Conserved responses to oxygen deprivation

Commentary

See related article, pages 685–693.

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Genetically tractable model organisms such as the fruit fly offer powerful experimental systems that have illuminated conserved biological phenomena and so guided research in human biology. Still, researchers concerned with the pathologies of stroke and myocardial infarct might consider their fields beyond the reach of even the most fundamental discoveries that could be made in an insect. Recent work, including a paper appearing in this issue of the *JCI* (1), suggests otherwise.

Given the obvious anatomical differences between the nutrient and oxygen delivery systems of mammals and arthropods, the etiologies of stroke and myocardial infarct are unlikely to be mimicked in the fruit fly *Drosophila*. Still, the pathologies caused by an interruption in blood supply are fundamentally the result of a shortage of oxygen, and the cellular responses to hypoxia in an insect may well be relevant to normal and pathological responses to oxygen deprivation. Interestingly, many organisms tolerate hypoxia without the devastating damage incurred by human heart and brain tissue (2, 3). Turtles, for example, hibernate buried in mud, with little access to oxygen. We understand little of the differences among species regarding tolerance to hypoxia, but identification of the relevant parameters could aid clinical management of acute interruptions in blood flow.

However great the relevance of cellular adaptations to hypoxia to stroke or heart attack, *Drosophila* might not appear an obvious choice of system for studying these pathologies. However, those of us who witnessed how the powerful genetic strategies available in *Drosophila* pried open the profoundly difficult problem of embryonic patterning have become champions of this organism. Its attributes can be used to probe a wide range of problems. Still, a fly is a long way from a human. Or is it?

Unsuspected parallels in development

The genes that pattern the embryo of the fly have led us to homologous genes in vertebrates and the realization that these genes guide the development of a human embryo. For example, analysis of the *eyeless* gene of *Drosophila*, local expression of which directs the development of an eye, has led to the recognition of an entire cascade of regulatory molecules that controls development of both the fly eye and the morphologically very different vertebrate eye (4, 5). New studies on the development of oxygen delivery systems in fruit flies and in our own species have brought to light other intriguing parallels that may be more pertinent to the study of ischemia.

A branching tubular system of trachea delivers oxygen to the tissues of insects. Consistent with its roles in primary oxygen uptake and oxygen delivery to the periphery, the development of insect trachea shows parallels to the branching development of the mammalian lung and to angiogenesis. Mutations revealed that BRANCHLESS, a homolog of mammalian FGF, is required for tracheal cell migration and morphogenesis (6). A detailed and dynamic program of BRANCHLESS expression in tissues surrounding the tracheal primordium directs tracheal development in a stereotyped pattern of branches by acting on BREATHLESS, an FGF receptor homolog, expressed in tracheal cells. Similarly, mouse mutations have shown that FGF-10 is required for the development of the bronchial tree and the mammalian lung, as well as for the branching morphogenesis of other glands (7, 8). As has occurred in the study of eye development, I expect that the ongoing genetic dissection of branching morphogenesis in *Drosophila* will uncover conserved pathways that guide the analogous developmental events in mammals.

The cellular morphogenesis by which insect tracheal cells produce fine terminal branches resembles capillary formation by mammalian endothelial cells. Furthermore, like capillaries, the branching of terminal trachea is not stereotyped, but is regulated by the availability of oxygen (9). The extent of tracheal tube extension and arborization is induced by local tissue hypoxia. The FGF homolog BRANCHLESS is again an important mediator of this process. While we cannot yet assess the extent of the parallels, it is intriguing that FGF is also an important mediator of hypoxia-promoted angiogenesis in mammals. Hence, despite the extensive differences in their designs, the oxygen delivery systems of insects and mammals may well have evolved from a common primitive oxygen delivery system present in an evolutionary predecessor of both organisms.

Cellular response to oxygen deprivation

If parallels between organisms extend to the development of the diverse oxygen delivery systems, surely there will be analogies in the cellular responses to oxygen shortage. Cellular responses to oxygen levels probably evolved early, as a result of competition among microorganisms for available oxygen and the need to survive periods of oxygen shortage. Indeed, modern unicellular organisms possess sophisticated responses to oxygen levels. As with other useful mechanisms that must have appeared during premetazoan evolution, responses to oxygen deprivation may be widely conserved among the metazoan phyla. Recent work has taken us beyond this a priori argument by producing an example of a mechanistic parallel between hypoxic responses in flies and humans.

If *Drosophila* are suddenly made severely hypoxic, the embryos arrest, the larvae wander away from their food, and the adults fall over (10–13), but all survive transient hypoxia. Indeed, the embryos survive remarkably well if they are a few hours old (10, 11). Their spectacular tolerance to hypoxia (which probably contributes to survival in their natural setting, where embryos must compete for oxygen with the microbes

growing on rotting fruit) is associated with dramatic cellular responses. When embryos are made hypoxic, the cell cycle arrests within minutes, and development stops (10, 11, 13). Even after a week in the near absence of oxygen, arrested embryos recover and develop when oxygen is restored. This embryonic arrest and survival requires that hypoxia block a myriad of dynamic events synchronously, possibly by pathways analogous to those conferring hypoxia tolerance in other organisms. The special advantage of *Drosophila* is that it provides a powerful genetic system in which to dissect the mechanisms of these remarkably rapid responses.

In mammals, one of the rapid responses to local hypoxia is vasodilatation, a response driven by nitric oxide (NO), but there has been little consideration of the possible evolutionary origins of the use of NO as signal for hypoxia. A trail of unusual clues suggested that NO is also used as a signal for hypoxia in flies. Thus, *Drosophila* larvae, when confronted with hypoxic conditions, wander away from their food and become highly active for a few tens of minutes before turning sluggish and arresting movement (13). This hypoxia-induced wandering resembles a less dramatic roving behavior that is seen even under normal oxygen levels during feeding by larvae of certain wild-type *Drosophila* strains (termed "rovers"). Other strains ("sitters") remain in place during feeding, and Osborne et al. (14) identified a genetic polymorphism that apparently underlies this behavioral difference. Remarkably, sequence variation at a locus encoding protein kinase G (PKG) leads not only to the behavioral difference between the two strains, but also to a markedly weaker response to hypoxia by strains carrying the sitter variant (13).

Since PKG is involved in the response to NO in mammals, we tested whether NO might also be involved in the responses to hypoxia in *Drosophila*, and we found that induction of an NO synthase (NOS) transgene renders flies hypersensitive to a drop in O_2 level. Moreover, providing NO donor compounds in the presence of O_2 provokes behavioral, cell cycle, and developmental responses resembling those elicited by hypoxia (10, 13), whereas inhibitors of NO accumulation block or blunt some of the responses to hypoxia. We therefore concluded that, as in mammals, NO contributes to the response to hypoxia and might well be a central mediator of these responses (13). While much needs to be learned about the mechanism of NO involvement in the responses to hypoxia, it appears that genetic analysis of *Drosophila* offers the prospects of identifying genes contributing to adaptation to hypoxia in both flies and humans.

A genetic approach to hypoxia

In flies, the unbiased way to identify genes that direct an event is to screen for mutations crippled in the process of interest. The first such screen was carried out by Haddad et al. (12), and the first molecular characterization of a hypoxia-sensitive mutant introduces an unanticipated possibility. The identification of the affected gene implicated RNA editing, a mechanism that modifies decoding of genetic information, in neuronal function and perhaps in the response to hypoxia (1). While some of the weight of this finding had been preempted by Palladino et al. (15), who identified mutations in this same gene while pursuing a very different question, the finding nonetheless opens up numerous avenues of enquiry regarding both the function of RNA editing and its possible role in modifying responses to hypoxia. Before exploring these details, let me review the origin of the mutant.

Haddad and colleagues focused their screen on the X chromosome, because sex linkage facilitates the work by revealing the phenotypes of mutations as defective males (12). Following X-irradiation, they isolated four mutants that were slow to recover mobility following a 5-minute period of hypoxia. Whereas wild-type flies righted themselves in about 5 minutes, the more severely defective mutants took about 10 minutes. The four mutations defined three genes, *hypnos-1*, *-2*, and *-3*, *hypnos-2* being "hit" twice. This screen is not biased by prediction of candidate genes, but screens are usually limited in scope — in this case, limited to X-linked genes influencing rapid responses in the adult. Additionally, screens usually sweep in a relatively large panel of only peripherally relevant genes. For example, flies that are "lazy" or crippled might not begin moving as quickly as normal flies upon restoration of oxygen even if their attributes are constitutive rather than related to hypoxia. Nonetheless, buried in the outcome of such screens are surprises that can teach us how the organism copes with specific kinds of challenges.

A role for mRNA editing in neuronal hypoxia?

In this issue of the *JCI*, Haddad's group reports the molecular identification of the gene *hypnos-2* (1). Aided by new genomic tools and a lot of hard work, the authors showed that *hypnos-2* encodes *a*denosine *d*eaminase *a*cting on *R*NA (ADAR). The adenosine deaminase part of this name suggests that the product is a metabolic enzyme, but this enzyme has a renegade activity, which, if unrestrained, would wreak havoc in the orderly decoding of information by the classical pathway through which DNA makes RNA makes protein. By deaminating adenosine nucleotides within an RNA molecule to produce inosine (which for purposes of the translational machinery is equivalent to guanosine), this enzyme alters the information encoded in RNA. The havoc that would be produced by randomly changing adenosines is avoided by limiting the modification. ADAR specifically targets sites that are double-stranded, and its expression is largely restricted to the nervous system (1). Editing in vivo is also considerably less promiscuous than that seen with synthetic RNA sequences in vitro, although the basis of the greater specificity in the cell is not clear. The result is a limited editing of neuronal RNAs, with most of the known target mRNAs encoding ion channels. But why do flies edit neuronal transcripts, and what is the consequence of the failure to edit these transcripts?

It was these questions that motivated Palladino et al. (15) to take a directed approach to retrieve mutations in the *ADAR* gene.

One surprise from this work was that *Drosophila* live with a complete absence of ADAR function (15). However, ADAR-deficient flies do not live the good life, even without imposed hypoxia. The males fail to exhibit mating behavior, and flies of both sexes have defects in posture, mobility, and activity. All this is not surprising as their brains show extensive neurodegeneration. The ADAR-deficient flies also have the expected biochemical phenotype: they fail to edit sites in the RNAs encoding at least three channels, a voltage-gated Ca^{++} channel (CACOPHONY), a sodium channel (PARALYTIC), and a glutamate-gated Cl^- channel

(GluCl-α). It is inferred that the failure to edit one or several of the target genes has functional consequences that lead to neuronal degeneration and the behavioral defects.

What has the *hypnos-2* mutant of *Drosophila* told us about why transcripts are edited? Regardless of the uncertain etiology of the final phenotype, the findings show that the major roles of *hypnos-2* are in the nervous system. The neuronal specificity of the *hypnos-2* phenotype supports a recent model suggesting a function for the particular type of editing catalyzed by ADAR (16). There are several types of editing reactions that act in phylogenetically and physiologically diverse contexts, but the adenosine-to-inosine editing carried out by ADAR appears to be devoted largely to altering the functional diversity of neuronal ion channels. In mice (17), squid (18), and flies, ion-channel mRNAs are edited at multiple sites. In many cases, because these sites are modified at less than 100% efficiency, editing produces heterogeneity in the mRNA sequence and, ultimately, in the encoded channels. As a result of alternative editing and alternative splicing, a single gene can give rise to numerous channels differing in ion conductance or regulation.

It has been proposed that editing, by increasing the repertoire of channel functions, contributes to the diversity of neuronal types and to neuronal processing (16). However, because of the haphazard identification of targets of ADAR editing, the neuronal and ion channel specificity of ADAR was not on a firm footing until recently, when a beautiful genetic analysis in mice showed that a major role of ADAR2, one of three ADAR activities in this species, is satisfied by providing an already edited version of an AMPA receptor (17). While this supported the functional relevance of editing of this ion channel transcript, it did not test the roles of ADAR as a whole, because of the continued activity of ADAR1 and ADAR3. The phenotypes of the *Drosophila hypnos-2* mutations, which eliminate all ADAR in the fly, provide the first direct confirmation of the presumed neuronal specificity of the action of ADAR (15). It remains to be seen whether the defects can be directly attributed to a reduced diversity of channel types in the *hypnos-2* mutant.

What, then, is the connection between ADAR deficiency and tolerance to oxygen deprivation? Since the *hypnos-2* mutants have a phenotype in the absence of hypoxia, it is clear that ADAR function is not uniquely required for dealing with this class of stress. It is possible that its identification in the hypoxia screen was incidental and secondary to its compromised behavior. However, it is also possible that the diversity of channel types produced by editing might play particularly important roles in adaptation to stress. In addition, editing might influence the activity of a nonchannel gene important to hypoxia tolerance in the nervous system. While further study will show whether this gene has a central role in response to oxygen deprivation, careful characterization of this and other mutations with altered response to hypoxia will ultimately uncover the collection of genes and biochemical pathways by which cells respond to, adapt to, and survive hypoxia.

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