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SPOTLIGHT

Planar cell polarity: moving from single cells to tissue-scale biology

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ABSTRACT

Planar cell polarity (PCP) reflects cellular orientation within the plane of an epithelium. PCP is crucial during many biological patterning processes and for organ function. It is omnipresent, from convergent-extension mechanisms during early development through to terminal organogenesis, and it regulates many aspects of cell positioning and orientation during tissue morphogenesis, organ development and homeostasis. Suzanne Eaton used the power of *Drosophila* as a model system to study PCP, but her vision of, and impact on, PCP studies in flies translates to all animal models. As I highlight here, Suzanne's incorporation of quantitative biophysical studies of whole tissues, integrated with the detailed cell biology of PCP phenomena, completely changed how the field studies this intriguing feature. Moreover, Suzanne's impact on ongoing and future PCP studies is fundamental, long-lasting and transformative.

KEY WORDS: *Drosophila*, PCP, Cell mechanics, Cell polarity

Introduction

During developmental patterning and organogenesis, all tissues need to attain specific, characteristic shapes and sizes. While cell proliferation rates and cell fate induction events contribute to the coarse framework, or 'backbone', of a tissue or organ, the finer aspects, including cell positioning, polarity and shape, ultimately define not only the characteristics of a tissue and organ, but also its functionality. Mechanisms of planar cell polarity (PCP) establishment regulate and guide these latter aspects of most, if not all, events in tissue development and organ morphogenesis (Adler, 2012; Goodrich and Strutt, 2011; Humphries and Mlodzik, 2018; Tada and Heisenberg, 2012). The genetics of PCP establishment in *Drosophila* (originally called 'tissue polarity' in flies) paved the way for the discovery of core players of the process and their molecular definition (Adler, 2012; Goodrich and Strutt, 2011; Humphries and Mlodzik, 2018; Tada and Heisenberg, 2012). However, it was the addition of large-scale biophysical analyses of PCP phenomena at the tissue level, and the integration of these insights with other aspects of cell and epithelial biological behavior – as pioneered and established by Suzanne Eaton (1959-2019) – that put PCP firmly on the cell biological map in development and disease. Throughout Suzanne's career, she pushed technological boundaries and innovative thinking, including quantitative analyses and descriptions of cellular features and dynamics, and so generated novel and unexpected cell biological insights at the level of whole tissue morphogenesis. This Spotlight article is a personal view and remembrance of how Suzanne's thinking and work shaped and transformed the PCP field.

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Planar cell polarity as a cell biological feature

In its early days, PCP was regarded as a rather peculiar – yet fascinating – biological phenomenon, largely observed in insects (Gubb and García-Bellido, 1982; Lawrence et al., 1972). It was most obvious in features of the insect cuticle, the exoskeleton, as sensory organs embedded within it and all cuticle cells displayed a specific orientation, either along the anterior-posterior axis on the body wall or along the proximo-distal axis in appendages (Adler, 2012; Goodrich and Strutt, 2011; Humphries and Mlodzik, 2018; Tada and Heisenberg, 2012). Suzanne entered the field as a postdoctoral fellow at EMBL (working with Kai Simons), at a time when my lab was becoming interested in PCP as well. Whereas most of us in the (then rather small) field were looking for molecular insight through forward genetics, Suzanne focused on it as a 'cell biological problem'. With the hypothesis that it must be a cytoskeleton- and actin-mediated cellular feature, she dived right into it with a systematic analysis of how actin regulators of the Rac/Cdc42 GTPase subfamily affect the formation of polarized cellular hair structures in *Drosophila*. Her work defined different roles for Rac1 and Cdc42 during larval epithelial morphogenesis (Eaton et al., 1995) and, subsequently, distinct requirements for them during PCP-based actin hair outgrowth, which is promoted by Cdc42 and restricted by Rac1 (Eaton et al., 1996). By that time, Suzanne's work had already revealed that: (1) there is a link between PCP-based actin features, junctional integrity and cell shape, with junctional proteins reorganizing on the proximal and distal cell edges; and (2) there is a connection between junctional actin and an intricate microtubule network, in which apical junctions and microtubules are essential for structural aspects of actin-hair formation and outgrowth, with apical, proximo-distally oriented microtubules elongating to reach into the emerging wing hair (Eaton et al., 1996). Both of these observations (reviewed by Eaton, 1997) then guided – and still do today – how the field approaches PCP links to cytoskeletal elements in tissues of interest.

Although the involvement of microtubules and actin seems obvious in hindsight, this was not the case at the time, and Suzanne's work opened up many new ways to think about PCP in general and to refocus efforts on the cell biology of the process. For example, immediately subsequent to these seminal publications in the mid-1990s, much work from other labs was built upon Suzanne's observations, actively looking for links between the then known Frizzled (Fz)/core PCP factors and regulatory inputs into cytoskeletal elements. The characterization of a direct Fz-Dishevelled (Dsh) signaling link to actin regulators that followed in *Drosophila* (e.g. Boutros et al., 1998; Winter et al., 2001) or in vertebrates (e.g. Habas et al., 2001; to list just a few examples) was a direct consequence of Suzanne's work and led to a thorough review of the matter (Eaton, 1997). Similarly, although this information was overlooked for a few years, Suzanne's description of microtubule extensions into growing cellular hairs was ground-breaking and culminated with the recognition that PCP positions cilia in vertebrate cells (Wallingford, 2010; Carvajal-Gonzalez et al., 2016a) and that centrioles are positioned at the base of each

Development (2020) 147, dev186346. doi:10.1242/dev.186346

actin-based hair in the epithelial cuticle cells as an evolutionarily conserved feature of PCP establishment (Carvajal-Gonzalez et al., 2016b).

Establishing a planar cell polarity framework

A senior scholar at UC Berkeley once told me that most scientific fields have three phases: (1) an 'obscure' phase, when nobody knows or cares about it; (2) a 'heroic' phase, when it hits mainstream and many factors are discovered and pathways assembled; and (3) an 'academic' phase, when the big picture emerges and starts to make sense. This third (academic) phase is often characterized by incremental gains and detailed insights. Strikingly, Suzanne ended the obscure phase of PCP studies with her ground-breaking work mentioned above and pushed the field into the heroic phase. Similarly, Suzanne opened the door to the academic phase, possibly earlier than would have been reached otherwise. Most importantly though, the academic PCP phase, as established by Suzanne, was highlighted by anything but incremental gains, thanks to her transformative approaches and studies, applying quantitative biophysical approaches at the tissue level, yet not losing sight of individual cells. Indeed, the academic phase represented leaps of knowledge (discussed below).

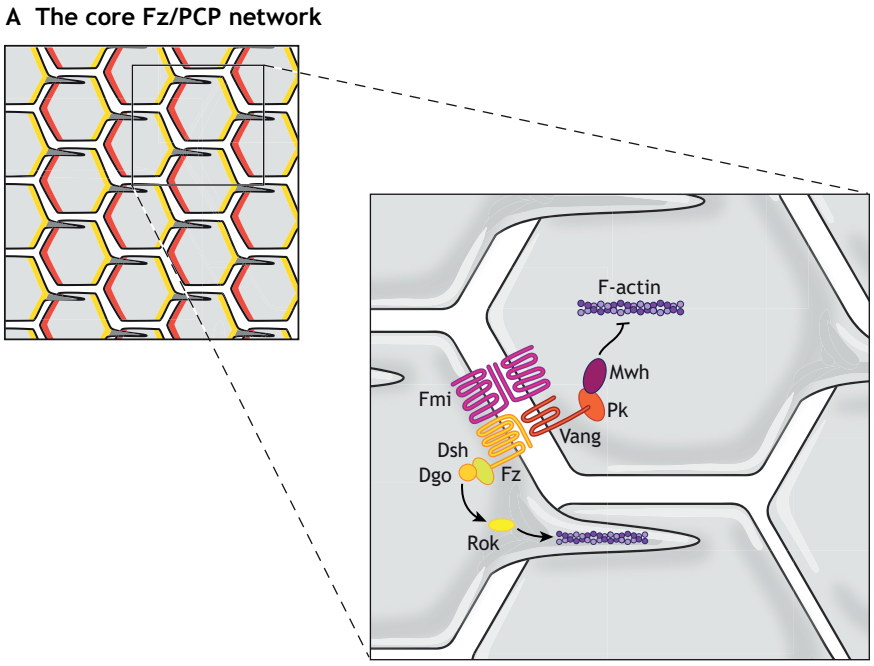

Fig. 1. The molecular logic and temporal progression of PCP establishment in the *Drosophila* wing blade epithelium. (A) Simplified schematic of the core Fz/PCP network that establishes planar polarity in wing cells and subsequently instructs formation of the actin-based hair that is characteristic of each cuticular cell. For simplicity, microtubules and additional regulators are not included. Suzanne's work helped to identify core factors (e.g. Dgo) and establish a link to cytoskeletal regulation. (B) Schematics of polarity establishment in the *Drosophila* wing. At early stages, cells are oriented towards the wing margin (top). Cellular flows and the rotation of wing blade cells, generated by the contraction of cells in the hinge region, then reorient the PCP pattern (middle). This eventually leads to the pattern of cell polarity observed in the mature, adult wing, which is aligned along the proximo-distal axis of the wing (bottom). h APF, hours after puparium formation. Schematized drawings are based on the original data figures in Aigouy et al. (2010) (J. Gregory © 2020 Mount Sinai Health System).

Development (2020) 147, dev186346. doi:10.1242/dev.186346

Suzanne also played a key part during the heroic phase, allowing the field to assemble pathway logic and discover key factors. Her approach to this was guided by the insight that many core PCP factors cause defects, even similar defects to loss-of-function mutants, when too much of them was present in any given cell. This was because tightly controlled subcellular localization and regulation could be overwhelmed by an excess of the protein in question. Hence, a gain-of-function Gal4-EP line screen (Rørth et al., 1998) seemed the most promising approach to interrogate this issue further. As Suzanne and I were lab neighbors at EMBL then, we joined forces and screened together, and this led to the identification of several additional factors, such as the core factor Diego (Feiguin et al., 2001; Das et al., 2004), also known as Inversin/Diversin in vertebrates, and other signaling pathway components (Hannus et al., 2002; Paricio et al., 1999). These screens, together with knowledge about known PCP factors and components, helped to define the core Fz/PCP factor network and pathway (Adler, 2012; Goodrich and Strutt, 2011; Humphries and Mlodzik, 2018; Tada and Heisenberg, 2012) (Fig. 1A).

PCP studies at the organismal and tissue level

Despite these insights and additions to the PCP network, Suzanne's real interest was always at the level of whole tissues and processes. Along these lines, I remember her saying back at EMBL that she was interested in 'studying processes not genes'. The natural progression was to apply this to PCP studies, and her approach transformed the field to this day.

Suzanne's parallel interest in tissue mechanics [see accompanying article by Dahmann and Classen (2020)], which was reinforced by her collaboration and scientific partnership with Frank Jülicher after her move to the Max Planck Institute in Dresden, guided this next phase of her efforts in dissecting PCP. Her vision led to a truly transformative approach of how PCP should be studied, and it profoundly changed the field and still has a huge impact today. The initial combination of her work on tissue mechanics and PCP was guided by her early observation that PCP, via actin, affects junctional stability and remodeling (Eaton et al., 1996). The work of Classen and Eaton (Classen et al., 2005) then demonstrated that the mature hexagonal cell packing, as seen in adult *Drosophila* wings, is generated from irregularly arranged epithelial cells during the time of PCP core factor interactions (just prior to cellular hair formation). The PCP core factors orchestrate directed junctional growth and shrinkage, and define cell neighbor exchanges via cadherin endocytosis. As such, hexagonal packing depends directly on the function of core Fz/PCP factors and their effects on the trafficking of cadherin-containing vesicles during junctional remodeling (Classen et al., 2005); this was an observation that also suggested this is likely a conserved and common function of the core Fz/PCP factor network.

Partnering with Frank Jülicher, and applying increasing levels of high-content quantitative biophysical analyses to studies of *Drosophila* wing PCP establishment, together with theoretical modeling, Suzanne subsequently made several paradigm-shifting discoveries. Establishing the vertex model as a 'tissue patterning analysis' resource (Farhadifar et al., 2007) allowed the next level of PCP patterning in the *Drosophila* wing epithelium to be dissected. Through elegant *in vivo* live-imaging studies and associated segmentation and quantitative biophysical data acquisition, Suzanne's lab showed that PCP is established much earlier than the field anticipated (Aigouy et al., 2010) (see also Classen et al., 2005; Sagner et al., 2012) and, strikingly, that the final proximo-distal orientation of cells as seen in the adult wing blade depends largely on the cellular behavior and biophysical properties of the contracting hinge, generating anisotropic tension on the wing blade cells (Aigouy et al., 2010). This effect causes very precise patterns of oriented cell elongation and rearrangement, which realign PCP to the proximo-distal axis (Fig. 1B). Suzanne's work was eye opening, revealing a whole new way of thinking about how polarity is generated and maintained – with it being both transient and dynamic. It also strikingly highlighted that tissue tension serves as a global PCP orientation cue.

Importantly, the Aigouy et al. (2010) study also added another paradigm-shifting insight. Suzanne's work revealed that hinge contraction is regulated by Dachsous (Ds), which, together with Fat and associated factors, works with the core Fz/PCP network (reviewed by Lawrence et al., 2007). This thus connected the Fat/Ds PCP system to the core Fz/PCP network in the developing wing. The study by Aigouy et al. (2010) demonstrated that Ds is essential for the anisotropic tension that re-orients the core PCP factor polarity and, importantly, that severing the wing from the hinge causes 'PCP defects' that are very similar to those seen in *ds* mutants. This insight also established that the Fat/Ds system might act in PCP via its tension-sensing features (reviewed by Grusche et al., 2010) and thus defects in Fat/Ds system mutants could 'collaborate' with and impact core Fz/PCP factor orientation via cellular tension. These studies were corroborated by an exciting follow-up paper addressing the coordination of cellular orientation by the Fat/Ds and core Fz/PCP systems (Merkel et al., 2014). Although the Aigouy et al. (2010) paper also influenced several recent papers by Suzanne addressing tissue level morphogenesis in general [see accompanying article by Dahmann and Classen (2020)], it remains to date the most important publication in PCP establishment across many species.

A personal perspective and Suzanne's legacy

It was my privilege and good fortune to have known Suzanne and to have had the opportunity to interact with her both during our overlapping time as postdocs [at UCSF and Berkeley, respectively, where I gladly shared enhancer trap lines with her when she was looking for compartment-specific gene expression, which led to her long-standing interest in Hedgehog signaling; see accompanying article by Prince et al. (2020)] and at EMBL, where we both started our independent careers and in part collaborated. Suzanne's vision of fascinating scientific problems and her dedication and uncompromising approaches to shedding light on these problems has always been personally inspirational. Her published work in the PCP field, and any field she worked in, has been trailblazing, transformative and inspirational to all colleagues exposed to it, and she always pushed the field – and all of us – to look past the obvious. With her detail-oriented curiosity, she opened doors that most of us did not know existed.

This article is part of a collection that commemorates the work of Suzanne Eaton. See also Dahmann and Classen (2020), Palm and Rodenfels (2020) and Prince et al. (2020) in this issue.

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Competing interests

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