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Development and diabetes on the fly

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ABSTRACT

We review the use of a model organism to study the effects of a slow course, degenerative disease: namely, diabetes mellitus. Development and aging are biological phenomena entailing reproduction, growth, and differentiation, and then decline and progressive loss of functionality leading ultimately to failure and death. It occurs at all biological levels of organization, from molecular interactions to organismal well being and homeostasis. Yet very few models capable of addressing the different levels of complexity in these chronic, developmental phenomena are available to study, and model organisms are an exception and a welcome opportunity for these approaches. Genetic model organisms, like the common fruit fly, *Drosophila melanogaster*, offer the possibility of studying the panoply of life processes in normal and diseased states like diabetes mellitus, from a plethora of different perspectives. These long-term aspects are now beginning to be characterized.

1. Introduction

Diabetes mellitus, the "old" disease characterized by glucose present in urine (used as a litmus test to diagnose the ailment) led to wasting and death. Insulin was discovered in the early twentieth century in Canada by a group of researchers, including notably Banting and Best, and proved to be a successful treatment for diabetes mellitus (Roth et al., 2012). Mammals, particularly, are dependent on insulin secretion to metabolize the sugar glucose, and, ultimately, survival. Those whose insulin secretion was altered died quickly. Insulin injections allowed them to live and recover substantially. It became clear that insulin controlled blood sugar, and in more of a hazy way, nutrition and health (Najjar, 2001).

The immediate cellular effects of insulin have been worked out in the main: insulin binding to its cellular plasma membrane receptor triggers a signaling cascade that results in the glucose transporter Glut4 to be deployed at the plasma membrane from its location at intracellular vesicles, allowing glucose to be transported inside the cell. Insulin also favors the synthesis of glycogen, and prevents glycogen breakdown. This work established insulin as a critical anabolic hormone. It was thought that through its control of glucose transport and homeostasis, insulin somehow controls metabolism (Foley et al., 2011; Gonzalez-Sanchez and Serrano-Rios, 2007).

Diabetes mellitus was soon recognized as grouping, in reality, two very similar but not identical illnesses: diabetes mellitus type one, stemming from a lack of insulin production in the body, and diabetes mellitus type two, due to relative insensitivity of cells to insulin. The first type could strike and affect patients fairly quickly, but could be treated with daily insulin injections, while the second type normally had a slow evolution, typical of a degenerative disease, disrupting glucose metabolism and leading ultimately to several complications and death. This last type was normally associated with old age (Zaccardi et al., 2016).

From a biological point of view, aging can be defined as a deleterious, progressive, and intrinsic process inevitably happening to all living organisms resulting in a decline in physiological integrity and function, leading to a deterioration of homeostasis. Aging affects all biological levels of complexity, from molecular interactions, cellular functions, and tissue structure and function to organismal and systemic physiological well-being. Due to this decline, aging is the primary risk factor for major human pathologies such as cancer, type 2 diabetes, cardiovascular disorders, and neurodegenerative diseases (Lopez-Otin et al., 2013; Niccoli and Partridge, 2012).

The study of human aging and related diseases represents a formidable challenge for research due in part to the complexity of interrelated factors that play a role, with a strong influence of both genetics and environment, highly variable elements that many times are difficult if not downright uncontrollable for individual subjects. In recent decades, however, scientists have developed the use of model organisms such as the budding yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the mouse, and especially the fruit fly *Drosophila melanogaster*, to study the evolutionarily conserved genetic and biochemical processes influencing aging and disease (Alic et al., 2014b; Gems and Partridge, 2008; Gems and Partridge, 2013; Gems et al., 2002; Greer and Brunet, 2008b; Kenyon, 2010; Lapierre and Hansen, 2012; Vanhooren and Libert, 2013). These studies have played and continue to play a prominent role in the accruement of knowledge about the molecular, cellular, and organismal basis of aging and its

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consequences on healthy and diseased states. Yet much remains to be learned.

Studies in model organisms have made a novel and important connection between insulin aging and insulin signaling. The first model organism used in modern times for aging studies was the nematode worm *Caenorhabditis elegans*. Animals with viable mutations in genes that altered dauer formation were isolated and characterized. These mutants had an extended lifespan, and it turned out that these mutations were in fact in coding sequences for genes of the insulin pathway of the worm (Friedman and Johnson, 1988; Kenyon et al., 1993).

In particular, the formation of dauer larva, a facultative stage that develops in response to adverse environmental conditions, was key in elucidating the "new" role played by the insulin-signaling pathway. It became clear that besides very precise glucose homeostasis, especially after food ingestion, the insulin pathway had a prominent role in overall growth and proliferation. This role is a general feature of this evolutionarily conserved pathway, even in organisms with different blood sugar metabolism (Barbieri et al., 2003). It also meant that nutrition (or alterations of it) affect growth and lifespan, implicating the insulin pathway in nutritional state-dependent abnormal lifespans (Greer and Brunet, 2009). These insulin-signaling effects on lifespan and nutrition are evolutionarily conserved in many animal groups, including mammals (Barbieri et al., 2003).

Recently, the common fruit fly (*Drosophila melanogaster*) has emerged as an excellent genetic model system to study insulin signaling and diabetes. What justifies using *Drosophila* for insulin research? Firstly, *Drosophila* has many advantages compared to others models, like a short and well-defined life cycle with eating and 'fasting' periods, easy and inexpensive laboratory maintenance (Greenspan, n.d.), harmless to humans, farm animals and agriculture, and being a sophisticated multicellular eukaryote, with a sequenced genome annotated to a very high quality. In addition, fly repositories harbor mutant flies for almost every gene including insulin and insulin related ones, coupled with sophisticated genetic and molecular techniques, and a wealth of studies conducted over a vast range of topics for over one hundred years (Brookes, 2001; Greenspan, n.d.; Kohler, 1994). Secondly, flies like yeast, worms, and mammals have evolutionarily conserved genetic pathways and biochemical processes influencing growth, maturation, and nutrition, altering development, lifespan, and quality of life. Thirdly, flies possess a short lifespan of about sixty days, making possible the study of effects of genetic and/or environmental manipulations on development and growth throughout the life cycle in a relatively short time frame. The relevant literature emphasizing some of these aspects is very extensive and reflects the advantage of the aforementioned benefits (Alic et al., 2014a; Bjedov et al., 2010; Giannakou et al., 2007; Giannakou et al., 2004; Giannakou and Partridge, 2004; Giannakou and Partridge, 2007; He and Jasper, 2014; Padmanabha and Baker, n.d.; Toivonen and Partridge, 2009).

2. Development and diabetes

2.1. Diabetes on the fly

As stated, *Drosophila* has been instructive for studying age-associated diseases such as cancer, diabetes mellitus and neurodegenerative pathologies (Gonzalez, 2013; Jaiswal et al., 2012; Teleman et al., 2012). It has also been possible to model human diseases in the fly through genetic manipulations and/or modified environmental conditions. In particular, diabetic flies generally have been obtained by two main approaches: by rearing wild-type animals on a high-sugar and/or high fat diet (Birse et al., 2010; Diop et al., 2015; Morris et al., 2012; Musselman et al., 2011; Pasco and Leopold, 2012; Skorupa et al., 2008), or by genetic perturbations generating individuals with partial loss-of-function in the insulin pathway (IIS) genes, or associated target of rapamycin (TOR) genes (complete lack of function of central genes in this signaling cascade are embryonic lethal) (Bohni et al., 1999; Tatar et al., 2001). Both strategies result in hyperglycemic animals with insulin resistance and with an abnormal accumulation of fat and carbohydrates, the hallmarks of diabetes mellitus. These models include states equivalent to diabetes type 2, but also type 1, where, for example, ablation of insulin secreting cells can mimic type 1 diabetes (Broughton et al., 2005; Rulifson et al., 2002).

The IIS/TOR pathway is conserved in *Drosophila* (Fig. 1). Molecular and genetic studies have demonstrated that insulin signaling is highly conserved, both structurally and functionally. The fly genome encodes eight insulin-like peptides (known as dilp or Ilp 1–8), homologs of mammalian insulin and insulin-like growth factors (Brogiolo et al., 2001; Ikeya et al., 2002; Nassel and Broeck, 2016). Mainly secreted from a small group of neurons in the brain (the insulin producing cells, or IPCs (Broughton et al., 2005; Cao and Brown, 2001)) and transported via the hemolymph to cells, dilps 1–7 bind to and activate the insulin receptor (InR) (Fernandez et al., 1995). dilp8 is somewhat divergent from the other Ilps, encoding an insulin-like relaxin peptide (Colombani et al., 2012; Garelli et al., 2012) that regulates growth asymmetries and maturation binding to Lgr3, a relaxin-type membrane receptor (Colombani et al., 2015; Vallejo et al., 2015).

The activated InR autophosphorylates, allowing the binding and phosphorylation of Insulin Receptor Substrate (IRS)-like proteins Chico and Lnk (Almudi et al., 2013; Bohni et al., 1999; Slack et al., 2010; Werz et al., 2009). The fly phophatidylinositol-3kinase (PI3K) homolog then binds Chico and the InR. The PI3K catalytic subunit (Dp110) phosphorylates the membrane lipid phosphatidylinositol (4,5) bisphosphate (PIP2) to produce phosphatidylinositol triphosphate (PIP3) (Leevers et al., 1996). The Susi protein negatively regulates PI3K by binding to the p60 PI3K regulatory subunit, keeping PI3K activity in check in a circadian fashion (Wittwer et al., 2005). PTEN also counters PI3K activity (Huang et al., 1999). PI3K produced by PIP3 recruits the PDK1 and Akt kinases to the membrane (Leevers et al., 1996; Rintelen et al., 2001; Verdu et al., 1999). Activated Akt regulates growth and metabolism via diverse protein targets (Scanga et al., 2000; Verdu et al., 1999), including Tsc1 and 2 (Potter et al., 2002; Schleich and Teleman, 2009), Rheb GTPase, d4E-BP, and the TOR, S6K, GSK-3 kinases (Miron et al., 2003; Montagne et al., 1999; Saucedo et al., 2003), as well as the transcription factor FOXO (Junger et al., 2003; Kramer et al., 2003). FOXO, a forkhead class of transcription factor, counters insulin signaling, thought of as a main catabolism-promoting element (Alic et al., 2014a; Combettes-Souverain and Issad, 1998; Garofalo, 2002; Taniguchi et al., 2006; Teleman, 2010). There is also crosstalk between pathways; one such example is the *Drosophila* phospholipase C gene *smallwing* (Murillo-Maldonado et al., 2011b).

In flies with partial loss- or gain-of-function of IIS or TOR genes, others and we have demonstrated altered metabolism and growth. Beginning with pioneering studies, the effects of disrupted insulin signaling at all life stages in the fly starting from growth defects at embryogenesis have been looked at (Bohni et al., 1999). Intriguingly, cells, tissues, organs, and even whole organisms show an independent requirement of the insulin pathway for normal growth (cell, tissue, organ or organismal size) and proliferation (cell number) (Huang et al., 1999; Junger et al., 2003; Montagne et al., 1999; Oldham et al., 2000; Oldham and Hafen, 2003). Mutant flies also have impaired brain and retinal functions mimicking some aspects of human diabetic complications, like retinopathy and neuropathy (Murillo-Maldonado et al., 2011a).

2.2. Insulin signaling and development

Some genes change expression patterns and levels during development and adult life, and may serve as markers of aging. Previously, work done identifying expression patterns using enhancer trap technology revealed dramatic expression changes taking place during aging in an adult structure (the antennae), and opened the field for studies of