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Heterochromatin: On the ADAR Radar?

Vigilin proteins, the absence of which is known to cause abnormalities in heterochromatin, have been found to bind edited RNAs. Molecular complexes including vigilin comprise proteins involved with RNA editing and with DNA repair, making connections between these processes and RNA-based silencing mechanisms.

H.R. Fernandez, H.H. Kavi, W. Xie and J.A. Birchler

In a typical eukaryotic cell, certain parts of the genome — notably, those around the centromeres and telomeres — are transcriptionally silent and packaged safely away in cytologically distinct units known as heterochromatin, contrasting with transcriptionally active euchromatin regions. The formation and maintenance of heterochromatin is characterized by addition of specific modifying groups to histones, which are consequently bound by heterochromatin protein 1 (HP1), a non-histone component of chromatin [1].

Recent years have seen a huge explosion in work on a phenomenon that at first might seem quite distinct from heterochromatin formation: RNA interference (RNAi). But it turns out the phenomena are closely connected and the RNAi machinery is clearly implicated in the establishment of heterochromatin in a broad evolutionary spectrum of eukaryotes [2]. Emerging evidence [3–5] now suggests that RNA editing and DNA repair processes also impact heterochromatic silencing, although the relationship among these remains unknown.

In a paper published last year in *Current Biology*, Huertas *et al*. [6] present evidence that the *Drosophila* dodeca centromeric binding protein (DPP1) is involved in the structure and assembly of heterochromatin. DDP1 is a multi-KH-domain protein, the KH domains of which facilitate single-stranded nucleic acid binding. KH domain proteins homologous to DDP1 have been isolated in organisms from yeast to humans. DDP1 had previously been shown to colocalize with HP1 to the heterochromatic chromocenter, as well as to euchromatic sites [7]. *Drosophila* mutants of DDP1 showed a strong reduction in histone H3-K9 methylation, one of the characteristics of heterochromatin structure, and HP1 deposition. DDP1 mutations were also shown to be dominant suppressors of position effect variegation [6], the phenomenon whereby a normally euchromatic gene, when placed in the vicinity of heterochromatin, shows a mosaic expression pattern. Work over many years has shown that position effect variegation is often suppressed by mutations of genes associated with heterochromatin function. The implications are that the nucleic acid binding capability of DDP1, particularly its RNA binding activity, might be important for its involvement in the formation and maintenance of heterochromatin.

As reported in this issue of *Current Biology*, Wang *et al*. [5] have uncovered other cellular functions of these KH-domain proteins that seemingly depend on the RNA-binding ability (Figure 1). They found that, in cell extracts, the human DPP1 homolog vigilin, and DDP1 itself, bound to promiscuously adenosine-to-inosine (A-to-I) edited RNAs. Site-specific RNA editing, catalyzed by the adenosine deaminases (ADARs), converts adenosine residues to inosine, so that different translated proteins can be made from the same transcribed RNA molecule [8]. When double-stranded RNA is non-specifically targeted by an ADAR, promiscuous editing occurs, converting as much as 50% of the RNA's adenosines to inosine [8]. Wang *et al*. [5] suggest that this targeting of double-stranded RNA provides a potential link between RNA-mediated gene silencing and RNA editing.

Promiscuously edited double-stranded RNA molecules had previously been shown to be retained in the nucleus, where they associate with three proteins, identified as the inosine-specific binding protein $p54^{nrb}$, the protein associated splicing factor (PSF), and the inner nuclear matrix structural protein, matrin 3 [9]. Wang *et al.* [5] isolated further proteins that bind inosine-containing RNA (I-RNA) by affinity chromatography, which uncovered vigilin and DDP1. They found that vigilin associates with heterochromatic α and β satellites, and that abrogation of DDP1 activity in *Drosophila* cells leads to mislocalization of HP1 and disrupted heterochromatin structure, consistent with the data reported earlier by Huertas *et al*. [6].

Wang *et al.* [5] identified two further proteins that co-purified with vigilin by I-RNA chromatography. The first was DNA-dependent protein kinase (DNA-PK), an important component of the machinery that repairs double-strand DNA breaks [10]. DNA-PK consists of a heterodimer of DNA-binding subunits Ku70 and Ku80 (or Ku86), and a catalytic subunit, $DNA\text{-}PK_{CS}$ [10]. The recovery of this protein suggests an interesting link between heterochromatin and DNA repair. Indeed, human Ku70 has previously been shown to interact with HP1α in human cells [11]. One of the targets of the kinase activity of DNA-PK is histone H2AX, one of three forms of histone 2A, which is rapidly phosphorylated in response to DNA damage, leading to the recruitment of repair factors [12]. The vigilin complex was found to have an RNA-dependent kinase

activity, and H2AX was subsequently shown to be phosphorylated in this complex.

The second co-purifying protein is RNA helicase A (RHA). RHA is a RNA- and DNA binding protein involved in transcription and RNA transport [13]. RHA has also been shown to be an RNA-dependent phosphorylation target of DNA-PK. Furthermore, it is a binding partner of BRCA1, a tumor suppressor gene product that participates in many DNA damage repair pathways [14]. BRCA1 is rapidly recruited to H2AX following DNA damage [12], and cells deficient in BRCA1 have defects in transcription-coupled repair, non-homologous end joining and homologous recombination. Inhibition of BRCA1 by overexpression of RHA has been shown to coincide with reduction in poly(ADP-ribose) polymerase-1 (PARP-1) activity and defects in ploidy [15]. PARP-1 and PARP-2 are involved in the immediate response to DNA damage in mammalian cells [16], and the *Drosophila* homolog of PARP-1 has been shown to be required for organizing heterochromatin structure during development [17].

The interplay of proteins involved in DNA repair, on the one hand, and heterochromatin formation and maintenance, on the other, is a developing story. This was also highlighted recently by the discovery of the *Arabidopsis* gene *BRU1*, mutation of which causes an extreme sensitivity to genotoxic stress as well as alterations to heterochromatin condensation [4]. Mutant *bru1* plants have a phenotype similar to that caused by mutation of chromatin assembly factor 1 (CAF1), another protein that participates in DNA repair, in conjunction with proliferating cell nuclear antigen (PCNA) [18].

CAF1 and PCNA are also both involved in DNA replication, and have been shown to interact with HP1α and suggested to be necessary for deposition of HP1 to heterochromatin [19]. HP1 has also been shown to interact with the *Drosophila* homolog of another important DNA repair protein, ATM [20]. Like DNA-PK, ATM is a kinase which is rapidly induced in response to DNA damage, and is one of the proteins responsible for the phosphorylation-mediated activation of p53, the product of a tumor suppressor gene with a central role in the DNA damage response.

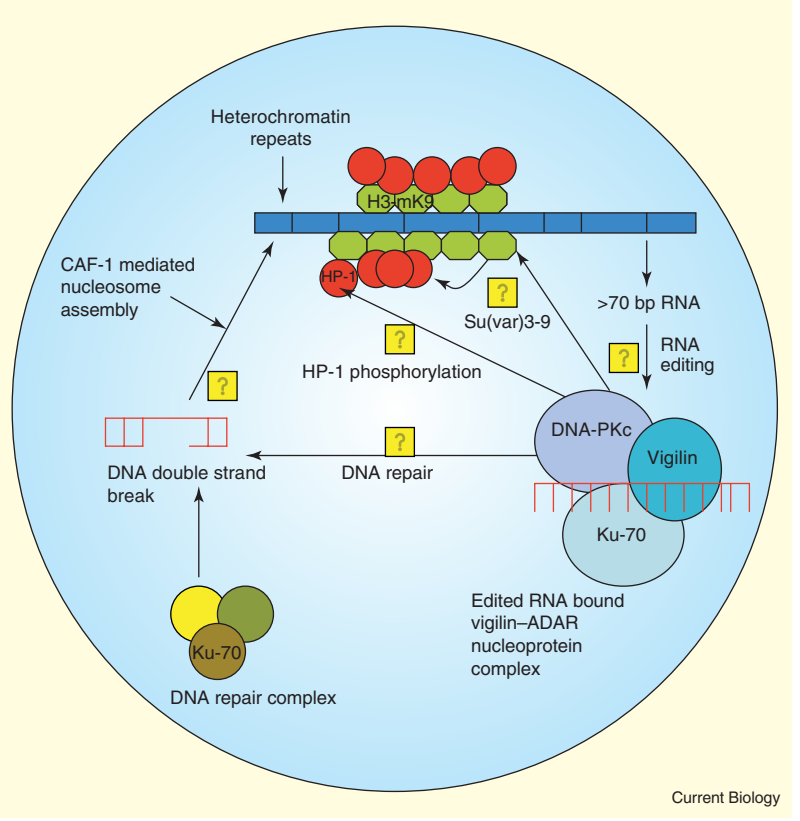

Figure 1. Possible roles of the vigilin complex in DNA repair and heterochromatin formation.

The low rate of transcription of the heterochromatin repeats in the nucleus might give rise to a low level of double-stranded RNA, which could be edited by ADAR. The edited double-stranded RNA — containing A-to-I base modifications — might be unwound by a helicase, possibly RNA helicase A. The resulting single stranded RNA could possibly be a part of the vigilin–ADAR complex as suggested by Wang *et al.* [5]. A potential future research direction would be to examine how the edited RNA (especially with its target base pairing complementarity compromised because of editing) might be targeting various histone modifications.

The involvement of ADAR in the silencing of repetitive sequences in heterochromatin raises the question of the relationship to RNAi. RNAi has been shown to be involved in heterochromatic silencing in fission yeast, *Drosophila*, chicken cells and *Arabidopsis* [2]. One proposed function of the RNAi machinery is as a defense mechanism against transposons and viruses. The mounting evidence that the RNA editing and DNA repair machineries function in heterochromatic silencing raises the issue of whether this process has been selected for such a defense role in parallel. While multiple processes may have been recruited to silence transposons and other repeats, both processes impact the histone H3-K9 modifications and HP1 association, suggesting that the mechanisms intersect.

References

1. Maison, C., and Almouzni, G. (2004). HP1 and the dynamics of heterochromatin maintenance. Nat. Rev. Mol. Cell Biol. *5*, 296–304.
2. Lippman, Z., and Martienssen, R. (2004). The role of RNA interference in heterochromatic silencing. Nature *431*, 364–370.
3. Frolov, M.V., and Birchler, J.A. (1998). Mutation in PO, a dual function ribosomal protein/apurinic/apyrimidinic endonuclease, modifies gene expression and position effect variegation in

Drosophila. Genetics *150*, 1487–1495.
4. Takeda, S., Tadele, Z., Hofmann, I., Probst, A.V., Angelis, K.J., Kaya, H., Araki, T., Mengiste, T., Scheid, O.M., Shibahara, K., *et al.* (2004). BRU1, a novel link between responses to DNA damage and epigenetic gene silencing in Arabidopsis. Genes Dev. *18*, 782–793.
5. Wang, Q., Zhang, Z., Blackwell, K., and Carmichael, G.G. (2005). Vigilins bind to promiscuously A-to-I edited RNAs and are involved in the formation of heterochromatin. Curr. Biol. 15, this issue.
6. Huertas, D., Cortes, A., Casanova, J., and Azorin, F. (2004). Drosophila DDP1, a multi-KH-domain protein, contributes to centromeric silencing and chromosome segregation. Curr. Biol. *14*, 1611–1620.
7. Cortes, A., Huertas, D., Fanti, L., Pimpinelli, S., Marsellach, F.X., Pina, B., and Azorin, F. (1999). DDP1, a single-stranded nucleic acid-binding protein of Drosophila, associates with pericentric heterochromatin and is functionally homologous to the yeast Scp160p, which is involved in the control of cell ploidy. EMBO J. *18*, 3820–3833.
8. Gerber, A.P., and Keller, W. (2001). RNA editing by base deamination: more enzymes, more targets, new mysteries. Trends Biochem. Sci. *26*, 376–384.
9. Zhang, Z., and Carmichael, G.G. (2001). The fate of dsRNA in the nucleus: a p54(nrb)-containing complex mediates the nuclear retention of promiscuously A-to-I edited RNAs. Cell *106*, 465–475.
10. Thacker, J., and Zdzienicka, M.Z. (2003). The mammalian XRCC genes: their roles in DNA repair and genetic stability. DNA Repair (Amst.) *2*, 655–672.
11. Song, K., Jung, Y., Jung, D., and Lee, I. (2001). Human Ku70 interacts with heterochromatin protein 1alpha. J. Biol. Chem. *276*, 8321–8327.
12. Paull, T.T., Rogakou, E.P., Yamazaki, V., Kirchgessner, C.U., Gellert, M., and Bonner, W.M. (2000). A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Curr. Biol. *10*, 886–895.
13. Zhang, S., and Grosse, F. (2004). Multiple functions of nuclear DNA helicase II (RNA helicase A) in nucleic acid metabolism. Acta Biochim Biophys Sin (Shanghai) *36*, 177–183.
14. Starita, L.M., and Parvin, J.D. (2003). The multiple nuclear functions of BRCA1: transcription, ubiquitination and DNA repair. Curr. Opin. Cell Biol. *15*, 345–350.
15. Schlegel, B.P., Starita, L.M., and Parvin, J.D. (2003). Overexpression of a protein fragment of RNA helicase A causes inhibition of endogenous BRCA1 function and defects in ploidy and cytokinesis in mammary epithelial cells. Oncogene *22*, 983–991.
16. Ame, J.C., Spenlehauer, C., and de Murcia, G. (2004). The PARP superfamily. Bioessays *26*, 882–893.
17. Tulin, A., Stewart, D., and Spradling, A.C. (2002). The Drosophila heterochromatic gene encoding poly(ADP-ribose) polymerase (PARP) is required to modulate chromatin structure during development. Genes Dev. *16*, 2108–2119.
18. Green, C.M., and Almouzni, G. (2003). Local action of the chromatin assembly factor CAF-1 at sites of nucleotide excision repair in vivo. EMBO J. *22*, 5163–5174.
19. Quivy, J.P., Roche, D., Kirschner, D., Tagami, H., Nakatani, Y., and Almouzni, G. (2004). A CAF-1 dependent pool of HP1 during heterochromatin duplication. EMBO J. *23*, 3516–3526.
20. Oikemus, S.R., McGinnis, N., Queiroz-Machado, J., Tukachinsky, H., Takada, S., Sunkel, C.E., and Brodsky, M.H. (2004). Drosophila atm/telomere fusion is required for telomeric localization of HP1 and telomere position effect. Genes Dev. *18*, 1850–1861.

Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211, USA.
E-mail: BirchlerJ@Missouri.edu

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Synapse Formation: Astrocytes Spout off

Trillions of synapses form as the brain develops. This intimate contact between two neurons has now been shown to be facilitated by thrombospondins — large extracellular matrix proteins secreted by astrocytes.

Michael D. Ehlers

The elaboration of neural circuits requires the formation of huge numbers of synapses. Navigating enormous distances and complex terrain in cellular brain space, axons and dendrites contact each other, culminating in the differentiation of presynaptic terminals and postsynaptic specializations [1,2]. This remarkable cellular conversion occurs most robustly during a short period of postnatal development and occurs with a spatial precision that ensures the right number of synapses are made in the right places. Because of their importance in establishing neural connectivity, the molecules and mechanisms involved in assembling synapses have been the objects of considerable inquiry.

Quite naturally, studies to date have focused on the intial recognition and adhesion events that occur between presynaptic and postsynaptic neurons [3]. Following contact, intraneuronal signaling pathways result in the organization of the presynaptic active zone and accumulation of synaptic vesicles in the presynaptic terminal, and trigger recruitment of postsynaptic scaffolding molecules and neurotransmitter receptors at the postsynaptic membrane [1,2]. Often considered mere voyeurs to this synaptic courtship, glial cells surround and ensheathe synapses, witness to the cellular changes that follow the first touch. The notion of glial cells as passive bystanders during synaptogenesis has been upended in recent years by the discovery that retinal ganglion cells in primary culture form few synapses unless they are co-cultured with a noncontacting feeder layer of astrocytes [4–7], pointing to a requirement for soluble glial-derived signals. One such signal is cholesterol secreted by astrocytes complexed with apolipoprotein E [5]. A recent paper from the laboratory of Ben Barres [8] now identifies thrombospondins as proteinaceous components of the synaptogenic material expectorated by astrocytes.

Christopherson *et al.* [8] began by fractionating astrocyte conditioned medium and showing that high molecular mass components (>100 kDa) elicited the same synapse promoting properties on cultured retinal ganglion cells as complete conditioned medium or astrocyte feeder layers. This suggested that a large molecule or molecular complex was responsible for the synapse promoting activity. Based in part on their observation that the synaptogenic activity bound heparin, the authors reasoned that the activity was likely an extracellular matrix protein. Testing a large panel of such proteins, they found that members of one particular