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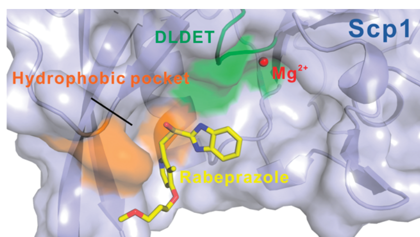
A Powerful Tool for Neuronal Stem Cell Research

RNA Polymerase II is a eukaryotic enzyme that catalyzes the transcription of DNA to mRNA and several small nuclear RNAs. The C-terminal domain (CTD) of this enzyme consists of 26−52 tandem heptapeptide repeats of the sequence Tyr_1-Ser_2-Pro_3-Thr_4-Ser_5-Pro_6-Ser_7, the phosphorylation of which regulates gene expression. Thus, CTD-specific kinases and phosphatases function as important regulatory factors for the transcriptional machinery in a cell. Among this class of phosphatases are the newly discovered, human small C-terminal domain phosphatases (Scp's), which specifically dephosphorylate serine residues at position 5 of the heptad repeats. Scp's function as transcriptional regulators for neuronal gene expression and, therefore, neuronal stem cell differentiation. In the current issue, Zhang *et al.* (DOI: 10.1021/cb100357t) describe the development of a specific small molecule inhibitor of Scp's that has significant implications to neuronal stem cell research.

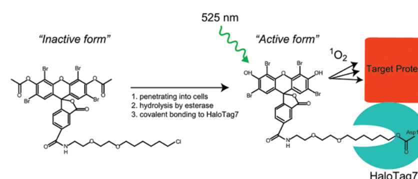
The authors screened ∼2400 compounds against the phosphatase Scp1 and identified rabeprazole as a specific inhibitor. The X-ray crystallographic structure of the compound bound to Scp1 revealed a hydrophobic binding site with residues unique to this subset of the phosphatase family. Consistent with this observation, rabeprazole was shown to specifically inhibit Scp's but not closely related phosphatases such as Fcp1 or Dullard, which play a role in cell survival. Thus this study provides the first specific lead compound for inhibition of Scp's and a starting point for research studying epigenetic silencing of neuronal gene expression.



Lighting the Way: Improved Functional Analysis of Proteins in Living Cells

Studying the function of specific proteins with high spatio-temporal resolution in living cells is challenging. Several chromophore-assisted light inactivation (CALI) techniques have emerged to address this need, but the tools are imperfect with drawbacks that range from inefficient generation of singlet oxygen by the photosensitizers to poor specificity when labeling target proteins. In this issue, Takemoto *et al.* (DOI: 10.1021/cb100431e) report the exciting development of antibody and Halo-Tag based CALI methodologies for studying *in vivo* protein function in a spatial and temporal context.

The authors describe an eosin dye, a membrane-permeable photosensitizer that, upon illumination, produces 11-fold more singlet oxygen than the classically used dye, fluorescein. The singlet oxygen production inactivates the target protein. The utility of the eosin dye was confirmed using an eosin-labeled anti-β-galactosidase antibody for the CALI of β-galactosidase. This eosin dye was further developed to covalently bind proteins with "HaloTag", tags which are known to bind cell-permeable dyes, permitting fluorescent tagging of proteins in living cells. This new CALI technique efficiently inactivated an organelle (mitochondria) or specifically HaloTag-Protein Kinase C-γ and Halo-Tag-Aurora B in living cells without phototoxicity. Moreover, less intense irradiation associated with this technology overcomes problems associated with nonspecific photodamage. Thus, the eosin-based CALI technique will be of great utility in the comprehensive assessment of function of localized proteins in living samples.



Allosteric Site for Cyclin-Dependent Kinase Inhibition

Cyclin-dependent kinases (CDKs) are a family of serine/threonine protein kinases known to regulate the cell cycle. Additionally, they are implicated in regulating key functions such as transcription, mRNA processing and nerve cell differentiation. The activity of CDKs is controlled by complex formation with a family of proteins termed cyclins. Each phase of the cell cycle is characterized by the expression of different CDK−cyclin complexes which regulate downstream substrates. One such complex, CDK2−cyclin A, is a key player in the G1/S2 transition checkpoint of the cell cycle, a period where the cell makes a commitment to dividing. This complex is a therapeutic target of high value to stop/start cell division. Betzi *et al.* (DOI: 10.1021/cb100410m) have unlocked a novel allosteric site that may serve as a target for inhibitors to disrupt CDK−cyclin complex formation.

Previously, the main target for CDK2 inhibition had been the ATP-binding site which is conserved among protein kinases. As a result, the development of inhibitors to targeting this site is nonspecific and none of the CDK2 inhibitors identified have been approved for clinical use. There is therefore a need to develop noncompetitive, allosteric inhibitors. To identify potential allosteric binding sites on CDK2, the authors employed a specific extrinsic 8-anilino-1-naphthalene sulfonate (ANS) fluorophore. X-ray crystallography showed that ANS binding to CDK2 caused a large conformational change that made cyclin binding unfavorable. Importantly, two ANS molecules were

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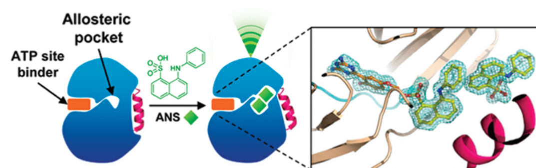
bound in an allosteric pocket away from the ATP-binding site. Thus, this ANS-binding pocket could be an important druggable site that inhibits complex formation between CDKs and cyclins.



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