

rium, with populations that depend on their relative free energies. Changes in the protein environment—such as a binding event—will alter the relative populations of the substates in the conformational ensemble (see the figure). In this context, induced fit and conformational selection are two extremes of a spectrum of possible protein binding mechanisms that can be categorized based on the initial binding interaction and the resulting structural changes in the energy landscape.

Indeed, a large body of structural work supports induced fit mechanisms (12), and kinetic signatures for both induced fit and conformational selection have been observed, sometimes in the same system (13–15). In a model that combines both mechanisms, the interaction proceeds through three steps: a diffusional encounter, recognition of complementary structures contained within the conformational ensembles of the free proteins, and conformational relaxation into the final bound state (16). As noted by Lange *et al.*, their results only characterize protein backbone structure and dynamics, and it is possible that minor backbone conformational changes or rotameric rearrangements of side chains may be induced after the initial interaction with a protein binding partner.

The analysis by Lange *et al.* provides much structural insight into the conformational ensemble of ubiquitin, but a more complete

Molecular recognition mechanisms in proteins. Induced fit (**top**) assumes an initial interaction between a protein and its binding partner, followed by conformational changes that act to optimize the interaction. In conformational selection (**bottom**), a weakly populated, higher-energy conformation interacts with the binding partner, stabilizing the complex. Relative populations of conformations are indicated by size. In the structural ensemble presented by Lange *et al.*, different conformations may interact with distinct protein-binding partners. The energy diagram depicted is the simplest case; binding partners may have affinity for a number of protein substates that would further modify the structural energy landscape.

picture of the energy landscape would require more detailed kinetic and thermodynamic information. What are the relative populations of the individual structures and the rate constants of exchange among the substates in the conformational ensemble? What is the nature of the thermodynamic barriers between conformations? The information gained about the conformational ensemble can be compared with a careful kinetic analysis of ubiquitin binding interactions to provide us with a richer understanding of the diversity of protein-protein binding mechanisms.

The findings by Lange *et al.* (3) also pose intriguing questions about the role of dynamics in protein evolution (17). Either the structural fluctuations of ubiquitin evolved to interact with various protein binding partners, or new binding interactions took advantage of the intrinsic protein dynamics. The second case would help facilitate new binding interactions without compromising the structural integrity and original function of the protein. Analysis of structural ensembles populated on time scales slower than molecular tumbling, as begun by Lange *et al.*, will lead to a better understand-

ing of evolution at the molecular level and may provide new approaches to protein engineering and drug design.

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DEVELOPMENTAL BIOLOGY

Sex and Poison in the Dark

Reinhard Fischer

A protein complex moves in and out of the nucleus in response to light, associating with proteins that control fungal development and metabolism.

Filamentous fungi are very successful organisms on our planet because of their metabolic versatility and potential to adapt to and survive extreme conditions. In this context, one important feature is their ability to produce different types of spores, for their dissemination in the environment and for resisting harsh conditions (1, 2). Another factor is their success in chemical warfare—fungi produce molecules that help them to compete with other microorganisms (2). The best-known of these compounds are antibiotics, which can benefit

one microorganism by inhibiting the growth of others. On the other hand, several other fungal metabolites, such as mycotoxins, cause millions of dollars in losses every year due to contaminated food and animal feed. If ingested by humans, mycotoxins, such as aflatoxin, may cause cancer or even death. Most interestingly, the phenomena of spore development and secondary metabolism are genetically linked (3). On page 1504 of this issue, Bayram *et al.* (4) unravel this association at a molecular level in the model fungus *Aspergillus nidulans* and show how this connection is controlled by light.

Most research with the filamentous fungus *A. nidulans* involves a strain in which the

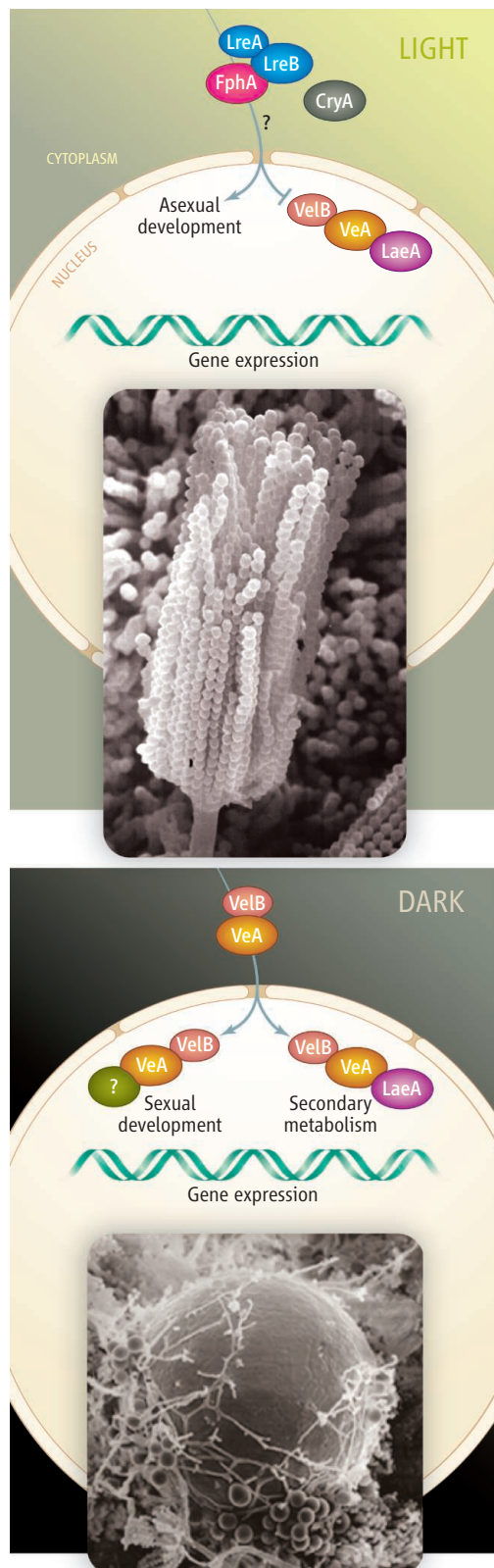
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gene encoding the light-responsive protein VeA is mutated (5). *A. nidulans* develops asexually in light and sexually in the dark, and a *veA* mutation causes a shift from sexual to asexual spore formation and renders asexual sporulation independent of light. Genetic data thus suggested that VeA regulates light-dependent development. In addition to this role, VeA controls secondary metabolism—the production of molecules that are not absolutely required for the survival of the organism (such as antibiotics and mycotoxins) (3, 6). For example, in the presence of light, *A. nidulans* produces less of the aflatoxin-related compound sterigmatocystin.

Orthologs of VeA have been characterized in several fungi, where the dual function in morphological and chemical differentiation appears to be conserved (7–9). Cloning of the *veA* gene reveals no indication that it encodes a transcriptional regulator or a light sensor (6, 10). However, VeA contains sequences for nuclear targeting and for fast protein turnover. VeA is found in both the cytoplasm and the nucleus, where it accumulates especially in the dark (11). Defects in the control of protein degradation impair the coordination of development and secondary metabolism in the presence or absence of light (12).

The genetic regulation of secondary metabolism is well studied in *A. nidulans*. Unlike most primary metabolism genes, genes encoding secondary metabolites are clustered in the genome (13). Expression of several of those gene clusters is coordinately regulated by a single protein, LaeA (14, 15). This global regulator is constitutively present in the nucleus and is presumably a methyltransferase, which modifies the chromatin structure of target gene clusters and activates their expression. The open question concerned how development and secondary metabolism are coupled and which role VeA and LaeA may play.

Bayram *et al.* have now solved this puzzle, showing that VeA forms a protein complex with VelB (a VeA-like protein) and LaeA. VeA and VelB appear to interact already in the cytoplasm and travel together into the nucleus to associate with LaeA. The trimeric protein complex was identified when *A. nidulans* was grown in the dark. Under light conditions, the VeA concentration in the cell was



Shifts in the light. Whereas asexual development of the fungus *A. nidulans* is stimulated by light, sexual development and secondary metabolite (mycotoxin) formation are repressed. The VeA-VelB protein complex plays a central role in transmitting the light signal to signaling pathways that control gene expression.

lower compared to that in the dark, and VeA interacted only with VelB (see the figure). Thus, the concentration of VeA in the nucleus appears to be one crucial parameter for secondary metabolite production and induction of the sexual developmental cycle. Because LaeA does not control sexual development, it is likely that other proteins are interacting with VeA and/or VelB to trigger this pathway.

This raises the question of how a light signal is transmitted to VeA. There are three possible upstream factors: a phytochrome, FphA; two blue-light receptor systems, LreA and LreB; and the cryptochrome, CryA (16–18). Although FphA interacts with VeA in the nucleus (16), a direct connection between any of the light regulators and LaeA has not been discovered through the biochemical approach of Bayram *et al.* This may indicate that interactions of VeA with light regulators are of a transient nature or that different protein interactions or protein complexes occur at different times in the cell. Whether and how the light regulators control the concentration or the activity of VeA is not yet known.

The challenge for future research will be to define specific functions for VeA and VelB in the discovered protein complex and to determine whether and how the light regulators are interlinked with the LaeA function. Insights into light signaling in *A. nidulans* may help to control mycotoxin formation or increase penicillin production.

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