix it



Unlike full-length TTDA (green, left), truncated TTDA (right) fails to recruit XPA (red) when immobilized onto chromatin.

**Z**iani et al. reveal how a large DNA repair complex assembles incompletely on undamaged DNA.

The nucleotide excision repair pathway begins when the proteins XPC and hHR23b recognize damaged DNA and recruit the multisubunit trans-

cription factor TFIIH. The transcription factor then recruits downstream repair factors such as XPA and the endonuclease XPF to form a preincision complex (PinC) that cuts out the damaged region of DNA. However, many components of the PinC, including XPC, have additional cellular functions that involve binding to undamaged stretches of DNA, suggesting that DNA lesions must somehow influence PinC assembly to promote the formation of active repair complexes.

Ziani et al. studied the PinC's ability to assemble after tethering various components of the complex to undamaged chromatin. Immobilized XPC recruited several TFIIH subunits but failed to enlist the downstream factors XPA and XPF, indicating that damaged DNA is required to assemble a complete PinC. Immobilizing the TFIIH subunit XPB, on the other hand, bypassed the need for XPC or a DNA lesion, inducing the recruitment of XPA and XPF to undamaged chromatin.

The ability to recruit XPA and XPF depended on whether or not the small TFIIH subunit TTDA was incorporated into the PinC. Some patients with trichothiodystrophy produce a truncated version of TTDA that lacks 15 amino acids at its N terminus. Ziani et al. found that TTDA required these amino acids to bind XPA and recruit the repair protein to both damaged and undamaged DNA. Senior author Frédéric Coin now wants to investigate how other mutations in TTDA affect the protein's function in nucleotide excision repair.

Ziani, S., et al. 2014. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201403096>.

## A replication origin gets SNP'd out in fragile X syndrome

**T**he alteration of a single nucleotide could initiate fragile X syndrome (FXS) by inactivating a replication origin, Gerhardt et al. reveal.

FXS is the most common inherited form of mental retardation and is caused by the expansion of a series of CGG repeats in the 5' untranslated region of the *FMR1* gene. Mothers carrying the FXS premutation have 55–200 repeats, but, during oogenesis or early embryogenesis, the number of repeats can expand until the *FMR1* gene is silenced. Gerhardt et al. previously found that a replication origin located ~50 kb upstream of the CGG repeats is inactivated in FXS embryonic stem cells. The majority of the repeats are therefore replicated from the opposite direction, which could pose problems that lead to repeat expansion.

Intriguingly, a single-nucleotide polymorphism (SNP) linked to an increased risk of repeat expansion has also been identified ~50 kb upstream of the repeat locus in FXS patients within haplogroup D.

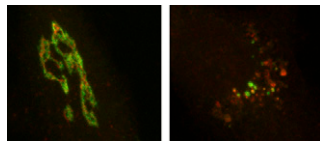
Gerhardt et al. discovered that this SNP is located within the replication origin inactivated in these FXS embryonic stem cells.

Normal embryonic stem cells had an active replication origin and a thymine base at the SNP locus. FXS cells, in contrast, had a cytosine base and an inactive origin. The researchers also derived embryonic stem cells from mothers carrying the FXS premutation. These cells had a thymine base and a normal replication pattern and, accordingly, showed no tendency to expand their repeat number over time.

The substitution of cytosine for thymine therefore appears to inactivate the replication origin during oogenesis/early embryogenesis, increasing the risk of repeat expansion and FXS. The authors now want to investigate whether the critical replication origin is reactivated later in development, which would explain why the number of CGG repeats stabilizes in adult tissues.

Gerhardt, J., et al. 2014. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201404157>.

## Sphingomyelin is the master of its domain



Sialyltransferase (green) and TGN46 (red) overlap in a control cell (left) but are segregated in a cell treated with D-cer-C6 (right).

**V**an Galen et al. reveal that the lipid sphingomyelin (SM) organizes proteins into functional domains at the trans-Golgi network.

Along with cholesterol, SM forms rigid, liquid-ordered domains within cell membranes, but whether these domains

control cellular functions by clustering specific transmembrane proteins together is unclear. Van Galen et al. perturbed SM homeostasis by treating cells with a short-chain ceramide, D-cer-C6, that is converted by enzymes at the trans-Golgi network and plasma membrane into a short-chain SM incapable of forming liquid-ordered domains.

D-cer-C6 treatment quickly disrupted the organization of Golgi membranes. Golgi proteins such as sialyltransferase and

TGN46 segregated away from each other instead of colocalizing at the trans-Golgi network. This physical separation prevented sialyltransferase from glycosylating TGN46, demonstrating that SM-based membrane domains organize the components of simple biochemical reactions. SM may also promote more complex processes, such as vesicle biogenesis, by clustering multiple proteins into the same domain.

D-cer-C6 disrupted Golgi form as well as function, causing the normally flat cisternae to curl up into concentric rings. Rigid, SM-rich membrane domains may therefore concentrate Golgi-resident enzymes in the flattened center of Golgi cisternae. Substrate proteins, in contrast, would only transiently associate with these domains as they move through the Golgi on their journey along the secretory pathway. Senior author Vivek Malhotra now wants to investigate how the transmembrane regions of Golgi enzymes and their substrates determine their affinities for SM-rich membrane domains.

van Galen, J., et al. 2014. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201405009>.