What can you do with a luciferase Reporter Assay?

Protein Interaction Analysis Applications

Presented Fall 2009

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Have a question?
Ask a Scientist

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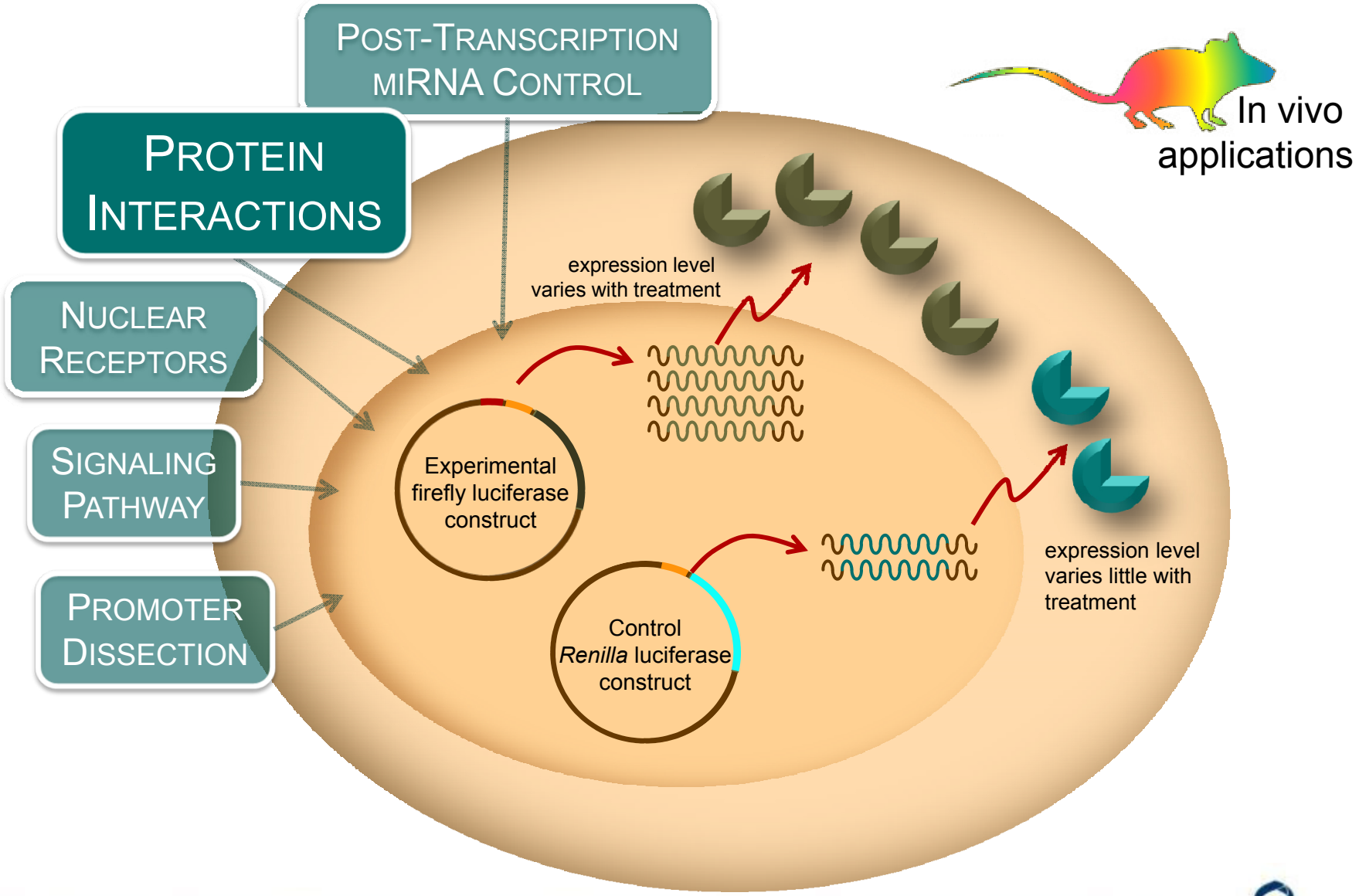
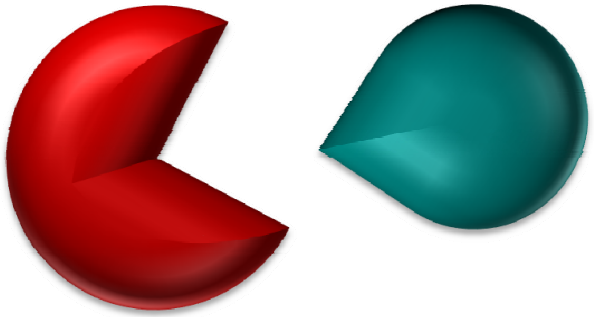
Application Overview



Do these proteins interact in mammalian cells?

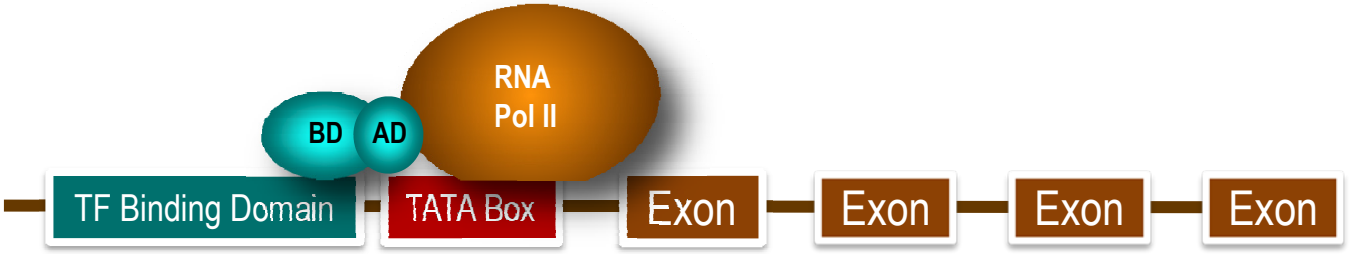


A yeast two-hybrid study says these two proteins interact.

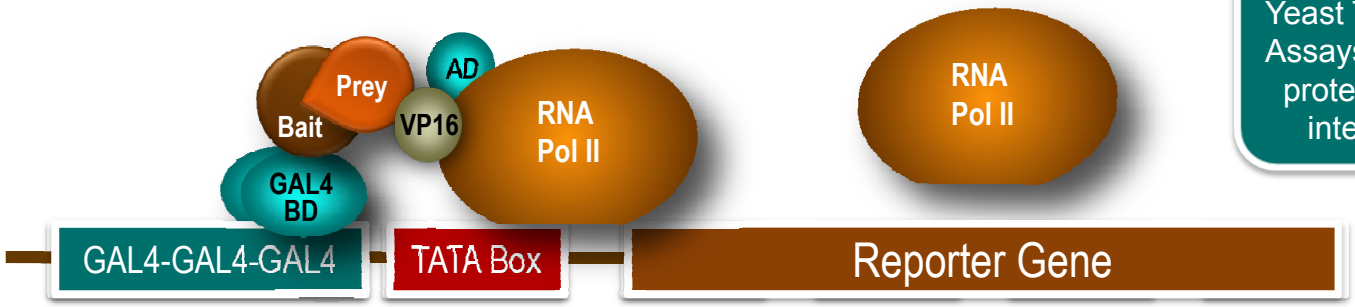
Do they still interact in a mammalian background?

Reporter assays can provide a rapid method to confirm interactions and even a way to investigate the interactions in more detail.

Exploiting Transcription Factor domains for protein interaction studies



- Transcription Factors have a **DNA-binding domain** (**BD**) that brings it next to the gene and a **transcription activation domain** (**AD**) that complexes with RNA Polymerase II to begin transcription



1st used for Yeast Two-Hybrid Assays to identify protein:protein interactions

- Removal/mutagenesis of either the binding domain or the activation domain yields a non-functional transcription factor
- You can bridge a binding domain and an activation domain with two proteins that interact and activate transcription.

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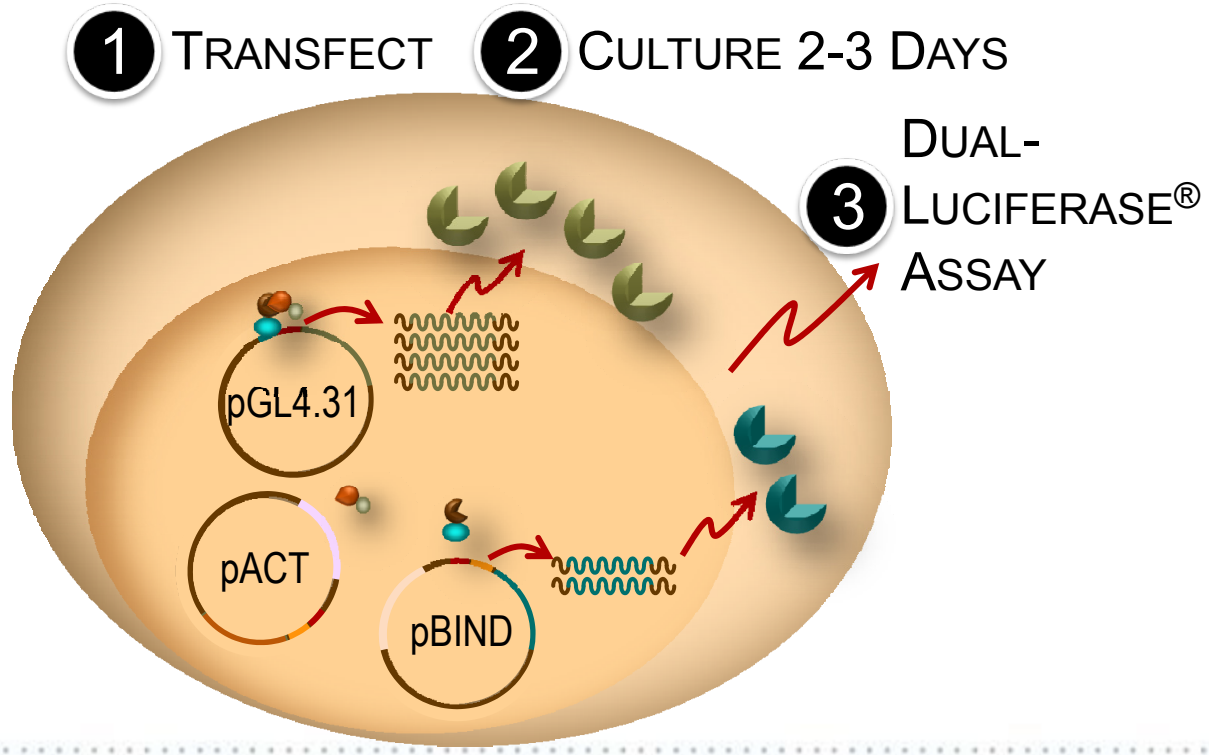
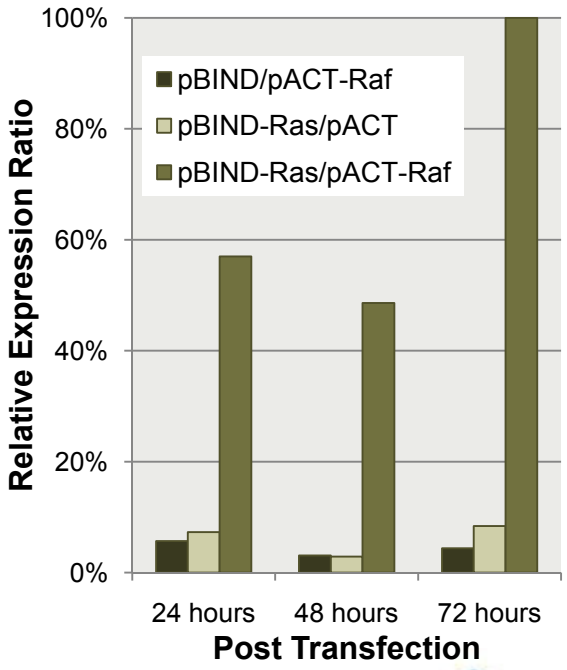
CheckMate uses Yeast Two-Hybrid principles in Mammalian cells





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Case Study: cdk3 interaction with ATF1

Research Article

Cyclin-Dependent Kinase 3–Mediated Activating Transcription Factor 1 Phosphorylation Enhances Cell Transformation

Duo Zheng, Yong-Yeon Cho, Andy T.Y. Lau, Jishuai Zhang, Wei-Ya Ma, Ann M. Bode, and Zigang Dong

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Abstract

Cyclin-dependent kinase (cdk)-3, a member of the cdk family of kinases, plays a critical role in cell cycle regulation and is involved in G_0-G_1 and G_1-S cell cycle transitions. However, the role of cdk3 in cell proliferation, as well as cell transformation, is not yet clearly understood. Here, we report that the protein expression level of cdk3 is higher in human cancer cell lines and human glioblastoma tissue compared with normal brain tissue. Furthermore, we found that cdk3 phosphorylates activating transcription factor 1 (ATF1) at serine 63 and enhances the transactivation and transcriptional activities of ATF1. Results also indicated that siRNA directed against cdk3 (si-cdk3) suppresses ATF1 activity, resulting in inhibition of proliferation and growth of human glioblastoma T98G cells in soft agar. Importantly, we showed that cdk3 enhances epidermal growth factor–induced transformation of JB6 C141 cells and si-cdk3 suppresses Ras^{G12V}/cdk3/ATF1–induced foci formation in NIH3T3 cells. These results clearly showed that the cdk3-ATF1 signaling axis is critical for cell proliferation and transformation. [Cancer Res 2008;68(18):7650–60]

Introduction

Cyclin-dependent kinases (cdk) are serine/threonine protein kinases that play essential roles in the control of cell cycle progression by interacting with a variety of regulators and substrates (1). In eukaryotic cells, cell cycle progression is driven by the sequential and periodic activation of cyclin/cdks, and dysregulation of the cell cycle is associated with cancer development (2). Very few mutations in cdks have been noted in human cancers with the exception of a $p16^{Ink4a}$-insensitive cdk4 mutation in human familial melanoma (3). However, dysregulation of cdk activity is found in many human cancers because of mutations or epigenetic alterations in upstream regulators of cdks or their substrates (1, 4). Overexpression of cdk6 has been observed in lymphomas, leukemias, and melanomas due to chromosomal translocation (5, 6). When cotransfected with activated H-Ras, cdk4 displayed oncogenic potential by provoking foci formation in primary rat embryo fibroblasts (7) and generating malignant human epidermal tumorigenesis (8). Cdk4 kinase activity was shown to be required for proliferation of breast cancer cells and mammary tumorigenesis in cdk4-null mice (9, 10) and kinase-deficient cyclin D1 knock-in mice (11). Hemizygous disruption of cdc25 was shown to inhibit cell transformation and mammary tumorigenesis in mice (12). Activation of cdk2 was required for human fibroblast proliferation and foci formation induced by cyclin E/SV40 small T antigen coexpression (13). Knockdown of cdk2 was shown to inhibit proliferation and colony formation of human melanoma cells (14), and ablation of cdk2 decreased Ras/cdk4-dependent malignant progression in mouse skin tumorigenesis models (15). Thus, cdks are closely associated with human cancer pathogenesis.

Cdk3 is an important regulator of cell cycle. The activity of cdk3 is first observed in early G_1 phase (16) and reaches a peak in mid-G_1 (17). A dominant-negative cdk3 was shown to induce G_1 arrest, which could not be rescued by cdk2, indicating that cdk3 plays an important role for G_1 exit to S entry (18, 19). Recently, cdk3 was found to form a complex with cyclin C and phosphorylate the retinoblastoma protein (pRb) at serine 807/811, which is required for G_0-G_1 transition (20). Furthermore, cdk3 seems to be expressed in various normal human tissues and cancer cell lines including glioblastoma and neuroblastoma cells (20–22). Ectopic overexpression of cdk3, but not cdk2, enhanced the proliferation and anchorage-independent growth of Rat1 cells, which was associated with activation of Myc (17). The genetic locus of cdk3 was mapped to the chromosome *17q22-qter* region, where cdk3 was involved in a chromosome rearrangement in a breast cancer cell line possibly resulting in an alteration of *cdk3* gene expression or an abnormal transcript (23). However, although cdk3 might have an effect on cell proliferation and transformation, the precise role of cdk3 in carcinogenesis has not been clearly elucidated.

The activating transcription factor 1 (ATF1) is a member of a well-known transcription factor family, the cyclic AMP (cAMP) response element (CRE)-binding protein (CREB) family, which includes ATF1, CREB1, and the cAMP response element modulator (CREM; ref. 24). Both ATF1 (25) and CREB1 (26) are expressed ubiquitously, whereas CREM (27) is highly expressed in neuroendocrine tissues. In response to a variety of growth factors, stress signals, neurotransmitters, and other agents that elevate intracellular cAMP or Ca^{2+} levels, CREB family members are activated and promote the expression of a large number of cellular target genes that contain CRE elements in their promoters, including proto-oncogenes such as *c-fos* and *c-jun*; cell cycle genes such as *cyclin D* and *cyclin A*; and other genes related to cell growth, proliferation, and neuronal activities (28, 29).

Phosphorylation of ATF1 at serine 63 in its kinase-inducible domain (KID) by serine/threonine kinases enhances its transactivation activity by promoting recruitment of the coactivator CREB-binding protein/p300 (30). Furthermore, overexpression of ATF1 was found in lymphomas, transformed lymphocytes (31), and may contribute to the growth of these tumor cells. ATF1 was shown to be up-regulated in metastatic melanoma cells, and

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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- cdk3 highly expressed in glioblastoma tissues and cell lines
- cdk3 is a ser/thr kinase
- Could the cdk3 be targeting transcription factors in the glioblastomas?
- Utilized the CheckMate System to find the answers.

Zheng, D., *et al.* (2008) *Cancer Res.* **68**, 7650-60.

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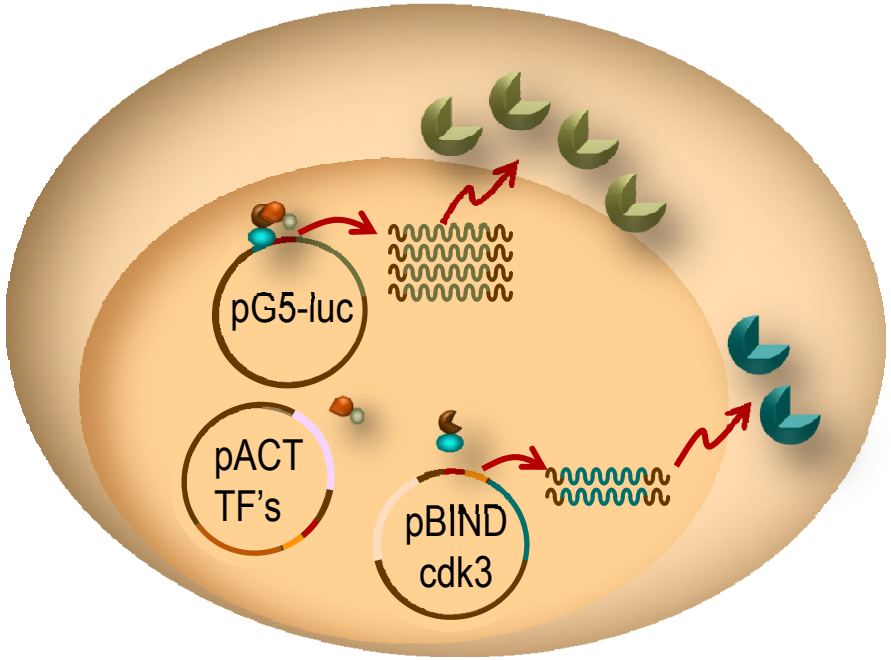
Case Study:
Zheng, D., et al.

ATF1:cdk3 found with CheckMate System

CheckMate System used to explore whether or not cdk3 interacted with selected transcription factors



Transcription Factor	Result
E2F1 (– control)	basal activity
E2F2 (+ control)	>3-fold increase
ATF1	>15-fold increase
ATF2	basal activity
ATF3	basal activity
STAT2	basal activity

Interaction confirmed by immunoprecipitation & cdk3 phosphorylates ATF1 Ser63

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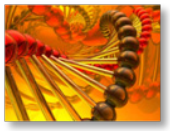
More Information



Protocols & Applications Guide:
Protein:Protein Interaction Analysis: In Vivo and In Vitro Methods

Schenborn, E., *et al.* (2006) The next-generation assay for mammalian protein interactions: The CheckMate™/Flexi® Vector Mammalian Two-Hybrid System. *Promega Notes* **94**, 12-16.

CheckMate™ System citations from HighWire Press®

CheckMate™ System citations from Nature.com

Please note, the CheckMate™/Flexi® Vector Mammalian Two-Hybrid System is an improved version of the original CheckMate™ Mammalian Two Hybrid System.

The original pBIND and pACT vectors are in the CheckMate™ Negative Control Vector Set