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Can telomerase be put in its place?

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The Cajal body is an intriguing nuclear structure present in a great variety of plant, animal, and some fungal cells. Recent work on the ribonucleoprotein enzyme telomerase has indicated an unanticipated degree of intranuclear dynamics of both its RNA and protein subunits. In this issue, Jady et al. place the Cajal body on the intranuclear traffic route of telomerase RNA (Jady et al., 2004).

Telomerase presents intriguing features of heterotypic enzyme design. Its evolutionary success has combined the high specificity RNA–DNA base pairing for substrate recognition with a "conventional" protein-orchestrated, metal-catalyzed chemical step. This strategy of RNA protein enzyme architecture is ancient, and exploited the advantages of each of the two kinds of molecules collaborating for improved reaction specificity or speed. Only a few such ribonucleoprotein enzymes are still with us today, to be cherished. Telomerase is mechanistically a reverse transcriptase but to the grateful genomes on which it operates it is a chromosome end-specific DNA polymerase. The acclaimed role of telomerase in the growth properties of some cells has given it tabloid status on both the oncology and biotechnology industry radar screens, but only time will tell if that rush has been warranted. As "merely" an interesting enzyme, telomerase enjoys a smaller crowd of admirers—but beautiful people we are, we cell biologists. Now, we learn that the RNA component of telomerase associates with a nuclear structure called the Cajal body (Jady et al., 2004). This finding is a new twist on the intranuclear location and dynamics of telomerase in mammalian cells, but we still have much to learn about how telomerase and its subunits roam around in the nucleus as related to the enzyme's on- and off-duty work cycles.

My father, an accomplished architect, would have likely called the end-game of DNA replication "structurally unpleasant." As every well-educated graduate student knows—and some accelerated high school students too—DNA is a gorgeous dyad, but its replication is aesthetically less elegant (although as molecular biology, it is profoundly elegant). One strand gets copied in a standard 5′-to-3′ linear polynucleotide assembly mode, zippering nucleotides right along one after the other. The other strand produces its copy in a chock-a-block fashion, assembling the new strand from bits and pieces. This all works except for one difficulty: the latter process leaves a cheerleader, a RNA primer, standing at the starting line of the template. When this RNA later leaves, its promotional job done, a little spot of single-stranded template DNA is exposed—a dangerous dangle, potentially activating chromosome and even cellular destruction. To complete the 5′ end of the product DNA strand, evolution came up with telomerase (as well as a different, recombination-based mechanism that is very important in many cells and creatures but does not figure in the work we are looking at here).

The focus of most research on telomerase in protozoa (gloriously), fungi, and plant and animal metazoa has been on the enzyme's molecular components—once thought to consist of just a subunit of RNA and another one of protein, but now probably several of the latter. How is telomerase put together in the cell and where does it hang out in the nucleus, whether or not DNA replication is underway ? Two recent publications direct our attention to Cajal bodies in the nucleus of mammalian cells as a place the RNA component of telomerase visits.

First things first: what is a Cajal body? Santiago Ramon y Cajal was a Madrid cytologist who shared the 1906 Nobel Prize in Physiology or Medicine with the Italian anatomist Camillo Golgi, of Pavia. In his studies of vertebrate neuronal cells, Cajal noted an "accessory body" within the nucleus (Cajal, 1903). Almost a century passed before this observation was rekindled by the discovery that there is a nucleolus-proximal body (more widely dispersed in the nucleus in some cells) in most vertebrate cells that corresponds to Cajal's "accessory body." This nuclear body has been molecularly defined in our present era by the laboratories of Eng Tan (Scripps Research Institute) and Joseph Gall (Carnegie Institution), the latter investigator proposing that this structure be called the "Cajal body," a term that, happily, has now been widely adopted. (This renaming was useful in two ways, as Gall first emphasized in his renomenclature campaign: the new name honors the discoverer; and many Cajal bodies do not show a coil-like internal fine structure that was the basis of the previous name.) Cajal bodies have been found to contain numerous transcription factors and small RNA visitors shuttling in and out (Gall 2000; 2003; Handwerger et al., 2003.)

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In their present study, Jady et al. (2004) located some of the endogenous RNA subunit (hTR) of telomerase in Cajal bodies in HeLa cells by in situ nucleic acid hybridization. They also transiently expressed wild-type and two mutant forms hTR in HeLa cells and identified a sequence within it (actually a single nucleotide, vide infra) that is required for Cajal body localization. The main value of this study, together with another important paper published a few weeks earlier (Zhu et al., 2004), is to direct our attention to an expanding repertoire of RNAs that visit Cajal bodies. These two new publications help to define that RNA traffic, but the puzzling cell biological nuances of telomerase remain before us.

Prior to the two recent studies (Jady et al., 2004; Zhu et al., 2004) the intranuclear localization of endogenous telomerase RNA (hTR) had been investigated in only three studies. Telomerase RNA was observed both in the cytologically recognizable DNA replication band as well as in nucleoplasmic foci in two ciliated protozoa (Fang and Cech, 1995). These latter intranuclear foci also were shown to contain trimethylguanosine-capped RNA (Fang and Cech, 1995), as well as ribosomal RNA (Cech, T.R., personal communication; discussed further in Pederson [1998]). In a subsequent study, telomerase RNA was identified in highly purified nucleoli from HeLa cells (Mitchell et al., 1999.) Finally, human telomerase RNA injected into the nucleus of *Xenopus* oocytes was observed to become localized in the nucleoli (Lukowiak et al., 2001). This is where things stood when the work being reviewed here was published.

In the first of the two recent papers, Zhu et al. (2004) showed that hTR is concentrated in Cajal bodies in HeLa cells as well as in several other tumor cell lines, and that hTR is less concentrated in Cajal bodies of cells that are not expressing the catalytic protein subunit of telomerase (hTERT). They also observed that hTR appears in Cajal bodies when hTERT is stably overexpressed and that, in such cells, a small percentage of the expressed hTERTis also present in Cajal bodies.

The work by Jady et al. (2004) in this issue is in part confirmatory of Zhu et al. (2004), but it also adds important new information. Jady et al. undertook their study in the context of having previously identified a Cajal body localization element (CAB box) in other small nuclear RNAs. This alerted them to the presence of a CAB box-like element in hTR. They transiently expressed wild-type hTR and two hTR mutants in HeLa cells and identified a nucleotide (G414) in the putative CAB box that is required for Cajal body localization. They also investigated hTR localization in Cajal bodies in relation to the cell cycle and reported that this association peaks in S phase. Finally, Jady et al. (2004) found that hTR contains a 5′ trimethylguanosine cap. Because this 5′-end structure is similar to the special 5′ end of other small nuclear RNAs known to traffic through Cajal bodies, and because Cajal bodies are known to contain the enzyme that produces this 5′ modification, they proposed that hTR undergoes this 5′-end modification in the Cajal body, a very plausible but as yet unproven hypothesis. In a previous study, human hTR was not observed to undergo appreciable cap hypermethylation after microinjection, as a monomethylG-capped transcript, into *Xenopus* oocytes (Lukowiak et al., 2001).

These two recent, concurrent studies add telomerase RNA to the group of small RNAs that visit Cajal bodies. An important issue is the true intranuclear concentration of hTR in Cajal bodies, nucleoli and nucleoplasm. There are potential quantitation issues in both studies, and the level of hTR in other nuclear quarters, such as the nucleolus, may have been underestimated somewhat. Finally, given that at least some hTR is present in Cajal bodies in the steady-state, there is no evidence that telomeres are positioned to embrace Cajal bodies in the S phase, or vice-versa (Pederson, 2004). Therefore the association of hTR (and presumably hTERT) with Cajal bodies does not appear to be related to the function of this enzyme in building chromosome ends.

The authors of these two recent studies have postulated different models for the Cajal body association of hTR, each consistent with their results. Zhu et al. (2004) speculate that a prior association of hTR with hTERT is required for the RNA's Cajal body localization, whereas Jady et al. (2004) suggest that hTR localization in the Cajal body is independent of its complexing with hTERT. These two ideas were reached on the basis of somewhat different experiments and this will have to be sorted out in further work.

Little if anything is known about whether hTR contains any internal RNA modifications. If it does, they (or its cap hypermethylation) might take place in Cajal bodies. Alternatively, it is possible that the substantial steady-state Cajal body association of hTR, visualized by in situ hybridization, is not related to either its covalent modification or even its assembly into a functional ribonucleoprotein enzyme for that matter, but reflects some unknown aspect of telomerase dynamics in the nucleus. In phrasing the question in this latter, fuzzy way, it is painfully obvious that we do not really have any specific (i.e., chemically rigorous) ideas. The two recent publications discussed here introduce the Cajal body to us on the circuit of telomerase RNA in the nucleus of mammalian cells, hopefully a launch pad for new research. Among several questions is the obvious key issue of the extent to which telomerase cycles among distinct, functionally relevant stations within the nucleus. It is a good thing to see cell biologists (e.g., Wong et al., 2002; Zhu et al., 2004; Jady et al., 2004) turning to this problem in the life history of telomerase.

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