RNA INTERFERENCE

Nuts and bolts

Although rapid progress is being made in understanding the key steps in the RNA interference (RNAi) pathway, it is clear that many of the 'nuts and bolts' of the silencing machinery are still unknown. Craig Mello and colleagues now report, in *Current Biology*, the characterization of the *Caenorhabditis elegans* protein RDE-3, and propose a role for this new member of the polymerase-β nucleotidyltransferase superfamily in RNAi.

Mello and co-workers had previously genetically mapped the *rde-3* locus in an RNAi-deficient strain. Further mapping and examining the candidate genes within the mapped region for mutations led them to the K04F10.6 gene — rescue experiments confirmed that K04F10.6 was indeed *rde-3*. Three presumed *rde-3* loss-of-function mutants showed similar levels of resistance to RNAi when they were injected with double stranded (ds)RNA. The mutants also failed to accumulate small interfering (si)RNA, which implies that RDE-3 functions upstream of siRNA accumulation during RNAi.

RDE-3 contains a conserved nucleotidyltransferase domain, NTP transferase-2, with two conserved features — a helical turn that contains a Gly-Ser motif and an aspartic-acid triad motif. Two *rde-3*-null alleles encode a mutation in each of these motifs, which implies that the polymerase activity of RDE-3 is likely to be important for its function.

So, how does RDE-3 function in RNAi? Although the authors can't answer this question just yet, they do suggest several interesting possibilities. They propose that RDE-3 might function as a polyadenylation (poly(A)) polymerase and that, in the absence of functional RDE-3, aberrant transcripts with short poly(A) tails accumulate — these could compete for limiting factors that are required for efficient RNAi.

Alternatively, Mello and colleagues suggest that RDE-3 could be a direct component of the RNAi pathway, which is required for the amplification of the RNAi response that is induced by small amounts of dsRNA. The initially produced, low-abundance, primary siRNAs trigger the first round of cleavage of the target mRNA. RDE-3 is then proposed to polyadenylate the cleavage product,

SIGNAL TRANSDUCTION

A stabilizing modification

Signal transducer and activator of transcription (STAT) proteins are latent in the cytoplasm until extracellular signals — such as cytokines or growth factors — induce their activation, dimerization and translocation to the nucleus. Here, the STAT dimers bind to DNA and promote the transcription of various genes. In response to the extracellular signals, STATs are phosphorylated and this post-translational modification is thought to be necessary for STAT activation. However, as unphosphorylated STATs can still dimerize and induce transcription, Chin and colleagues investigated whether other modifications might be important for STAT activation, and they report their results in *Science*.

Proteins of the cAMP-response-element-binding protein (CREB)-binding protein (CBP)/p300 family have intrinsic histone-acetylase activity, and they are known to associate with various STATs and promote STAT-mediated transcription. So, the authors looked at whether STATs can be acetylated, and found that STAT3 acetylation was induced by treating cells with extracellular signalling proteins. In addition, they showed that this acetylation was increased by transfecting cells with CBP or p300, and that neither STAT3 phosphorylation, nor the activity of the Src-homology-2 domain of STAT3, were required for this modification.

A broad inhibitor of histone deacetylases (HDACs) increased STAT3 acetylation in response to p300 transfection or cytokine treatment, and Chin and co-workers found that STAT3 deacetylation could be mediated by type-I HDACs. It therefore seems that p300 and type-I HDACs, which are present in most cell types, might control cytokine-induced STAT3 acetylation/deacetylation.

Next, the authors showed that Lys685 of STAT3, which is conserved in several STATs from various species, was acetylated by p300, and they investigated the effect of this modification on STAT3 activity. They showed that the dimerization of wild-type STAT3 was stabilized by cytokine treatment and that, by contrast, no dimerization could be detected for Lys685Arg-mutant STAT3 proteins after cytokine treatment, even though these mutant proteins were phosphorylated. Lys685 acetylation therefore seems to be important for STAT3 dimerization. Furthermore, the authors showed that cytokine treatment could induce the formation of complexes between DNA and wild-type STAT3, but not DNA and Lys685Arg STAT3.

In the final part of their study, Chin and colleagues looked at the effect of STAT3 acetylation on cell growth *in vivo*, as STAT3 regulates several cell-growth- and cell-survival-related genes. In a cell line that lacks *STAT3*, the expression level of three such genes was low, with or without cytokine treatment, but, after cytokine treatment, cells that had been transfected with wild-type STAT3 — but not Lys685Arg STAT3 — showed increased expression of these genes. They also showed that wild-type, but not Lys685Arg, STAT3 could promote cell-cycle progression and cell growth in response to cytokines.

So, this work indicates that STAT acetylation is necessary for the stable dimerization of STATs and for them to activate transcription. Furthermore, as CBP/p300 is known to associate with various STATs, "…it is possible that all STAT family members are tightly regulated by the acetylation and deacetylation cycle."

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Yuan, Z.-L. *et al.* Stat3 dimerization regulated by reversible acetylation of a single lysine residue. *Science* **307**, 269–273 (2005)

FURTHER READING O'Shea, J. J. *et al.* Cell signaling: Stat acetylation — a key facet of cytokine signaling? *Science* **307**, 217–218 (2005)

WEB SITE

Y. Eugene Chin's laboratory:
http://www.brown.edu/Divisions/Medical_School/andera/person.php?id=299&returnURL=%2FDivisions%2FMedical_School%2Fandera%2Ffaclist.php%3Ffirst%3DC

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

Chen, C.-C. G. *et al*. A member of the polymerase β nucleotidyltransferase superfamily is required for RNA interference in *C. elegans*. *Curr. Biol.* 13 Jan 2005 (doi:10.1016/S0960982205000370)

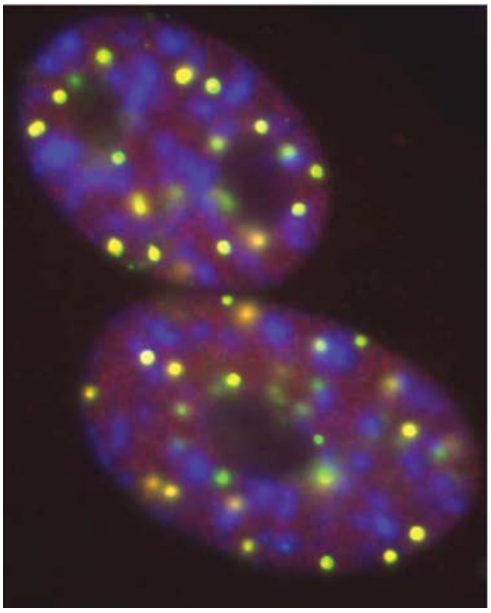
NUCLEAR ORGANIZATION

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)