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VIRAL INFECTION

How histones go viral

Nucleosomes are loaded onto pre-existing eukaryotic chromatin to maintain the structure of nuclear DNA. However, how retroviral DNA that enters cells during infection acquires nucleosomes *de novo* is unclear. Wang, Wang and Goff now show that histones are loaded onto unintegrated Moloney murine leukaemia virus (MLV) DNAs shortly after they have entered the nucleus.

The authors infected mouse embryonic fibroblasts (MEFs) with a MLV reporter virus and carried out chromatin immunoprecipitation using a histone H3-specific antibody. They observed that histone H3 was associated with total viral DNA (which includes linear, circular and integrated proviral DNA) as early as 12 h post-infection and that it was present at a level similar to that associated with glyceraldehyde-3-phosphate dehydrogenase (GAPDH; monitored as a control) 24 h post-infection. Two-long terminal repeat circles (2-LTR circles), the presence of which indicates that retroviral DNA has entered the nucleus, were associated with histone H3 at levels similar to the control at 12 h. These data suggest that histones are rapidly loaded onto retroviral DNA following infection and that this may occur in the nucleus.

Indeed, preventing viral DNA from entering the nucleus of NIH 3T3 cells decreased the amount of histone H3 associated with viral DNA 10-fold. Furthermore, the total viral DNA in cells that were infected with Mason–Pfizer monkey virus (MPMV), which is unable to enter the nucleus of mouse cells, showed a sixfold decrease in their association with histone H3 compared with MLV DNA. Interestingly, viral DNAs still became associated with histones in cells that were infected with an integrase-deficient MLV and in cells that were treated with an integrase inhibitor, which demonstrates that retroviral DNA does not need to be integrated into the host genome for histone loading.

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Histones can be epigenetically modified by various post-translational modifications. F9 mouse embryonic carcinoma cells silence integrated proviral DNA and mark proviral nucleosomes through the methylation of histone H3 on lysine 9 (H3K9me3; a marker of silenced chromatin). However, this mark was low on viral DNA until 6 days post-infection. In MEF cells, which are permissive to retroviral DNA, the acetylation of histone H3 (H3Ac; a marker of active chromatin), was not observed on viral DNA until 6 days post-infection. Together, these data suggest that epigenetically modified histones appear on viral DNA later than core histones are loaded. Furthermore, the authors showed that the low levels of gene expression from unintegrated MLV genomes are probably owing to the fact that they rapidly load unmodified core histones rather than those (such as H3Ac and H4Ac) that recruit RNA polymerase to promote transcription.

In short, unmodified histones are rapidly loaded onto unintegrated, nuclear retroviral DNA after infection. The authors also show that histone modifiers can be used to manipulate the expression of unintegrated retroviral DNA; treating cells that were infected with an integrase-deficient MLV with a histone deacetylase inhibitor increased the level of H3Ac associated with total viral DNA and also the expression of a reporter gene.

Katharine H. Wrighton

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