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leading to the downregulation of telomerase at the 3′ end (Loayza and de Lange, 2003). Does POT1 also affect the state of the telomere in a binary manner? Are long human telomeres visited less often by telomerase, as in yeast? Or are long telomeres more likely to limit the extent of their elongation? Given that human cells lack the nifty features that the Lingner group used to address this problem, it may be a while before we know.

The next challenge in telomere length regulation in yeast will be to define the nature of the nonextendible state: Is the telomere folded so that the 3′ end of chromosomes is hidden from telomerase? Is telomerase always at the telomere, but gets locally inhibited as they get longer? In any event, we are now much closer to seeing the light at the end of the (chromosome) tunnel.

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# Hitchhiking without Covalent Integration

**In eukaryotes, many latent viruses attach to mitotic chromosomes noncovalently for effective partitioning in dividing cells. For different viruses, the *cis* and *trans* elements encoded by the episomes have been effectively defined but the chromosomal "receptors" for such tethering have remained elusive. In this issue of *Cell*, You et al. (2004) give us a first insight into the cellular protein machinery important for animal papillomavirus retention.**

In a historically important work, Lwoff and Gutman (Lwoff and Gutmann, 1950) showed that with lysogenic strains of bacteria, each cell harbored within a noninfective structure, a prophage, that enabled the organism to give rise to virus particles without exogenous infection. It was Alan Campbell who first proposed that the prophage came about by genetic recombination between a vegetative phage and the host chromosome (Campbell, 1962). This paradigm system provided a marvelous springboard for the study of among other things site-specific recombination, gene expression, and DNA replication. It also provided models for thinking about latent viral infections in higher eukaryotes. I can remember being inspired by the papers of Renato Dulbecco as a graduate student while studying phage λ molecular biology in Hatch Echols' class. Dulbecco, with his students, had used the prophage ideas to attack the question of how the small DNA viruses such as Polyoma and SV-40 managed to infect certain mammalian cells and essentially disappear although persistently changing the growth properties of the infected cell. The analogy to the classical prophage was not a very good one in the end and by and large covalent integration is a dead end for all nondefective animal DNA viruses so far studied.

The exception to this generalization seems to be provided by the defective or so called "dependoviruses" such as the human adeno-associated virus (AAV) that encode for a site-specific endonuclease/helicase required for viral replication and establishment of latency. AAV can establish a latent infection by integrating into chromosome 19 as tandem head to tail copies. The provirus then waits for subsequent rescue by a superinfecting helper adenovirus (Muzyczka and Berns, 2001).

A large number of very successful viruses—in that there are many different evolutionary variants infecting many different cell types—including the papillomaviruses (Pv) and the γ-herpesviruses do establish a natural latency as plasmids in the nuclei of dividing cells. During the initial infection phases, these viruses attempt to get their nucleoproteins into the nucleus and once there, an essential function is to keep the viral episomes in the nuclear space. This is especially important upon the breakdown of the nuclear membrane during the mitotic phase of the cell cycle. This maintenance is established by hooking onto another structure that has the ability to persist in the nuclear space even without the membrane. Mutations in the viral factors that mediate this function lead to catastrophic loss of the plasmids, even for high copy number plasmids such as those maintained in Bovine papillomaviral (BPV-1) transformed cells. As this viral plasmid can amplify its genome after segregation mistakes, it would seem that this rapid curing reflects the inefficient re-entry of the genome back into the nucleus if the hitchhiking fails.

The mechanism used by the Epstein-Barr virus, the human herpesvirus-8, and BPV-1 is attachment to the cellular chromosomes. All of these viruses encode for site-specific DNA binding proteins that recognize repeated DNA motifs near the viral origins of plasmid replication. Further, these viral site-specific binding proteins play dual functions that are critical for plasmid replication during interphase and for tethering during mitosis. In the case of BPV-1, the enhancer protein E2 serves as a matchmaker, bringing the DNA replication initiator/helicase E1 to the viral origin during S phase. During mitosis, an intriguing sandwich is made. Cytological approaches reveal that E2 must be bound to both the viral plasmid and the cellular chromosome throughout mitosis. The E1 protein likely competes for E2 activation domain binding with a cellular protein(s) important for tethering as overexpression of E1 pulls E2 off the chromosomes (Voitenleitner and Botchan, 2002). How does this tethering mechanism work, is it regulated, and what

insights about cellular activities can be gleaned from deciphering this process? Moreover, from what evolutionary branches does the mechanism descend? Answers to these questions require a knowledge of the cellular machinery to which the viral protein binds.

You et al. have used a biochemical approach to discover a critical cellular protein required for BPV-1 hitchhiking. They have expressed a tandem tagged E2 in mammalian cells and used the Flag and HA tags to purify E2 with a binding partner and identified the cellular protein as Brd4. E2 colocalizes with Brd4 on mitotic chromosomes and chromatin immunoprecipitation experiments show that Brd4 is bound to viral plasmids established in cells. You et al. have also mapped the Brd4 protein domain that mediates the binding to the activation domain of E2 and have found that expression of this portion alone serves to block BPV-1 transformation and keeps the plasmid from ever attaching to chromosomes. These experiments establish that Brd4 binding to E2 is necessary for stable plasmid maintenance and give us a handle on the cellular apparatus.

Brd4 is a member of the double bromodomain family of proteins and as such binds to acetylated histones H4 and H3 with much higher avidity than would single bromodomain proteins such as GCN5. Dey et al. (2003) have shown that Brd4 will bind to di and tetra-acetylated but not to monoacetylated histone tail peptides, so it would seem to be a player in deciphering the "histone code." Most importantly, deletion studies of Brd4 show that its binding to chromosomes (presumably through attachment to the tails protruding from core nucleosomes) is dependent upon the bromodomains, and an inhibitor of histone-deacetylase activity increases Brd4 avidity for chromatin. Brd4 attachment to cellular chromatin through the bromodomains would not interfere with E2 binding as the two domains are far apart on the protein. In uninfected human or mouse cells, Brd4 binds to mitotic chromosomes in a rather diffuse manner. Why should a bromodomain protein be bound to mitotic chromosomes in the first place, as one generally thinks of the histones in condensed chromatin as being hypoacetylated? Ectopic expression of Brd4 leads to cell cycle arrest before S phase (Maruyama et al., 2002) and this is likely due to blocking the functions of the PCNA loader RFC by a direct association. However, this doesn't seem to give us much insight into a mitotic function for Brd4. Houzelstein et al. (2002) have shown that Brd4 is an essential gene in mice and intriguingly report that by sequence analysis, Brd4 might be a member of the brd-like element found in the *mediator* complex reported for the human transcriptional coactivator. Perhaps this presages a role for Brd4 in transcriptional control (either in a positive way for the next G1 or a negative way in shutting down G2 transcription in mitosis) and might even link PV gene expression to tethering.

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# The Spindle Gets Bigger

**Although cortical nonmuscle myosin II has long been implicated in cytokinetic aspects of cell division, there has never been strong evidence that it plays a role in the organization of the mitotic spindle. Rosenblatt et al. (2004, [this issue of *Cell*]) use a number of methods to show that cortical myosin II is in fact important for spindle assembly in higher eukaryotic cells, specifically for the complete separation of centrosomes after nuclear envelope breakdown.**

During the year before I started graduate school, I used to argue with Daniel Mazia as to whether or not the mitotic spindle should be considered as a true cytoplasmic "organelle". Dan argued that it should, primarily on the grounds that the spindle could be isolated, and his isolation of the spindle in the 1950s ultimately led, among other things, to one of the early characterizations of the protein tubulin (Kiefer et al., 1966). With further advances in our understanding of the cell cycle, mitotic control, and the cytoskeleton, most of us might currently favor the view that formation of the mitotic spindle involves a transient but wholesale restructuring of cytoplasmic organization at the level of the entire cell. But where, then, are the boundaries of the spindle within the cytoplasm? In this issue of Cell, the realm of the spindle becomes significantly bigger, as Rosenblatt et al. (2004) describe an important role for cortical myosin II in the assembly of the mitotic spindle in higher eukaryotic cells. Myosin II has long been recognized as important for generating the contractile forces required for cytokinesis in conjunction with actin microfilaments and associated proteins in the cell cortex. Other studies have also implicated interactions between microtubules and the cell cortex as playing a role in positioning the spindle for both asymmetric and symmetric cell divisions. However, until now there has been little evidence that cortical myosin might play an active role in spindle assembly per se.

Rosenblatt et al.'s (2004) work begins with the observation that disruption of the actin cytoskeleton in Ptk2