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ing transcription factors. It is possible that a similar strategy of targeting MOF to promoters with specific transcription factors or transcription machinery is present in the fly.

Several important questions with general implications are brought to light by the Kind et al. study. What are the apparently independent mechanisms of MOF targeting to the 5′ and 3′ ends of genes? Given that MOF acetylates H4K16 at both sites, what is the molecular function of H4K16 acetylation at these two regions? MOF is undoubtedly representative of many enzymes and chromatin-associated proteins that perform different functions through regulated targeting. The answers to the questions raised by the new work should help to elucidate general mechanisms for how specificity in targeting allows the same protein to perform distinct and independent regulatory functions.

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Follow the Monomer

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Capping proteins limit actin filament growth, but paradoxically increase actin-based cell motility. This has been attributed to funneling of actin monomers to the filament ends that remain uncapped. Using a reconstituted motility system, Akin and Mullins (2008) now demonstrate that filament capping increases Arp2/3-based nucleation and branching, rather than elevating the rate of filament elongation.

Although actin can polymerize into filaments on its own, cells use an army of proteins to control the starting and stopping of this reaction, as well as to organize the filaments into useful structures. Studying the function and regulation of these actin-modulating proteins has been the work of many labs for several decades. The control of actin dynamics is not a mere academic curiosity, but plays a key role in physiological processes such as morphogenesis and immune system function as well as in diseases such as metastatic cancer.

Two important factors for controlling actin dynamics are the Arp2/3 complex, which nucleates new filaments and concomitantly anchors them to the sides of existing filaments, and the capping protein, which binds to the rapidly growing barbed ends of filaments and terminates their growth (reviewed in Pollard and Borisy, 2003). Although these factors have been extensively studied in vitro, the relationship between their biochemical activities and their effects on motility is complex. This is particularly true of capping proteins that block filament growth yet enhance cell motility in vitro and in vivo (van der Gucht et al., 2005; Hug et al., 1995). One explanation for this paradoxical set of observations is the "actin funneling hypothesis," which posits that capping proteins enhance motility by capping most actin filaments in the reaction and funneling the increased number of free actin monomers onto a small subset of filaments that grow with higher rates of elongation (Carlier and Pantaloni, 1997) (Figure 1A).

In their new study, Akin and Mullins (2008) re-examine this question and come to a strikingly different conclusion about the role of capping protein in enhancing motility. They used an established in vitro motility system (Loisel et al., 1999) comprising polystyrene beads coated with the Arp2/3-activator ActA. These beads were incubated in a precise mixture of purified protein components including nonmuscle actin, the

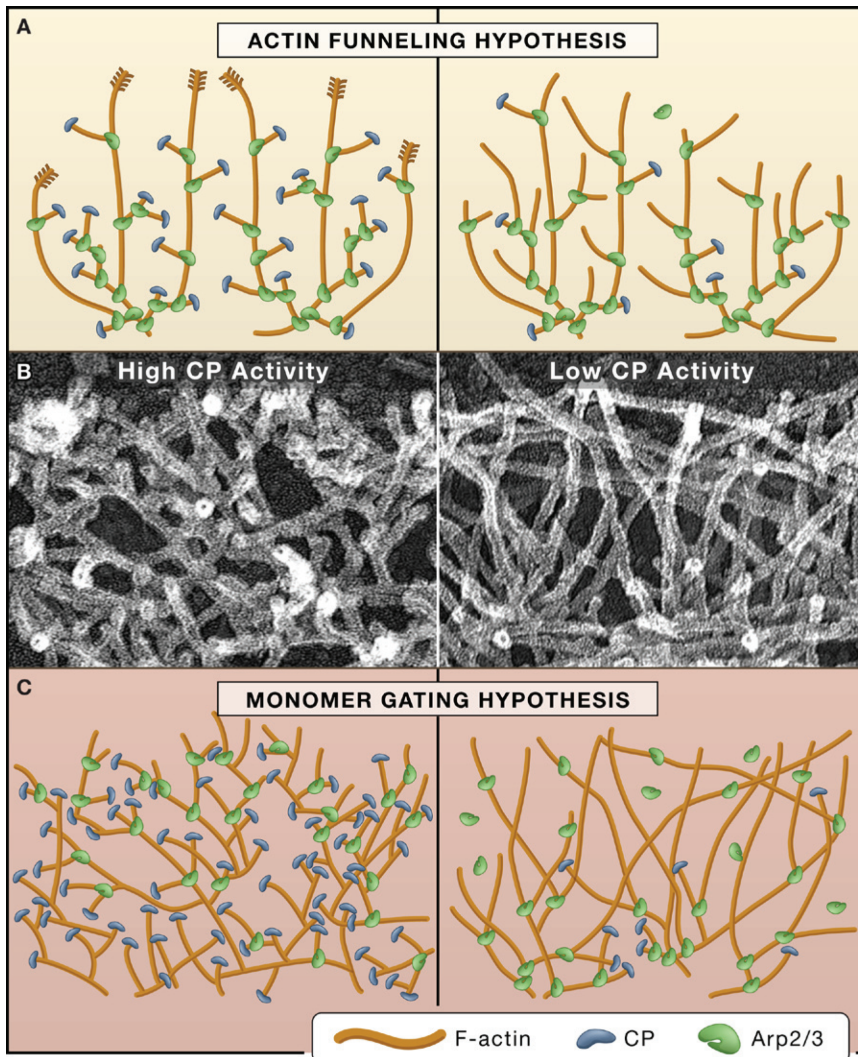

Figure 1. Capping Protein (CP) Activity and Actin Architecture
(A) The actin funneling hypothesis proposes that capping protein enhances cell motility by capping most actin filaments in the reaction and funneling the increased number of free actin monomers onto a small subset of filaments that grow with higher rates of elongation. The enhanced elongation rate on the uncapped filaments is represented by chevrons on the barbed ends.
(B) Electron micrographs of lamellipodia showing varying CP activity.
(C) Based on their analysis of actin filament nucleation and elongation in a motility system reconstituted in vitro, Akin and Mullins (2008) propose the monomer gating hypothesis. They observe that the ratio of the Arp2/3 complex to actin increases with higher levels of CP, indicating an increase in nucleation. This effect arises due to higher local availability of actin monomers when more filaments are capped. Given that actin monomers are essential for Arp2/3-based nucleation, more monomers means more nucleation rather than increased barbed-end elongation. (Electron micrographs from Bear et al., 2002.)

Arp2/3 complex, capping protein, cofilin, and profilin. This system allowed them to visualize the initiation of actin assembly that occurs in a shell around the beads and the subsequent symmetry breaking event that leads to sustained movement of the beads on rocket tails of branched, polymerizing actin filaments.

Using this approach, they systematically varied the concentration of the Arp2/3 complex and capping protein to test several tenets of the actin funneling hypothesis. Consistent with previous work, they found that an increase in capping protein increased the rate of bead motility. One clear prediction of the actin funneling hypothesis is that increasing capping protein should increase the concentration of actin monomers; this in turn should lead to faster rates of filament elongation on the few uncapped filaments in the reaction. On the contrary, increasing capping protein had no effect on actin monomer concentration in these reactions despite the clear increase in bead movement. Two other predictions of the actin funneling hypothesis are that the rate of filament growth on the bead surface should increase and that the number of filaments contributing to motility will decrease with increased concentration of capping protein. Again, the rate of filament elongation and the number of filaments were unaffected by increased capping protein.

So why does increasing the capping protein concentration lead to increased motility? The authors postulate an intriguing alternate hypothesis: Increased capping protein leads to enhanced nucleation mediated by Arp2/3. In their experiments, increasing the capping protein concentration led to an increased number of capped filaments, as expected. However, the total number of filaments remained constant, suggesting that increased nucleation must be occurring. Indeed, the ratio of the Arp2/3 complex to actin increases with higher levels of capping protein, indicating that more nucleation is happening. This effect arises due to higher local availability of actin monomers at the bead surface when more filaments are capped. Given that actin monomers are essential for Arp2/3-based nucleation, more monomers means more nucleation rather than increased barbed-end elongation. The authors term this the "monomer gating model" (Figure 1C).

This notion of momomer gating has important implications for interpreting some previous studies and will influence future work in this area. Factors such as the Ena/VASP proteins that inhibit capping of actin filaments though an anticapping effect would be predicted to indirectly decrease Arp2/3 branching. Indeed, this is entirely consistent with studies where Ena/VASP proteins are targeted to the plasma membrane or added to in vitro reconstitution systems and branch frequency is reduced (Figure 1B) (Bear et al., 2002; Samarin et al., 2003). It will be interesting to see if other anticapping proteins such as formins also decrease branching in reconstitution systems that include Arp2/3 and capping protein.

Although this study breaks important new ground, it will be useful to further verify this effect in the more complex environment found inside cells. A key experiment will be to analyze the effect of inositol phospholipids, such as PIP_2, on this process given that they regulate both Arp2/3-activating proteins (such as N-WASP) and capping protein. It will also be exciting to see these ideas incorporated into future biophysical models of actin polymerization-induced force generation.

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p53 Regulation Orchestrates the TGF-β Response

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Among its multiple functions, p53 is a critical regulator of TGF-β responses. Sasai et al. (2008) now identify a new p53 inhibitory protein, XFDL156. During embryonic development, this factor is expressed in the ectoderm germ layer and maintains the pluripotency of ectodermal cells by inhibiting TGF-β target genes that promote mesoderm specification.

The signals delivered by members of the transforming growth factor β (TGF-β) protein family are central to the specification of germ cell identity in embryonic development. In the endoderm, maternal factors activate the expression of TGF-β ligands that in turn induce overlying cells to become mesoderm (Figure 1). Yet, how this induction remains confined to cells lying at the equator of the embryo leaving the ectoderm pluripotent remains unclear (Niehrs, 2004). Recent embryological and molecular evidence argues that the development of a pluripotent ectoderm appears to result from an active molecular process in which cells constantly keep TGF-β signaling in check. For example, prior to gastrulation, the embryo requires an ectoderm-specific maternal determinant, Ectodermin/Tif1γ, that ubiquitinates Smad4, inhibiting its activity (Dupont et al., 2005). At the end of gastrulation, once germ layers have been induced, TGF-β then takes on other duties. At the gastrula stage, TGF-β ligands signal through Smad2 to start dividing the ectoderm into neural and non-neural territories along the dorsoventral axis (Figure 1) (Chang and Harland, 2007). Paradoxically, this event is not temporally isolated from mesoderm induction (Camus et al., 2006). It is unclear how embryos seamlessly orchestrate what appear to be diametrically opposing needs: avoiding TGF-β-mediated transformation of the ectoderm into mesoderm while at the same time using TGF-β for ectodermal patterning.

The work of Sasai et al. (2008) in this issue of *Cell* provides an elegant solution to this conundrum (Sasai et al., 2008). They clone, from the frog *Xenopus*, a new ectoderm-specific gene encoding XFDL156, which has the remarkable capacity to uncouple TGF-β responses from mesodermal differentiation. The real surprise and excitement about this discovery comes from its mechanism of action—XFDL156 is a new antagonist of the p53 tumor suppressor that leaves the TGF-β/Smad pathway operational.

Expression cloning in *Xenopus* embryos was the route taken to this discovery (Smith and Harland, 1992). It is remarkable that in the era of short-interfering RNA (siRNA) libraries and genome-wide mutagenesis screens, this straightforward and purely gain-of-function approach continues to uncover some of the most interesting new genes, suggesting that we are still far from saturation. As in any screen, the experimental strategy is critical. Sasai et al. searched almost 20,000 genes specifically expressed at the end of gastrulation and assayed for those able to inhibit TGF-β induction of mesoderm in explanted ectoderm cells.