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YelA, a putative *Dictyostelium* translational regulator, acts as an antagonist of DIF-1 signaling to control cell-type proportioning

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Abstract

DIF-1 is a polyketide, that is produced by *Dictyostelium* prespore cells and that induces initially uncommitted cells to differentiate as prestalk cells. Exposure of cells to DIF-1 causes transitory hypo-phosphorylation of seven serine residues in YelA; a protein with a region of strong homology to the MIF4G domain of eukaryotic initiation factor eIF4G. Based upon its domain architecture, which in one important aspect closely resembles that of Death-Associated Protein 5 (DAP5), we predict a role in stimulating internal ribosome entry driven mRNA translation. The two paradigmatic DIF-1 inducible genes are *ecmA* and *ecmB*. In support of a YelA function in DIF-1 signaling, a YelA null strain shows greatly increased expression of *ecmA* and *ecmB* in response to DIF-1. Also, during normal development in the null strain, the two genes are accelerated in their expression. This is particularly evident for *ecmB*, a marker of stalk tube and supporting structure differentiation. Mutants in DIF-1 bio-synthesis or signaling display a rudimentary or no basal disc and, conversely, YelA null mutants produce fruiting bodies with a highly enlarged basal

disc that ectopically expresses a stalk tube-specific marker. Thus YelA acts as an antagonist of DIF-1 signaling, with a consequent effect on cell type proportioning and it is predicted to act as a translational regulator.

Running title: Translational regulation by DIF

Keywords:

Dictyostelium, DIF-1, MIF4G domain, Translational regulation, DAP5

Introduction

Dictyostelium discoideum is an amoebozoan that undergoes a remarkable change, from unicellular growth to multicellular development, when its food source is depleted. The end point of development is a fruiting body, composed of a ball of spores supported by a cellular stalk that is embedded into a basal disc which is also composed of dead, vacuolated stalk cells. These two terminal stalk cell types derive from different precursor prestalk sub-types: pstA, pstAB, pstO, pstU and pstB cells (Gaudet et al., (2008), Yamada et al., (2010) and see Fig. 5A). Differentiation into prestalk and prespore cells is regulated by extracellular signaling molecules; cAMP induces prespore differentiation (Wang et al., 1988) and DIF-1, a polyketide, acts antagonistically to cAMP to induce prestalk differentiation (Kay et al., 1999). The cells that respond to DIF-1 by becoming prestalk cells appear to be a pre-enriched

population: exposed to starvation at a particular phase of the cell cycle and hyper-sensitive to DIF-1(Thompson and Kay, 2000).

DIF-1 signaling is normally assayed in monolayer cells developing under buffer in a petri dish. Under such conditions it is not possible to decide which kind of prestalk cells are induced. However, whole organism analysis of mutants deficient in DIF-1 production or DIF-1 signaling suggest that pstB and pstO differentiation are affected but to different degrees; PstB cells form the basal disc while pstO cells form the upper part of the stalk (Fig. 5A). Basal disc tissue itself is greatly reduced in DIF-1 mutants but the pstO region is maintained, albeit with a different pattern of gene expression (Keller and Thompson, 2008; Saito et al., 2008: Yamada et al., 2011). The defect in pstO cells derives from an analysis of *ecmA* and *ecmB,* the markers that originally defined the cell types and that encode two closely related proteins of the extracellular matrix (Jermyn et al., 1989; McRobbie et al., 1988; Williams et al., 1987).

At least two discrete transcriptional signaling pathways mediate prestalk gene expression (Yamada et al., 2013), and these are best understood for the *ecmA*-promoter. When multimerised and placed upstream of basal promoter elements, a 22 nucleotide element from within the ecmA promoter directs generic prestalk expression, and is DIF-inducible (Fukuzawa et al., 2006). The element contains an essential binding site for the Myb transcription factor MybE but there is genetic and biochemical evidence for the involvement of three other transcription factors in *ecmA* gene transcription: the b-Zip proteins, DimA and DimB, and the GATA factor GtaC (Huang et al., 2006; Keller and Thompson, 2008; Thompson et al., 2004; Zhukovskaya et al., 2006) reviewed by (Fukuzawa, 2011). DimA, DimB and GtaC

accumulate rapidly in the nuclei of cells induced with DIF-1 and DimB contains a phosphorylation site, very near the C terminus, that displays an increased level of modification in DIF-treated cells (Yamada et al., 2013). The signaling steps upstream from DimB phosphorylation are, however, entirely unknown.

In order to identify potential signaling intermediates, we previously used global proteomic methodology to identify sites that changed phosphorylation level within minutes of exposure to DIF-1 (Sugden et al., 2015). The results identified many proteins that undergo a transient net dephosphorylation at specific sites. The gene encoding one of these, *yelA,* was originally discovered in an insertional mutagenesis screen and was found to play an essential role in terminal differentiation on both the stalk and spore pathways (Osherov et al., 1997)*.* We identify seven DIF-regulated phosphorylation sites in YelA, all of which are dephosphorylated rapidly in response to DIF-1.

YelA contains an MIF4G domain (Osherov et al., 1997), a sequence initially identified as a sub-domain of eIF4G but now known to be present in several other proteins of diverse function (see legend to Fig. 3). eIF4G is a scaffold protein that recruits key components of the translational machinery to the mRNA; the MIF4G domain in eIF4G includes the binding sites for the eIF4A RNA helicase and for eIF4E, the 5' methyl G cap-binding protein (Fig. 1). Here we present evidence that YelA has a predicted role in setting the ratio of cap-site dependent to cap-site independent translation. Also, we characterize a deletion mutant of *yelA* that further implicates it in DIF signaling. This is the first suggestion that, in addition to its role as a regulator of transcription (Williams et al., 1987), DIF-1 functions as a translational regulator.

The identity of the regulated mRNA(s) is unknown but the *yelA* deletion mutant has an excessively large basal disc and is hyper-sensitive to DIF for gene expression suggesting that it or they encode negatively acting pathway components.

Results

Seven sites of serine phosphorylation in YelA become rapidly hypo-phosphorylated in cells exposed to DIF-1

In our previous study we performed a global quantitative screen for phosphorylation changes that occur within the first few minutes after addition of DIF-1, using a triple-label SILAC approach (Sugden et al., 2015). Several thousand peptides were detected that changed phosphorylation level at least two-fold in response to DIF-1. Only a few of these proteins were characterized further: the two forms of Protein Kinase B, PKBA and PKBR, the MAP kinase Erk2 and calcineurin. Here we characterize one of the other proteins, YelA. We identify seven DIF-regulated sites in YelA and they all follow the most common pattern, whereby DIF-1 causes net dephosphorylation (Fig. 2A-D).

Residues surrounding the phosphorylated serine do not show a strong consensus sequence. However arginine at P-3 and serine at P+2, P+4 and P+6 are common as is proline at P+1 (all numbered relative to the phosphorylated residue) (Fig. 2C, 2D).

YelA has the characteristics of a translational regulator

YelA contains a MIF4G domain, immediately proximal to its N-terminus and therefore well separated from the sites of DIF-1 inhibitable phosphorylation (Fig. 2B and (Osherov et al., 1997). The MI4FG domain was discovered in the eIF4G scaffolding protein and is also present in other components of the translational machinery. It is also, however, present in proteins involved in RNA splicing and in nonsense codon-mediated mRNA decay (see legend to Fig. 3). *Dictyostelium* encodes orthologues of all these proteins. Fig. 3 panel A is a "SMART" analysis (Letunic et al., 2012) of the seven membered *Dictyostelium* MIF4G domain family and panel B is the tree that derives from an alignment of the MIF4G domains. The YelA MIF4G domain clusters most closely with that of eIF4G itself and with a very close orthologue that we term eIF4G-like.

The YelA null strain forms aberrant fruiting bodies in which prestalk and stalk markers are mis-expressed and that have highly enlarged basal discs

In order to determine whether YelA does indeed have a role in DIF signaling, we generated a deletion strain by homologous recombination, removing most of the MIF4G domain (Fig. 4). The mutant forms aberrant fruiting bodies over approximately the same time course as the parental, Ax2 strain. This is in radical contrast to the published phenotype (Osherov et al., 1997). YelA was identified in a REMI screen for developmental mutants. Both the original REMI mutant and a freshly generated insertion mutant, with a more cap site proximal insertion point, arrest development when cells form mound shaped aggregates prior to slug

formation. These mounds contain environmental insult-sensitive cells that accumulate yellow pigment. There are two straightforward explanations for this disparity. It could reflect the fact that different parental strains were used: AX4 in the original study and Ax2 here. There are other examples where disruption of the same gene produces a more severe phenotype in AX4 (Nelson et al., 2000; Schilde et al., 2004). Alternatively, it may result from differences in the genetic lesion; the original REMI mutant and the re-disrupted mutant were single site insertions while the new mutant is a deletion mutant that removes a significant part of the gene including most of the MIF4G domain (Fig. 4). An important feature of the latter explanation is the implication that a more extensive mutation gives a weaker phenotype. This could most simply be explained if the previously described insertional mutants display a dominant negative effect that is not shown by the deletion mutant.

As mentioned, the series of morphological changes that shape the fruiting body, such as tip formation at the apex of the mound, occur at approximately the same times in the YelA null (*yelA*-) strain as in the parent. However, the final fruiting body is aberrant. The spore head fails to ascend the stalk, there is often a clearly demarcated band of cells near the apex and the basal disc is greatly over-sized. These features can best be visualized and understood using cell-type specific markers.

CotC is a protein of the spore coat and the gene is selectively expressed in prespore cells. In slugs or first fingers expressing cotC:gal, there is an unstained region at the posterior that will at culmination contribute to the outer part of the

basal disc. This region is greatly expanded in the *yelA-* strain (Fig. 5B). We also have positive staining data, confirming our designation of the pre-basal disc cells and identifying the apical band of cells. This derives from analyses of *ecmA* and *ecmB* expression. The ecmA:gal marker is, in parental structures, selectively expressed in pstA and pstO cells. They populate the front one-fifth of the slug and also constitute scattered cells within the prespore region: the ALC. It is also expressed in the prebasal disc region; weakly, but strongly enough to delineate the structure. In agreement with the *cotC* data the stained region is much larger in the yelA- strain (Fig. 5C). This was confirmed using the pstB and pstAB marker, ecmB:gal.

In parental Ax2 cells at the first finger stage ecmB:gal is expressed in ALC and selectively in pstB cells at the base. Once a migratory slug is formed, the pstB population disappears, presumably either deposited onto the substratum or dispersed within the slug, and a cone of *ecmB*-expressing cells appears in the tip. Because these cells also express *ecmA* they are called pstAB cells. At very long times of staining like that shown in Fig. 6A, there is apparent weak expression in the region of the pstO zone. Staining in this position increases dramatically at culmination to form a structure called the upper cup and there is most often a lower cup, below the spore head. The inner part of the basal disc is formed by the downward movement of the stalk, which embeds itself into the cone of pre-basal disc cells that form the outer basal disc. In the *yelA-* strain there is a much higher apparent level of ecmB:gal expression and it is highly precocious. Staining occurs in minutes rather than hours and there is considerable, apparently ectopic, expression. The upper cup is particularly prominent and premature; it stains strongly during the

finger stage and through into culmination. Again, as is visualized with the *cotC* and *ecmA*-derived markers, the prebasal disc and outer basal disc are expanded.

The above effects on cellular differentiation, although pronounced, are quantitative rather than qualitative. We therefore analysed a less widely expressed *ecmB* promoter-derived marker, ST:gal (Ceccarelli et al., 1991). This is normally expressed only within the stalk tube cells but in the *yelA-* strain it is also expressed in the outer part of the basal disc (Fig. 6B). Thus there is qualitative ectopic gene expression in the mutant.

ecmA and ecmB are prematurely expressed in the *yelA-* strain

Staining of the ecmA:gal and ecmB:gal expressing structures gives a rough guide to the relative timing and extent of expression in the parental vs the *yelA-* strain. However, analysis of the endogenous *ecmA* and *ecmB* transcripts can be made truly quantitative. Thus RT-qPCR was performed on parental and *yelA-* RNAs isolated every two hours from 8hr to 16hr of development (Fig. 5D and Fig. 6C). In the case of *ecmA* the rise in mRNA abundance occurs just one or two hours earlier in the *yelA-* strain, the peak is achieved earlier and the decline is much more rapid. In the case of *ecmB* there is a much more radical difference. A barely detectable rise in abundance in parental cells does not occur until 14hr while in the *yelA-* strain the mRNA is at 50% of its peak value by 10 hr. When the staining times are taking into consideration, these PCR data seem to be in accord with the marker expression patterns.

ecmA and _ecmB_ are hyper-inducible by DIF-1 in the _yelA-_ strain

The _ecmA_ and _ecmB_ genes are both inducible by DIF-1 in a monolayer assay. Given the effects of the _yelA_ null mutation on gene expression and gross anatomy, it was of interest to compare sensitivity to DIF-1. In the assay cells were rendered competent to respond to DIF by starvation as a monolayer, then exposed to vehicle (ethanol) alone or to DIF-1 at 30nM or at 100nM. In Fig. 7A the qPCR results for _ecmA_ and _ecmB_ are in each case normalized to the maximum value obtained; i.e. when the _yelA-_ strain is induced at 100nM. This reveals a major difference between the parental and the _yelA_ null, with the mutant showing gross over-expression of both markers. The mutant is not however entirely DIF insensitive. This becomes apparent when the same data set is normalized to the value at 100nM DIF-1 for each strain separately (Fig. 7B). As the concentration of DIF-1 rises from zero the level of expression also rises in the mutant strain.

Discussion

The seven sites where DIF-1 treatment exerts an effect on YelA phosphorylation

It is important to know whether the same kinase modifies YelA at the seven regulated sites. Although there is no strong consensus sequence surrounding the phosphorylated serine residue, there are some common features such as arginine at P-3, serine at P+2, P+4 and P+6 and proline at P+1 (Fig. 2C, D). Proline at P+1 was

also identified as a prominent residue in many other of the DIF-1 regulated phosphorylation sites (Sugden et al., 2015). This is often indicative of proline-directed kinase regulation such as extracellular-regulated kinase-2 (ERK2) and cyclin-dependent kinases (Sugden et al., 2015). They vary in their maximum response but it is impossible to give precise kinetics for all seven, because the sampling is by its nature highly stochastic. However, they were all rapid, transient and the largest decreases were in the order of 10-fold (Fig. 2A).

Insights into the possible function of YelA

We have performed a bio-informatic analysis of the *Dictyostelium* MIF4G family of proteins. Based upon MIF4G domain sequence alignments it would appear that YelA is most closely related to eIF4G itself and also to a related protein (termed here the "eIF4G-like" protein) that contains a von Willebrand Factor A domain ("VWA" in Fig. 3A). The latter fact is not however particularly informative because proteins containing a VWA domain are involved in a very wide range of processes. There is one glaring difference between the domain organization of eIF4G and YelA. The N-terminus proximal region of eIF4G contains the binding sites for eIF4E, the 5' cap binding protein, and PABP, the polyA-binding protein. However, the equivalent region is almost entirely absent from YelA; the MIF4G domain initiates a mere six amino acids from the initiation codon. Such an organization mirrors that of DAP5/P97/NAT1; a metazoan translational regulator that resembles eIF4G through most of its length but lacks sequences homologous to the approximate N terminal one third of eIF4G. The lack of an eIF4E association domain means DAP5 cannot

recruit mRNAs by binding to their caps, but retains the ability to initiate cap-independent translation via any IRES elements contained within the mRNA (Lee and McCormick, 2006; Lewis et al., 2008; Liberman et al., 2008; Liberman et al., 2015; Weingarten-Gabbay et al., 2014).

YelA is only a partial orthologue of DAP5

Although there is this striking similarity of architecture and detailed sequence of the MIF4G domain, YelA is not a complete orthologue of DAP5. Metazoan DAP5 and eIF4G both possess a C terminal domain that binds the Mnk-1 kinase. This is absent from both *Dictyostelium* YelA and eIF4G. YelA also lacks an MA3 domain, downstream of the MIF4G domain, such as is frequently found in MIF4G containing proteins including DAP5 (Fig. 3A). However this may be misleading because the MA3 domain in DAP5 does not bind eIF4A (Imataka and Sonenberg, 1997). Thus there are potentially significant differences between YelA and DAP5. However, we strengthen the case that YelA is the closest orthologue of DAP5 by showing that, although there are direct equivalents of most of the MIF4G containing proteins, there are, within the sequences of the MIF4G domains, no closer matches to DAP5. Thus, by a process of exclusion, we can be confident that there are no better candidates for the role of DAP5.

How does YelA regulate DIF-1 signaling?

The necessity to utilize IRES elements, rather than the cap site, confers potential selectivity on the translational process and DAP5 is believed to act in this

way to facilitate translation of cell cycle regulated and stress regulated mRNAs (Lewis et al., 2008; Liberman et al., 2009; Marash et al., 2008). These include Bcl-2, Cdk1 and p53. In the case of YelA, its domain structure suggests strongly that it is a translational regulator that recognizes IRES's in one or more unknown mRNAs encoding components of a DIF signaling pathway. Also, there is the implication that DIF-1 controls this activity via one or more of the phosphorylation events.

YelA as a repressor of DIF-1 signalling

The increased sensitivity to DIF-1 in the *yelA* null inversely correlates with the effects of genetically inactivating components of the DIF-synthesis or response machinery; the latter mutations result in reduced basal disc formation while the YelA null mutation results in increased basal disc differentiation. Precocious expression is more prominent with ecmB, a gene that is highly expressed in pstB cells. Therefore *yelA* functions to supress pstB cell differentiation. DIF-1 seems to antagonize this effect in the precursors of pstB cells, presumably by controling the phosphorylation of YelA. Analysis of a marker that is normally only expressed in the stalk tube confirms that expression is truly ectopic in the *yelA-* mutant. We do not know the target of the proposed translational control or how it impinges on the DIF signaling pathway. The most telling observation is the increased sensitivity to DIF but we do not know the identity of any other of the <u>direct</u> signaling intermediates, aside from DimB where there is ChIP data for promoter recruitment in response to DIF-1 (Zhukovskaya et al., 2006; Yamada et at., 2011)

Materials and Methods

Cell culture and development

Axenically grown cells, strain Ax2 Gerisch isolate, were used. Development, transformation and staining for lacZ expression is done as before (Fukuzawa et al., 2006).

Construction of the *yelA* knockout plasmid

For the *yelA* gene knockout, the *yelA* gene was amplified with primers GAATTCAGCCAATCAGATCCCTTAAACG and CTCGAGTTACAAAACAAATACATCAGACCCTAAATC and cloned into the pJet1.2 blunt cloning vector (Thermo Scientific, Ltd.). Region from 5' end to EcoRV site at nucleotide 2040 was replaced with a fragment from nucleotide -749 to 281 that was amplified with primers GAATTCGTCTGTATATTTGTCTATTTGGTTGGC and GATATCGATGATGGTTCCTCAACCACC, creating EcoRV site at the junction. The SmaI fragment of the blasticidin-resistant cassette from pLPBLP (Faix et al., 2004) was inserted into the EcoRV site.

Gene expression

RNA was prepared from cells and analysed by RT-qPCR as described previously (Sugden et al., 2011). Primers used were as follows. ecmA;

CCGTAAACTGTGAATGTGATGACC and GTCTTGGAATCGCAACTATCAGC,

ecmB; CTCTTGATTCATGTTGTTCAACTG and CATCGCCACATTTTCCAAATG,

Ig7; TTACATTTATTAGACCCGAAACCAAGCG and

AACAGCTATCACCAAGCTTGATTAGCC.

DIF sensitivity assay

$2x10^6$ cells were plated in 2.5 ml of stalk buffer (10mM MES-KOH pH 6.2, 10mM KCl, 2mM NaCl, 1mM $CaCl_2$) in a 6 cm dish and exposed to 4mM cAMP in fresh stalk buffer containing 50μM cerulenin for 1 hr. After incubating for 7 hours to attain competence to respond to DIF, cells were induced in fresh stalk buffer containing 50μM cerulenin and different concentrations of DIF-1. After 2 hours RNA was prepared and gene expression was analysed by RT-qPCR.

**Protein domain structure and phylogeny analysis**

Protein domain architecture was analysed with SMART (Letunic et al., 2012). Sequence of MIF4G domains (SM00543) is extracted and aligned using COBALT (Papadopoulos and Agarwala, 2007). Phylogeny is constructed by Bayesian inference using a mixed amino acid model with rate variation between sites estimated by a gamma distribution. Analysis was run for $4x10^5$ generations.

Acknowledgements

Fig 1
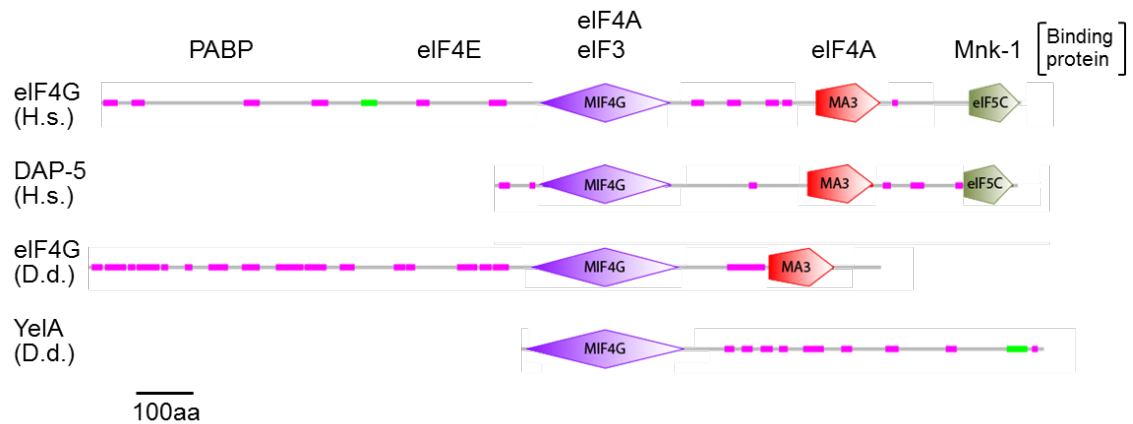
Figure 1. The domain architecture of human and *Dictyostelium* MIF4G containing proteins. MIF4G is shown in purple. Some proteins contain MA3 (red) and eIF5C (green) domains. The MA3 domain of *Dictyostelium* eIF4G does not register in SMART but is identified as a Pfam domain. Low complexity (pink) and coiled-coil (green) regions are shown. Proteins that interact with different part of human eIF4G are indicated at the top.

Fig 2
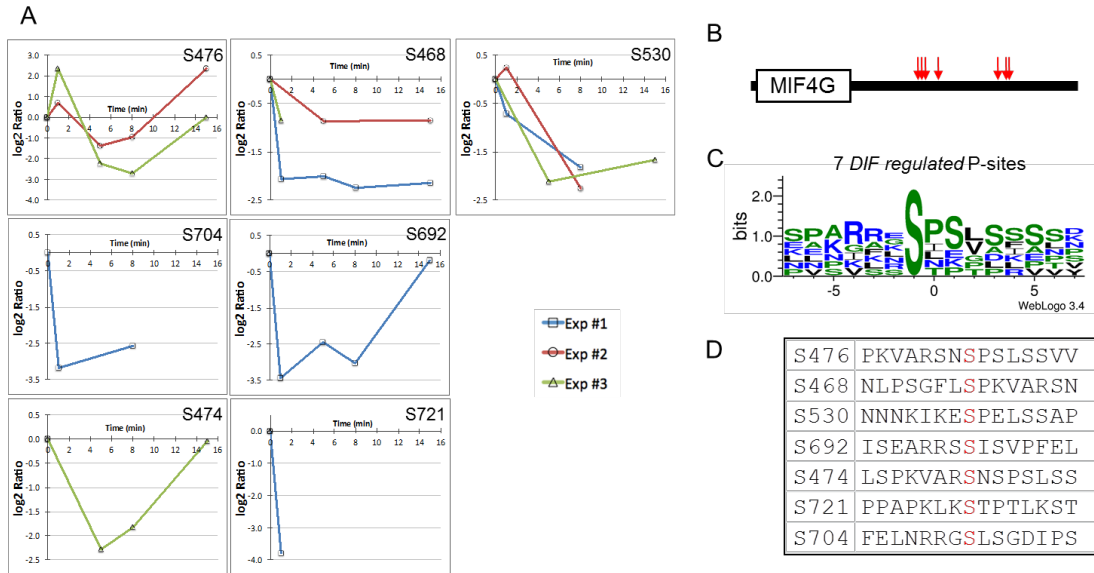
Figure 2. DIF-regulated phosphorylation sites in YelA. (A) Temporal profile of phosphorylation changes in response to DIF-1 stimulation. Level of phosphorylation is expressed as log2 ratio relative to pretreatment. Results from three biological experiments are shown in different colours. (B) Diagram of the positions of the DIF-regulated phosphorylation sites (red arrows) in the YelA protein. (C) A sequence logo of the seven phosphorylation sites in which amino acids are color coded according to their hydrophobicity: hydrophobic; black, neutral; green, hydrophilic; blue. (D) Amino acid sequences of the phosphorylation sites. The phosphorylated serine residue is shown in red.

Fig 3
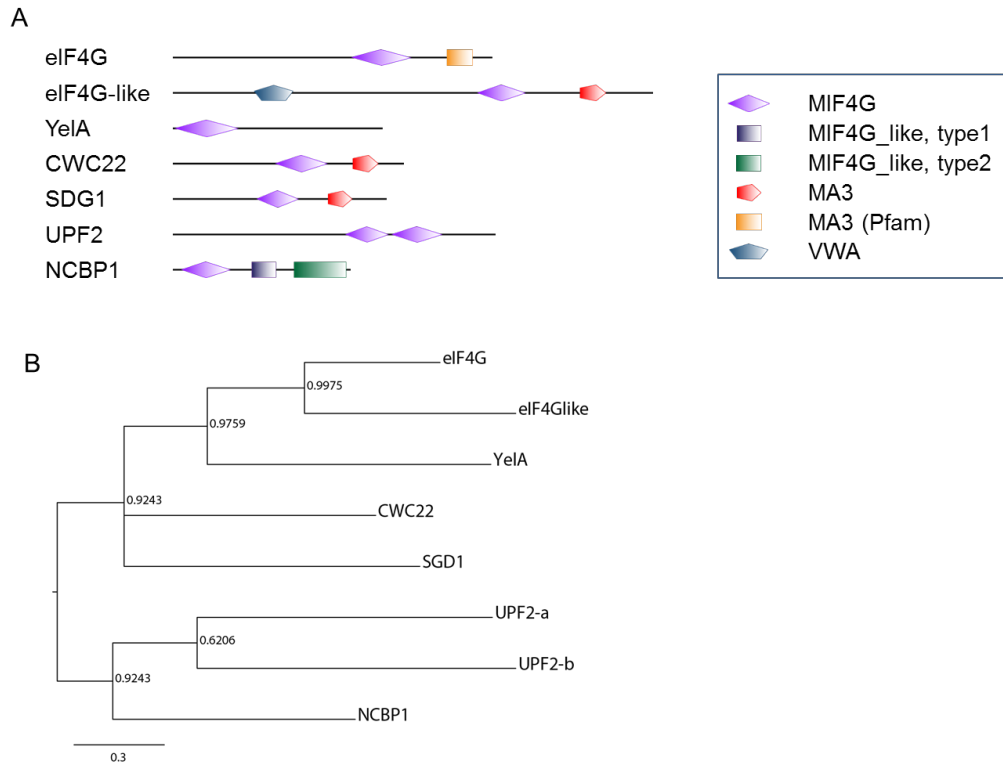
Fig. 3 *D. discoideum* **MIF4G containing proteins.** The identities of the proteins were assigned by machine annotation at dictyBase. (A) The domain architectures of the proteins were analysed using SMART. Domains are shown with different colours as indicated in the panel. Regions of low complexity and coiled coil are not shown for simplicity. (B) The sequences of the MIF4G domains were aligned using COBALT and phylogeny was constructed by Bayesian inference. Numbers at nodes indicate probability. The two MIF4G domains of Ufp-2 are shown as Ufp2-a and b.

eIF4G; DDB_G0275395, is the protein designated eIF4G at dictyBase.

eIF4G-like; DDB_G0286969.

CWC22; DDB_G0267796, is a pre-mRNA splicing factor that is associated with the spliceosome.

SGD1 (NOM1); DDB_G0284539 is a nucleolar protein that is required for 18S rRNA biogenesis.

NCBP1 (CBP80); DDB_G0269814, Nuclear cap binding protein 1 is responsible for a "pioneer" round of translation designed to detect defective mRNAs.
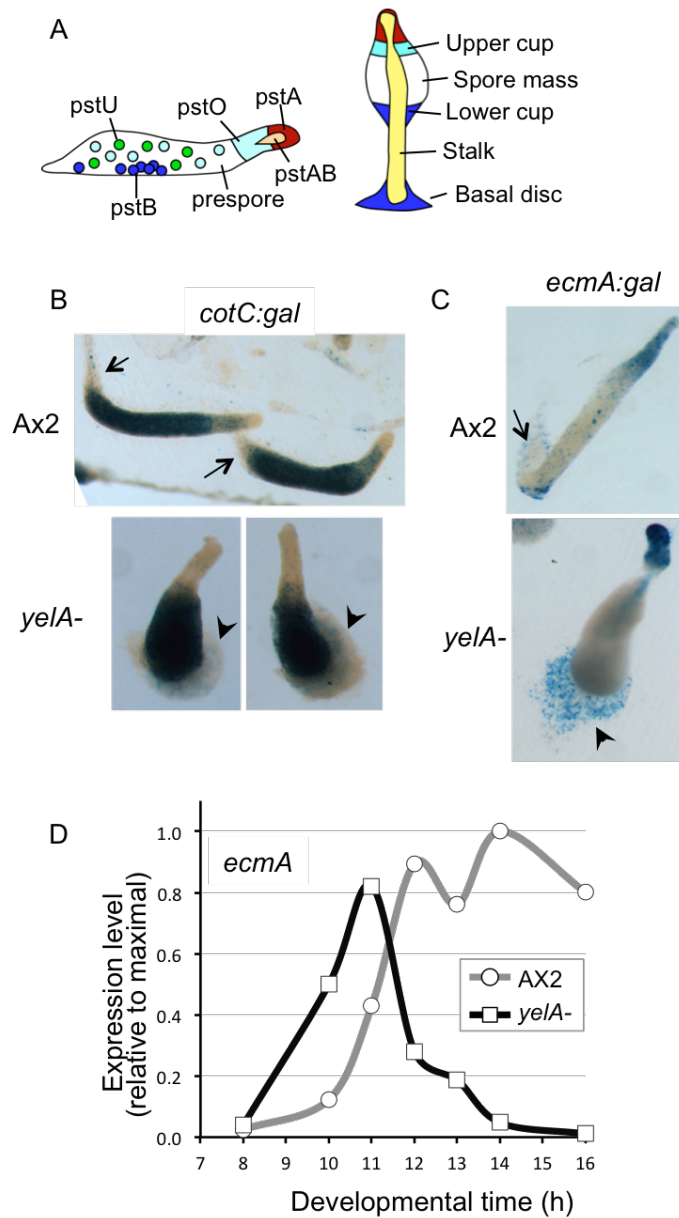
Upf2; DDB_G0281623, is a nuclear protein that mediates the decay of mRNAs encoding a premature stop codon.

Fig 4
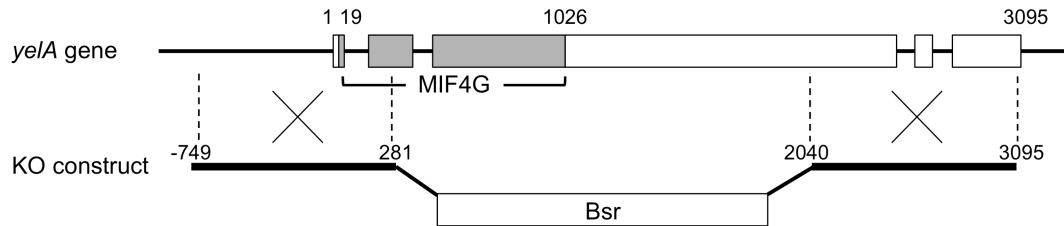
Figure 4. Disruption of the *yelA* gene. The genomic structures of *yelA* and the *yelA* knockout (KO) construct are shown. Bsr; blasticidin resistant cassette. The MIF4G domain (grey) encompasses nucleotides 19 to 1026.

Fig 5

Figure 5. Expression of prespore and prestalk marker genes in Ax2 and *yelA-* cells.

(A) Schematic diagram of cell type patterning in a slug and a culminating structure. The stalk derives from prestalk cells that occupy the anterior region of the slug: pstA cells and pstO cells. Within the anterior region is a core of pstAB cells that will form

the inner part of the basal disc. The outer region of the basal disc is formed from pstB cells, which reside at the rear, substrate proximal region of the slug. Some of the pstB cells also form a lower cup that supports the spore cell mass, whereas some of the pstO cells and another sub-type, the pstU cells form an upper cup above the spore mass (Yamada et al., 2010). (B, C) Ax2 and *yelA-* cells expressing cotC:gal (B) or ecmA:gal (C) were developed for 14.5 hours, fixed and stained for β-galactosidase. Prebasal disc region at the rear of Ax2 slug (arrow) and the corresponding region in yelA- (arrow head) are indicated. (D) RNA was prepared from Ax2 (circle) or *yelA-* (square) cells at the indicated times of development and analysed for *ecmA* expression by RT-qPCR. Expression is shown relative to the maximal expression observed, that of Ax2 cells at 14.5hr.

Fig 6
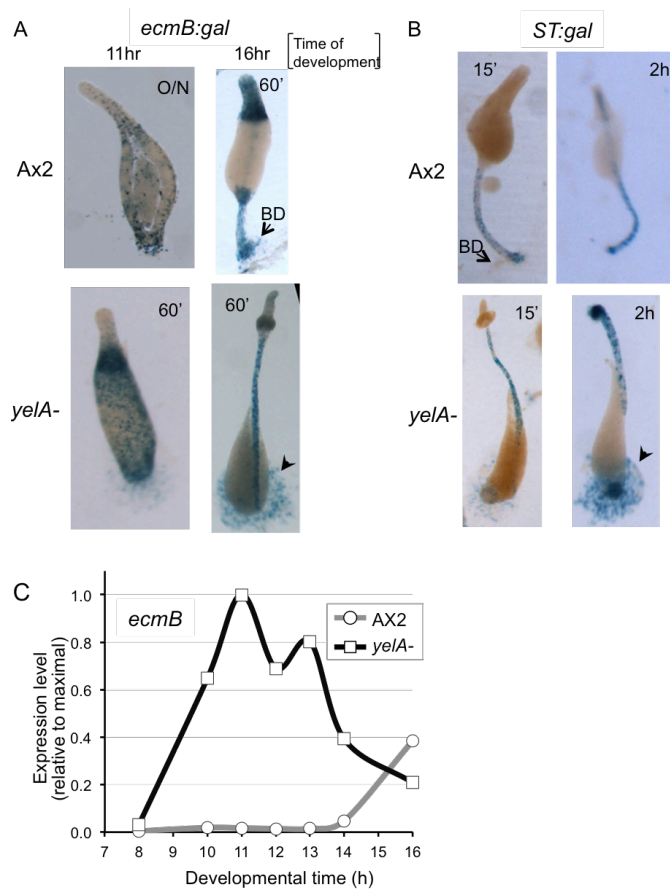
Figure 6. Expression of *ecmB* gene in Ax2 and *yelA-* cells. Ax2 and *yelA-* cells transformed with ecmB:gal (A) or a stalk tube specific ST:gal (B) were developed, fixed and stained for β-galactosidase. In (A), cells were developed for indicated times and the 11 hour-structure of Ax2 was stained overnight (O/N), while other structures were stained for 60 min. In (B) 17 hour-developed structures were used. The staining times are indicated on each panel. In both (A) and (B), basal disc of culminating structures of Ax2 (arrow) and the corresponding region of *yelA-* (arrow head) are indicated. (C) RNA was prepared from Ax2 (circle) or *yelA-* (square) cells at the indicated times of development and analysed for *ecmB* expression by RT-qPCR. Expression is shown relative to the maximal expression (yelA at 11 hr).

Fig7
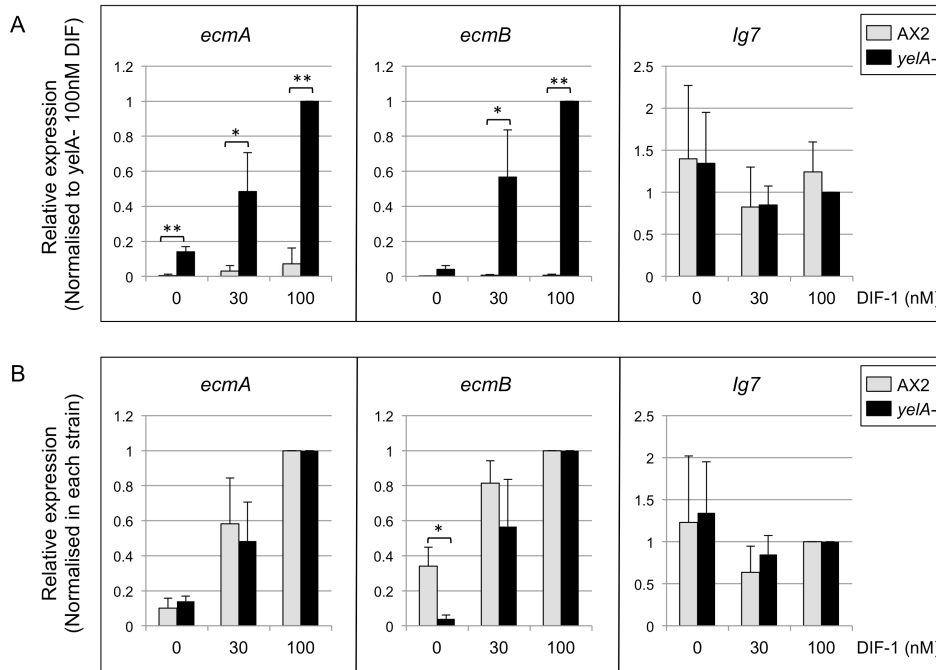
Figure 7. DIF sensitivity of prestalk gene induction in Ax2 and *yelA-* cells. Ax2 and *yelA-* cells were incubated in monolayer with different concentration of DIF-1. After 2 hours, RNA was extracted and analysed for gene expression by RT-qPCR. In panel (A), expression is normalised to expression in *yelA-* cells treated with 100nM DIF-1. In (B), the same result is presented as a fraction of expression with 100nM DIF-1 within each strain. Ax2; grey bars, *yelA-*; black bars. Where the p-value of the t-test was <0.01 (**) or <0.1 (*) is indicated by asterisks.

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