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Inhibitors Target Actin Nucleators

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In this issue of *Chemistry and Biology*, Rizvi and colleagues indentify a small molecule that inhibits formin-mediated actin assembly. Together with recently characterized inhibitors of the Arp2/3 complex (Nolen et al., 2009) and formins (Gauvin et al., 2009), these small molecules provide useful laboratory tools to dissect the link between actin nucleators and actin-based structures in living cells.

Polymerization of actin filaments initiated by actin nucleators power a large number of cellular processes, including morphogenesis, the establishment of polarity, and motility (Pollard and Borisy, 2003). However, the basic mechanisms that control and coordinate the action of the different nucleators in a living cell remain one of the central questions in cell biology. Identification of the small molecules that inhibit selectively these actin nucleators may help to elucidate their respective, and even synergistic, role in this complex biological process.

At the molecular level, de novo nucleation through the action of actin nucleators, such as the well-characterized formins or the Arp2/3 complex, is the predominant mechanism that initiates actin assembly in living cells (Figure 1). Actin filaments are structurally and thermodynamically polarized polymers. Although actin filaments exhibit a fast growing "barbed" end, their spontaneous nucleation is a limiting step. Therefore, actin nucleators bypass this thermodynamically unfavorable reaction and stimulate actin assembly (Figure 1). Despite major progress in understanding their mechanism of action either in vitro or in vivo, it remains difficult to generate a complete view of actin nucleator activities and correlated actin-based structure formation in metazoan cells. The limitations in achieving this goal were clearly identified and discussed in an elegant review by Chhabra and Higgs (2007). First, routine imaging techniques for real-time visualization of the dynamics of actin filaments within overlapping actin-based structures are lacking. Second, there is a large number of nucleator isoforms with functional redundancy. The development of a super resolution imaging technique may help our understanding of the molecular coordination between actin structure and actin nucleators (Fernandez-Suarez and Ting, 2008). However, the time resolution of these techniques remains a limitation in studying such a dynamic processes as actin assembly. Fortunately, in yeast, the link between the four different actin-based structures and their respective actin nucleators is well characterized (Moseley and Goode, 2006). Indeed, the Arp2/3 complex is involved in building endocytic actin patches, whereas formins are responsible for contractile ring assembly during division, actin cable initiation, and the formation of the mating projection (Moseley and Goode, 2006). Yeast is therefore an ideal model system to validate potential small molecules inhibitors of actin nucleators in vivo that had been already identified through in vitro screening assays.

In this issue of *Chemistry and Biology*, Rizvi and colleagues (2009) screened the effect of about 10,000 small molecules in vitro on the assembly of actin filaments initiated by formins. Formin inhibitors were selected for their ability to slow down formin-mediated actin assembly by at least 50% at a concentration between 500 and 1000 times higher than the formin concentration. Similar assay was recently performed to identify inhibitors of the Arp2/3 complex (Nolen et al., 2009) and formins (Gauvin et al., 2009).

The small molecule inhibitor of formin homology 2 domains (SMIFH2) was found to satisfy the above criteria. SMIFH2 is a generic target of formin isoforms from an evolutionary diverse organism. Formin family members are characterized by the presence of two different formin homology domains (FH1 and FH2) that modulate the multiple activities of these proteins on actin assembly (Pruyne et al., 2002; Sagot et al., 2002). The FH1-FH2 domains are sufficient to account for the formins nucleation activity, which consists in a processive binding to the actin filament barbed end and stimulation of its elongation. The FH2 domain binds specifically to the barbed end, while the FH1 domain increases elongation by recruiting actin monomers from the medium (Kovar et al., 2006). SMIFH2 likely interacts with the FH2 domain since it inhibits both FH2 and FH1FH2 constructs to the same extent. Additionally, SMIFH2 was found to prevent the interaction of formin with the barbed ends of actin filaments without affecting the elongation of filaments at the same end in the absence of formin. In agreement with its effect on formin in vitro, low micromolar concentrations of SIMFH2 disrupted all formin-mediated actin structures in yeast. In the future, it will be critical to carefully determine to what extent SMIFH2 interacts with targets other than formin.

Further progress in understanding the molecular mechanism of the inhibition of formins by this compound will require structural characterization of the complex between the FH2 domain and SMIFH2. Structural information will be useful to design better inhibitors with stronger affinity, formin specificity,or isoform selectivity. Interaction of formin at the barbed ends during actin filament elongation is a complex mechanism that small molecules may help to elucidate. In any case, the characterization of formins or the Arp2/3 complex inhibitors is a great opportunity for cell biologists to study their relative effect on actin-based structure organizations in living cells. We should all encourage such approaches because, as these inhibitors will become

more efficient and specific, we will have tools to finely tune cell-dependent actin processes.

Does This Strategy Have Pharmacological and Therapeutic Implications?

Formin activities seem tightly correlated with tumor cell transformation and metastasis. For example, formins are required for invapodia formation and invasion of breast adenocarcinoma cells (Lizarraga et al., 2009). Rizvi and colleagues (2009) demonstrated that compounds targeting formins in animal cells lead to the disruption of specific formin-mediated actin structures. They therefore highlight the potential antiproliferative effect of SIMFH2 as a result of its effect on cell division and motility processes. Although these major findings validate formins as a promising target in anticancer therapies, the development of antiformin drugs will have to overcome standard obstacles as those faced by any new pharmacological agent, including cellular uptake, nonspecific interactions, catabolism, and toxicity. Additionally, formin isoform selectivity and potency must be improved.

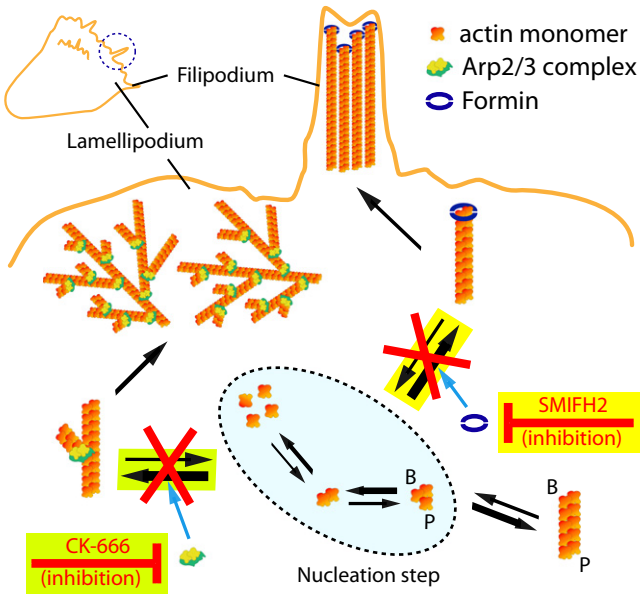

Figure 1. Effect of Inhibition of Actin Nucleators by Small Molecules
Formins and the Arp2/3 complex are actin nucleation factors that promote actin assembly by bypassing the unfavorable nucleation reaction and/or by enhancing the rate of monomer association at the fast growing barbed end. The Arp2/3 complex catalyzes the formation of branched actin filaments, while formins generate long unbranched actin filaments. Interestingly, the small molecules SMIFH2 (Rizvi et al., 2009) and the CK-666 (Nolen et al., 2009) inhibit specifically these two actin nucleators. In vivo, these small molecules are important tools to correlate actin nucleator activities and actin-based structure formation (B, barbed end; P, pointed end). Recently, other formin inhibitors have been characterized but not tested in live cells (Gauvin et al., 2009).

REFERENCES

Chhabra, E.S., and Higgs, H.N. (2007). Nat. Cell Biol. *9*, 1110–1121.

Fernandez-Suarez, M., and Ting, A.Y. (2008). Nat. Rev. Mol. Cell Biol. *9*, 929–943.

Gauvin, T.J., Fukui, J., Peterson, J.R., and Higgs, H.N. (2009). Biochemistry *48*, 9327–9329.

Kovar, D.R., Harris, E.S., Mahaffy, R., Higgs, H.N., and Pollard, T.D. (2006). Cell *124*, 423–435.

Lizarraga, F., Poincloux, R., Romao, M., Montagnac, G., Le Dez, G., Bonne, I., Rigaill, G., Raposo, G., and Chavrier, P. (2009). Cancer Res. *69*, 2792–2800.

Moseley, J.B., and Goode, B.L. (2006). Microbiol. Mol. Biol. Rev. *70*, 605–645.

Nolen, B.J., Tomasevic, N., Russell, A., Pierce, D.W., Jia, Z., McCormick, C.D., Hartman, J., Sakowicz, R., and Pollard, T.D. (2009). Nature *460*, 1031–1034.

Pollard, T.D., and Borisy, G.G. (2003). Cell *112*, 453–465.

Pruyne, D., Evangelista, M., Yang, C., Bi, E., Zigmond, S., Bretscher, A., and Boone, C. (2002). Science *297*, 612–615.

Rizvi, S.A., Neidt, E.M., Cui, J., Feiger, Z., Skau, C.T., Gardel, M.L., Kozmin, S.A., and Kovar, D.R. (2009). Chem. Biol. *16*, this issue, 1158–1168.

Sagot, I., Rodal, A.A., Moseley, J., Goode, B.L., and Pellman, D. (2002). Nat. Cell Biol. *4*, 626–631.

