DBT/ CDFD, Hyderabad

"Back-pyrophosphorylation assay to detect in vivo InsP7-dependent protein pyrophosphorylation in mammalian cells"

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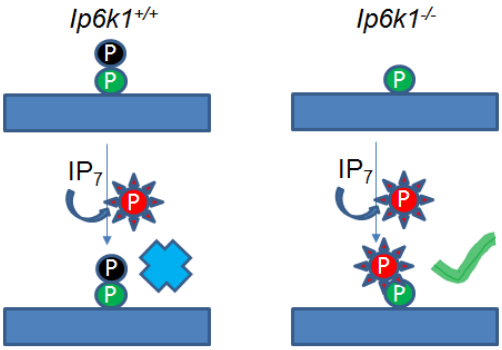
New Delhi: Signals from the environment are transmitted within a cell by the use of small molecules called second messengers. One such messenger is a molecule called InsP7, which consists of a sugar (inositol) with seven phosphate groups (Ins=inositol; P7=seven phosphates). The levels of InsP7 in a cell are approximately 1,000 fold lower than the levels of ATP, but like ATP, it carries high energy, and regulates many processes in a cell, including DNA repair, insulin release, spermatogenesis, and blood clotting.

One mechanism by which InsP7 regulates cellular functions is by transferring one of its phosphates to proteins, by a process called protein pyrophosphorylation. Although this protein modification was discovered over a decade ago, monitoring pyrophosphorylation inside cells has been a difficult task.

A new paper published in the series Methods in Molecular Biology by CDFD scientists describes a simple technique that all researchers can use to detect pyrophosphorylated proteins inside cells.

This method is based on the idea that a target protein isolated from cells with lower levels of InsP7 has reduced intrinsic pyrophosphorylation compared with the same protein isolated from cells with normal levels of InsP7. Isolation of the target protein from both cell types, followed by comparison of the extent of back-pyrophosphorylation of both proteins in a test tube will reveal the extent of pyrophosphorylation of the target protein inside cells (see Figure).

Using this method, any scientist can find out whether a protein of their interest is pyrophosphorylated inside cells, and then study the effect of this modification on the behaviour of that protein.



Back-pyrophosphorylation

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