Genome Biology

Review

How to build a paraspeckle

Yasnory TF Sasaki and Tetsuro Hirose

Address: Functional RNomics Team, Biomedicinal Information Research Center, National Institute of Advanced Industrial Science and Technology (AIST), 2-42 Aomi, Koutou, Tokyo 135-0064, Japan. Email: y.t.f.sasaki@aist.go.jp; tets-hirose@aist.go.jp

Abstract

Noncoding RNAs have recently been identified as essential components of the nuclear suborganelles called paraspeckles. This finding will facilitate our understanding of the molecular dynamics and physiological role of these enigmatic macromolecular structures.

Discovery of paraspeckles

Paraspeckles are large ribonucleoprotein structures around 0.5 μm in diameter that can be detected in nuclei with a light microscope and appropriate antibody staining, and are currently of unknown function. They were discovered quite unexpectedly as recently as 2002 [1,2]. Lamond and colleagues conducted a large-scale mass-spectrometric analysis of nucleoli isolated from HeLa cells, which identified 271 nucleolar proteins. Of these proteins, more than 30% were novel or uncharacterized [1]. The localization of a subset of the novel proteins fused with yellow fluorescent protein (YFP) for visual detection was then determined [2]. Surprisingly, one of those fusion proteins was found to co-localize not to the nucleolus itself, but to a novel nuclear compartment or suborganelle.

The protein was found to be ubiquitously expressed in all human cell lines examined [2], and is localized in granular foci often adjacent to 'splicing-speckles', which are implicated as the reservoir of various splicing factors. Hence, the newly discovered foci were dubbed 'paraspeckles' and the newly characterized protein was named paraspeckle protein 1 (PSP1) [2]. Mass spectrometric analysis of nucleolar proteins demonstrated that a small fraction of this protein, undetectable by fluorescence microscopy, transiently associated with the nucleolus, which explained its original detection as a nucleolar protein [1].

The number of paraspeckles per interphase nuclei in human cell lines varies between 10 and 20, and their typical size is 0.5 μm in diameter. In addition to PSP1, three proteins, $p54^{nrb}$ (also known as NONO, non-POU domain containing octamer-binding protein), polypyrimidine tract-binding protein-associated splicing factor (PSF), and paraspeckle protein 2 (PSP2), exhibit a punctate nucleoplasmic distribution, co-localizing to paraspeckles as seen by immunnostaining using antibodies against corresponding proteins [2,3].

These paraspeckle proteins each contain two RNA-recognition motifs (RRMs). The properties and interaction behavior of PSF, $p54^{nrb}$, and their homologs in species ranging from *Drosophila* to mouse have been extensively characterized. PSF and $p54^{nrb}$ interact with a nuclear receptor and with RNA, and also with both single- and double-stranded DNA [4-9]. Both $p54^{nrb}$ and PSF are multifunctional proteins that are implicated in nuclear processes such as transcriptional control, splicing regulation, mRNA 3'-end formation, DNA repair and recombination, and nuclear retention of hyperedited RNAs in various human and mouse cell lines [4-9]. Chromosomal translocations involving the genes encoding PSF or $p54^{nrb}$ can produce chimeric proteins that cause tumorigenesis (see [4] and references therein). Furthermore, if transcription is inhibited by actinomycin D, all the paraspeckle proteins relocate to a perinucleolar cap [10]. There are several more proteins that meet some of the above criteria, and the list of paraspeckle proteins is therefore expected to expand in the near future. Indeed, Cardinale *et al.* [11] recently reported that a pre-mRNA 3'-end processing factor, mammalian cleavage factor I (CF I_m68), localizes to paraspeckles. The protein contains one RRM instead of two and moves to the perinucleolar cap when transcription is inhibited [11].

The identification of paraspeckle proteins immediately prompted investigations of the molecular mechanism by which this membraneless suborganelle is assembled. Fox *et al.* [3] reported that PSP1 heterodimerizes with $p54^{nrb}$ both *in vivo* and *in vitro*, and that the functioning RRM domains are critical for targeting PSP1 to the paraspeckle. Furthermore, the paraspeckle structure is sensitive to RNase, indicating that RNA is also an essential structural component [3].

Noncoding RNAs as 'architectural RNAs'

Given that the paraspeckle was predicted to be a large ribonucleoprotein complex [3], the presumed RNA-protein interactions have become a focus of research into the molecular mechanisms underlying paraspeckle formation. Three groups have now independently identified the long-sought architectural RNAs [12-14]. These groups began working from different research perspectives but eventually found the same noncoding RNAs (ncRNAs) -

two isoforms, *MENε* and *MENβ*, which are transcribed from the same RNA polymerase II promoter but differ in the location of their 3' ends, and the functions of which are largely uncharacterized [15]. Our laboratory [12] identified *MENε* and *MENβ* from the LeLa cell nuclei as a component of the paraspeckle-enriched fraction by biochemical purification. Sunwoo *et al.* [13] identified some 200 ncRNAs that are either up- or downregulated during differentiation of the C2C12 mouse myoblast cell line into myotubes [13]. They narrowed down their target to *Menε/β* by manual examination and subcellular localization analyses. Looking for nuclear-retained abundant ncRNAs in both humans and mouse cells, Clemson and colleagues [14,16] identified three: the inactivated X-chromosome transcript *XIST*, and two ncRNAs they called nuclear-enriched abundant transcripts 1 and 2, *NEAT1* and *NEAT2*. *NEAT1* is identical to *MENε* and *NEAT2* to the noncoding ncRNA *MENα*, which resides downstream of *Menε/β* in the MEN locus.

In humans, two *MEN* isoforms, *MENε* (3.7 kb) and *MENβ* (approximately 23 kb), are transcribed from a single promoter at the *MENε/β* locus at chromosome 11q13.1; similarly, the mouse counterparts, *Menε* (3.2 kb) and *Menβ* (approximately 20 kb), share the same promoter at chromosome 19qA [12-14]. In both human and mouse, the shorter transcript, *MENε*/*Menε*, is polyadenylated at its 3' end; however, the 3' end of the longer isoform, *MENβ*/*Menβ*, is formed by RNase P cleavage [13]. The physiological significance of this noncanonical 3'-end processing is not yet clear. In all cases, the exclusive paraspeckle localization of *MENε/β* was confirmed by RNA fluorescence *in situ* hybridization analysis combined with immunofluorescent detection of paraspeckle marker proteins [12-14] (Figure 1).

The *MENε/β* depletion phenotype was also examined in both human and mouse cells, using knockdown with chimeric antisense oligonucleotides [12,13] or small interfering RNA (siRNA) [14]. *MENε/β* knockdown resulted in disruption of the paraspeckles but not of other intranuclear bodies [12-14] (Figure 1). Importantly, there is no degradation of paraspeckle proteins in these knockdowns and no paraspeckles remained intact without *MENε/β*. Furthermore, the reassembly of paraspeckles disassembled by treatment with an RNA polymerase II inhibitor, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), was suppressed in *MENε/β*-depleted cells [12,13]. These results strongly support the hypothesis that *MENε* and *MENβ* are essential for the integrity of the paraspeckle structure.

The physical associations of *MENε/β* RNAs with paraspeckle proteins have been investigated using immunoprecipitation and the following RNA-protein interactions have been reported: *MENβ* and $p54^{nrb}$ and *MENβ* and PSF [12], *Menε/β* and $p54^{nrb}$ [13], and *MENε* and $p54^{nrb}$ and *MENε* and PSP1 [14]. Clemson *et al.* [14] demonstrated

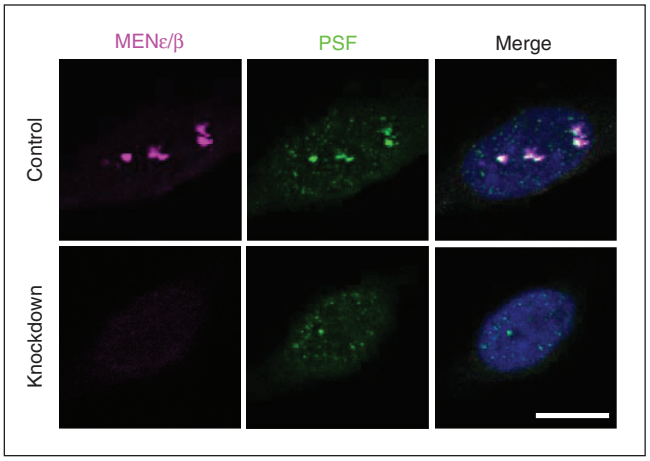

Figure 1

Knockdown of *MENε/β* ncRNAs leads to disintegration of the paraspeckles. Confocal images of HeLa cells treated either with a control scrambled antisense oligonucleotide (upper panels) or with a *MENε/β* knockdown antisense oligonucleotide (lower panels). Upper panel: *MENε/β* ncRNAs (magenta) co-localize to paraspeckles defined by PSF immunofluorescence (green). Lower panel: the paraspeckle-associated PSF signal disappeared when the *MENε/β* ncRNAs were successfully depleted, indicating that the paraspeckles have disintegrated. Note that the nucleoplasmic PSF signal remains intact. The HeLa cell nuclei were counterstained with DAPI (blue). Scale bar, 10 μm.

that deletion of the RRM domains of PSP1 abrogates its association with *MENε* in paraspeckles. Our group [12] examined the effect of paraspeckle protein depletion on *MENε/β* RNA levels and paraspeckle structure. We found that depletion of either $p54^{nrb}$ or PSF preferentially decreases *MENβ* but not *MENε*, and disrupts paraspeckle structure. Notably, PSP1 depletion did not affect either *MENε/β* levels or paraspeckle structure. These results suggest that PSP1 plays a role in paraspeckle organization distinct from $p54^{nrb}$ and PSF. Despite some discrepancies among the reports of the three research groups, the consensus that the ncRNAs *MENε/β* are essential to paraspeckle formation via interactions with the RRM domains of each paraspeckle protein is clear.

Prasanth *et al.* [17] have proposed a role for paraspeckles in the posttranscriptional regulation of expression of cationic amino acid transporter 2 (*CAT2*) gene mRNAs. An RNA called CTN-RNA is transcribed from the protein-coding mouse cationic amino acid transporter 2 gene through alternative promoter and poly(A) site usage and is retained in the nucleus [17]. Under stress, this RNA can be cleaved to produce the protein-coding *CAT2* mRNA. However, CTN-RNA is thought to be retained in the nucleus as a result of A-to-I RNA editing in the 3' untranslated region [17], whereas *MENε/β* RNAs do not appear to be edited [12-14].

With the currently available knowledge, what else can we determine regarding the physiological function of paraspeckles? The ubiquity of paraspeckles across different tissues must be taken into consideration. Given that most paraspeckle components have previously been identified as involved in transcriptional regulation and RNA processing, it is tempting to speculate that paraspeckles control gene expression. However, the mechanism of paraspeckle action is open to question, as the 'paraspeckle proteins' in fact seem to function primarily in nuclear compartments other than *MENε/β*-containing paraspeckles [4-10]. One plausible assumption, as has been hypothesized for other intranuclear compartments such as the nucleolus and splicing speckles, is that paraspeckles serve as a warehouse for a number of regulatory proteins that are sequestered in the paraspeckle until required in response to physiological conditions [18-21]. Thus, the availability of regulatory proteins at a target gene locus can be strictly controlled by the paraspeckle.

Paraspeckle dynamics

The remarkable dynamics of paraspeckle proteins have been noted since the discovery of paraspeckles, as proteomic analyses also identified all these proteins in the perinucleolar compartment [1,2]. When paraspeckle proteins relocate to the perinucleolar compartment, the *MENε/β* RNAs have dissociated, and are degraded [12] or relocate to either splicing speckles [13] or the nucleolus [14]. Paraspeckle proteins diffuse across the nucleoplasm in the absence of the *MENε/β* RNAs [6,12,13]. It is possible that posttranslational modifications such as phosphorylation and methylation could alter the interaction between the *MENε/β* RNAs and paraspeckle proteins, and could increase the affinity of paraspeckle proteins for the perinucleolar compartment.

The number of paraspeckles varies with the cell cycle: paraspeckles increase during interphase, disappear at telophase, when paraspeckle proteins translocate to the perinucleolar compartment, and reappear early in G1 [3] (Figure 2). This variation in paraspeckle number coincides with the transcriptional activity of RNA polymerase II, and, hence, perhaps with the expression level of the *MENε/β* RNAs. Intriguingly, Clemson *et al.* [14] reported paraspeckle formation at transcriptionally active *MENε/β* loci. Newly generated *MENε/β* foci seem to be larger than those found later in the cell cycle, and are constrained within a nuclear subvolume, most probably in the vicinity of the *MENε/β* locus [14]. These data imply that nascent *MENε/β* transcripts are concentrated in the vicinity of the *MENε/β* loci and serve as a platform for paraspeckle protein recruitment (Figure 2). Consistent with the above observation, stable expression of ectopic *Menε* causes an increase in paraspeckle number [14], whereas transient expression does not [12].

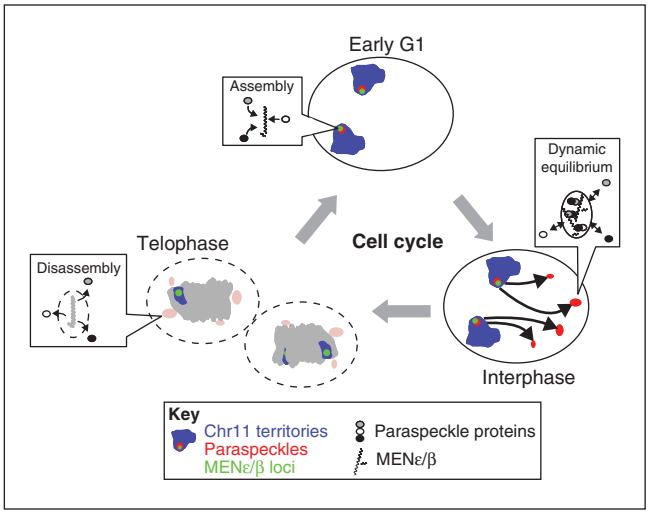

Figure 2

Paraspeckle dynamics. A model illustrating paraspeckle dynamics in the cell cycle. Three representative stages are shown: early G1; interphase; and telophase. The localization and behavior of paraspeckles throughout the cell cycle are highly dynamic. Early G1 (top): the nucleus of a human cell (large oval) contains two *MENε/β* loci (green circle), one on each chromosome 11q13 (blue territories). Paraspeckles (red circles or ovals) are generated at the transcriptionally active *MENε/β* loci, where paraspeckle proteins (smaller white, grey and black ovals in inset) associate with nascent *MENε/β* RNAs (black helices) to generate the paraspeckle. Interphase (lower right): the number of paraspeckles increases, typically to between 10 and 20 per nucleus. Newly generated paraspeckles are first localized to the *MENε/β* loci and then become distributed throughout the nucleus (indicated by arrows) by an unknown mechanism. Intact paraspeckles appear to be in a dynamic equilibrium, in which the flux of constituents between paraspeckles and nucleoplasm is balanced. The trajectories of redistribution of paraspeckles throughout the nucleus may be random as paraspeckles roam the interchromatin space by scanning specific target sites. Telophase (lower left): RNA polymerase II transcriptional activity is undetectable at this stage and, therefore, the levels of *MENε/β* decrease, which in turn causes paraspeckle disassembly. Paraspeckles are reassembled once *MENε/β* transcription restarts in the daughter cells.

There is an apparent difference in the number and distribution pattern of paraspeckles in the nucleus between the G1 phase and the rest of interphase. In addition, each cell line that has been observed displays a unique paraspeckle distribution pattern, which may represent the physiological status of the cells. These observations inevitably raise questions as to the precise mechanisms of paraspeckle formation and translocation. Is an individual paraspeckle formed on the *MEN* locus, or is a large paraspeckle precursor formed and then subsequently divided into several daughter paraspeckles? How do paraspeckles depart from the *MENε/β* loci? Do paraspeckles roam through the nucleus or are they destined for specific target locations? These questions are inextricably intertwined if both the formation and movement of

paraspeckles are dependent on the nuclear domains with which paraspeckles associate, that is, the *MENε/β* loci and putative target gene loci. In addressing these questions, comparisons with the formation of other nuclear bodies may be useful. The nucleolus is formed at the nucleolar organizer region (NOR) containing the rRNA genes, and its formation is dependent on rRNA transcription. Additional nucleoli can be formed by introducing extrachromosomal NORs [22]. Cajal bodies, involved in small nuclear ribonucleoprotein (snRNP) and small nucleor RNP (snoRNP) biogenesis, also closely interact with particular gene loci such as those for spliceosomal small nuclear RNAs (snRNAs) and histones, and are recruited or formed *de novo* in a microenvironment in which the local concentration of their substrates, snRNAs, is elevated [23]. Thus, gene loci provide nucleation sites for nuclear body formation and may be a target for transcriptional regulation or modulation by nuclear bodies [18-21]. Interestingly, the RRM protein NonA, the *Drosophila* counterpart of $p54^{nrb}$, forms a complex with other RNA-binding proteins in developmentally regulated 'puffs' on polytene chromosomes [7]. It will be of great interest to determine whether paraspeckles also target particular gene loci in specific physiological conditions (Figure 2).

Having ncRNAs as part of their structure gives paraspeckles unique properties; for example, unlike other intranuclear bodies, paraspeckle structure persists during most of mitosis, with the exception of telophase, in the absence of association with condensed chromatin [3]. This observation implies that long ncRNAs can themselves function as a scaffold for nucleation. In contrast, nucleoli and Cajal bodies disassemble when cells enter mitosis because association with their target loci is a prerequisite for nucleation [24,25]. It should be noted that RNAs associated with these nuclear bodies (for example, pre-rRNA and snRNA) are relatively small compared to *MENε/β*). The biogenesis of Cajal bodies exhibits the hallmarks of stochastic self-organization [26]. An important focus of future investigations will be to determine to what extent paraspeckle formation is consistent with the self-organization model.

The identification of *MENε/β* as a component of paraspeckles has raised many more questions, rather than simply answering the question of what a paraspeckle is. The depletion of *MENε/β* RNA profoundly affects the structural integrity of paraspeckles, which does not necessarily exclude the possibility of the presence of other structural/functional RNAs in paraspeckles. Transcriptome analysis of isolated paraspeckles, for example, may lead to the identification of ancillary RNA components. Through mechanical and functional characterization of paraspeckles, with emphasis on the RNA components, we will gain substantial insights into the dynamic nature of these nuclear bodies - in particular, how they are assembled into large ribonucleoprotein complexes and how they find their targets on chromatin and/or in particular nuclear domains. These insights should be relevant to our understanding of the dynamics of other nuclear bodies as well.

Acknowledgements

We thank members of the Hirose laboratory, in particular T Naganuma, K Aoki and T Kawaguchi for helpful discussions. We also thank K Watanabe and T Misteli for their continuous support and encouragement.

References

1. Andersen JS, Lyon CE, Fox AH, Leung AKL, Lam YW, Steen H, Mann M, Lamond AI: **Directed proteomic analysis of the human nucleolus.** *Curr Biol* 2002, **12:**1-11.
2. Fox AH, Lam YW, Leung AKL, Lyon CE, Andersen J, Mann M, Lamond AI: **Paraspeckles: A novel nuclear domain.** *Curr Biol* 2002, **12:**13-25.
3. Fox AH, Bond CS, Lamond AI: **P54nrb forms a heterodimer with PSP1 that localizes to paraspeckles in an RNA-dependent manner.** *Mol Biol Cell* 2005, **16:**5304-5315.
4. Shav-Tal Y, Zipori D: **PSF and p54(nrb)/NonO - multi-functional nuclear proteins.** *FEBS Lett* 2002, **531:**109-114.
5. Auboeuf D, Dowhan DH, Li X, Larkin K, Ko L, Berget SM, O'Malley BW: **CoAA, a nuclear receptor coactivator protein at the interface of transcriptional coactivation and RNA splicing.** *Mol Cell Biol* 2004, **24:**442-453.
6. Dong X, Sweet J, Challis JRG, Brown T, Lye SJ: **Transcriptional activity of androgen receptor is modulated by two RNA splicing factors, PSF and p54nrb.** *Mol Cell Biol* 2007, **27:**4863-4875.
7. Reim I, Stanewsky R, Saumweber H: **The puff-specific RRM protein NonA is a single-stranded nucleic acid binding protein.** *Chromosoma* 1999, **108:**162-172.
8. Zhang Z, Carmichael GG: **The fate of dsRNA in the nucleus: A $p54^{nrb}$-containing complex mediates the nuclear retention of promiscuously A-to-I edited RNAs.** *Cell* 2001, **106:**465-475.
9. Bladen CL, Udayakumar D, Takeda Y, Dynan WS: **Identification of the polypyrimidine tract binding protein-associated splicing factor p54(nrb) complex as a candidate RNA double-strand break rejoining factor.** *J Biol Chem* 2005, **280:**5205-5210.
10. Shav-Tal Y, Blechman J, Darzacq X, Montagna C, Dye BT, Patton JG, Singer RH, Zipori D: **Dynamic sorting of nuclear components into distinct nucleolar caps during transcriptional inhibition.** *Mol Biol Cell* 2005, **16:**2395-2413.
11. Cardinale S, Cisterna B, Bonetti P, Aringhieri C, Biggiogera M, Barabino SML: **Subnuclear localization and dynamics of the pre-mRNA 3' end processing factor mammalian cleavage factor I 68-kDa subunit.** *Mol Biol Cell* 2007, **18:**1282-1292.
12. Sasaki YT, Ideue T, Sano M, Mituyama T, Hirose T: **MEN*ε/β* noncoding RNAs are essential for structural integrity of nuclear paraspeckles.** *Proc Natl Acad Sci USA* 2009, **106:** 2525-2530.
13. Sunwoo H, Dinger ME, Wilusz JE, Amaral PP, Mattick JS, Spector DL: ***MENε/β* nuclear-retained non-coding RNAs are up-regulated upon muscle differentiation and are essential components of paraspeckles.** *Genome Res* 2009, **19:**347-359.
14. Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, Lawrence JB: **An architectural role for a nuclear noncoding RNA: *NEAT1* RNA is essential for the structure of paraspeckles.** *Mol Cell* 2009, **33:**717-726.
15. Guru SC, Agarwal SK, Manickam P, Olufemi SE, Crabtree JS, Weisemann JM, Kester MB, Kim YS, Wang Y, Emmert-Buck MR, Liotta LA, Spiegel AM, Boguski MS, Roe BA, Collins FS, Marx SJ, Burns L, Chandrasekharappa SC: **A transcript map for the 2.8-Mb region containing the multiple endocrine neoplasia type 1 locus.** *Genome Res* 1997, **7:**725-735.

16. Hutchinson JN, Ensminger AW, Clemson CM, Lynch CR, Lawrence JB, Chess A: **A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains.** *BMC Genomics* 2007, 8:39.
17. Prasanth KV, Prasanth SG, Xuan Z, Hearn S, Freier SM, Bennett CF, Zhang MQ, Spector DL: **Regulating gene expression through RNA nuclear retention.** *Cell* 2005, **123:**249-263.
18. Misteli T: **Protein dynamics: Implications for nuclear architecture and gene expression.** *Science* 2001, **291:**843-84719.
Misteli T: **Concepts in nuclear architecture.** *BioEssays* 2005, **27:**477-487.
20. Shav-Tal Y, Darzacq X, Singer RH: **Gene expression within a dynamic nuclear landscape.** *EMBO J* 2006, **25:**3469-3479.
21. Misteli T: **Physiological importance of RNA and protein mobility in the cell nucleus.** *Histochem Cell Biol* 2008, **129:**5-11.
22. Oakes M, Aris JP, Brockenbrough JS, Wai H, Vu L, Nomura M: **Mutational analysis of the structure and localization of the nucleolus in the yeast *Saccharomyces cerevisiae.*** *J Cell Biol* 1998, **143:**23-34.
23. Dundr M, Ospina JK, Sung M-H, John S, Upender M, Ried T, Hager GL, Matera SG: **Actin-dependent intranuclear repositioning of an active gene locus in vivo.** *J Cell Biol* 2007, **179:**1095-1103.
24. Leung AK, Gerlich D, Miller G, Lyon C, Lam YW, Lleres D, Daigle N, Zomerdijk J, Ellenberg J, Lamond AI: **Quantitative kinetic analysis of nucleolar breakdown and reassembly during mitosis in live human cells.** *J Cell Biol* 2004, **166:**787-800.
25. Carmo-Fonseca M, Ferreira J and Lamond AI: **Assembly of snRNP-containing coiled bodies is regulated in interphase and mitosis - evidence that the coiled body is a kinetic nuclear structure.** *J Cell Biol* 1993, **120:**841-852.
26. Kaiser TE, Intine RV, Dundr M: **De novo formation of a subnuclear body.** *Science* 2008, **322:**1713-1717.

Published: 16 July 2009
doi:10.1186/gb-2009-10-7-227