thereby stabilizing it and allowing RNA-dependent RNA polymerase (RdRP) to amplify the response by generating abundant secondary siRNAs. This possibility is consistent with data indicating that RDE-3 is not required for RNAi that is initiated by large amounts of transgene-expressed dsRNA. In addition, the fission yeast RDE-3 homologue Cid12 interacts with RdRP, and the Mello group has unpublished data showing that the detectable accumulation of siRNA during RNAi requires RdRP activity. Clearly, other explanations are possible and functional studies are needed to resolve this question.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPER

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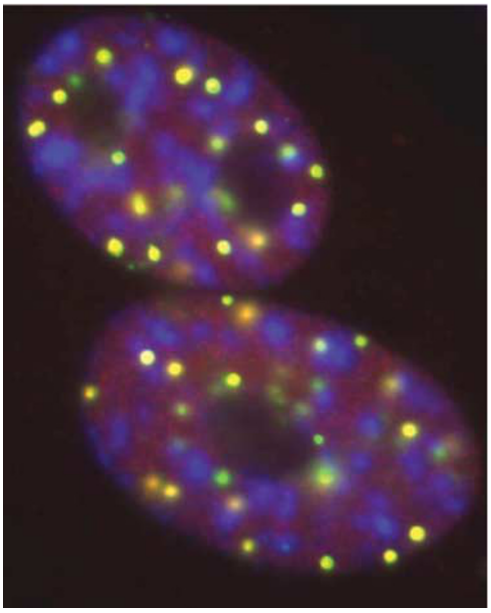
NUCLEAR ORGANIZATION

An organized exit

It is well known that the extensive passaging of most normal cells in culture induces a state of irreversible cell-cycle arrest that is known as cellular senescence. Previous studies have shown that the entry of cells into senescence is accompanied by marked changes in nuclear morphology — the chromatin of senescent cells condenses to form several transcriptionally inactive senescence-associated heterochromatin foci (SAHF) that are enriched in heterochromatin protein-1 (HP1). It has been suggested that the genes that promote cell proliferation are stably silenced by sequestration to SAHF, but the precise contribution of SAHF to the permanent cell-cycle arrest that typifies senescence is unclear. Now, by characterizing the composition and assembly of SAHF, Peter Adams and colleagues have shed light on the relationship between chromatin structure and senescence and they report their findings in *Developmental Cell*.

Adams and co-workers induced senescence in primary human fibroblasts, and analysed the changes that took place in the nucleus of these cells using immunofluorescence microscopy. They found that, in addition to HP1, SAHF contained the variant histone macroH2A. MacroH2A is enriched on the mammalian inactive X chromosome and is strongly associated with transcriptional repression. So, SAHF might represent sites in the nucleus where proliferation-promoting genes are packaged into repressive heterochromatin — thereby ensuring that these genes are refractory to mitogenic stimuli.

Interestingly, the promyelocytic leukaemia (PML) body — a nuclear organelle that has been implicated in the promotion of senescence — seems to have a role in the formation of SAHF. Adams and his team observed that, shortly after the induction of senescence, HP1 proteins accumulated in PML bodies. Subsequently, HP1 relocated from PML bodies to SAHF. Furthermore, the authors observed that the histone-chaperone protein HIRA localized to PML bodies coincident with HP1. HIRA forms a complex with another histone-chaperone protein ASF1a, and the yeast orthologues of HIRA and ASF1a are implicated in the assembly of heterochromatin. The authors therefore proposed that the HIRA–ASF1a complex might promote the formation of SAHF. Consistent with this prediction, the overexpression of HIRA and ASF1a in primary human fibroblasts induced the formation of macroH2A- and HP1-containing SAHF. And, when human fibroblasts were induced to senesce, the knockdown of ASF1a by short hairpin RNA inhibited the formation of SAHF and delayed cell-cycle exit. This supports the view that the HIRA–ASF1a-mediated formation of SAHF is necessary for efficient cell-cycle exit during senescence.



Two senescent primary human fibroblasts showing SAHF (blue), HIRA (red) and PML bodies (green). The HIRA and PML bodies colocalize (yellow). Image kindly provided by Peter Adams, Fox Chase Cancer Center, Philadelphia, USA.

So how does the HIRA–ASF1a complex promote the deposition of macroH2A in SAHF? As HIRA seemingly does not interact directly with macroH2A, the authors reasoned that this complex is unlikely to directly incorporate macroH2A into chromatin. Instead, they suggest that human ASF1a and HIRA function at a point upstream of macroH2A deposition, which indirectly facilitates the subsequent incorporation of macroH2A by other histone chaperones. They also suggest that the localization of HP1 and HIRA to PML bodies, prior to SAHF formation, modifies these proteins in some way to facilitate SAHF assembly. Importantly, the participation of PML bodies in the reorganization of chromatin that accompanies senescence might explain the tumour-suppressor activity of these enigmatic nuclear bodies.

Shannon Amoils

References and links

ORIGINAL RESEARCH PAPER Zhang, R. *et al.* Formation of macroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. *Dev. Cell* **8**, 19–30 (2005)

FURTHER READING Schulz, L. & Tyler, J. Heterochromatin focuses on senescence. *Mol. Cell* **17**, 168–170 (2005)