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Previews

Striking it Rich by Data Mining

In this issue of *Cell*, Lamb et al. have used a combination of molecular genetics, DNA microarray, and data mining of human tumor expression databases to identify a cdk-independent mechanism by which the interaction of cyclin D1 with the transcription factor C/EBPβ may mediate tumorigenesis.

Although the protooncogene cyclin D1 is overexpressed in approximately 45% of breast cancers (Hui et al., 1996) as well as a broad range of other human tumor types, the mechanisms through which cyclin D1 mediates its effects on tumorigenesis have not yet been elucidated. The D-type cyclins represent a fundamental link between signal transduction pathways and the cell cycle machinery; thus, it has been suspected that cyclin/cdk complexes act not only as essential regulators of cell division, but are also responsible for the oncogenic effect of cyclin D1. However, several recent studies, including the analysis of cyclin D1-deficient mice, have suggested that cyclin D1 may indeed have other activities separate and distinct from its role as a cdk regulatory subunit and regulator of Rb (reviewed in Coqueret, 2002). Lamb et al. (2003) have now taken a molecular genetic approach to identify alternative cyclin D1 activities by generating a mutant incapable of activating cdk4 and then using DNA microarrays to screen for target genes differentially activated by overexpression of this mutant in comparison with wild-type cyclin D1. Using this approach, they have identified an expression signature for the putative cdk-independent function of cyclin D1 in MCF-7 breast cancer cells and then validated many of the target genes identified by RNase protection assays in both MCF-7 and another breast cancer cell line, MDA-MB468.

In addition, these investigators have also used a more powerful and novel approach involving data mining to query the expression patterns of thousands of genes across 190 primary human tumors of 14 different histological types to determine if the biologically relevant cyclin D1 target genes were frequently coexpressed with endogenous cyclin D1. Using the Kolmogorov-Smirnov (KS) nonparametric rank statistic, Lamb et al. (2003) first validated the set of 21 genes. Surprisingly, no E2F target genes were found in the set of genes affected by overexpression of wild-type or mutant cyclin D1. Furthermore, using KS in a data mining exercise with the cyclin D1 expression signature in additional prostate, lung, and tumor cell databases, they consistently observed coexpression of the transcription factor CCAAT enhancer binding protein (C/EBP) β across more than 500 human tumors and cell lines. C/EBPβ is a member of the basic leucine zipper family of transcription factors. Encoded by an intronless gene, *cebpb* is expressed as several distinct protein isoforms whose expression is regulated by the differential use of a number of in-frame translation start sites. Increased expression of C/EBPβ has been detected in breast cancer, ovarian tumors, and colorectal tumors (reviewed in Zahnow, 2002). In contrast, C/EBPβ null mice are refractory to Ras-mediated skin tumorigenesis (Zhu et al., 2002). Thus, disruption of signaling through C/EBPβ appears to contribute to malignant transformation.

To provide independent evidence that supports the role of C/EBPβ in the mechanism of cyclin D1 action, Lamb et al. (2003) conducted a functional analysis of target gene promoters using conventional transfection assays. Cyclin D1-responsive regions were mapped in the promoters of six genes, including *HSP70-2*, which the original microarray analysis showed to be induced with similar kinetics by both wild-type and mutant cyclin D1. Consensus C/EBPβ binding sites were present in each of these promoters, and mutation of these sites resulted in a higher basal activity. Surprisingly, these results suggest that C/EBPβ acts as a constitutive repressor of cyclin D1 targets and that cyclin D1 antagonizes this repressor function. Additional support was provided through the use of a dominant-negative isoform of C/EBPβ, LIP, which is translated from the third in-frame AUG and therefore lacks most of the aminoterminal transactivation domain, but still contains the DNA binding and leucine zipper dimerization domains. LIP can recapitulate the effects of cyclin D1, suggesting an alternative mechanism of regulation of target gene expression. The direct or indirect association of cyclin D1 with C/EBPβ was demonstrated by coimmunoprecipitation experiments. Finally, the regulation of target gene expression by cyclin D1 was abrogated in C/EBPβ null mammary epithelial cells, providing genetic evidence for the importance of this interaction. Thus, by integrating experimental and tumor expression data, these investigators have been able to provide novel insights into proteins that function in a common pathway with cyclin D1 and may participate in its mechanism of action, an approach heretofore used primarily in yeast.

Several important questions are raised by these intriguing studies. First, by what mechanism does C/EBPβ repress the expression of the cyclin D1 target genes and second, how does the interaction with cyclin D1 counteract this repression? In order to answer these questions, it will be critical to determine the occupancy of specific coactivators and corepressors on the cyclin D1 target promoters. Full-length C/EBPβ LAP1, whose translation is initiated from the first AUG in C/EBPβ mRNA, has been shown to interact with the SWI/SNF ATPase/helicase chromatin-remodeling complex (Kowenz-Leutz and Leutz, 1999). Thus, it is conceivable that this activity, usually involved in gene activation, is actually involved in repression of certain target gene promoters. Alternatively, C/EBPβ is acetylated at a unique lysine motif within its DNA binding domain. Recruitment of HDAC1 by Stat5 has been shown to lead to C/EBPβ deacetylation, increased DNA binding, and activation of transcription (Xu et al., 2003). Interestingly, cyclin D1 has been reported to interact with both histone deacetylases, such as HDAC3, and histone acetylases

such as P/CAF and SRC1 (reviewed in Coqueret, 2002) depending on the promoter context, so it will be critical to determine the acetylation status of both C/EBPβ and histones on cyclin D1 target gene promoters. A third important question is why should LIP activate these target genes? Does it merely displace or antagonize the transcriptional activity of the bound LAP isoforms? LIP has been reported to exhibit an increased DNA binding affinity relative to the LAP isoforms of C/EBPβ. Does this then imply that there is a reciprocal relationship between cyclin D1 and LIP expression in cancer? In breast cancers, LIP was reported to be predominantly overexpressed in ER-negative tumors (Milde-Langosch et al., 2003; Zahnow et al., 1997), while cyclin D1 is usually elevated in ER-positive tumors. Curiously, overexpression of the translation initiation factor eIF4e has been shown to increase cyclin D1 expression, while decreased expression of eIF4e and eIF2α has been correlated with increased LIP expression (Calkhoven et al., 2000). However, other mechanisms involving both gene amplification and transcriptional activation are involved in the overexpression of cyclin D1 in cancer. A more thorough examination of the mechanisms regulating the expression of different C/EBPβ isoforms and their activities on different target gene promoters is, however, clearly warranted. Finally, what are the functions of the specific cyclin D1 target genes identified in this study in the etiology of cancer? Thus, while numerous interesting questions remain to be answered, the future for combining molecular genetic and data mining approaches to discover novel interactions and pathways in human cancer appears bright.

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Selected Reading

Calkhoven, C.F., Muller, C., and Leutz, A. (2000). Genes Dev. *14*, 1920–1932.

Coqueret, O. (2002). Gene *299*, 35–55.

Hui, R., Cornish, A.L., McClelland, R.A., Robertson, J.F., Blamey, R.W., Musgrove, E.A., Nicholson, R.I., and Sutherland, R.L. (1996). Clin. Cancer Res. *2*, 923–928.

Kowenz-Leutz, E., and Leutz, A. (1999). Mol. Cell *4*, 735–743.

Lamb, J., Ramaswamy, S., Ford, H.L., Contreras, B., Martinez, R.V., Kittrell, F.S., Zahnow, C.A., Patterson, N., Golub, T.R., and Ewen, M.E. (2003). Cell *114*, this issue, 323–334.

Milde-Langosch, K., Loning, T., and Bamberger, A.M. (2003). Breast Cancer Res. Treat. *79*, 175–185.

Xu, M., Nie, L., Kim, S.H., and Sun, X.H. (2003). EMBO J. *22*, 893–904.

Zahnow, C.A. (2002). Breast Cancer Res. *4*, 113–121.

Zahnow, C.A., Younes, P., Laucirica, R., and Rosen, J.M. (1997). J. Natl. Cancer Inst. *89*, 1887–1891.

Zhu, S., Yoon, K., Sterneck, E., Johnson, P.F., and Smart, R.C. (2002). Proc. Natl. Acad. Sci. USA *99*, 207–212.

TFIIS and GreB: Two Like-Minded Transcription Elongation Factors with Sticky Fingers

How the structurally distinct transcription factor TFIIS from eukaryotes and its bacterial counterpart GreB act to convert their cognate RNA polymerases into ribonucleases has been a longstanding question. Now, two new structures of these factors bound to their respective RNA polymerases (Opalka et al. and Kettenberger et al. [this issue of *Cell*]) suggest how they accomplish this feat.

Eukaryotic TFIIS (also known as SII) and its bacterial counterpart GreB are unique among all transcription factors: they are the only known transcription factors capable of restarting arrested RNA polymerases (Wind and Reines, 2000; Uptain et al., 1997). TFIIS is expressed ubiquitously in eukaryotes, where it acts specifically to reactivate arrested RNA polymerase II to ensure efficient synthesis of mRNA. GreB performs a similar task in bacteria.

The tendency to arrest is an inherent property of RNA polymerases. Upon arrest, an RNA polymerase stops transcribing, refuses to budge even in the presence of sufficient concentrations of ribonucleoside triphosphates to support further transcript elongation, and clings tenaciously to its DNA template and nascent transcript, presenting a potential impediment to other RNA polymerases.

Arrest occurs when the 3′-end of a nascent transcript loses critical base pair contacts with the DNA template and is displaced from, or in some cases, completely extruded from the polymerase active site through a pore or channel that is situated directly beneath the primary catalytic magnesium ion and through which incoming ribonucleoside triphosphates are believed to enter the active site (Komissarova and Kashlev, 1997; Nudler et al., 1997; Cramer et al., 2000, 2001; Zhang et al., 1999). Elegant biochemical studies have shown that TFIIS and GreB restart arrested RNA polymerases by a remarkable mechanism that proceeds with TFIIS- or GreB-promoted ribonucleolytic cleavage of the displaced 3′-end of the nascent transcript, producing a new 3′-end that is properly base paired with the DNA template in the active site and, thus, can be extended by polymerase (Wind and Reines, 2000; Uptain et al., 1997). TFIIS and GreB are capable of promoting endonucleolytic removal of as many as 17 nucleotides from the 3′-ends of nascent transcripts in arrested RNA polymerase elongation complexes while still allowing efficient reextension of those transcripts by polymerase. In part because pyrophosphorylysis—chemically the reverse of the polymerization reaction—can also result in removal of large oligonucleotides from the 3′-ends of transcripts in arrested elongation complexes, it was proposed that transcription elongation factor-promoted removal of the 3′-ends of nascent transcripts is carried out by the polymerase's own active site (Rudd et al., 1994).

Exactly how TFIIS and GreB can produce in their cog-