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REVIEW

Aspergillus nidulans in the post-genomic era: a top-model filamentous fungus for the study of signaling and homeostasis mechanisms

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Abstract

The accessibility to next-generation sequencing (NGS) techniques has enabled the sequencing of hundreds of genomes of species representing all kingdoms. In the case of fungi, genomes of more than a thousand of species are publicly available. This is far from covering the number of 2.2–3.8 million fungal species estimated to populate the world but has significantly improved the resolution of the fungal tree of life. Furthermore, it has boosted systematic evolutionary analyses, the development of faster and more accurate diagnostic analyses of pathogenic strains or the improvement of several biotechnological processes. Nevertheless, the diversification of the nature of fungal species used as model has also weakened research in other species that were traditionally used as reference in the pre-genomic era. In this context, and after more than 65 years since the first works published by Pontecorvo, *Aspergillus nidulans* remains as one of the most referential model filamentous fungus in research fields such as hyphal morphogenesis, intracellular transport, developmental programs, secondary metabolism, or stress response. This mini-review summarizes how *A. nidulans* has contributed to the progress in these fields during the last years, and discusses how it could contribute in the future, assisted by NGS and new-generation molecular, microscopy, or cellular tools.

Keywords *Aspergillus nidulans* · Polar growth · Nucleo-cytoplasmic transport · Development · Secondary metabolism · Stress response

Introduction: *Aspergillus nidulans* in the pre- and post-genomic eras

A great proportion of the millions of fungal species populating the globe is characterized by the generation of filamentous structures called hyphae (Hawksworth and Lücking 2017; Spatafora et al. 2017). Hyphae grow polarly, a unidirectional extension mode that is based on the acropetal transport of the compounds that form the plasma membrane and the cell wall to the growth region (the apex of the tip; see Fig. 1A; see next section). Hyphae also branch, generating new growth axes that lead to the formation of tridimensional multicellular networks called mycelia. Although there is a great diversity of morphogenetic patterns among hyphae (i.e., some are mononuclear while others multinucleate; some branch apically while others laterally), the final aim of the mycelium is the invasion of a substrate or the search for nutrients (Nguyen et al. 2017).

Hyphae-forming fungi are known as filamentous fungi and are found in most clades of the fungal tree of life (Spatafora et al. 2017). One of these clades corresponds to the genus *Aspergillus* (subkingdom: Dikarya; phylum: Ascomycetes; Subphylum: Pezizomycetes; class: Eurotiomycetes; order: Eurotiales; family: Aspergillaceae), which comprises multiple industrially and medically important species. It probably constitutes the genus with the highest number of sequenced genomes (see the MycoCosm website; https://genome.jgi.doe.gov/programs/fungi/index.jsf; see also (de Vries et al. 2017; Kjærbølling et al. 2018)). This has furthered great progress in the genome analysis and study of the life-cycle/infection mechanisms of these economically important *Aspergilli*. However, a non-pathogenic *Aspergillus* species has attracted the attention of multiple fungal researchers during more than 65 years.

Aspergillus nidulans was first established as a reference organism by Pontecorvo and his seminal studies on the bases

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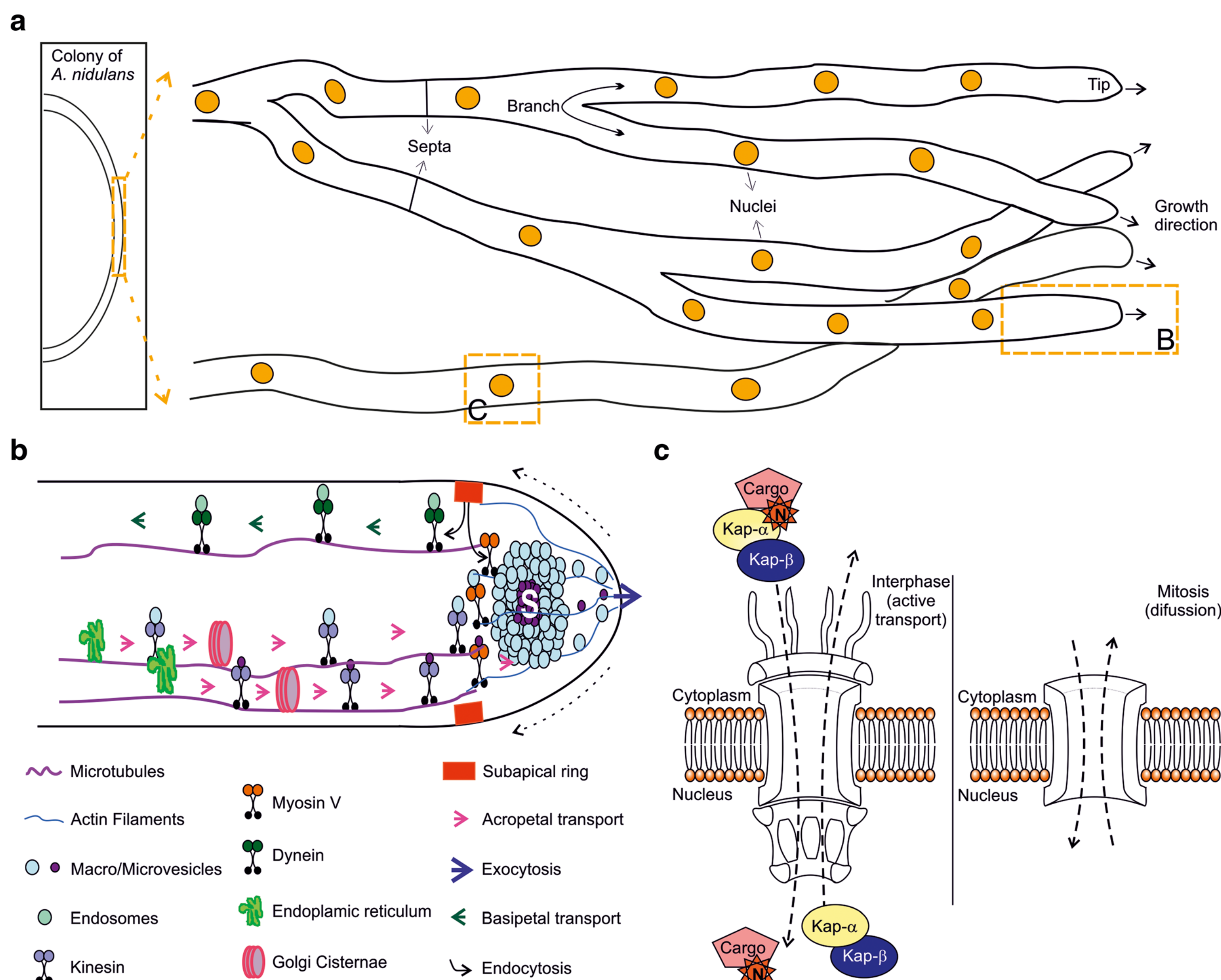


Fig. 1 Growth and nucleo-cytoplasmic transport in *A. nidulans* hyphae. (A) Scheme of the different regions within a colony (left), with an amplification of peripheral, polarly growing hyphae (right). Septa (open rings that separate, but not isolate, cells within hyphae), branching sites, nuclei, tips, and the direction of hyphal growth are indicated. (B) Model for the distribution of the machinery controlling polar growth at the region between the apex and the closest nucleus. The nature of each player is indicated below the image. Pink and green arrows indicate the direction of vesicle flow. See main text for the main events. (C) Simplified structure of an NPC during interphase (assembled) and in mitosis (partially disassembled; the core of NPC remains assembled). Transport of cargos across the NPC during the interphase requires energy and nucleo-cytoplasmic transporters, such as the dimer importin-α/β (KapA/B in *A. nidulans*). Importin-α binds the NLS (N) of the cargo and importin-β through the importin-β-binding domain. Once in the nucleus, the complex is disassembled and KAPs are exported again to the cytoplasm. The absence of the cytoplasmic and nucleoplasmic extensions of the NPC during mitosis allow diffusion

of classical genetics and the parasexual cycle, a mechanism for the transfer of genetic material without sex (Pontecorvo 1952; Pontecorvo et al. 1953; Oliver and Schweizer 1999). In the subsequent decades, several authors consolidated this fungus as a model for the study of, i.e., sexual/asexual/parasexual cycles, nucleotide or amino acid metabolism, the cell cycle, and so on. Research focused on *A. nidulans* also resulted in important breakthroughs, such as the discovery of γ-tubulin, a protein carrying out multiple cytoskeletal functions in all eukaryotes (Oakley and Oakley 1989; Oakley et al. 2015). Besides the previously mentioned non-pathogenic nature, there were several reasons to prioritize *A. nidulans* as a reference organism in those fields. For example, at that time, it was the only *Aspergilli* with a reproducible sexual cycle at the lab scale, which enabled meiotic crosses of strains with the same (homothallic) or different (heterothallic) genetic backgrounds in an acceptable limit of time. Basic procedures were developed in order to reproduce its growth and reproduction, obtain mutants, and perform reverse/forward genetics experiments. These, and the advantage of a reproducible sexual cycle, also enabled the mapping of multiple markers throughout the eight chromosomes of the *A. nidulans* genome (http://www.fgsc.net/Aspergillus/gene_list/).

The adaptation of *A. nidulans* to the era of molecular biology was highly successful, and several genes were (and are still being) isolated and sequenced and their translation products

characterized. The implementation of fluorescence microscopy techniques significantly improved our knowledge of the life cycle of *A. nidulans* and the role of those proteins. These procedures initially used fluorescent dyes to track in wild-type or mutant strains specific organelles such as nuclei, the plasma membrane, or the cell wall. Sequencing of *A. nidulans* genome (Galagan et al. 2005) furthered the development of a second-generation set of tools for *A. nidulans* molecular biology such as the fusion-PCR technique (Yang et al. 2004). This methodology enables the generation of strains expressing epitope-labeled wild-type or mutant forms of the protein of interest. The use of strains with the Δ*nkuA* background guaranteed a high percentage of homologous recombination among transformants (Nayak et al. 2006), which in combination with more powerful fluorescence microscopes (see references within (Etxebeste and Takeshita 2015)), furthered the in vivo analysis of the localization, dynamics, and function of the corresponding proteins. The application of massive sequencing techniques such as LC-coupled mass spectrometry or RNA sequencing has been reported during the last decade (see the first RNAseq reports on *A. nidulans* or the TAP-tagging of the velvet protein VeA to identify interactors; (Bayram et al. 2008; Sibthorp et al. 2013; Garzia et al. 2013)).

Initiatives such as the 1000 fungal genomes project intend to extend our understanding of fungal diversity by the sequencing of at least two reference genomes from the more than 500 recognized families of fungi (http://1000.fungalgenomes.org/home/). The genomes of more than a thousand of species are currently available (last release of the MycoCosm database; August, 2018; https://genome.jgi.doe.gov/programs/fungi/index.jsf), increasing the number of fungal species that can be used as reference organisms. Accordingly, this trend should be accompanied by a decrease in the impact of the research based on *A. nidulans* as a model organism. Nevertheless, it remains as a key reference organism in multiple research fields. Due to length restrictions, this mini-review will focus only on some of these research fields: polar growth, nucleo-cytoplasmic transport, secondary metabolism, developmental programs, and pH- or cation-stress response. Finally, we discuss how *A. nidulans* could contribute to these and additional research fields in the future.

A tunnel-boring machine at the tip of hyphae: how *A. nidulans* grows polarized

Lee and Adams described that, on solid surfaces, *A. nidulans* colonies formed radially symmetric superficial structures that expanded at a constant rate of about 0.5 mm/h at 37 °C (Lee and Adams 1994b; Adams et al. 1998). A later study described that tips of *A. nidulans* wild-type hyphae extended more or less straight at an average pace of 0.506 μm per minute at 25 °C (Horio and Oakley 2005). The first figure corresponds to a macroscopic observation while the second one to a microscopic one. In addition, the difference in both values probably reflects the importance of environmental conditions such as the temperature in hyphal growth, being much faster in *A. nidulans* at 37 °C than at 25 °C. However, in the second case, one month would be enough to almost completely colonize a volume equivalent to a human eyeball while, in the first one, eleven days would result in the colonization of a volume corresponding to a human brain. These figures certainly represent over-estimations of the growth pace of hyphae in the environment because (1) they were measured under favorable culture conditions, (2) they do not consider the nature of the substrate, and (3) they overlook in case of pathogenesis the effect of the immune response of the host to infection. Nevertheless, they are representative examples of the potential of *A. nidulans* in particular but also filamentous fungi in general to colonize a substrate.

Together with filamentous fungal species such as *Neurospora crassa* or *Ustilago maydis*, *A. nidulans* is one of the most important reference organisms for the study of hyphal extension. Hyphal growth means that a substrate must be penetrated, whatever the nature of this substrate is (i.e., tissues, organs, agar, or liquid medium), and this occurs by exerting high turgor pressure at hyphal tips through the massive but coordinated delivery (exocytosis) of vesicles of variable size and composition (Fig. 1A and B). The fusion of exocytic vesicles with the plasma membrane is not a continuous process in fungi. The oscillatory nature of hyphal growth was described in the 1990s (López-Franco et al. 1994) but the underlying molecular mechanism (transient polarity assembly model) was elegantly described recently using *A. nidulans* as the reference (Takeshita et al. 2017). Briefly, pulsed growth is the consequence of a repetition of events which include the assembly/disassembly of cell-end marker complexes, actin polymerization/depolymerization, and exocytosis (Riquelme et al. 2018).

The protein complexes, structures, and organelles required for the acropetal transport of apical materials are also polarized (Fig. 1B). This means that they show a gradient-like distribution within the region between the apex and the closest nucleus (*A. nidulans* hyphae are multinucleate). According to the accepted model of hyphal growth, cargoes initially transit through the network defined by the endoplasmic reticulum (ER) and the Golgi apparatus (Fig. 1B). Several markers have been generated for the general processing and transport steps within the ER-Golgi network. In general, the results obtained using *A. nidulans* hyphae support the Cisternal Maturation Model, in which early Golgi cisternae generated with materials originated at the ER progressively modify their identity into late Golgi cisternae, leading to secretory carriers which depart towards the tip (for a review see (Pantazopoulou 2016)). *A. nidulans* have also been used as the reference system in the study of the role of clathrin and the adaptor complex AP-1, both required for the invagination and coating of secretory vesicles generated at the late Golgi (Schultzhaus et al. 2016a; Martzoukou et al. 2018).

RabE is a GTPase used as marker of the secretory vesicles that feed the apex of hyphae (Pantazopoulou et al. 2014). Two main transport steps can be differentiated (Fig. 1B). The first one is dependent on microtubules (MTs), cytoskeletal filaments that nucleate at complexes embedded in the nuclear pore complex (microtubule-organizing centers; MTOCs) and grow towards the hyphal tip, and the kinesin motor KinA (Peñalva et al. 2017). The MT-based transport of secretory vesicles continues until MTs spatially overlap with actin filaments, which nucleate at hyphal apices and grow towards distal regions (Schultzhaus and Shaw 2015; Schultzhaus et al. 2016b; Peñalva et al. 2017; Riquelme et al. 2018). This occurs preferentially at a subapical region (3–4 μm far from the apex) where endocytosis is a massive process: the endocytic collar (also known as the dynein loading zone; see below; (Upadhyay and Shaw 2008; Abenza et al. 2009)).

There, secretory vesicles are transferred to actin cables and transported by the myosin motor MyoE (Fig. 1B) (Taheri-Talesh et al. 2012; Peñalva et al. 2017). They accumulate in a pseudo-organelle known as *Spitzenkörper* (S in Fig. 1B), originally described in the Agaricomycete *Polystictus versicolor* and supposed to act as a supplier of secretory vesicles that feed the apex (Girbardt 1957; Bartnicki-Garcia et al. 1989). The site of exocytosis is determined by cell-end marker proteins such as TeaA and TeaR (Fischer et al. 2008) and vesicle fusion with the membrane and the cell wall at the apex is mediated by different proteins and complexes such as the polarisome or the exocyst. Although some of the polarisome/exocyst proteins have been functionally characterized in *A. nidulans*, the activity and dynamics of these complexes were (or are being) originally characterized using other fungal species as model (see references within (Schultzhaus and Shaw 2015; Riquelme et al. 2018)).

The apex of hyphae is estimated to receive through secretory vesicles more material than the one required to build the membrane and the cell wall (Riquelme et al. 2018). The massive (but pulsed) delivery of these vesicles also makes apical compounds become subapical. Furthermore, the polysaccharides of the cell wall are rapidly modified after exocytosis. These features generate a need for endocytosis that is satisfied at the endocytic collar by actin patches, actin-binding proteins (Abps), the type I myosin MyoA (required also for actin-filament nucleation and secretion), and auxiliary complexes (McGoldrick et al. 1995; Yamashita and May 1998; Yamashita et al. 2000; Liu et al. 2001; Araujo-Bazán et al. 2008; Upadhyay and Shaw 2008). A recent study on the second adaptor complex of *A. nidulans*, AP-2, suggested that there are at least two endocytic pathways operating at the subapex of hyphae (Martzoukou et al. 2017). One of those pathways would be independent of clathrin and dependent on the AP-2 complex as well as Abps and SlaB, while the second pathway would be independent of the AP-2 complex and dependent on clathrin- and α-arrestin (Lau and Chou 2008; Martzoukou et al. 2017).

There are two options for endocytosed materials (black arrows in Fig. 1B). The *A. nidulans* v-SNARE protein synaptobrevin, SynA, joins again the exocytic pathway (Taheri-Talesh et al. 2008) while most materials are incorporated into Rab5-marked early endosomes (Abenza et al. 2009), which are basipetally transported by dynein and its auxiliary proteins to distal regions for their transformation/recycling (green arrows in Fig. 1B) (see the reviews by (Xiang et al. 2015; Reck-Peterson et al. 2018)).

Avoiding traffic jams in the nuclear envelope: control of nucleo-cytoplasmic transport

Besides being important for the bi-directional transport of cargos and thus for polar extension, the MT cytoskeleton also plays a key role in the positioning and distribution of organelles such as nuclei (Oakley and Morris 1980; Xiang et al. 1994). The main feature defining eukaryotic cells is, indeed, the development of nuclei as a compartment to preserve the genetic information. The nucleus is delimited by the nuclear membrane or envelope, a bilayer that extends into the endoplasmic reticulum. However, the presence of the nuclear envelope increases the complexity of the connection between DNA-associated processes and other cellular processes such as translation or signal transduction. This is why the nuclear envelope is porous, and these pores are formed by protein complexes known as nuclear pore complexes or NPCs (Fig. 1C) (Kim et al. 2018). Molecules smaller than 30 kDa cross the NPC passively, without any energy requirement (Görlich and Kutay 1999). Bigger macromolecules require a specific machinery capable of transporting them actively into the nucleus (nuclear import; importins; Fig. 1C) or to the cytoplasm (nuclear export; exportins) (Ghavami et al. 2016).

One characteristic of *A. nidulans* hyphae is that they contain multiple nuclei that are regularly distributed from the tip to distal regions (Fig. 1A). Of course, all of these nuclei receive in the form of proteins information from the cytoplasm and cytoplasmic organelles, such as the endoplasmic reticulum or the tip (Bat-Ochir et al. 2016; Etxebeste and Espeso 2016). They also incorporate proteins required for DNA-associated processes. The most widely used fungal reference in the field of nucleo-cytoplasmic transport and NPCs undoubtedly is *Saccharomyces cerevisiae*. Nevertheless, the presence of multiple nuclei, their regular distribution along hyphae, and their semi-synchronous division make *A. nidulans* hyphae a suitable coenocytical model for the study of the composition/dynamics of the NPC during the cell cycle as well as the localization/dynamics of nuclear transporters, which in almost all cases belong to the family of karyopherins (KAPs). The first case has been and is currently being studied by the group of S.A. Osmani, while the systematic characterization of *A. nidulans* KAPs was carried out by the group of

E.A. Espeso (De Souza and Osmani 2009; Markina-Iñarrairaegui et al. 2011).

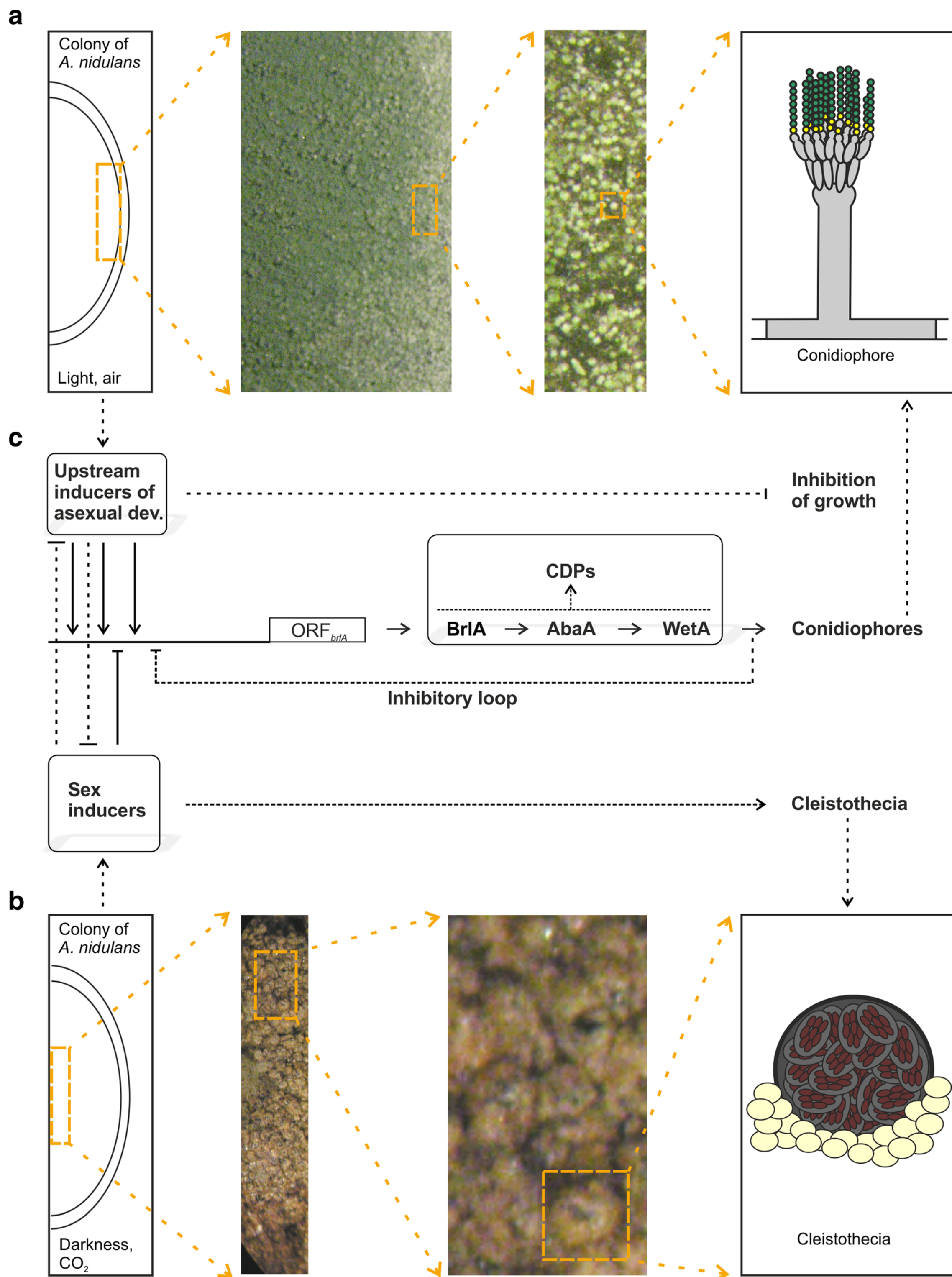
The proteins constituting the NPC are known as nucleoporins (NUPs) and assemble into sub-complexes that form the higher-order structures characteristic of the NPC (Fig. 1C) (see references within (Kim et al. 2018)). All of them generate the specific environment inside the pore for the translocation of transporter/cargo heterocomplexes in or out of the nucleus (Markina-Iñarrairaegui et al. 2011). NPCs are active during the interphase of higher eukaryotes but disassemble during mitosis, being one of the main events leading to the collapse of the nuclear envelope and enabling the interaction between centrosomes and kinetochores through kinetochore MTs. One of the main findings in the functional characterization of *A. nidulans* NUPs is that NPCs maintain their core structure during mitosis while peripheral NUPS generally disperse (with some exceptions, such as Gle1) (Fig. 1C) (Osmani et al. 2006; Chemudupati et al. 2016). This led the authors to define *A. nidulans* mitosis as partially open, allowing free diffusion of macromolecules between nuclei and the cytoplasm at this stage (Osmani et al. 2006; De Souza and Osmani 2007, 2009). Through the characterization of NUPs and related proteins, or nucleolar proteins, the same group was also able to describe the dynamics of nucleoli during *A. nidulans* mitosis (Ukil et al. 2009; Chemudupati et al. 2016) or the dependence with DNA of NPC segregation to daughter nuclei (Suresh et al. 2017).

Seventeen nuclear transporters were identified by in silico analyses in *A. nidulans*, a lower number than in higher eukaryotes (Markina-Iñarrairaegui et al. 2011). Three of them belong to the NTF family of transporters and 14 to the karyopherin family (thus, they were named KapA-N). The absence of 11 of these 17 transporters led to viable colonies. Eight of the deletants showed a wild-type phenotype while three showed growth or developmental defects (see, for example, (Etxebeste et al. 2009b)). The six essential transporters are grouped into four transport pathways, such as the most widely characterized import pathway defined by importins-α and importins-β, KapA/B (Fig. 1C), or the export pathway defined by exportin Crm1, KapK. The presence of only four essential nuclear transport pathways strongly suggests that there is a functional redundancy among nucleo-cytoplasmic transport pathways in *A. nidulans*, making the assignment of a function to each KAP highly complex (Markina-Iñarrairaegui et al. 2011). This hypothesis correlates with the presence of multiple nuclear localization signals (NLSs) in key transcriptional regulators of metabolism or stress response, such as PacC, CrzA, or AreA (see below; our unpublished work on PacC; (Hernández-Ortiz and Espeso 2013; Hunter et al. 2014)), suggesting that transcription factors are usually imported into nuclei using different transport pathways. Unfortunately, the knowledge on the nature and characteristics of the NLSs bound by each KAP within their cargos is scarce and is generally limited to the importin-α/β dimer (see for example the NLStradamus and cNLSmapper algorithms; (Kosugi et al. 2009; Nguyen Ba et al. 2009)). This is why more experimental work is essential in order to determine the set of KAPs that each TF can bind.

All nuclear carriers of *A. nidulans* show a regular distribution in all nuclei within hyphae during interphase, suggesting that all nuclei possess the required elements to mediate active transport (Markina-Iñarrairaegui et al. 2011). During mitosis, modifications in the localization and dynamics of multiple KAPs were described, as for example, the re-localization of KapI/Kap121/Pse1 to nucleoli. This observation is probably directly related to the NPC modifications that occur during mitosis and suggests that KAPs carry out specific functions at this stage of the cell cycle (Markina-Iñarrairaegui et al. 2011). A further work also showed that KAPs have a role beyond the nuclear periphery. Importins-α and importins-β move bidirectionally along the cytoplasm in *A. nidulans*, reaching the hyphal tip and distal regions. This movement depends on the MT cytoskeleton, dynein, and probably kinesin-3 UncA, suggesting that KapA and KapB could be involved in the long-distance transport and nuclear import of cargos, as occurs in other types of polarized cells such as neurons (Etxebeste et al. 2013).

The ESC key is in the genome: asexual multicellularity in *A. nidulans*

The development and optimization of resources enabling growth within a specific niche are key for species to thrive. In this sense, the previous sections of this review have shown how *A. nidulans*, and filamentous fungi in general, manages growth and nucleo-cytoplasmic transport machineries in order to generate mycelia which are highly successful structures in terms of substrate colonization and adherence to it. But from an ecological point of view, as important as growth is the ability to propagate to new niches when environmental conditions are suboptimal (abiotic stress) or other organisms threat survival (biotic stress). Filamentous fungi behave as factories of thousands of cells, asexual spores, specially programmed with that aim: dispersion to a more favorable environment. The diversity of types and morphologies of fungal asexual spores is overwhelming (Kirk et al. 2008). Those produced by *A. nidulans* are known as conidia and are produced in complex multicellular structures called conidiophores (Fig. 2A) (Mims et al. 1988). The formation of conidiophores comprises stages of polar and isotropic growth, as well as budding. *A. nidulans* is the most widely used reference for the study of the genetic and molecular control of asexual development (Meyer et al. 2016). Most of the regulators of *A. nidulans* conidiophore development were identified in mutant screenings in the 1980s and the 1990s, and functionally



characterized in the last 30 years (reviewed by (Adams et al. 1998; Etxebeste et al. 2010; Oiartzabal-Arano et al. 2016)).

Recent reports suggest that the known regulators of asexual development in *A. nidulans* emerged progressively in evolution (de Vries et al. 2017; Ojeda-López et al. 2018). This stepwise emergence would rewire developmental pathways by establishing among the regulators hierarchical/democratic relationships not necessarily conserved in the rest of filamentous fungi, resulting in a vast array of possible spore morphologies (Etxebeste et al., unpublished). In *Aspergilli*, the transcription factor BrlA plays a central role in the induction and control of conidiophore development (Fig. 2C) (Adams et al.

◀ **Fig. 2** Control and balance of developmental cycles in *A. nidulans*. (A) In an agar medium, light and air induce the generation of conidiophores in *A. nidulans* colonies. Conidiophores are developed at subperipheral and central regions of the colony, while peripheral hyphae continue growing outwards (see Fig. 1). Each conidiophore bears thousands of asexual (meiotic) spores called conidia, which are the result of a limited number of morphological transformations. (B) Stimuli such as darkness and low O_2 concentrations induce the production of cleistothecia. Each cleistothecia bears multiple sacs called *asci* and each *ascus* contains eight meiotic spores called ascospores. Cleistothecia are surrounded by Hülle cells, which are supposed to feed sexual structures with nutrients. (C) Simplified model for the control of the balance between asexual and sexual developmental programs. On the one hand, upstream activators of asexual development induce the expression of *brlA*, and thus the central pathway that will control the morphological transformations leading to conidia production. Upstream activators also inhibit growth and sexual development. On the other hand, sex inducers bind the promoter of *brlA* to inhibit conidiophore development and induce the morphological transformations and nuclear rearrangements leading to cleistothecia and ascospore production. For simplification, the upstream reading frame in the promoter of *brlA*, the effect of chromatin remodelers, or the alternative splicing event have been intentionally omitted. See main text for more details

1988). It constitutes a bottleneck between the upstream developmental signaling pathways that converge at the promoter of *brlA*, deciding its induction/repression, and the central pathway that regulates all the morphological transitions leading to the production of approximately 10,000 conidia per conidiophore (Oiartzabal-Arano et al. 2016). Interestingly, *brlA* emerged in evolution after most of these upstream and downstream regulators (our unpublished results; see also (de Vries et al. 2017)), and developed the ability to recruit some of them to its promoter (both inducers and repressors; see below) and transcriptionally control the expression of the rest.

Research published in the last ten years has significantly improved our understanding of the molecular mechanisms that control the localization, dynamics, and activity of specific upstream regulators. Some of them show a close relationship with the subcellular transport machinery described in previous sections. The transcription factor FlbB and its partner FlbE must be first located at the tip of hyphae as a prerequisite for the induction of conidiation (Etxebeste et al. 2008, 2009a; Garzia et al. 2009; Herrero-Garcia et al. 2015). After that, FlbB purportedly convey apical signals to nuclei and directly induces the expression of *brlA* in coordination with a second transcription factor known as FlbD (Garzia et al. 2010; Herrero-Garcia et al. 2015). A third transcriptional regulator, FlbC, is the last effector of a second pathway essential for the induction of *brlA* expression (Kwon et al. 2010). The RGS protein FlbA participates in the induction of conidiation by inhibiting the polar growth machinery (Yu et al. 1996; Park and Yu 2012). Several proteins required for the synthesis of metabolites that induce *brlA* expression and thus conidiation have also been identified (Lee and Adams 1994a; Soid-Raggi et al. 2005, 2016; Márquez-Fernández et al. 2007; Rodríguez-Urra et al. 2012; Etxebeste et al. 2012).

A great number of the reports on the central developmental pathway (CDP) of conidiation were published principally by the group of W. E. Timberlake and after him T. H. Adams (Adams et al. 1998). Besides the activity of transcriptional regulators at its promoter, there are additional mechanisms for the control of *brlA* expression/translation: chromatin remodeling, the existence of two *brlA* transcripts that are required at different stages of conidiophore development, an upstream open reading frame or regulatory loops through late-CDP factors (Fischer and Kües 2006; Ni and Yu 2007; Twumasi-Boateng et al. 2009; Cánovas et al. 2014; Oiartzabal-Arano et al. 2016). Nevertheless, our understanding of how all these mechanisms are coordinated and modified during development is limited and deserves future collaborative efforts.

Due to its centrality in the induction and the control of the progression of the formation of conidiophores, BrlA was defined as sufficient and necessary to direct conidiophore development in *A. nidulans* (Adams et al. 1988). It was also considered that the activation of *brlA* irreversibly led to the completion of all morphological changes required for conidia production. Later, it was shown that this holds true if the environmental conditions that triggered development were maintained (Skromne et al. 1995; Etxebeste et al. 2009a).

Together with BrlA, the transcription factors AbaA and WetA constitute the basic structure of the CDP pathway (Fig. 2C). The general model draws a hierarchical relationship among these three regulators (BrlA > AbaA > WetA). AbaA controls late stages of conidiophore formation while WetA regulates conidial gene expression and the production/deposition of diverse metabolites into asexual spores, being thus essential for their integrity and pigmentation (Sewall et al. 1990; Marshall and Timberlake 1991; Andrianopoulos and Timberlake 1994; Wu et al. 2018). Nevertheless, AbaA and WetA are also supposed to control *brlA* expression through regulatory loops, directly or by additional transcription factors that inhibit *brlA* in the end of the process (Ni and Yu 2007).

With or without you: balance between sexual and asexual programs

While conidia are mass-produced mitotic (genetically identical) spores, the second type of spores produced by *A. nidulans*, ascospores, are meiotic (recombinant) spores contained in sexual complex multicellular structures called cleistothecia (Fig. 2B) (Todd et al. 2007). Morphologically, cleistothecia are much more sophisticated structures than conidiophores and, consequently, include more cell types. Conidiophore formation is thus faster and precedes cleistothecia development. Conidia constitute the main vehicle for propagation, while ascospores are programmed for long-term survival and genetic exchange (Etxebeste et al.

2010). There is also opposite correlation among the stimuli that induce or inhibit each developmental cycle. While signals such as light and the exposure of colonies to the air stimulates conidiophore development, darkness and high CO_2 concentrations favor cleistothecia production (Fig. 2A and B) (for a review, see (Dyer and O'Gorman 2012)). This has been interpreted as a mechanism of adaptation to environmental conditions, since considering that *A. nidulans* is a soil inhabitant, light and air would inform of nutrient limitation, inducing conidia production. Darkness and CO_2 (low O_2) would have the opposite meaning, favoring ascospore production. Taking the abovementioned features into consideration, both developmental cycles seem as mutually exclusive and, indeed, the activity of specific transcription factors in the induction/repression of each developmental cycle supports this hypothesis (Fig. 2C). It has also been suggested that the sexual cycle of *A. nidulans* may have fitness effects, since deleterious mutations accumulate at a lower rate in the sexual pathway than during conidiation (Bruggeman et al. 2003).

Again, *A. nidulans* is the main species within its genus in the study of sexual development (Dyer and O'Gorman 2012), due probably to the fact that its sexual cycle is being successfully reproduced at laboratories since the 1950s. Thus, the morphological transitions and the position of the compartments within cleistothecia are well known. Several transcriptional regulators involved in this process are also known, some of which establish functional/genetic links with other cellular processes such as the study of protein degradation through the COP9 signalosome (Braus et al. 2010) or secondary metabolism (see next section). However, in this review, we will focus on the multiple layers of crosstalk and mutual exclusion between both developmental programs (Fig. 2C). This means that *A. nidulans* invests a great deal of genetic and molecular resources in controlling the timing of each cycle, always depending on the signals received.

A first layer is exerted at the metabolic level through a group of compounds known as psi (precocious sexual inducer) factors. These oxylipins are polyunsaturated fatty-acid derivatives whose importance for *A. nidulans* sexual development was first described in the late 1980s (Champe et al. 1987; Champe and El-Zayat 1989). The group of N. P. Keller characterized the role of the psi-producing oxygenases in the previous decade, describing that the inactivation of any of the corresponding genes derived in altered levels of specific psi factors and the conidia-to-ascospore ratio (reviewed in (Tsitsigiannis and Keller 2007)). Furthermore, deletion of *fluG*, which codes for an upstream regulator of asexual development, causes an inhibition of conidia production due to the lack of an endogenously produced metabolite (Lee and Adams 1994a). The group of U. Ugalde showed that the aconidial phenotype of a null *fluG* strain was reversed by the combination of two compounds: dehydroaustinol, the active metabolite, and diorcinol, which prevented crystal formation of the former on the surface of aerial hyphae (Rodríguez-Urra et al. 2012).

Multiple transcriptional regulators act as balancers of development. Proteins such as VeA and VelB modify their interaction/localization patterns to control developmental cycles in response to light signals. The velvet complex shows strong genetic/molecular connections with regulators of sexual and asexual development as well as secondary metabolism (Ruger-Herreros et al. 2011; Bayram and Braus 2012). NsdD is key for cleistothecia production but at the same time represses *brlA* expression by targeting multiple sequences within its promoter (Han et al. 2001; Lee et al. 2016). Unlike that of *nsdD*, deletion of either *osaA* or *urdA* inhibits conidia production and prematurely induces sexual development (Alkhayyat et al. 2015; Oiartzabal-Arano et al. 2015).

Mining in the search of active compounds: secondary metabolism in *A. nidulans*

Species of the genus *Aspergillus* have a significant biotechnological impact. For example, *A. niger* is the main source of citric acid worldwide (Meyer et al. 2016). *Aspergilli* also produce antibiotics such as the β-lactam penicillin or mycotoxins such as aflatoxins (Espeso et al. 1993; Brakhage et al. 2009; Alshannaq and Yu 2017). Filamentous fungi in general were an important source of antibiotics in the previous century and it is estimated that they will be a key source of new ones in the future. In a context in which the emergence of bacterial strains resistant to antibiotics will become one of the major threats to humans, it is important to understand how fungi produce secondary metabolites and develop molecular tools to maximize or redesign the synthesis of bioactive compounds.

Rokas et al. (2018) defined secondary metabolism as the part of metabolism involving pathways associated with the production of small, bioactive molecules, such as mycotoxins, pigments, and antibiotics. Thus, fungal secondary metabolites can be considered chemical resources produced by fungi in order to obtain an advantage in a niche with respect to other (micro)organisms (Keller 2015). The ability to synthesize these compounds in fungi is coded in the genome by groups of genes that are clustered (Fig. 3). Secondary metabolite gene clusters code all or almost all of the information required for the synthesis and modification, cellular compartmentalization, and secretion of one or more active compounds (Nützmann et al. 2018). And we say *all or almost all information* because the organization of secondary metabolite genes clusters in fungi is variable. As reviewed recently by Nützmann et al. (2018), a metabolic cluster can include all the abovementioned genes but in other cases satellite subgroups may be located outside of the main cluster (even as unlinked genes). Chiang et al. (2015) recently described that collaboration between clusters was also possible in *A. nidulans* and that,

for example, the lipopeptide aspercryptin B was the product of two compounds produced by two separate gene clusters. Overall, these observations modify the initial hypothesis of *one gene cluster-one compound* to a more flexible one in which the authors suggest a coordinated and flexible control of the expression of secondary metabolite gene clusters to increase the variety of metabolites produced.

A fungal secondary metabolite gene cluster is (in most of the cases) defined by the presence of a gene coding for an enzyme that builds the basic structure of an end product (backbone enzyme; Fig. 3) (Inglis et al. 2013; Nützmann et al. 2018). These enzymes can belong to the families of polyketide synthases (PKSs), nonribosomal peptide synthetases (NRPSs), prenyltransferases, or terpene synthases and, thus, define the type of end product that may be generated. They use to be flanked by genes encoding auxiliary enzymes involved in the modification of the first precursor, transporters required for secretion or to avoid self-poisoning, and transcription factors that specifically control the expression of the genes of that cluster (Fig. 3). Inglis and colleagues proposed the presence of more than 65 secondary metabolite gene clusters in *A. nidulans*, while Rokas et al. have recently limited this number to 60 (Inglis et al. 2013; Rokas et al. 2018). Considering the possibility of collaboration among clusters for the synthesis of combined products, the number of bioactive compounds produced by *A. nidulans* will be probably significantly higher.

Some metabolic clusters of *A. nidulans* have a narrow taxonomic distribution, a general characteristic of fungal clusters, which reach the species- or even strain-specific level (see references within (Kjærbølling et al. 2018; Nützmann et al. 2018; Rokas et al. 2018)). Two main hypotheses have been proposed to explain the origin of secondary metabolite clusters, horizontal gene transfer (HGT), and de novo assembly through gene duplication and genome rearrangements. HGT has been proposed as the main mechanism for the presence in the genome of *A. nidulans* of at least the *dba* cluster (proposed source, *Talaromyces stipitatus*; all *dba* genes matched the position and orientation of their orthologs in *T. stipitatus*) and the An2030-2038 cluster (proposed source, *Metarhizium robertsii*; one gene lost and two genes interchanged their position) (Nguyen et al. 2015; Oiartzabal-Arano et al. 2015).

One of the main problems for the genetic/molecular characterization of metabolic clusters as well as the isolation of the associated compounds is the low expression levels of the cluster genes at laboratory conditions. This is in all probability due to the optimal growth and nutritional conditions used for culturing fungi at the lab as well as the absence of any competing (micro)organism. Different strategies for the induction of gene clusters, the identification of secondary metabolites, the

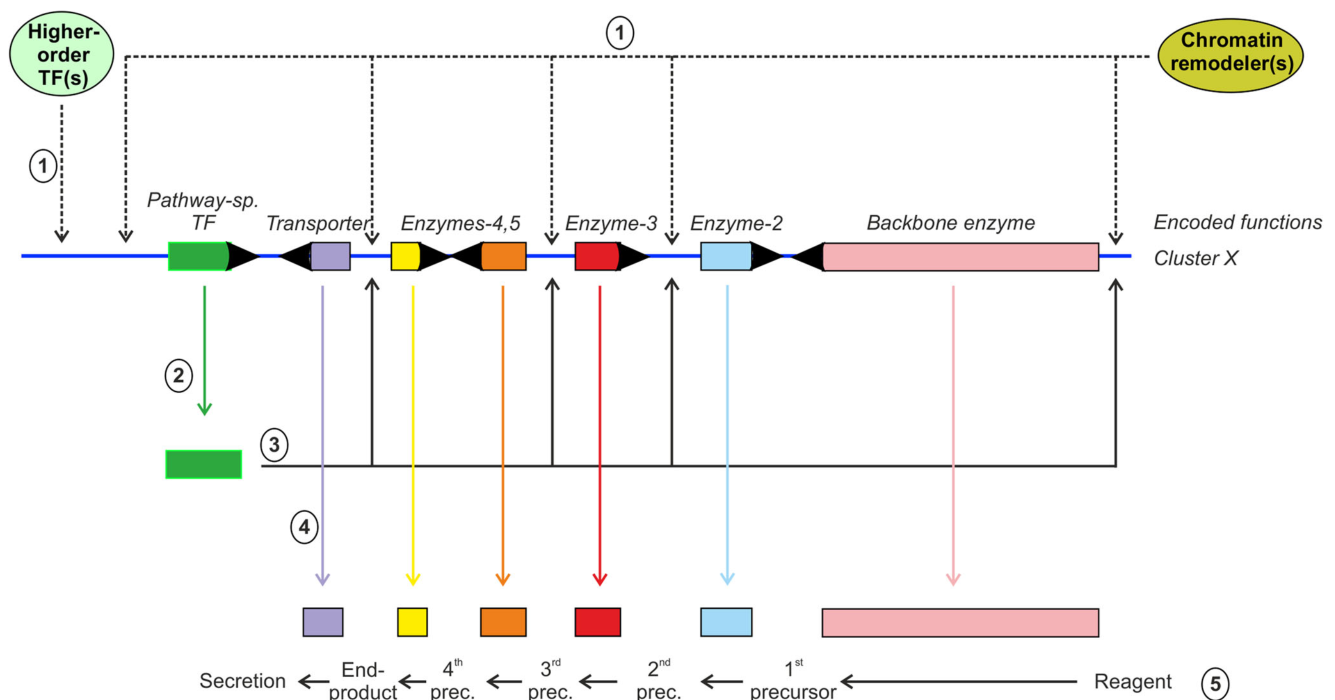


Fig. 3 A representative model for the control of the expression and the activity of secondary metabolite gene clusters. In this case, the cluster is composed of seven genes, encoding all the functions required for the synthesis and secretion of the active compound. (1) Higher-order transcriptional regulators (TF) induce the expression of the gene coding for the pathway-specific TF. Chromatin remodelers modify the conformation of the DNA to allow the induction of the genes of the cluster. (2) Synthesis of the TF and its import into nuclei. (3) Induction of the expression of the rest of the cluster genes. (4) Synthesis of the corresponding proteins. (5) Reaction pathway, starting from an initial compound (reagent) that is transformed by the backbone enzyme (PKS, NRPS,…) into the first precursor of the end product. Additional enzymes transform this precursor into the end product, which is secreted across a specific transporter

elucidation of the chain of chemical reactions that lead to their synthesis, or the characterization of their secretion pathway have been developed during the last years. In all of these research lines, *A. nidulans* (and other *Aspergilli*) has been an important reference organism.

The first and probably one of the easiest strategies is the modification of growth conditions, either by culturing the samples under sup-optimal nutritional conditions (abiotic stress) or by inducing developmental programs. This is, for example, a way to induce the sterigmatocystin (stc) gene cluster (mycotoxin production) and the *wA* gene cluster (spore pigmentation) (Kurtz and Champe 1982; Brown et al. 1996; Inglis et al. 2013). Directly related to this approach is the use of mutant backgrounds in which development or other key cellular processes are delayed or halted, as for example the absence of FlbB, FlbD, or AslA (transcriptional regulators of conidiation; see before); CsnE (COP9 signalosome component), MtfA, and McrA (zinc finger–type transcription factors); or the chromatin remodelers KdmB, RmtA, and EsaA (Soukup et al. 2012; Ramamoorthy et al. 2013; Gerke and Braus 2014; Oiartzabal-Arano et al. 2015; Gacek-Matthews et al. 2016; Oakley et al. 2016; Satterlee et al. 2016; Kim et al. 2017). More sophisticated approaches have deleted/overexpressed specific genes within a cluster, heterologously expressed secondary metabolite genes in *A. nidulans* or swapped domains within specific PKSs (see, for example, Chiang et al. 2013; Yeh et al. 2013). Finally, an elegant strategy designed by the group of A. Brakhage for the induction of secondary metabolite gene clusters is the exposure to biotic stress exerted by other microorganisms such as specific bacterial species (Schroeckh et al. 2009). Through this approach, the authors were able to induce the expression of a set of more than 30 genes that included three sequential secondary metabolite gene clusters. In addition, this work supported the view that fungi and bacteria establish transcriptional, peptidic, and metabolic relationships from which compounds with potential bioactivity can be isolated if the corresponding gene clusters are induced (Chiang et al. 2011).

Did you think I would crumble? No, I will survive (a tribute to Aretha): adaptation to ambient pH and cation stress conditions

It was in 1965 when Gordon Dorn published a manuscript describing the number and the regulation of phosphatases in *Aspergillus nidulans* (Dorn 1965). Perhaps he did not realize that what he was publishing was the first genetic analysis of a regulatory system based on sensing the ambient pH. Then, twenty years later, in 1986, H. N. Arst and his then student M. X. Caddick published their work rescuing Dorn's mutants and isolating new elements of the, today well-known, PacC-Pal regulatory pathway (Caddick and Arst 1986; Caddick et al. 1986a, b). Since then, several works have been published using *A. nidulans* (and also other fungal species) as reference organism. These works showed that PacC function goes beyond the regulation of the expression of extracellular proteins such as alkaline and acid phosphatases, and includes the production of antibiotics and mycotoxins as well as the modulation of virulence of yeast and filamentous fungi.

PacC is a zinc finger transcription factor that recognizes the target sequence 5′-GCCAAG-3′ within those promoters under its direct regulation (Tilburn et al. 1995). Initial genetic analyses gave some contradictory phenotypes that were understood when biochemical analyses were performed. Two classes of mutations can be found in PacC, those exhibiting a phenotype mimicking growth of the fungus at acidic pH (low levels of extracellular alkaline phosphatase, but high levels of acid phosphatase) and, on the other hand, mutations displaying the opposite phenotype (Tilburn et al. 1995). Mutations leading to the first type of phenotype were classified as loss-of-function mutations because the null *pacC* strain exhibits a similar phenotype. The second group of mutations was designated as $PacC^c$, constitutive mutations of PacC. The explanation to these opposed phenotypes came from the elucidation of the mechanism that activates PacC (Fig. 4A) (Orejas et al. 1995). Three forms of PacC are present in cells grown in neutral pH conditions: a primary form of 72 kDa ($PacC^{72}$), a truncated version of 53 kDa ($PacC^{53}$), and a third version of 27 kDa ($PacC^{27}$). Mutations truncating the protein and mimicking $PacC^{53}$ or shorter versions up to $PacC^{27}$ display a $PacC^c$ phenotype. However, those mutations causing an early truncation or before residue 250 (limit of $PacC^{27}$) display a loss-of-function phenotype (Arst and Peñalva 2003a). The molecular machinery after these two proteolytic steps are the Pal pathway through PalB, a cysteine protease of calpain family, and the proteasome (Fig. 4A) (Denison et al. 1995; Hervás-Aguilar et al. 2007). Pal products mediate the only pH regulated proteolytic step of PacC, from the 72-kDa to the 53-kDa form. Then, $PacC^{53}$ is committed to proteasome degradation to the $PacC^{27}$ form. This process has been shown to be conserved among species, although some differences have been found in yeast systems, in which PacC is designated as RIM101 (Li and Mitchell 1997; Li et al. 2004; Peñalva et al. 2008). The group of M. A. Peñalva has been referential in the study of PacC and the Pal signaling pathway and established a link between the machinery controlling the internal trafficking of vesicles and the pH regulatory system (Vincent et al. 2003; Galindo et al. 2007; Rodríguez-Galán et al. 2009), although this signaling pathway does not require endocytosis (Lucena-Agell et al. 2015).

Many groups have described possible phenotypes associated with defects in the putative PacC/RIM101-mediated pH regulatory system in other reference organisms. Nevertheless, some phenotypes were contradictory and evidenced the presence of additional regulatory systems accounting for the response to changes in ambient pH, especially when alkaline (Arst and

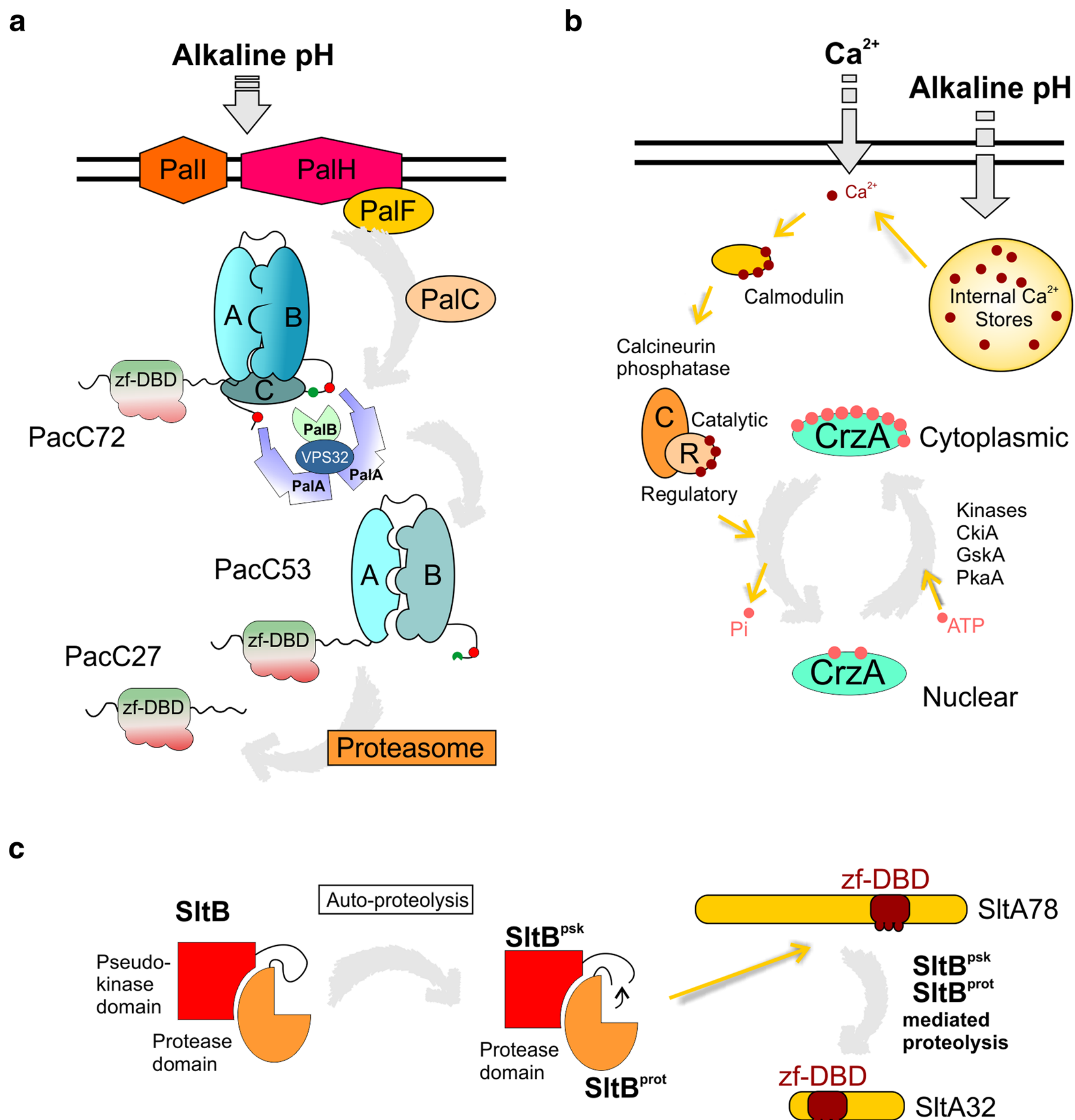


Fig. 4 Ambient pH and cation stress responsive pathways. (A) Schematic overview of the PacC/Pal regulatory pathway (see main text and the reviews by Arst and Penalva for an extensive explanation). (B) Signaling model for the calcineurin/CrzA pathway. Subcellular localization of CrzA is determined by its phosphorylated state depending on calcineurin phosphate and diverse kinases activities (see Hernández-Ortiz and Espeso 2013). (C) Representation of the two proteolytic steps of the Slt pathway. Autoproteolysis of SltB renders two subunits, one of them with a SltA-specific protease activity, leading to the generation of the active SltA[32] form (see Mellado et al. 2016). Zf-DBD: zinc finger DNA-binding domain

Peñalva 2003b). Further studies in *A. nidulans* have demonstrated that other two general regulatory systems are required for the fungus to tolerate alkaline ambient pH: (1) the calcineurin-response pathway mediated by the zinc finger transcription factor CrzA, a homolog of Crz1p in *S. cerevisiae*, and (2) the filamentous fungi-specific zinc finger transcription factor SltA.

Deletion of CrzA has two major effects in *A. nidulans* (also in *A. fumigatus*), increasing sensitivity to ambient alkaline pH and to elevated concentrations of extracellular calcium (> 10 mM) (Hagiwara et al. 2008; Soriani et al. 2008; Spielvogel et al. 2008). The regulation of CrzA activity depends on its phosphorylation levels (Hernández-Ortiz and Espeso 2013). Calcineurin (CN) is a calcium-dependent protein phosphatase and one of its targets is CrzA. Highly phosphorylated CrzA, mainly observed when calcineurin is inactive, is located at the cytoplasm and excluded from nuclei (Fig. 4B). A sudden elevation of extracellular calcium causes activation of CN by interaction with calmodulin (CaM), another calcium-binding protein. Active CN docks to and subsequently dephosphorylates CrzA. This causes the activation of the nuclear import mechanisms and inactivation of nuclear export signals, and consequently CrzA locates into the nucleus (Fig.

4B) (Hernández-Ortiz and Espeso 2013). Nuclear localization of CrzA is transient and depends on the intensity of calcium signaling, and other signals such as alkalinity, light, or even mechanical contact (Hernández-Ortiz and Espeso 2017). Importantly, alkaline pH signal must be converted into a calcium signal since, in the absence of calcineurin, CrzA remains cytoplasmic after extracellular alkalinization. Phosphorylation of CrzA is mediated by different kinases; among them are CkiA (casein kinase I) as well as GskA (glycogen synthase kinase) (Hernández-Ortiz and Espeso 2017), and probably PkaA (protein kinase A; our unpublished results). Because cellular localization of these kinases is different, they may act at different levels at CrzA, causing differential phosphoversions of CrzA to modulate its activity.

The third member of this group of transcription factors required for tolerance to alkalinity in *A. nidulans* is SltA. This zinc finger transcription factor was first described by O'Neil et al. (2002) when search for mutants defective in response to numerous abiotic stresses. SltA peculiarity is that it is only found in genomes of fungi of the Pezizomycotina subphylum and its degree of sequence conservation is very low outside the DNA-binding region, even among *Aspergilli* (Spielvogel et al. 2008; Mellado et al. 2015). SltA activity is required in *A. nidulans* for growth in the presence of elevated concentrations of many mono and divalent cations, such as sodium, lithium, potassium, cesium, and magnesium, but not calcium (Spielvogel et al. 2008). In fact, storage of calcium is greatly increased in a null *sltA* mutant. It is important to note the genetic interaction with mutations in the gene coding for the kinase HalA (Espeso et al. 2005; Findon et al. 2010). HalA participates in the homeostasis of sodium and potassium, and a double Δ*sltA*, Δ*halA* mutant requires elevated concentrations of extracellular calcium to display normal colonial growth. This phenotype was designated as "calcium auxotrophy" and further genetic studies demonstrated that it was a result of impairment of the correct storage and release of calcium from vacuoles (Findon et al. 2010). Suppressor mutations of the calcium-auxotrophy phenotype mapped in genes coding for sodium (*nhaA*) and potassium (*trkB*) transporters indicating a connection between calcium and sodium/potassium homeostasis.

As PacC (see above), SltA function is also regulated by proteolysis (Mellado et al. 2016). Two SltA forms are detected in cell protein extracts, the full-length version of 78 kDa ($SltA^{78}$) and a truncated version of 32 kDa ($SltA^{32}$) (Fig. 4C). $SltA^{32}$ is also phosphorylated by a still unknown mechanism. The protease responsible of SltA processing is SltB, a 1272 amino acid protein with two well-defined domains: (1) an N-terminal pseudo-kinase domain structurally related to kinases but lacking conserved functional residues at the catalytic site, and (2) a C-terminal domain related to the chymotrypsin family of serine proteases (Mellado et al. 2015). Directed mutagenesis and characterization of loss-of-function mutations in *sltB* have defined the sequence of events required for SltA activation (Mellado et al. 2016). SltB is first activated by autoproteolysis, separating the pseudo-kinase domain from the serine protease domain. Then, the serine protease domain processes $SltA^{78}$ to render $SltA^{32}$, the functional form that will control *sltB* expression, cation homeostasis, and probably intracellular traffic (Fig. 4C) (Spielvogel et al. 2008; Mellado et al. 2016; López-Berges et al. 2017).

Future prospects: *A. nidulans* in the near future

A brief search in the NCBI website (https://www.ncbi.nlm.nih.gov/) using the keywords *Aspergillus nidulans* plus the year (from 1995 to 2018) showed a minimum of 125 publications entries in PubMed for the year 2017. An equivalent search for the year 2018 rendered 152 entries. Compared to an estimated average of 159 entries in PubMed per year for this 24-year period and a peak of 190 entries in 2008, we found an average of 165 entries per year for the period 2010–2018. Overall, these figures suggest that the number of publications that used *A. nidulans* as a reference organism did not decline significantly since 2010. Of course, *A. nidulans* cannot be considered a suitable reference fungus in the study of, for example, effector synthesis and pathogenesis, but we predict that it will continue making major contributions in the fields reviewed above and additional ones. This demands the adaptation of recently developed techniques to the cellular and molecular biology of this model ascomycete and the improvement of previously existing ones. In this sense, some recent reports show the way towards this scenario.

A first example can be the improvement of the procedures for the generation of recombinant strains. Until recently, genetically engineered strains of *A. nidulans* could combine a limited number of site-directed mutations/deletions or gene tags. This was due to the limited availability of selection markers (mainly *A. fumigatus pyrG*, *pyroA*, and *riboB*). The group of B. R. Oakley have recently developed recombinant multi-marker strains with up to seven selectable markers, which will undoubtedly facilitate multiple sequential transformations in the future (Dohn et al. 2018). The combination of these markers with the use of photo-convertible/switchable tags and more-powerful fluorescence microscopy techniques such as super-resolution microscopy (adapted to *A. nidulans* by the groups of N. Takeshita and R. Fischer) will significantly improve our understanding of the localization/dynamics/reorganization of protein complexes (Perez-de-Nanclares-Arregi and Etxebeste 2014; Etxebeste and Takeshita 2015; Ishitsuka et al. 2015). Although site-directed mutagenesis procedures in *A. nidulans* are highly efficient and easily reproducible, the adaptation of CRISPR/Cas9 technology is a must,

as has been already done for other *Aspergilli* (Fuller et al. 2015; Nødvig et al. 2015; Zhang et al. 2016; Al Abdallah et al. 2018). Similarly, the study of protein complexes and the elucidation of their structure also require improved proteomic procedures, such as the one developed for the isolation of NPCs of *S. cerevisiae* and the determination of the type/amount of each NUP or the morphology of the entire pore (Kim et al. 2018).

A. nidulans has been used as a model for the study of non-essential genes/proteins that indeed are essential in plants and animals. This occurs for example with the characterization of the constituents of the COP9 signalosome in *A. nidulans*, carried out by the group of G. H. Braus et al. (2010). Considering the advantages of using a haploid system optimal for genetic manipulation as *A. nidulans*, this trend should be strengthened in the future. In this context, the characterization of *A. nidulans* NudF, required for the initiation of dynein-driven transport of cargos, contributed to the understanding of the role of its human ortholog Lis1 in neurological disease (Morris et al. 1998; Egan et al. 2012). Similarly, there are several parallels in the mechanisms enabling communication of polarity sites and nuclei in neurons and *A. nidulans* hyphae (Etxebeste and Espeso 2016), raising the possibility of using the latter as model for long-distance retrograde transport of cargos for neurons.

Massive analyses of *A. nidulans* development would enormously benefit from the coupling of single-cell microdissection to RNAseq or mass spectrometry, as was done previously in the Agaricomycete *Sordaria macrospora* (Teichert et al. 2012). This would enable a more accurate analysis of the genes up- or downregulated at each stage of the development of conidiophores or cleistothecia (even Hülle cells), and the subsequent study of their role. An equivalent experimental design applied to different sections of hyphae, as for example growth versus distal regions or most apical (closest to the tip) versus distal nuclei, would render valuable information on how hyphal asymmetry (polarization) is shaped or whether all nuclei within a hypha are transcriptionally/proteically non-equivalent.

As mentioned previously in this review, sexual and asexual developmental cycles of *A. nidulans* are probably the most widely extended system for the study of the genetic/molecular mechanism that control fungal spore production. Consequently, most of the regulators and pathways controlling the initiation or progression of development that have hitherto been identified and characterized belong to *A. nidulans*. Phylostratigraphy studies of these regulators, that is, the identification of the most phylogenetically ancient classes in which orthologs of a specific query protein could be detected (Krizsan et al. 2018), suggested that they emerged gradually in evolution (Etxebeste et al., under review). Thus, *A. nidulans* is a suitable model to study how democracy/hierarchy relationships within the transcriptional networks controlling development were shaped in each fungal species as well as the importance of network rewiring in determining the vast array of types of multicellular structures formed fungi.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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