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Switch-like reprogramming of gene expression after fusion of multinucleate plasmodial cells of two *Physarum polycephalum* sporulation mutants

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ABSTRACT

Nonlinear dynamic processes involving the differential regulation of transcription factors are considered to impact the reprogramming of stem cells, germ cells, and somatic cells. Here, we fused two multinucleate plasmodial cells of *Physarum polycephalum* mutants defective in different sporulation control genes while being in different physiological states. The resulting heterokaryons established one of two significantly different expression patterns of marker genes while the plasmodial halves that were fused to each other synchronized spontaneously. Spontaneous synchronization suggests that switch-like control mechanisms spread over and finally control the entire plasmodium as a result of cytoplasmic mixing. Regulatory molecules due to the large volume of the vigorously streaming cytoplasm will define concentrations in acting on the population of nuclei and in the global setting of switches. Mixing of a large cytoplasmic volume is expected to damp stochasticity when individual nuclei deliver certain RNAs at low copy number into the cytoplasm. We conclude that spontaneous synchronization, the damping of molecular noise in gene expression by the large cytoplasmic volume, and the option to take multiple macroscopic samples from the same plasmodium provide unique options for studying the dynamics of cellular reprogramming at the single cell level.

1. Introduction

It has been suggested that dynamic processes play an important role in the regulatory control of the reprogramming of stem cells, germ cells, and somatic cells [1,2]. Here we provide experimental evidence that multinucleate plasmodial cells of *Physarum polycephalum* obtained by fusion of two genetically and physiologically different individuals choose between two clearly different gene expression patters suggesting switch-like control mechanisms.

P. polycephalum like *Dictyostelium discoideum* and *Entamoeba histolytica* belongs to the amoebozoa group of organisms [3–7]. The life cycle of *P. polycephalum* comprises several cell types of specific morphology, function, and gene expression pattern that occur in temporal order as regulated by environmental conditions [8,9]. One stage is the so-called plasmodium, a multinucleate single cell. Since the early 1970s the *Physarum* plasmodium was used as a model organism to study different cell biological phenomena including cell cycle, cell differentiation, DNA replication, and cancer [10,11]. Plasmodia can be easily grown to a diameter of 10 cm. Even such large cells which then may contain 10^7–10^8 nuclei display natural synchrony in cell cycle and differentiation [12,13]. Plasmodia of compatible fusion type [14] spontaneously fuse upon cell contact and the cellular content mixes through the vigorous rhythmic cytoplasmic shuttle streaming [15]. When two fusing plasmodia are in different phases of the cell cycle, the nuclear populations synchronise over the entire plasmodium within a short period of time [12]. By quantitative evaluation of these phenomena the regulatory control of the cell cycle has been studied early on [13].

Upon starvation, macroscopically visible plasmodia (macroplasmodia) develop into a veined network [16]. Also in this physiological state, the cytoplasm is vigorously pumped forth and back through the veins by the rhythmic contraction of actin–myosin fibres [15,17,18]. Starved plasmodia are competent for being induced to sporulation by brief pulses of blue light [19,20] or far-red light received by specific blue light or phytochrome-like photoreceptors, respectively [21,22].

When a light stimulated plasmodium is fused with an unstimulated plasmodium, the developmental decision to sporulate depends on the physiological states of the two fusion partners and will be all-or-none for the entire plasmodium [21,22].

When two mutant plasmodia that are unable to sporulate in response to light fuse with each other, the ability to sporulate can be recovered as one fusion partner contributes the wild type gene product which is missing in the other partner and *vice versa* [23,24]. Fusion of plasmodia of different genotype in general leads to the formation of so-called heterokaryons in which genetically different populations of nuclei stably coexist. The different genetic identity of the nuclei is preserved as the nuclear membrane

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remains intact even during the cell cycle which occurs in the form of a closed mitosis [8].

Although we have previously shown that experimental fusion of a light stimulated plasmodium with an unstimulated plasmodium results in an all-or-none response in terms of sporulation [25–27], molecular events that are associated with the commitment of the cell were unknown. Accordingly, it was unclear to which extent the processes triggered by the light signal spread over the entire heterokaryon. In the present work we show that plasmodial fusion of two different sporulation-deficient mutants, one plasmodium light-stimulated the other not stimulated, results in the establishment of a common cytoplasmic state as characterised by the switch-like expression of developmentally regulated genes.

2. Materials and methods

2.1. Growth and preparation of plasmodia

For stock cultures, plasmodia of wild-type and mutant strains were hatched from spherules and taken into liquid shaken culture to be grown in the form of microplasmodial suspensions at 24 °C as described [24]. Subculturing was performed every 3.5 days by inoculating 50 ml Daniel and Baldwin liquid medium [28] in a 500 ml baffled flask with cotton plug. Plasmodia for gene expression studies were grown in a 5 L fermenter (Minifors, Infors HT, Bottmingen, Switzerland) with 1.5 L of growth medium [28] which was inoculated with 2 vol.% of a 3.5 days old shaken culture. Plasmodia were grown for four days at 24 °C, supplied with 1 L of air per minute, and stirred at 250 rpm with a marine propeller. Microplasmodia were harvested, washed twice with salt medium, and applied to starvation agar plates (9 cm diameter) with niacin and niacinamide [29] as described [30]. A ring of 1 g of cell paste (fresh weight) was applied to the centre of each plate with the help of a motor-driven 50 ml syringe coupled to an automatic device for rotating the agar plate around its axis. Plates were incubated for 8 days at 22 °C in complete darkness. During this time period, one multinucleate macroplasmodium develops on each plate. During the starvation period, wild type plasmodia become competent for sporulation.

2.2. Light stimulation of plasmodia and preparation of samples for RNA isolation

For stimulation with light, plasmodia were exposed to a 30 min pulse of far-red light (30 min, $\lambda \geqslant 700$ nm, 13 W/m^2), which was generated by Concentra Weißlicht lamps (Osram, Munich, Germany) and passed through an Orange 478 combined to a Blue 627 plexiglass filter (Röhm, Darmstadt, Germany) [27]. After irradiation, plasmodia were returned to the dark and incubated 22 °C. At approximately 6 h after the onset of the light pulse the plasmodia were harvested with a small glass spoon (Roth, Karlsruhe, FRG) and each individual plasmodium was separately shock-frozen in liquid nitrogen and stored at −80 °C for RNA isolation and gene expression analysis. Control plasmodia were treated identically except that the light pulse was omitted (dark controls). All manipulations were done under sterile conditions and under dim green safe light as described [31].

2.3. Fusion of mutant plasmodia

Plasmodia of strain PHO26 starved for 8 days in the dark were exposed to a 30 min pulse of far-red light. At 5.5 h after the onset of the pulse, the agar plates carrying the plasmodia were cut into 8 sectors each using a scalpel. In parallel, PHO1 plasmodia, which had not been irradiated, were also cut into 8 equal sectors. One sector carrying a PHO26 plasmodium was transferred into an empty Petri dish and placed adjacent to a sector carrying a PHO1 plasmodium so that the two agar slices touched each other and that large veins were brought into close proximity as much as possible. The sectors were marked according to the strain they were carrying and the plates were incubated in the dark for another 5 h. Subsequently, the two sectors in each plate were separated again and all plasmodial halves were frozen separately in liquid nitrogen for gene expression analysis. All manipulations were done under sterile conditions and under dim green safe light.

Table 1
Genes encoding transcripts that were quantified in the present study and their orthologs in the Uniprot database. For further details see [32].

Gene	Similarity	Uniprot entry
anxA	Annexin-B12	P26256
ardA	Actin, plasmodial isoform	P02576
arpA	Probable basic-leucine zipper transcription factor G	Q54RZ9
cdcA	Cell division control protein 31	P06704
cudA	Putative transcriptional regulator cudA	O00841
damA	DNA damage-binding protein 1a	Q9M0V3
dspA	Dual specificity protein phosphatase 12	Q9JIM4
ehdA	EH domain-containing protein 1	Q641Z6
gapA	Probable GTPase-activating protein 8	Q8H100
hcpA	Histone chaperone ASF1A	Q2KIG1
hstA	Probable histone H2B 4	Q27876
ligA	Checkpoint protein hus1 homolog 1 (LigA)	Q54NC0
meiB	Meiosis protein mei2	Q64M78.1
nhpA	Non-histone chromosomal protein 6	Q4PBZ9
pakA	Serine/threonine-protein kinase pakC	Q55GV3
pcnA	Proliferating cell nuclear antigen	Q43124
pikB	Phosphatidylinositol 3-kinase 2	P54674
pikC	Phosphatidylinositol 4-kinase beta	Q49GP3
pksA	Serine/threonine-protein kinase phg2	Q54QQ1
pldA	Phosphatidylinositol-glycan-specific phospholipase D	Q8R2H5
pldB	Phosphatidylinositol-glycan-specific phospholipase D	P80108
pldC	Phospholipase D	Q9LRZ5
pptA	Phosphatase DCR2	Q05924
pptB	Protein phosphatase 2C POL	Q8RWN7
psgA	Physarum specific gene	
pumA	Pumilio homolog 2	Q80U58
pwiA	Piwi-like protein 1	Q96J94
ralA	Circularly permutated Ras protein 1	Q75J93.1
rasA	Ras-related protein RABD2a	P28188
rgsA	Regulator of G-protein signaling 2	O08849
ribA	60S ribosomal protein L38	Q1HRT4
ribB	60S ribosomal protein L4-2	Q54Z69
spiA	Protein spire	Q9U1K1
tspA	Tumor suppressor p53-binding protein 1	P70399
uchA	Programmed cell death protein 2	Q2YDC9

2.4. Isolation of RNA and GeXP multiplex reverse transcription polymerase chain reaction (RT-PCR)

RNA was isolated from the plasmodial samples according to a standard protocol [30]. After reverse transcription, the transcripts of 35 genes were coamplified in each sample by PCR and the fluorescently labelled fragments separated and quantified on a Beckman Coulter 8-capillary sequencer (CEQ 8800) exactly as described previously [32]. With this method, repeated measurements of the same sample give identical results as the abundances of the individual transcripts are determined relative to each other [30,32].

3. Results

3.1. Gene expression patterns in the non-sporulating mutants PHO1 and PHO26 are altered as compared to the wild type

Two strains of *Physarum polycephalum*, PHO1 and PHO26, isolated in screens for sporulation-deficient mutants display a strongly reduced probability to sporulate [27,30] in response to a