Optogenetic Control of Biological Forces

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Abstract

The boom of optogenetics, the art of making biological processes "light sensitive", is influencing many different fields in biology. In this primer, we aim to summarise how this new methodology is being especially useful for the study of those processes in which mechanical forces are involved. For the first time, we can test and direct the assembly of cytoskeletal components with high spatial and temporal resolution, which enables controlled production of forces at different scales. Many new promising applications arise, such as the control of tissue morphogenesis.

Introduction

Perhaps one of the most exciting aspects of studying biology is exploring processes that we can't see with our own eyes nor handle with our own hands. From understanding how cells organise as multicellular beings, to learn how microscopic processes condition our lives, biologists need to find methods to study processes that escape the resolution of our own senses. Luckily for us, many scientists before us have already put great efforts into making the invisible visible. From the invention and improvement of microscopy, that led to the first observations of cells and microscopical life, to the fine tuning of methods that measure bulk and single cell properties (such as western blot or single cell RNA-Seq), these techniques have enhanced our ability to perceive biological phenomena. As in the rest of the scientific fields, in order to further increase our understanding of complex problems, new techniques must be invented.

In this sense, while all different kinds of microscopy have greatly expanded the resolution at which we can observe biological processes, the methods by which biologists have altered these systems have been more limited in space and time. This is because perturbations in the microscopic scale have been produced mainly through the use of chemical compounds that would affect, for example: transcription, protein activity or cell behaviour. These perturbations are limited by the diffusion speed of the compound used and how fast it can be washed away. In the 20th century, very few of the techniques used were able to alter biological systems with high spatio-temporal control, and their applications were limited to very specific purposes (e.g. Atomic Force Microscopy, laser ablation or the use of optical tweezers). The gap created by the absence of an advanced technique that can allow for increased temporal and spatial resolution at which we can interact with biological systems paved way for the arrival of a new method at the beginning of the 21st century: Optogenetics.

Optogenetics is the field that, through genetic modifications, uses photoreceptor proteins to make biological processes sensitive to light. The field started originally with the use of channelrhodopsins - membrane ion channels that are naturally sensitive to light (Figure 1a). These membrane proteins were used to activate or inhibit single neurons, because illumination of cells expressing channelrhodopsins can induce membrane depolarisation [Deisseroth (2015), Mohan (2017)]. Precise application of optogenetics depended on technologies that could deliver beams of light precisely at the region of interest. Combining optogenetics with techniques such as confocal microscopy thereby allowed scientists to show that light could be used to control biological processes very precisely. Taking this as a proof of concept, several research groups across the globe started working to make other biological processes "light sensitive". In 2009, Yazawa and colleagues used a different plant photoreceptor to induce protein binding, which permitted them to control protein localisation and gene transcription upon light illumination [Yazawa et al. (2009)]. This was the start of non-neuronal optogenetics.

Non-neuronal optogenetics makes use of plant and microbiological photoreceptors to induce binding or clustering of proteins of interest upon light illumination. In Yazawa's study, the photorreceptor FKF1 from *Arabidopsis Thaliana* was used together with its binding partner Gigantea. Upon blue light illumination, FKF1 naturally changes conformation and binds to Gigantea (Figure 1b). The authors took advantage of this light-inducible binding by genetically adding new parts to the sequences of these components, so protein localisation could be controlled in mammalian cells by adding a membrane anchoring motif (Figure 1c), or controlling transcription by including a DNA binding and a transactivation domain on each part of the system. Nowadays, a wide range of photoreceptors has been used to study very different biological processes. These receptors can be activated with different wavelengths (Figure 1b), they have their own biochemical properties that must be taken into account for their use and they have been used for a wide range of purposes, such as control of protein localisation, clustering, secuestration, activation or protein heterodimerisation. Some very useful reviews have been written proposing which criteria must be followed to choose an optogenetic system [Tischer and Weiner (2014), Hallet et al. (2016)].

In fact, while spatial resolution relies mainly on the accuracy of the microscope used to deliver light

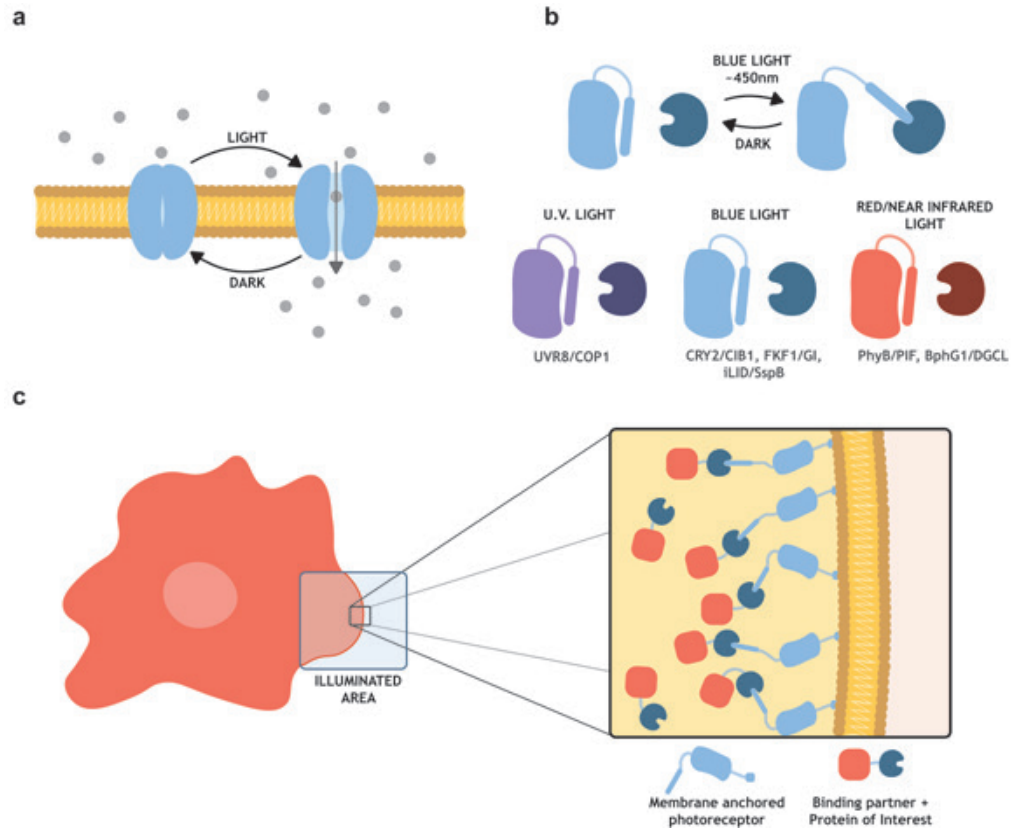

Figure 1. Optogenetics enables tight control of celullar processes. a, Mechanism of function of Channelrhodopsins, which permit ion flux upon light illumination and go back to their initial state in absence of light. b, Light controlled heterodimerization is achieved by a light-induced conformational change, which permits protein-protein interaction with a binding partner. Multiple naturally existing and synthetically developed systems are available. Some examples are given for the main wavelength ranges used. c, Light induced protein translocation to plasma membrane can be achieved by adding a membrane anchor protein motif to the photoreceptor, so proteins of interest can be translocated by genetically engineering its gene with binding partner's gene.

in a confined area, the choice of photoreceptor will determine the temporal resolution of the experiment. The reason is that, although the time required to activate photoreceptors is generally very short (in the range of seconds), the time taken for half of the photoreceptors to inactivate once light is turned off (reversion half-life) will vary greatly depending on the tool used [Repina et al. (2017)]. For instance, the improved Light-Induced-Dimer (iLID) has an approximate reversion half-life of ≈1 minute depending on the conditions [Guntas et al. (2014), Hallet et al. (2016)]. Hence, a full iLID activation-deactivation cycle is limited by these parameters and can't be shorter than 1 minute in any case. The scale of the reversion half-life must then match with the timescale of the biological process of interest. There are also cases where a longer half-life binding is more appropiate. For instance, the light-inducible transcription factor GAVPO, with a half-life reversion of ≈2 hours [Wang et al. (2012)], can allow the user to induce transcription with evenly spaced short pulses of light (in Wang and colleagues' study, one pulse of 10s every 8 minutes), reducing the need to illuminate target cells so often. In cases were higher temporal resolution is necessary, the PhyB/PIF system stands out because it can be turned off with a 750nm wavelength pulse in less than 5 seconds, acting like a toggle switch [Levskaya et al. (2009)]. In addition, the publication of new tools and the discovery of point mutations that can change the half-life duration of previously existing tools keeps expanding the chances to find a system that matches the necessities of the user.

In the last 10 years, a wide range of biological processes have been studied taking advantage of the unprecedented spatiotemporal precision that optogenetic methods can achieve. Some examples are control of transcription, signal transduction, and developmental processes [Mühlhäuser et al. (2017), Muller et al. (2015), Johnson and Toettcher (2018), Gugliemi et al. 2016, Krueger et al. (2019)]. In this primer, our aim is to highlight how this young field is enabling for the first time to control biological mechanical forces at a microscopical level. This is bound to have a great impact on the field of mechanobiology, which studies the processes by which cells sense, integrate, generate and transmit mechanical forces [Wang et al. (2006), Eyckmans et al. (2015)]. With optogenetics, activation or inactivation of components involved in force generation can be induced locally in pulses of controlled duration, while monitoring how these perturbations affect other components in the system and by which mechanisms they are sensed by the cell. Here, we will review how these local perturbations in the generation of cellular forces can lead to discoveries on the molecular, cellular, and even tissue scales. Moreover, we'll discuss how this approach is not only proving useful to improve our understanding of how biological forces are generated, but also opening the way for new fields like synthetic morphogenesis, which aims to induce tissue shape synthetically [Davies (2008), Krueger et al. (2019)].

Optogenetic control of cellular forces

Molecular scale

Optogenetic tools are designed by taking advantage of the conformational change that light induces on photoreceptors. This change can make parts of the photoreceptor freely available for binding, or induce structural changes that affect protein behaviour. On the molecular level, these "controllable" interactions can be used to tune or alter protein activity.

Nakamura and colleagues used the light-induced conformational change of LOV2 photoreceptor from *Avena Sativa* to control actin and microtubule-based motors' speed and directionality in vitro [Nakamura et al. (2014)]. The authors engineered MyosinVI and the Non-claret disjunctional protein (Ncd), two motor proteins that use a swing lever arm mechanism, to move along actin and microtubules respectively. By genetically engineering these proteins with the LOV2 photoreceptor and a "leverage" domain (derived from α-actinin), they were able to alter their speed and directionality upon blue light illumination (Figure 2). This example shows how optogenetics can be used on the molecular level to make biological functions tunable. These applications not only improve our understanding of the molecular mechanisms that are used by these proteins, but also create new possible ways to disturb biological systems in a controlled and specific way. How would cellular processes like mitosis be affected by having slower or reversed molecular motors in specific stages or areas?

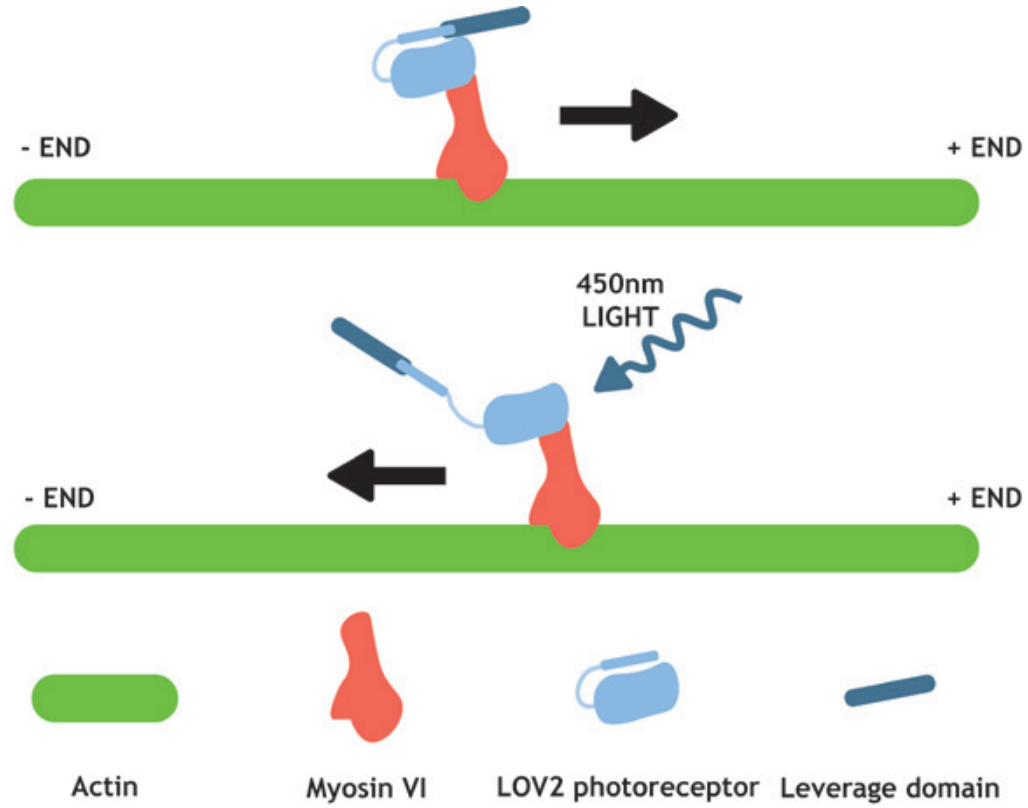

Figure 2. Light sensitive MyosinVI (MyLOV2) mechanism. In the dark state, MyLOV2 moves towards the (+) end of the actin filament. Upon illumination, the change of conformation in the photoreceptor makes swing lever arm mechanism to produce movement in the opposite direction, towards the (-) end.

Cellular level

Eukaryotic cells are characterised by their compartmentalisation. Reactions need to be separated in space and time to allow coordination of cellular processes. This is also true for cellular forces: in order to achieve cell division, migration, changes in cell shape or even to control the localisation of some inner components, cells need to make use of the cytoskeleton in a tightly controlled manner. With optogenetics, scientists can get closer to this resolution, studying how components related to production of biological force interact in space and time. Here, we highlight some of the studies that have shown how optogenetics enables not only the study of how cells exert force, but how these forces can be controlled to study other processes.

Inner cell organisation:

In the last decade, several tools have been developed to understand how different components come together in specific cell locations to regulate the cytoskeleton or alter inner cell distribution by making use of the cytoskeleton.

For example, an optogenetic tool was developed to control the activity of an auto-regulatory domain of the protein mDia1, a diaphanous related formin which has a function related to actin nucleation and elongation [Rao et al. (2013)]. The authors put the function of this protein domain under the control of light by binding it to LOV2 photorreceptor. This

is done using a "caging" approach: the inactive state of LOV2 inhibits sterically the function of the protein of interest, while the light induced conformational change recovers protein function. This revealed how this domain of mDia1 can increase thickness of stress fibers without force induction or creation of new fibers. Similar studies have been performed on proteins that alter microtubule polymerisation [Adikes et al (2018), van Haren et al. (2018)]. In these cases, the advantage of this approach is that the dynamics of these components can be observed through live microscopy, together with the reversibility of the processes.

Moreover, these tools can be used not only to test the functionality of the protein being studied, but also to use cellular forces on other cell components and perturb intracellular processes or their distribution. A remarkable example of this has been the use of molecular motors coupled with photoreceptors to alter organelle distribution inside of the cell.

Bergeijk and colleagues made use of the heterodimerisation of the LOV/ePDZb1 pair by binding one component to Kinesin3 or Myosin-V and the second component to organelle specific markers to create light inducible shuttling of organelles (Figure 3a). This way, the organelles of interest (in the article: peroxisomes, endosomes or mitochondria) were shipped either to the edge of the cell or the perinuclear area. Through these techniques, organelles can be delivered to specific areas of the cell for defined time durations to interrogate how organelle localisation affects cellular functions. In this case, it was demonstrated that endosome localisation can enhance or suppress axon growth in primary rat hippocampal neurons [van Bergeijk et al. (2015)].

Cell migration:

Cell migration is a process that makes use of a combination of signaling cascades and tight control of the cytoskeleton. To migrate, cells must be able to interpret chemical and physical gradients, which must be translated in an intracellular gradient for asymmetric distribution of the cytoskeleton. Optogenetics emerges as a useful method to test how spatially restricted activation of components of this signaling cascades affects cell polarity and movement.

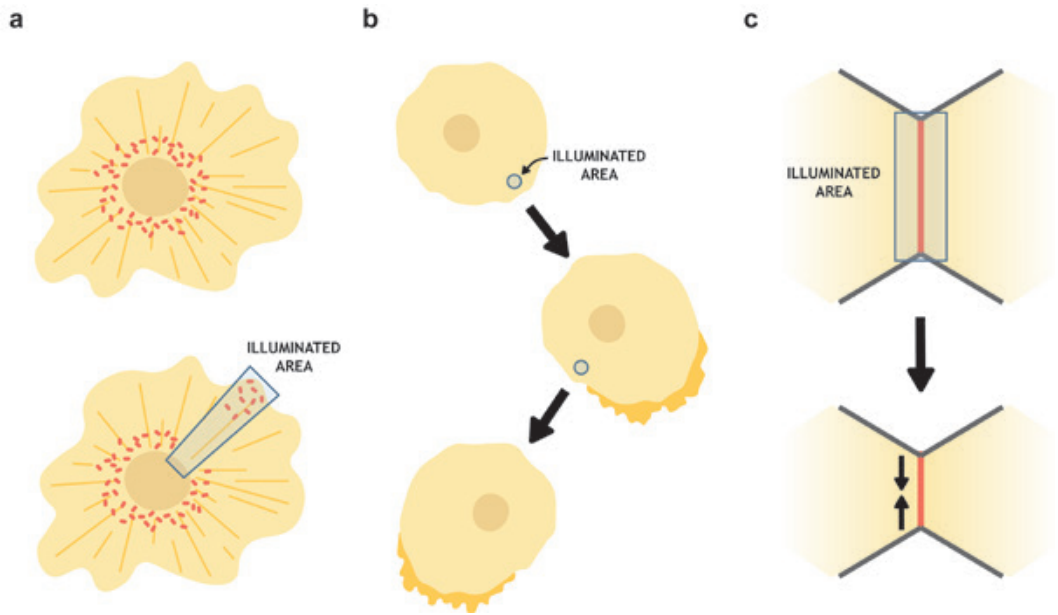

Figure 3: Use of optogenetics to control cellular forces. a, By fusing one of the components of a heterodimerisation tool to a motor protein and the other to a protein motif specific of an organelle membrane, the organelle can be shuttled along actin or microtubule polymers. b, PA-Rac1 asymmetric activation was demonstrated to induce lamellipodia protrusions and cell migration in the direction of the activated border. c, Activation of contractility through a Rho-GEF optogenetic tool in Caco-2 cell junctions allowed the discovery of a junction remodeling mechanism.

For instance, Y. Wu and colleagues created a photoactivatable version of the GTPase Rac1 (PA-Rac1) also by using a caging approach. Upon blue light illumination, the Jα helix domain of the LOV2 photoreceptor liberates Rac1's binding domain, which is then able to interact with its effector PAK. Hence, PA-Rac1 was used to test how different localised pulses of RAC activation inhibited RhoA activity and induced lamellipodial protrusions, but also demonstrated that these pulses could be used to direct cell migration [Wu et al. (2009)] (Figure3b). With other optogenetic approaches, other proteins that can interact with this signaling cascade such as Cdc42 or a blue opsin have been demonstrated to be able to drive migration in different cell lines [O'Neil et al. (2016), Karunarathne et al. (2013)].

Although the original objective of these experiments was to improve our understanding of the structure of the signaling cascades that guide cell migration, gaining control over cell migration can now allow us to address new questions related to a higher level of organisation: tissue biology.

Cell shape

During metazoan development, cells need to acquire complex shapes to achieve cell specialisation and morphogenesis, which is accomplished through the use of the cytoskeletal machinery. Elongation, constriction and flattening are examples of processes that require asymmetric use of biological forces. Therefore, optogenetics has come out as a great tool to find out which components and under which spatiotemporal regulations are needed to induce cell shape changes.

Until now, most efforts have focused on the application of the heterodimerisation concept to recruit to plasma membrane proteins that take part in the regulation of actomyosin contractility [Levskaya et al. (2009), Guglielmi et al. (2015), Wagner et al. (2016), Valon et al. (2017)]. One remarkable strategy has been the recruitment of Guanine Exchange Factors (Rho-GEF) to the plasma membrane to activate RhoA, a small GTPase that eventually leads to myosin phosphorylation and acto-myosin contraction. Hence, light induced recruitment of Rho-GEF to activate RhoA has been used in several contexts: to induce cytokinetic furrows in mammalian cells [Wagner et al. (2016)], to induce contractility in drosophila embryos [Izquierdo et al. (2018)], and to increase or reduce cell contractility in MDCK cells. This last approach demonstrated that this variations in cell tension can affect translocation of YAP, a transcriptional regulator that can act as mechanosensor [Valon et al. (2017)].

Moreover, a recent publication also used an optogenetic version of Rho-GEF to induce cell junction shortening (Figure 3c), which has enabled the study of junction remodeling dynamics [Cavanaugh et al. (2020)]. In this case, the tool was only activated at the interface of two cells, and stimulated with variable duration and frequency of irradiation. The possibility to precisely variate the contraction and resting time, lead to the discovery of the activation of junction remodeling processes through the endocytosis of cell membrane and E-cadherin.

As stated before, development of optogenetic tools to study cell shape change processes is not only useful to increase our understanding of this processes, but also gives us control over them, which allows us ask questions about how these processes come together to form tissues.

Tissue level

Morphogenesis, the process by which complex tissue architectures develop, is based on the finely regulated application of biological forces. The combination of these forces produces specific tissue-level processes such as tissue invagination, folding or cell migration, which can now be studied using optogenetics.

In this regard, we must highlight the work of Izquierdo and colleagues, that used a RhoA-GEF based optogenetic tool to induce apical constriction and provoke tissue invagination in *Drosophila* embryo. The combination of this optogenetic tool with a fine delimitation of the activated area using two-photon microscopy permitted to recruit the RhoA-activating component only to the apical membrane of cells in the dorsal side of *Drosophila* embryo, causing apical

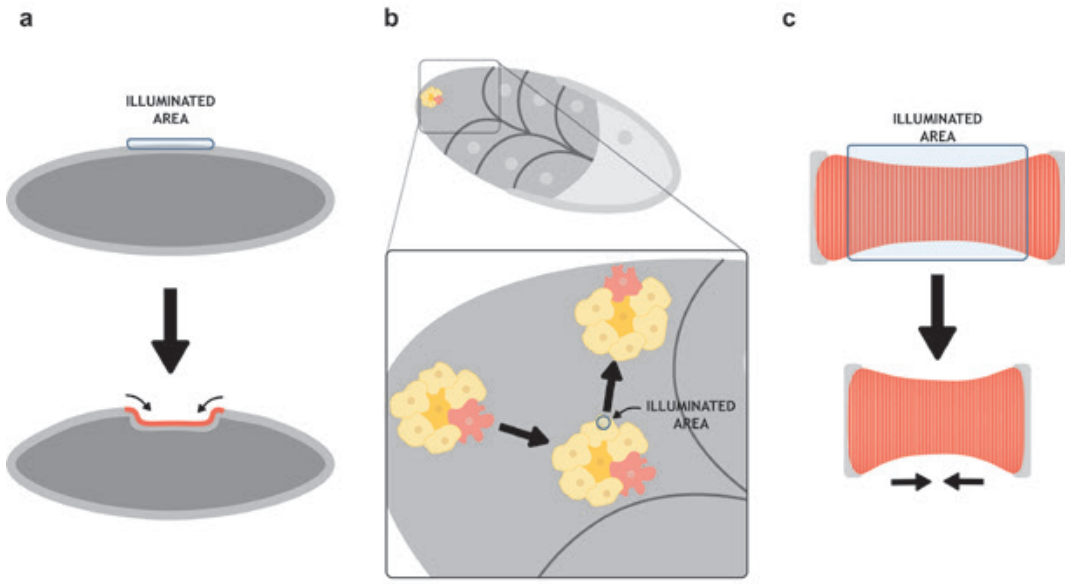

Figure 4. Optogenetic control of forces on the tissue level. a, Two photon illumination can achieve activation of the apical plane in epithelial cells of the dorsal side of the Drosophila embryo. This, coupled with the induced expression of a Rho-GEF optogenetic tool can be used to reproduce the first steps of tissue invagination. b, Application of PA-Rac1 optogenetic tool to switch the leader cell (in red) of the border cells in the egg chamber of Drosophila. This enables control over group cell migration in an in vivo setting. c, Mice muscle explants contractility can be controlled with the expression of Channelrhodopsin-2, an approach that can be used to contract muscle selectively in vivo.

constriction (Figure 4A). This induced constriction reconstituted the first steps of ventral furrow invagination, a milestone in *Drosophila* development. The approach demonstrated that an increase in apical tension is sufficient to induce the beginning of an invagination in the dorsal side of *Drosophila* embryos. Moreover, the spatial control of this tool allowed the study of how different irradiation geometries could lead to different invaginations [Izquierdo et al. (2018)]. This work is a proof of concept that optogenetically controlled cellular forces can be used to control tissue shape.

Moreover, the optogenetic approach used to control single cell migration with PA-Rac1, proved to be useful also for the study of group migration. The tool was applied to study migration of *Drosophila* border cells, [Wang et al. (2010)]. This group is composed of 6-8 cells that migrate along the nurse cells of the egg chamber. This study demonstrated that asymmetric Rac1 photo-activation could induce a change in the leading cell of the group, and therefore direct migration of this group of cells (Figure 4B). It was demonstrated how JNK signaling was necessary for group migration and how EGFR and PVR receptors are used for guidance of the migration of this group. Remarkably, this tool was also used to map the areas of the egg chamber that the migratory group could access and those where cells couldn't migrate, an experiment that could not be achieved without spatiotemporal control of migration. To sum up, this approach enabled better understanding of the components necessary for group migration and found new clues pointing where migratory barriers and mechanisms of control could be found in the egg chamber.

Finally, optogenetic control of biological forces can also be applied to non-developing tissues. For instance, control of muscle contraction can be achieved with light irradiation by making use of classic neuronal optogenetic tools such as channelrhodopsins, which can induce membrane depolarisation of muscle cells and therefore contraction (Figure 4C). This approach has been achieved for both skeletal and cardiac muscle fibres, [Bruegmann et al. (2010)]. Bruegmann et al. (2015). For example, control of pharyngeal muscle contraction was achieved both in explants and *in vivo* through the use of Channelrhodopsin-2 (Chr2). Light induced contractions achieved up to 84% of the maximal contraction force induced with electrodes, while they were precise enough to activate specific muscles provoking opening and closing of vocal cords. The optogenetic approach may be more promising for *in vivo* applications than traditional use of electrical currents for contraction induction, since it offers a clean, pain-free and more specific control over muscle contraction.

Concluding remarks

Although optogenetics was initially aimed to be applied to activation or inactivation of neurons in neurobiology, it has opened a vast field of possibilities by enabling spatiotemporal control of biological processes. Many fields are already benefiting from this, such as the study of signal transduction pathways or developmental biology. As we have seen, the study and application of biological forces has been greatly influenced by these new methods. The optogenetic revolution is enabling, for the first time, the control of cellular forces in vivo with an unprecedented precision and accuracy that permits not only the interrogation of mechanisms by which these cellular forces are normally produced, but also to use and control these forces for other applications. In this primer we have seen a few of the many examples of how optogenetics is bringing spatiotemporal resolution to the interrogation of biological processes. From the molecular to the tissue scale, we can now induce mechanical forces in a specific area and moment. From controlling sub-cellular structures such as trafficking of endosomes within neuronal axons, to the probing of supra-cellular processes such as induction of tissue invagination in the *Drosophila* embryo, optogenetic control of cellular forces can be applied to learn about processes of very different scales. The limits of this new field lie both in how precisely our microscopes can deliver light, and how efficiently we can engineer proteins to become light sensitive.

In conclusion, optogenetics is opening new ways to interrogate and use biological forces from the molecular to the tissue level. An exciting range of possible application goes from regenerative medicine, to the exploration of the new field of synthetic morphology, learning to create new tissues by shaping them with light.

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Guillermo is a PhD student at Miki Ebisuya's lab (EMBL Barcelona). Guillermo's interest for optogenetics started during an internship at the department of Photobiology of Université Pierre et Marie Curie in Paris, where he worked on the development of a transcriptional optogenetic tool in yeast. Now, he is using a synthetic biology approach to study and reproduce mechanisms of mammalian tissue morphogenesis in vitro. More precisely, his project is focused on epithelial morphogenesis, a field for which the development and application of optogenetic tools are becoming of great interest.