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Powered by Gas—A Ligand for a Fruit Fly Nuclear Receptor

The difficulty in identifying ligands for nuclear hormone receptors remains an obstacle to understanding their function. For example, in the fruit fly *Drosophila melanogaster*, only one of its nuclear receptors has a known ligand. In this issue of *Cell*, Reinking et al. (2005) report that the fruit fly E75 nuclear receptor contains heme in its ligand binding pocket and that the oxidation state of this molecule controls E75 activity. They also show that E75-heme responds to the small diatomic gases, nitric oxide and carbon monoxide. This study sheds light on how heme, gas signaling, and nuclear receptors interact to control metabolic and developmental pathways.

Nuclear receptors are transcription factors that are regulated by their ligands. They act at the crossroads of key biological processes including embryonic development, maturation, aging, homeostasis, and metabolism. Nuclear receptors are defined by the presence of two functional domains, a highly conserved DNA binding domain that targets the receptor to specific response elements in the genome, and a ligand binding domain (LBD) that often interacts with small lipophilic compounds. Binding of ligand triggers an allosteric shift in LBD conformation that can lead to changes in the subcellular localization of nuclear receptors, their DNA binding affinity, their ability to dimerize, recruit cofactors, and their transcriptional activity. The ligands that regulate these nuclear-receptor activities include well-known hormones such as steroids, retinoic acid, and thyroid hormone. The realization that nuclear receptors are highly conserved through evolution has led to genomic definitions of this family, which includes 48 genes in humans, 284 genes in the nematode *Caenorhabditis elegans*, and 18 genes in *Drosophila* (Maglich et al., 2001). The rapid discovery of these receptors has paved the way for a new field of "reverse endocrinology" where orphan nuclear receptors are used to identify their cognate ligands and thus define new signaling pathways. Given the critical role that nuclear receptors play at the interface between chemical signaling and transcriptional control, identifying their ligands has become a central focus for the field of nuclear-receptor research.

During the past few years, crystallographic studies of nuclear-receptor LBDs have provided a major means of ligand identification, enabling the detection of small compounds buried within the lipophilic pocket. These "fortuitous ligands" copurify with the LBD and provide critical clues to natural ligands for the corresponding nuclear receptor. Many such studies have graced the pages of Cell Press journals. A paper in this issue of *Cell* by Reinking et al. (2005), however, reports the discovery of a ligand for the *Drosophila* E75 nuclear receptor and provides several new twists to the field of reverse endocrinology. The first surprise is the elegance of their discovery, depicted in the first figure of the paper as a photograph of a BioRad Econo-Column with the bright red E75-LBD purified from bacteria. The startling color led the authors to guess that this nuclear receptor might bind to a heme molecule (protoporphyrin IX bound to iron), a hypothesis they confirmed by biophysical techniques. They demonstrate a 1:1 stoichiometry for heme binding to E75 and a remarkable avidity. The investigators were unable to detect E75 protein in the absence of heme, and the levels of stable E75-LBD varied in parallel with the amount of heme, suggesting that this compound is required for E75 protein stability (Figure 1A). In an unprecedented step for nuclear-receptor ligand identification, the authors extended their studies to full-length E75 protein purified from SF9 and S2 insect cell lines as well as from developing fruit fly pupae. They demonstrated that E75 is always conjugated with heme in stoichiometric amounts. Like other heme-containing proteins, the heme group in E75 can be reversibly oxidized and reduced, leading the authors to propose that this nuclear receptor might act as a redox sensor. They show that the ability of E75 to interact with the AF2 domain of its regulatory partner, the DHR3 orphan nuclear receptor, is dependent upon the presence of a reduced Fe(II) heme group. This links the oxidative state of the E75-heme group to its functional interactions with DHR3 and thus the transcriptional activity of the DHR3-E75 complex (Figure 1B).

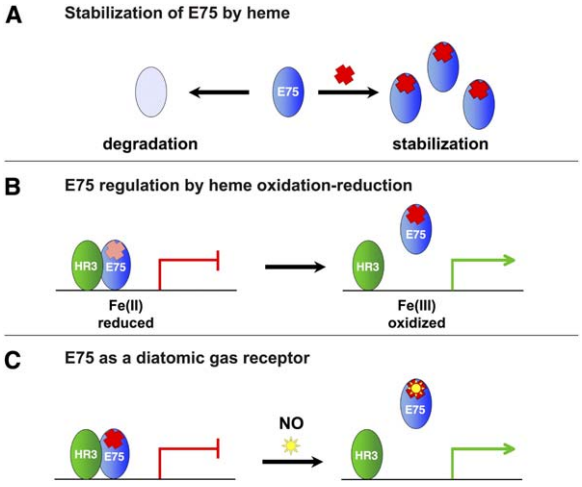

Figure 1. Three Ways to Regulate the Fruit Fly E75 Nuclear Receptor

(A) Binding of heme (red cross) stabilizes the E75 nuclear receptor (blue oval), resulting in an overall increase in E75 levels.

(B) Possible regulation of DHR3 and its repressive partner E75 based on the redox state of the heme group bound to E75.

(C) Possible regulation of E75 and DHR3 based on binding of E75 to the small diatomic gas, NO (yellow star). The red bars and green arrows represent repression or induction of target gene transcription, respectively.

A key property of heme-containing proteins is their ability to be regulated by direct binding of the small diatomic gases, carbon monoxide (CO) and nitric oxide (NO). These gases act as short-lived signaling molecules that are locally synthesized and diffuse freely between cells. Reinking et al. (2005) found that either CO or NO can bind to the reduced form of E75 and that binding of CO blocks the ability of E75 to bind to the AF2 domain of DHR3. They also show, at least for NO, that the dissociation of DHR3 and E75 has functional consequences because DHR3 can now revert to being an activator in the absence of its E75 repressive partner (White et al., 1997; Figure 1C).

A challenge for the future is to bridge the gap between these new biochemical discoveries and the biological functions of E75. Transcription of the *E75* gene is induced by ecdysteroids, and fly mutants lacking *E75* have reduced ecdysteroid titers. These mutant flies die primarily during larval stages with defects in molting, suggesting that E75 acts in a feed-forward pathway to control ecdysteroid biosynthesis or release (Bialecki et al., 2002). Interestingly, among its many regulatory functions, NO has been implicated in insect steroid biosynthesis, acting through its critical downstream effector, cGMP (Maniere et al., 2003). Null mutations that affect nitric-oxide synthase (NOS), the key enzyme for NO production, result in early larval lethality, although the phenotype of these larvae has not been described (Regulski et al., 2004). It will be interesting to determine if NOS mutations are associated with ecdysteroid deficiencies and if NOS alleles display genetic interactions with *E75* mutants. Another possible point where E75 and NOS could regulate molting is through ecdysis-triggering hormone (ETH), which is a key regulator of larval molting behavior that also exerts its effects through cGMP (Kingan et al., 1997). In addition, studies in the tobacco hornworm, *Manduca sexta*, have shown that ecdysteroids can reduce NO levels in neuronal cells during metamorphosis, attenuating their inhibition of neuronal cell proliferation (Champlin and Truman, 2000). It would be interesting to determine if there are similar effects in *Drosophila* and if *E75* mutations impact these pathways.

As the authors note, one possible role for heme in E75 signaling emerges from studies in the yellow fever mosquito, *Aedes aegypti*. A pulse of ecdysteroids in the female mosquito, in response to a blood meal, triggers a cascade of gene expression that ultimately controls oogenesis and yolk production. The *E75* gene is one of the primary ecdysteroid-induced genes in this pathway (Pierceall et al., 1999). Given that heme is a major byproduct from the blood meal, this system provides an ideal context for testing the hypothesis that heme levels, or the state of heme oxidation, might regulate E75 function.

Heme binding by E75 can be disrupted by mutating the two most highly conserved histidine residues in the LBD, residues that are also present in the vertebrate ortholog of E75, Rev-Erbα. Rev-Erbα interacts with the vertebrate ortholog of DHR3, RORα, raising the possibility that studies of these nuclear receptors in one system will have direct implications for how they operate in all higher organisms. Both Rev-Erbα and RORα are critical components of the mammalian circadian clock, an elegant molecular circuit in which NO, CO, and heme are key regulatory molecules (Pardee et al., 2004). It will be interesting to determine if E75 and DHR3 contribute to circadian cycling in *Drosophila* and, if so, whether this provides a context for studying how heme and diatomic gas signaling regulate E75 function. An experimental direction that we can expect to see in the near future is to test whether the Rev-Erbα LBD binds to heme, as predicted.

The Reinking et al. (2005) study adds nuclear receptors to the list of transcription factors that bind to heme and depend on this molecule for their regulatory activity. In addition, this is the first description of a nuclear receptor that can act as a redox sensor and participate in diatomic gas signaling. These discoveries provide a new context for thinking about nuclear-receptor regulation and set the stage for studies of E75 and DHR3, with direct implications for the regulation of their orthologous mammalian receptors.

Carl S. Thummel
Department of Human Genetics
Howard Hughes Medical Institute
University of Utah School of Medicine
Salt Lake City, Utah 84112

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