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Determination of mechanical stress distribution in *Drosophila* wing discs using photoelasticity

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ABSTRACT

Morphogenesis, the process by which all complex biological structures are formed, is driven by an intricate interplay between genes, growth, as well as intra- and intercellular forces. While the expression of different genes changes the mechanical properties and shapes of cells, growth exerts forces in response to which tissues, organs and more complex structures are shaped. This is exemplified by a number of recent findings for instance in meristem formation in Arabidopsis and tracheal tube formation in Drosophila. However, growth not only generates forces, mechanical forces can also have an effect on growth rates, as is seen in mammalian tissues or bone growth. In fact, mechanical forces can influence the expression levels of patterning genes, allowing control of morphogenesis via mechanical feedback. In order to study the connections between mechanical stress, growth control and morphogenesis, information about the distribution of stress in a tissue is invaluable. Here, we applied stress-birefringence to the wing imaginal disc of *Drosophila melanogaster*, a commonly used model system for organ growth and patterning, in order to assess the stress distribution present in this tissue. For this purpose, stress-related differences in retardance are measured using a custom-built optical set-up. Applying this method, we found that the stresses are inhomogeneously distributed in the wing disc, with maximum compression in the centre of the wing pouch. This compression increases with wing disc size, showing that mechanical forces vary with the age of the tissue. These results are discussed in light of recent models proposing mechanical regulation of wing disc growth.

1. Introduction

As was recognized by D'Arcy Thompson as early as the beginning of the 20th century, mechanical forces play a fundamental role during morphogenesis (Thompson, 1917). However, the interplay between growth, mechanical forces and morphogenesis is much more intricate than he assumed. Expression levels of genes following a developmental programme determine the makeup and thus the mechanical properties of cells, thereby enabling them to exert the mechanical forces needed to collectively form spatial structures (Caussinus et al., 2008; Oster et al., 1983). These mechanical forces have in turn been shown to regulate gene expression and growth (Hamant et al., 2008; Ingber, 2006; Farge, 2003; Kahn et al., 2009). This enables the control of growth and patterning via feedback loops involving

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mechanical forces present in tissues, whereby non-uniform growth could relate back to itself via the development of mechanical stresses (Shraiman, 2005). This raises the experimental question of assessing the mechanical stresses in developing tissues or growing organs in order to correlate the stresses present with morphological changes and growth control. So far this has only been done indirectly, either by disturbing the tissues using laser ablation and studying the ensuing relaxations (Hamant et al., 2008; Caussinus et al., 2008) or by quantifying the strains present in the tissue by investigating the shape of the cells (Gorfinkiel et al., 2009).

A commonly studied model system for both growth and patterning is the wing imaginal disc of *Drosophila melanogaster*. A major morphogen and growth regulator in the wing disc is Decapentaplegic (Dpp), which is produced along the antero-posterior boundary and forms a gradient (Burke and Basler, 1996; Lecuit et al., 1996; Entchev et al., 2000; Teleman and Cohen, 2000). However, paradoxically, growth is uniform in the presence of this growth factor gradient (Milan et al., 1996), but becomes non-uniform when Dpp is expressed uniformly. In fact, in the latter case, growth rates are increased laterally and decreased medially (Rogulja and Irvine, 2005; Schwank et al., 2008). In light of the results in other organisms and the difficulties of purely morphogen-based approaches, models involving growth regulation via mechanical forces have recently been proposed for the wing disc (Shraiman, 2005; Hufnagel et al., 2007; Aegerter-Wilmsen et al., 2007).

Here, we employed photoelasticity on the tissue of the wing disc itself to determine the distribution of stress and its change during disc growth. The application of a mechanical stress to birefringent molecules leads to their orientation and stretching, subsequently resulting in a measurable change in birefringence, which is proportional to the applied stress. This change in birefringence corresponds to a change in the retardance of the light passing through the tissue (Majmudar and Behringer, 2005). We measured this retardance using a sensitive optical setup and hence studied the spatial distribution of stresses in the wing imaginal disc. Furthermore, in order to ascertain that a photoelastic response was measured as opposed to merely a change in material density or thickness, we relieved stress by incisions and applied stress by stretching. This lead to a corresponding change in retardance, thus showing that birefringence can indeed be used to measure stresses in biological tissues.

In the case of wing discs, we found that there is an inhomogeneous distribution of mechanical stress with high compression in the centre of the disc. This compression increases with increasing disc size, showing that stress increases during growth and could thus play a part as regulators of growth. These findings are compared in more detail to recent models of growth regulation via mechanical forces, with which they are in good agreement. Finally, we observed that compression is also present in the centres of other types of imaginal discs, offering the possibility of a common size regulatory mechanism.

2. Results and discussion

The spatial distribution of the retardance in a third instar wild-type wing disc is shown in Fig. 1a. The colourmap describes the magnitude of the retardance relative to that of insect Ringer's solution (IRS) of the same thickness. As can be seen in Fig. 1a, as well as Figs. 3 a-c below, the distribution of retardance in a wild-type wing disc has a characteristic pattern. There is a circular region of higher birefringence in the area of the wing pouch with a distinct maximum in the centre, while the values in the rest of the wing disc are nearly constant.

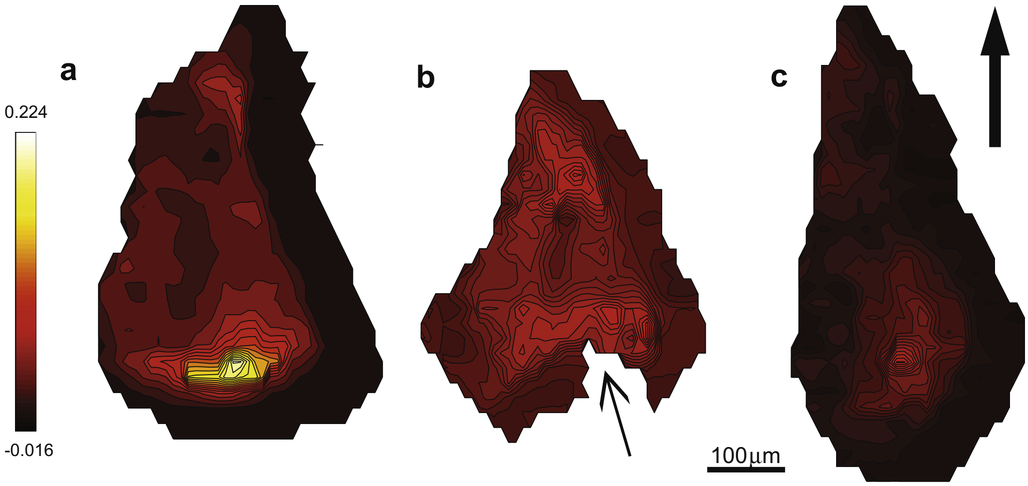

Fig. 1 – (a) Distribution of retardance in a normal, late stage third instar wing disc. The retardance is highest in the centre of the wing pouch and the direction of the main stress can be seen from the orientation of the increased retardance relative to the incoming polarization (see Fig. 2). (b) When a wing disc is cut, to relieve mechanical stresses, the retardance distribution changes accordingly. Here, the cut is indicated by an arrow. (c) Direct mechanical stimulation by stretching the disc in the direction of the arrow also leads to a release of compression in the centre of the wing pouch. The disc shown here was of comparable size (width and length) as that in (a) before stretching. The qualitative changes shown in (b) and (c) are observed in all stimulated wing discs (*n* = 6 for cut, *n* = 4 for stretched discs).