dered it inducible in fat bodies. This demonstrates vividly that the code is followed in larval fat bodies as predicted.

It remains to be determined which of the five *Drosophila* GATA factors are actually involved in regulating all these immunity genes. Serpent is the GATA factor being implicated (Petersen et al., 1999; Senger et al., 2004). Unfortunately, *serpent* mutants have serious defects in fat body and hemocyte development. This precludes a simple genetic analysis of the role of this GATA factor in antimicrobial response. Another noteworthy issue is that the immunity gene induction mechanisms in larvae may be different from those in adults. For instance, mutating the GATA site on the *cecropinA1* enhancer abolished the inducible expression in larvae but did not affect the responsiveness in adults (Petersen et al., 1999). As mentioned above, the requirements for Dorsal in activating *drosomycin* expression in larvae and adults are also different (Meng et al., 1999; Rutschmann et al., 2000). Further analysis of the enhancers described by Senger et al. in adult flies may provide insights into this mystery.

How does the GATA and NF-κB interaction regulate immunity gene expression? Although the REL-GATA code has also been observed in mammalian cells (Minami et al., 2003), no GATA and NF-κB complex formation has been reported in either system. It may be that the two proteins have weak affinity toward each other, which could explain why the binding sites have to be in close proximity. Another possibility is that the proteins do not interact with each other physically, but the simultaneous binding of the two factors to the promoter provides a synergistic effect on building the basal transcription machinery (Senger et al., 2004). This could explain why the relative orientation of the binding sites is important. Even though many questions remain, the work leads us to enjoy the simple idea that perhaps the various NF-κB dimers in conjunction with the five GATA factors constitute largely the codes of fly immunity.

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Destruction with a Box: Substrate Recognition by the Anaphase-Promoting Complex

Destruction boxes mark cyclin B and other proteins degraded in mitosis for ubiquitination by the anaphase-promoting complex (APC/C). In a paper in this issue of *Molecular Cell*, Yamano et al. show that destruction boxes directly bind to the APC/C in a cell cycle-regulated manner. Interestingly, this interaction does not require APC/C activators of the Cdc20 family, which were thought to be essential for recruiting substrates to the APC/C.

The discovery of cyclin by Hunt and colleagues in 1983 drew attention to selective protein destruction as a mechanism for cell cycle control. Cyclin is synthesized in fertilized sea urchin eggs and abruptly destroyed before each division. Selective proteolysis is mediated by ubiquitin ligases, which assemble poly-ubiquitin chains onto specific substrate proteins and thereby mark them for destruction by the 26 S proteasome. A multisubunit ligase called the anaphase-promoting complex or cyclosome (APC/C) is essential for progression through mitosis (reviewed in Harper et al., 2002). APC/C-dependent ubiquitination of the anaphase inhibitor Pds1/securin initiates sister chromatid separation whereas degradation of cyclin B is needed for exit from mitosis. The APC/C can only ubiquitinate substrates upon association of the core complex with a member of the Cdc20 family of WD repeat proteins. Cdc20/Fizzy activates the APC/C from metaphase until late anaphase whereas Cdh1/Hct1/Fizzy-related maintains activity during the G1 phase in somatic cells. Studies in budding yeast revealed that APC/C-Cdc20 and APC/C-Cdh1 have different substrate specificities. Pds1 is degraded preferentially through APC/C-Cdc20 and the cyclin Clb2 through APC/C-Cdh1 (Schwab et al., 1997; Visintin et al., 1997). Due to the sequential activation of the APC/C holo-enzymes, Pds1 is destroyed shortly before anaphase whereas most of Clb2 is degraded later, as cells exit from mitosis. Substrate specificity is not absolute, however. APC/C-Cdc20 can initiate but not complete Clb2's degradation during anaphase and APC/C-Cdh1 can overwhelm the low level of Pds1 expression in G1. Meanwhile, many more APC/C substrates have been discovered, including mitotic kinases, proteins controlling spindle behavior, and regulators of DNA replication. The ordered degradation of different substrates is crucial for cell cycle progression as exemplified by the observation that human cells coaxed to degrade cyclin B and Pds1 in the wrong order undergo fatal cytokinesis without chromosome segregation.

How the APC/C recognizes and then ubiquitinates substrates is still poorly understood. Proteins degraded during anaphase such as cyclin B contain destruction boxes (D boxes) with the consensus RxxLxxxxN/D/E, which are required but not sufficient for ubiquitination. D box-containing fragments of a few dozen residues, however, confer APC/C-dependent degradation to an otherwise stable protein. The requirements for sequences surrounding functional D boxes are not yet understood. While lysine residues are essential for ubiquitination, they are not required for the targeting function of D boxes and can even be provided by a foreign sequence. A different motif with related properties called the KEN box was found to target human Cdc20 for ubiquitination and degradation in G1 (Pfleger and Kirschner, 2000). KEN boxes have since been found in other APC/C substrates, often in the vicinity of a D box. Additional degradation motifs exist such as the A box recently identified in the Aurora A kinase. Analysis of substrates containing a single degradation motif suggested a simple rule for substrate specificity: ubiquitination by APC/C-Cdc20 requires a D box whereas APC/C-Cdh1 can recognize D and KEN boxes (Pfleger and Kirschner, 2000). The requirements of dual motif substrates are more difficult to predict. For example, in substrates such as cyclin A or the yeast kinase Hsl1 both D and KEN boxes are important for Cdc20-dependent degradation and both motifs function in concert rather than independently. The APC/C may use dual-motif recognition to tune degradation timing and kinetics between the extremes represented by single-motif substrates such as cyclin B and human Cdc20 (Harper et al., 2002).

The substrate specificity of Cdc20 and Cdh1 suggested that they might activate ubiquitination by recruiting substrates to the APC/C. This idea gained momentum with the discovery of activator-substrate interactions that correlated with the requirements for degradation (Burton and Solomon, 2001; Hilioti et al., 2001; Pfleger et al., 2001; Schwab et al., 2001). For example, Cdh1 binds Clb2 but not Pds1, which associates with Cdc20 instead. Studies on mutant versions of the dual-motif substrates Pds1, Clb2, and Hsl1 led to the following conclusions. (1) The degradation motifs are important for activator binding since D box mutations in Pds1 and Hsl1 prevent association of Cdc20 and a KEN box mutant of Hsl1 is defective in binding to Cdh1. (2) Activator binding is required for ubiquitination since mutations that disrupt the activator-substrate interaction result in stabilization of the substrate. (3) Activator binding is not necessarily sufficient for ubiquitination because a D box mutation stabilizes Clb2 in G1 but it does not affect binding to Cdh1. Likewise, mutations in the D or the KEN box stabilize Hsl1 but still allow binding to one or the other activator. This indicates that the degradation motifs provide essential functions beyond activator binding.

In this issue, Yamano et al. show that D boxes associate with the APC/C core complex, suggesting a mechanism of how substrates are bound in the vicinity of APC/C's catalytic center to allow processive ubiquitination. To isolate D box-interacting factors, Yamano et al. loaded frog egg extracts onto columns carrying N-terminal cyclin B fragments with or without D boxes. The D box column retained an activity required for cyclin B degradation, which was identified as the APC/C core complex. Most of Cdc20 was found in the flow through, which is consistent with the low affinity of N-terminal cyclin B fragments for activators. Significantly, APC/C purified away from Cdc20 still showed D box-dependent binding. Only APC/C from mitotic extracts bound to the column, suggesting that the APC/C harbors a D box receptor whose activity is cell cycle regulated.

Recent studies on the budding yeast APC/C point to the core subunit Doc1/Apc10 as a candidate for the D box receptor. APC/C lacking Doc1 is normal with respect to subunit composition, association with E2 enzyme, and binding to activators. Nevertheless, it fails to interact with substrates (Carroll and Morgan, 2002; Passmore et al., 2003), which is unexpected because substrates can bind to free activators, at least at elevated concentrations. In vivo, however, interactions with both activator and Doc1 might be required to allow processive ubiquitination. Strong interaction with one component might compensate for weak binding to the other, which could explain why N-terminal cyclin B fragments interact poorly with activators but are ubiquitinated efficiently. Doc1 is ideally suited to present substrates for ubiquitination because it directly binds to APC/C's catalytic center, the RING finger subunit Apc11. Whether Doc1 directly interacts with degradation motifs, or indeed with substrates, remains to be tested. The work of Yamano et al. justifies a reexamination of Cdc20's activating mechanism. While they might assist in substrate delivery, activators might also induce conformational changes in the APC/C to provide access to the catalytic center and/or the D box receptor. This is consistent with the finding that recombinant Apc11 but not activator-free APC/C can conjugate multi-ubiquitin chains to substrates. The construction of yeast mutants able to survive without APC/C function now allows manipulation of essential components at will (Thornton and Toczyski, 2003), which should help to unravel the inner workings of the APC/C.

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