

Discovery of a new ‘dynasore’

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Dynamin is a large GTPase that participates in the severing of membrane-bound vesicles. A small-molecule inhibitor specific for the dynamin family of GTPases has been identified and reveals new aspects of membrane dynamics.

Traditionally, inhibition of dynamin in cells has been performed through the expression of GTPase mutants affecting GTP binding or hydrolysis or, more recently, by small interfering RNA (siRNA)-mediated knockdown of dynamin. Both of these approaches are less than optimal for several reasons: first, they require many hours or even days to exert an effect, causing substantive concerns about spurious, indirect results; second, rapid cellular events are difficult to study with slow-acting probes; and, third, once the exogenous mutant protein is expressed, it may not be feasible to rapidly reduce its abundance in the cell. Now Macia *et al.*¹ have discovered a cell-permeable small molecule, termed ‘dynasore’, that inhibits dynamin endocytic functions in a matter of minutes (Fig. 1); and, significantly, the effects are reversible.

Members of the dynamin family, comprising dynamin, dynamin-like protein (known as Drp or DLP) and the Mx proteins—with dynamin being the best characterized—are known for their oligomerization and membrane-tubulating properties^{2,3}. Dynamin is particularly well known for its role in endocytosis but also functions in vesicle formation from other organelles⁴ and has additional roles in cell spreading, migration and invasion^{5,6}. The role of dynamin in the formation of clathrin-coated vesicles has been studied quite extensively; however, questions still remain about the exact stages in vesicle formation at which dynamin functions and the role of dynamin’s GTPase activity at these stages.

Small-molecule inhibitors of other enzymes, such as kinases and myosin motors, have been used extensively; however, relatively few chemical compounds have been identified that are specific for distinct classes

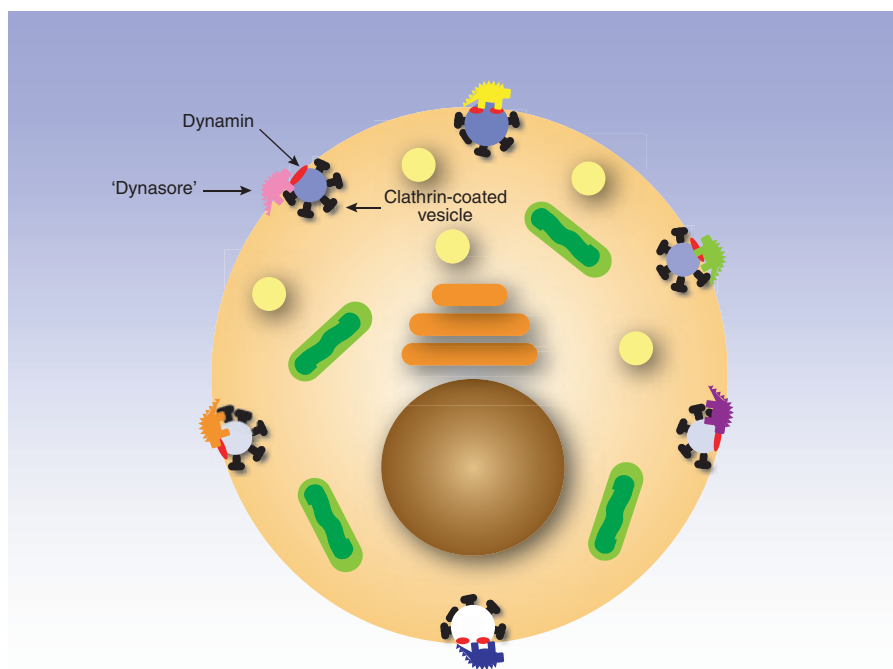


Figure 1 Previously, methods such as siRNA and expression of dominant-negative dynamin have been used to test dynamin-mediated processes. These methods are slow acting and could produce secondary effects. Now a new, fast-acting, reversible tool for analyzing dynamin has been discovered: the cell-permeable, small-molecule ‘dynasore’. Clathrin-coated pits (black) trapped at the plasma membrane as a result of inhibition of dynamin (red) by dynasore (colored dinosaurs) are shown (arrows highlight an example). Also shown are endosomes (yellow), mitochondria (green), the Golgi (orange) and the nucleus (brown).

of proteins involved in the various aspects of vesicle formation and trafficking. Now, as reported in *Developmental Cell*, Kirchhausen and colleagues¹ have discovered a new ‘dynasore’ in their dig through approximately 16,000 small molecules in search of a chemical inhibitor for dynamin. Dynasore functions as a noncompetitive inhibitor of dynamin 1 and dynamin 2 GTPase activity *in vitro* and blocks endocytic functions previously shown to require dynamin. At higher concentrations, equal to those used for *in vivo* experiments, dynasore also interfered with the *in vitro* GTPase activity of Drp1/DLP1 but not with that of MxA or the small GTPase Cdc42. Its effects on another dyna-

min-related large GTPase, guanylate-binding protein (GBP), were not tested.

Using this cell-permeable small molecule in conjunction with live-cell microscopy, Macia *et al.*¹ demonstrate that dynasore provides a means to rapidly block the formation of clathrin-coated vesicles, with the initial effects being observable within seconds and a more complete halt in coated-pit dynamics occurring in approximately 6.5 minutes. Later electron microscopic analysis revealed that clathrin-coated pits were blocked at two stages of vesicle formation, an early phase representing shallow pits and a later phase that seemed to represent late-stage vesicles that were unable to undergo the final scission event. Dynasore did

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not affect the ability of dynamin to oligomerize or bind lipids, indicating that it mediates its inhibitory effects through the GTPase domain of dynamin. Thus, the GTPase activity of dynamin seems to have a second function related to the early phase of coated-vesicle formation.

Vesicle formation and trafficking, especially at nerve terminals, as well as other membrane-based events involving dynamin, such as migration and cytokinesis, are very dynamic processes. Dynasore now provides a new tool for analyzing the role of dynamin, specifically its GTPase activity, in these processes in real time and assessing the effects of

rapid, short-term inhibition of dynamin. In addition, as dynamins but not small GTPases were particularly sensitive to dynasore, this provides another biochemical distinction between the large and small GTPase families in addition to their differences in nucleotide affinity and rates of GTP hydrolysis⁷. Perhaps structural studies incorporating dynasore will provide insight into the differences in the GTPase domains and mechanisms of hydrolysis between these different classes of GTPases. Certainly dynasore is a worthwhile find in the search for small-molecule inhibitors of mechanochemical enzymes that will

hopefully evolve into more specific as well as more varied inhibitors for the dynamins.

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