DNA Binding Is Not Sufficient for Nuclear Localization of Regulatory Proteins in *Saccharomyces cerevisiae*

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We showed by immunofluorescence that the procaryotic DNA-binding protein LexA and a chimeric protein that contains the DNA-binding portion of LexA (amino acids 1 to 87) and a large portion (amino acids 74 to 881) of the *Saccharomyces cerevisiae* positive regulatory GAL4 protein (GAL4 gene product) are not preferentially localized in the nucleus in *S. cerevisiae*.

Certain proteins are found only in the cell nucleus. Following their synthesis in the cytoplasm, these proteins move into the nucleus in a way we do not understand. One possibility is that proteins diffuse into the nucleus through the nuclear pores and are retained there by binding to DNA or chromatin (6, 7). Proteins that failed to bind would freely diffuse out and thus not be preferentially localized in the nucleus. Alternatively, a transport system might selectively transport nuclear proteins across the nuclear envelope. We know that portions of some nuclear proteins direct them to the nucleus (5, 8–10, 16, 19). For instance, the first 74 amino acids of the 881-amino-acid *Saccharomyces cerevisiae* GALA gene product are sufficient for its nuclear localization (19). These specific amino acid sequences could be recognized by a transport system or could be responsible for binding to a nuclear component once the protein is inside the nucleus. In this report, we test whether DNA binding alone is sufficient for two proteins to be localized preferentially in the nucleus of *S. cerevisiae*.

We have shown that chimeric proteins containing as few as the first 74 amino acids of the *S. cerevisiae* positive regulatory GALA protein (GALA gene product) fused to *Escherichia coli* β-galactosidase are localized in the cell nucleus when produced in *S. cerevisiae* (19) (Fig. 1A to C). By contrast, chimeric proteins lacking the first 74 GALA amino acids but containing most of the remainder of GALA fused to β-galactosidase are excluded from the nucleus, as determined by indirect immunofluorescence (19). From this analysis, we concluded that the information for nuclear localization of a chimeric GALA-β-galactosidase protein resides in the first 74 GALA amino acids [GALA(1–74)]. More recently, Keegan et al. (12) demonstrated that the chimeric GALA(1–74)-β-galactosidase protein binds to the upstream activator sequence of the *GAL1* and *GAL10* genes in vitro and in vivo. These results demonstrated that the information for specific DNA binding and concentration in the nucleus resides in the same portion of the GALA protein. It is thus not possible to determine whether localization in the nucleus is separable from the ability of the protein to bind DNA.

To examine in more detail the role of DNA binding in nuclear protein localization, we analyzed the intracellular distribution in *S. cerevisiae* of a procaryotic DNA-binding protein, LexA. LexA is a small (24 kilodalton) protein that represses the *E. coli* genes necessary for the response to DNA damage (SOS response) by binding to a 22-base-pair operator (2). LexA, when produced in *S. cerevisiae*, is capable of repressing transcription by binding to a synthetic operator placed appropriately in an *S. cerevisiae* promoter (1, 3). Thus, as judged by this functional criterion, LexA can gain access to the interior of the nucleus.

LexA made in *S. cerevisiae* at levels comparable to those of nuclear GALA(1–74)-β-galactosidase was not preferentially localized in the nucleus. Yeast cells producing LexA were examined by immunofluorescence with anti-LexA antibody (Fig. 1D to F). Immunofluorescence appeared to be distributed throughout the cell and was not localized in any specific intracellular compartment. LexA appeared in newly formed buds that did not yet contain nuclear DNA (compare Fig. 1D and 1E). From the distribution of the immunofluorescence, it was difficult to assess whether LexA was less concentrated in the nucleus than in the cytoplasm. At very dilute anti-LexA antibody concentrations, we could still not reliably detect any preferential localization of LexA in the cell (data not shown). The presence of the LexA operator in the genome did not affect the distribution of the LexA protein.

The amount of the LexA protein associated with nuclear and cytoplasmic fractions isolated from cells producing the LexA protein was consistent with the immunofluorescence results. We observed on immunoblots that the LexA protein remained intact (Fig. 2B, lane 2) when produced in *S. cerevisiae* and that almost all of the protein remained in the soluble fraction from lysed cells (Fig. 2B, lane 3). Very little of the LexA protein (less than 10%) remained associated with the nucleus-enriched fraction (Fig. 2B, lane 4). This behavior was similar to that of the cytoplasmic alcohol dehydrogenase, 97% of which was in the cytoplasmic fraction, and distinctly different from GALA(1–74)-β-galactosidase, of which 78% was in the nuclear fraction. We do not know if the LexA protein in the crude nuclear pellet reflected the amount of the LexA protein associated with the nucleus. Alternatively, most of the LexA protein was not tightly bound inside the nucleus and leaked out when the cells were lysed.

We also examined the intracellular distribution of LexA-GALA, a larger (99 kilodalton) hybrid protein that contains the LexA DNA-binding domain at its N terminus followed by the C-terminal 91% of GALA (4). This LexA-GALA hybrid protein lacks the GALA DNA-binding region, the same portion that we previously showed could localize β-galactosidase in the nucleus (19). However, it is capable of

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activating the transcription of yeast genes that have the LexA operator near the start of transcription, implying that the LexA-GAL4 hybrid protein must enter the nucleus (4).

Yeast cells producing the LexA-GAL4 hybrid at levels comparable to those of GAL4(1-74)-β-galactosidase and intact LexA were examined by immunofluorescence with anti-LexA antibody (Fig. 1G to I). As in the case of LexA alone, the immunofluorescence was distributed throughout the cell and was not concentrated only in the nucleus, as GAL4(1-74)-β-galactosidase was (compare Fig. 1A and 1G). The results of cell fractionation experiments were consistent with this conclusion. LexA-GAL4 produced in S. cerevisiae had the predicted size of 99 kilodaltons (Fig. 2A, lane 2). Approximately 75 to 85% of the total LexA-GAL4 recovered
from lysed cells remained in the soluble fraction. (Fig. 2A, lane 3). LexA-GAL4 found in the nucleus-containing fraction (Fig. 2A, lane 4) could be extracted with nonionic detergent (data not shown). We do not know if this small amount of LexA-GAL4 was associated with nuclei or other membranous material. Up to 75% of GAL4(1-74)-β-galactosidase was associated with the crude nuclear fraction, and more than half was retained by nuclei isolated in the presence of nonionic detergent (19). We could not reliably judge by immunofluorescence whether LexA-GAL4 was more concentrated in the nucleus than in the cytoplasm. As in the case of LexA alone, the presence of the LexA operator had no effect on the distribution of LexA-GAL4.

By the above analysis, we demonstrated that the small DNA-binding protein LexA, when made in S. cerevisiae, is not specifically localized in the nucleus. Moreover, a LexA-GAL4 hybrid protein which lacks the DNA-binding region of the GAL4 protein but contains the DNA-binding domain of LexA is also not preferentially associated with the nucleus. We conclude that DNA affinity alone is not sufficient for complete localization of this protein to the nucleus. Similarly, Paucha et al. (15) found that a mutant simian virus 40 T antigen that was not associated with the nucleus in mammalian cells still retained the ability to specifically bind DNA in vitro. This result led them also to conclude that DNA binding and localization in the nucleus are independent of one another. In E. coli, almost all of the lac repressor is nonspecifically associated with DNA (11). If LexA has a nonspecific affinity for DNA comparable to that of the lac repressor, it is not unreasonable to expect all of LexA in S. cerevisiae to be associated with nuclear DNA, if LexA has free access to every compartment in the cell.

We do not know how much LexA or LexA-GAL4 is actually in the nucleus. However, since both proteins are able to function as transcriptional regulatory proteins, some must gain access to the interior of the nucleus and recognize the LexA operator. Because of its larger size, one might not expect LexA-GAL4 to diffuse readily through the nuclear pores. It is possible that LexA-GAL4 enters the nucleus by association with another nuclear protein, such as intact GAL4, which contains the determinant for specific nuclear transport. The experiments reported here were done with cells producing intact GAL4.

Analyses of other nuclear proteins have led to the suggestion that some proteins may contain specific amino acid sequences that are responsible for directing them to the nucleus (9, 10, 16). The LexA protein does not contain any of these proposed nuclear target sequences.

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