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COMMENTARY

Excited-State Control of Protein Activity

At page 155 in this issue, Masterson *et al.* discuss a remarkable example of protein behavior in which all the action, both functional and regulatory, takes place in excited states.[1] In this situation of "excited-state control", a protein is maintained in equilibrium between a highly populated state, which is inactive, and a low-populated state, which instead is active. Furthermore, regulation is also carried out through events that happen in the excited state and that modulate its population. In this view, the ground state appears to act predominantly as storage for protein molecules, which can be readily activated on the fast timescale required for promoting them to their excited state.

Masterson *et al.* provide an example of an excited-state control mechanism by studying phospholamban,[1] an integral membrane protein that regulates the activity of sarcoplasmic reticulum calcium ATPase, a calcium pump essential in controlling cardiac contractility.[2] Previous studies indicated that phospholamban is most active in an excited conformation, which is capable of binding sarcoplasmic reticulum calcium ATPase.[3] It was also shown that an increase in the population of the excited state of phospholamban can be caused by phosphorylation, which is carried out by the cAMP-dependent protein kinase A.[4] What the new study now reveals is that the phosphorylation event itself takes place preferentially in the excited state of phospholamban.[1] Incidentally, to add interest to an already fascinating story, protein kinase A also acts on phospholamban from an exited state of its own.[5]

These results are particularly significant when considered in comparison to more extensively studied mechanisms of protein regulation. The most common of such mechanisms involves a change in the ground state of a protein in response to a signal, such as a posttranslational modification, or a small molecule, nucleic acid, or protein binding event. For example, in allosteric regulation, the binding of a ligand in an allosteric site changes the thermodynamic state of a protein, enabling or disabling its function.[6,7] In this type of events, the activity of a protein is carried out from its ground state, and a change in such activity upon regulatory events involves a transition to a new ground state. However, excited states can play a role also in these cases. Indeed, a view is gaining momentum according to which the binding events required for regulation can take place in conformations present at low populations in the initial state of the protein—that is, in excited states.[6,8]

Although we are accustomed at thinking about protein activity in terms of structural and dynamical properties of native states, increasing evidence suggests that nonnative states, including excited states and intermediates, do also play a significant role in many cellular processes.[1,3,6–8] It is early to establish just how common is for proteins to be regulated by tuning the populations of their excited states, in a way illustrated clearly in the example reported in this issue of *Journal of Molecular Biology*.[1] One of the problems is that it has been extremely challenging to make progress in this context because of the difficulties in obtaining atomic-level information about nonnative states.[6] Cases of protein activity in such states are starting to be uncovered at a pace that, perhaps not by coincidence, corresponds to the development of conceptual and technical methods capable of defining protein states present at low populations. Procedures that combine NMR spectroscopy and molecular simulations methods[9,10] are making it possible to achieve this result and to determine the structures of low-populated states.[11] We should thus expect even more surprising mechanisms of protein activity and regulation to emerge, many of which may involve nonnative states in some form.

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