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Previews

The Power of Pumping Together: Deconstructing the Engine of a DNA Replication Machine

The replicative DNA helicase lies at the heart of the eukaryotic replication machine, yet how it works remains puzzling. New structures of the viral replicative helicase SV40 T antigen (Gai et al., 2004 [this issue of *Cell*]) suggest that a novel concerted mode of nucleotide binding and hydrolysis powers conformation changes and DNA unwinding.

The basic machinery required to replicate DNA in eukaryotic cells was elucidated by reconstituting the replication of SV40 viral DNA with the viral helicase large T antigen (LTag) and ten proteins purified from human cell extracts (Simmons, 2000; Stenlund, 2003). LTag orchestrates this process: it recognizes the viral replication origin and assembles into a double hexamer that distorts the origin DNA locally. Subsequently, it unwinds the two strands bidirectionally and recruits the human initiation proteins. Bidirectional unwinding occurs when duplex DNA is pumped into the double hexamer from both ends and two single-stranded loops that serve as the templates for replication are extruded from the center. Mutations that specifically cripple the ability of the two hexamers to work together prevent bidirectional unwinding and replication without disabling the helicase activity of the individual hexamers (Weisshart et al., 1999). This deceptively simple replication machine is now beginning to provide a molecular blueprint for the eukaryotic replication machinery and its operation (Sclafani et al., 2004).

LTag serves as a paradigm for the MCM helicase, an essential component of the complex protein assembly that replicates the chromosomes of eukaryotic cells (Forsburg, 2004). Like LTag, the MCM helicase is thought to assemble into a double hexamer near start sites of replication, unwind the two parental DNA strands, and interact with other parts of the replication machine. MCM subunits, like the LTag helicase domain, contain a zinc domain followed by the AAA^+ domain. Remarkably, electron microscopic views of MCM double hexamers resemble LTag in size and shape despite their limited sequence similarity. However, until recently, the mechanisms that couple ATP hydrolysis to DNA unwinding in either LTag or MCM helicase have remained sketchy.

In contrast, kinetic and structural studies of the hexameric replicative helicases of bacteria and phage have revealed much about their operation (e.g., Singleton et al., 2000 and references therein). The hexameric T7 helicase binds to DNA in the central cavity and to nucleoside triphosphate (NTP) in the cleft between pairs of adjacent subunits. The T7 hexamer is asymmetric in spite of the fact that it is composed of six identical subunits because only some of the subunits in each hexamer are bound to NTP at any one time. The constraints of ring formation that limit NTP binding are thought to underlie a sequential mechanism of NTP binding and hydrolysis that "ripples" around the ring and is linked to DNA translocation through sequential binding of DNA to the loops in the cavity.

Given the fact that LTag is a homohexamer, it was expected to behave much like its prokaryotic cousins. However, in this issue of *Cell*, a family of new high-resolution crystal structures of the LTag helicase domain reveal a novel nucleotide binding mode for hexameric AAA^+ molecular machines: all six subunits in the hexamer bind to ATP or an ATP analog in concert (Gai et al., 2004). Similarly, ADP binds to the subunits of a hexamer in an all-or-none fashion, confirming and extending studies of LTag binding to fluorescent nucleotide analogs in solution (Huang et al., 1998). This concerted mode of nucleotide binding is in stark contrast to that previously observed for any other AAA^+ molecular machine. Gai et al. (2004) provide compelling evidence that concerted ATP binding is accompanied by conformation changes in each subunit in which the AAA^+ domain twists relative to the zinc domain, an "iris-like" motion that dramatically narrows the central channel in the hexamer. Upon hydrolysis and dissociation of the products, the rotational movement is reversed, restoring the maximal interior channel dimensions.

One of the biggest challenges in the helicase field has been to understand how ATP binding and hydrolysis drive translocation of DNA through the helicase ring. Gai et al. (2004) suggest that the "iris-like" constriction and restoration of the channel dimensions in response to ATP binding and hydrolysis are coupled to DNA unwinding. Several lines of evidence indicate that a basic β-hairpin loop located in the central channel binds to origin DNA in the double hexamer assembly of LTag and that this contact is needed for origin unwinding (Reese et al., 2004 and references therein). Importantly, the new set of LTag structures reveals that the basic hairpin moves 17 angstroms along the channel in response to ATP binding. Chen and colleagues propose that this concerted movement of the hairpins in each hexamer is the power stroke responsible for coupling nucleotide binding and hydrolysis to DNA translocation and unwinding.

How does the hairpin movement arise? The high resolution of the structures allows visualization of the detailed interactions between the bound nucleotides and helicase side chains. Nucleotides bind inside a pocket within each AAA^+ domain and at the interfaces between monomers, and form extensive interactions with side chains from the adjacent monomers ("*trans*" interactions). Upon binding nucleotide, the side chains from the *trans*-monomer undergo extensive repositioning. Interestingly, the degree of side chain movement in the *trans*-monomer is correlated with the type of nucleotide present: nucleotide-free, ground state; ADP bound, intermediate shift; and ATP bound, greatest shift. These *trans* residues are located at the base of the β-hairpin loops that protrude into the hexamer central channel,

and the distance moved by the β-hairpin loops correlates with the nucleotide-dependent movement observed in the *trans* side chains. These crystallographic snapshots along the reaction pathway suggest that the repositioning of the *trans* residues in response to ATP binding and hydrolysis powers the lever action of the β-hairpins.

Despite these exciting new insights, significant challenges to a full understanding of the DNA unwinding mechanism of LTag and MCM helicases lie ahead. The working model proposed by Chen and colleagues is one of several that are consistent with the data so far (Kaplan and O'Donnell, 2004). Elucidating the site of duplex unwinding in the hexamer, the coordination of the dual pumps in the bidirectional helicase machine and their interactions with other parts of the replisome will be needed to bring the mechanism into focus.

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Neuronal Differentiation: TOR and Insulin Receptor Pathways Set the Tempo

Target of rapamycin (TOR) and insulin receptor pathways regulate growth in metazoans. A recent study (Bateman and McNeill [2004], this issue of *Cell*) reveals a novel role for these pathways in controlling the timing of neuronal differentiation during *Drosophila* development, thus allowing a tight coordination between growth and differentiation programs.

During development, cells and tissues differentiate according to exquisite spatial and temporal programs. Signals responsible for setting up spatial cues have been identified in many model systems, and extensive studies have established that inappropriate spatial differentiation is detrimental to proper development. A limited number of signaling molecules required for differentiation are often repetitively used at different times in a given tissue. The reiterative use of common signaling pathways illustrates the need for a tight temporal regulation of the process of differentiation. A classical example of this strategy is observed during ommatidial differentiation in the *Drosophila* compound eye (Voas and Rebay, 2004). Each ommatidial unit is composed of eight photoreceptor cells and a fixed complement of nonneural accessory cells, which arise from a pool of undifferentiated cells produced after a short period of mitotic amplification. Differentiation of the eye starts during the third larval instar at the posterior edge of the imaginal disc (the structure that will give rise to the adult retina) and progresses toward the anterior. The differentiation front, marked by a tissue indentation called the morphogenetic furrow (MF) moves across the whole cellular field during a 2 day period. Interestingly, a similar propagation phenomenon is also found in the developing retina of the vertebrate zebrafish (Neumann and Nuesslein-Volhard, 2000). In the wake of the MF, reiterative use of the antagonistic Notch and EGFR pathways sets up the recruitment of cells in a stereotyped sequence of cell fate inductions, which leads to the characteristic lattice-like arrangement of the ommatidial field.

The molecular basis for the temporal control of these events, although critical for the specificity of the cell fate-determining cascades, is still elusive. A new twist to this phenomenon comes now with the finding by Bateman and McNeill (2004) that the timing of differentiation might be coupled to extrinsic growth-regulatory signals through the TOR and insulin receptor signaling pathways (Bateman and McNeill, 2004).

Ommatidial differentiation is accompanied by extensive cell growth posterior to the MF, making the fly eye a good tissue to identify growth defects. Using this system, researchers in the growth control field have established that interfering with the cell growth machinery changes the size of the eye and individual ommatidia without perturbing the differentiation process. Bateman and McNeill (2004) present a more refined examination of mosaic eyes mutant for the fly tuberous sclerosis complex 1 (TSC1) gene, which reveals that, in addition to the overgrowth defect characteristic of such mutants, the stereotyped arrangement of the photoreceptors in the ommatidia is perturbed due to a precocious differentiation of mutant cells. TSC1 and TSC2 genes are part of a growth control network comprising the TOR kinase pathway and the insulin receptor pathway (see Figure 1 and Leevers and Hafen [2004]). Both pathways are involved in coupling cell growth with extrinsic factors such as nutrition in *Drosophila* (Britton et al., 2002; Colombani et al., 2003). Bateman and McNeill (2004) make use of genetics to show unambiguously that up- and downregulation of TOR and InR pathways affect the timing of neuronal cell differentiation: their activation leads to precautious differentiation, while their inhibition delays differentiation. Importantly, this happens without