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Insects: Friends, foes, and models/*Insectes : amis, ennemis et modèles*

Talk summaries

Résumés des interventions

Session I. Insects as model organisms in biology

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How flies contribute to the discovery of human diseases and their pathogenic mechanisms

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An area of research that has developed rapidly in the past five years is the use of *Drosophila* to facilitate the diagnoses of rare human diseases. The technical developments in sequencing human genomes using whole exome (WES) or whole genome sequencing (WGS) have completely changed the landscape of human genetics. It is estimated that there are approximately 30 million people in the US and 400 million worldwide with rare diseases. Many of the patients who have these diseases undergo a diagnostic odyssey and remain undiagnosed for many years. By sequencing their genomes and those of their relatives we can now discover genetic variations in patients that are rare or ultrarare. However, considering that there are many polymorphisms in the population, assessing which variant(s) is/are related to the observed symptoms has remained a challenge. This is precisely where model organisms can contribute and *Drosophila* is now playing a prominent role in this strategy. Although there are nearly 4,000 genes annotated in OMIM, the pace of gene discovery has not shown any evidence of slowing down in the past few years.

The NIH launched the Undiagnosed Diseases Network (UDN) four years ago. Its goal is to enroll patients with undiagnosed diseases and try to determine their causes, which are typically genetic in origin. The patients who are enrolled are the most challenging cases in medicine, and 12 clinical sites, a sequencing center, a metabolomics core and two Model Organisms Screening Centers (MOSC) are supported to develop diagnoses. To date, over 3,000 patients have applied and about 30% of the accepted patients have been diagnosed by combining clinical phenotyping, WES or WGS of the proband and 2–3 members of the family, and performing functional studies in a model organism. The MOSC *Drosophila* Core at Baylor College of Medicine (BCM) has played a prominent role in this venture and has provided critical data for many human genetic diseases [1]. The MOSC at BCM has also collaborated with the Center for Mendelian Genomics to assess variant function using *Drosophila* and facilitate the discovery of new human disease-causing genes.

The first project discussed in this presentation was driven by WES of a patient and his parents and the discovery of a *de novo* point mutation in *ACOX 1* (*Acyl-CoA oxidase 1*). *ACOX1* encodes the rate-limiting enzyme in very-long-chain fatty acid (VLCFA) β -oxidation, and produces H_2O_2 in peroxisomes [2]. The proband was diagnosed at age 11 and now uses a wheelchair at age 18. Another patient with the same point mutation was diagnosed at age 9 and is now comatose at age 15. By studying the function of the *ACOX1* gene in flies using strains developed in my lab, we showed that homozygous loss-of-function mutations cause lethality and an autoimmune phenotype in which the animals activate their cellular and humoral immune responses. A comparison with the vertebrate literature suggests that autoimmunity may also be the main reason infants with an *ACOX1* deficiency die at a very young age, prior to the age of 5.

We then created flies with the patient's point mutation and observed a very different phenotype. Flies eclosed and died within one or two days but were able to survive, fly, mate, and live for extended periods of time when kept on food with a potent antioxidant, *N*-acetylcysteine. This is consistent with the fact that ACOX1 produces ROS in peroxisomes and that the point mutation is a gain-of-function mutation. However, we were surprised by the potency of the suppression. In addition, just expressing the variant in *Drosophila* wrapping glia but not any other tissue, is sufficient to kill animals, showing that the toxicity is mainly due to an expression of the mutant protein in glial cells. Importantly, expressing catalase in glia suppresses this lethality. We were therefore able to demonstrate that fly *ACOX1* is indeed abundantly expressed and mostly restricted to wrapping glia.

We then turned to rats and showed that *ACOX1* is abundantly expressed in Schwann cells (not in neurons). In addition, expression of the patient *de novo* mutation kills cultured Schwann cells and the antioxidant AD4 fully suppresses cell

death. Finally, nerve biopsies of the patient, who suffered severe axonal loss, showed that surviving Schwann cells are morphologically (TEM) and functionally aberrant (electrophysiology). This prompted the physicians to provide the patient with high doses of *N*-acetylcysteine and this new treatment is associated with a clear improvement in motility.

A second project was driven by a forward mutagenesis screen in *Drosophila* in which we previously identified variants in the fly *Ankle2* gene. We discovered that loss-of-function mutations of the human ortholog of ANKLE2 cause microcephaly in a child [3]. We then discovered, in collaboration with Nevan Krogan and Priya Shah, that a Zika virus protein, NS4A, interacts with and inhibits Ankle2 [4,5]. Our studies in *Drosophila* combined with human genetic studies allowed us to uncover a pathway that implicates several genes associated with primary microcephaly in human.

We identified five additional microcephaly patients with variants in *ANKLE2* and showed that these variants act as loss-of-function alleles in flies. Our data show that Ankle2 is an ER-localized protein essential for proper ER and nuclear envelope structure. Mutations in *Ankle2* affect cell division, spindle alignment, and localization of asymmetric determinants including the proteins of the Par complex. Ankle2 strongly interacts with the nuclear kinase Ballchen, the homolog of human VRK1, an established microcephaly locus. *Ankle2* mutants fail to maintain Ballchen/VRK1 in the nucleus, and we propose that this leads to a "gain-of-function" phenotype where Ballchen/VRK1 can ectopically interact with targets that it normally does not interact with. This results in severely reduced aPKC phosphorylation, which has previously been shown to have reduced kinase activity. The Ankle2 pathway also physically and genetically interacts with L(2)gl, an inhibitor of aPKC activity, which is consistent with the observed aPKC defects noted in *Ankle2* mutants. Finally, we show that expression of NS4A, which binds to and inhibits the function of Ankle2, phenocopies *Ankle2* mutant defects in neuroblasts, and these defects can be rescued by modulation of the Ankle2 pathway [3] (Link et al., 2019).

Our work highlights an important pathway required for proper human brain development: ANKLE2 and VRK1 are both associated with microcephaly; a member of the Par complex has been linked to brain defects in mice; and LLGL1, the homolog of L(2)gl, maps to the critical region of Smith Magenis Syndrome, a disease associated with microcephaly. Furthermore, we identified many novel variants associated with microcephaly in many of the human homologues of the fly genes described in this study.

Disclosure of interest The author declares that he has no competing interest.

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Drosophila research: From the genome to the proteome

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Starting from 1900, *Drosophila melanogaster* has been studied in the laboratory by scientists interested in many different aspects of animal development, physiology, and evolution. Over much of this more than hundred year period, genetic analyses have been at the basis of most studies, also those who led to the Nobel prices attributed to scientists working with *Drosophila*. More recently, fluorescent proteins and optogenetic tools have been added to the ever-expanding genetic toolbox allowing for a better understanding of basic cellular processes underlying complex developmental processes.

Even more recently, a novel approach is being added to the toolbox. Small protein binders can be used to directly target and manipulate proteins in their native environment, in cells of the living organism. The development of numerous antibody- and non-antibody-based scaffolds of protein binders (Fig. 1) has allowed the rapid identification of such small binding domain, recognizing virtually any target protein of interest. Such binding molecules can then be functionalized in many different ways, allowing for acute and direct protein manipulation

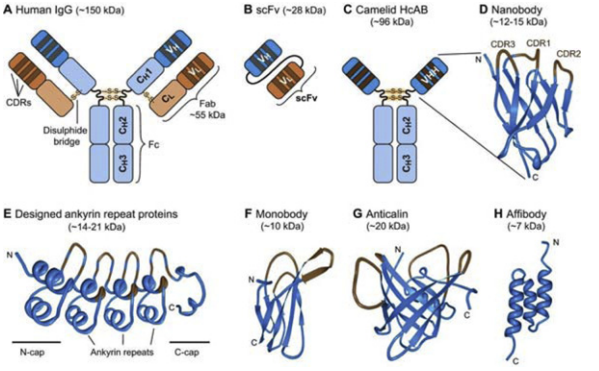

Fig. 1 A–H: Different protein binder scaffolds that can be used in developmental biology studies (see [1]).

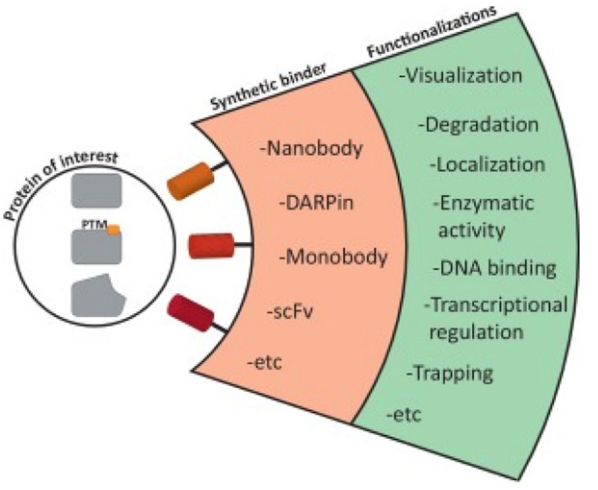

Fig. 2 Protein binders can be expressed as functionalized proteins in vivo (see [2]).