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Short Communication

Identification of new modes of Dd-STATa regulation of gene expression in *Dictyostelium* by *in situ* hybridisation

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ABSTRACT Signal Transducers and Activators of Transcription (STATs) are transcription factors which lie at the end of cytokine and growth signal transduction pathways. *Dictyostelium* Dd-STATa is a functional homologue of metazoan STATs. It is activated by cAMP and, at the slug stage, it translocates into the nuclei of the tip cells, which are a subset of the anterior, prestalk A (pstA) cells. Here we searched for novel Dd-STATa regulated genes by *in situ* hybridisation. A set of 54 cDNA clones whose gene expression patterns are known to be prestalk-specific (Maeda *et al.,* 2003), were chosen as probes and we compared their expression patterns in parental and *Dd-STATa*-null strains. We identified 13 genes which are candidates for direct induction by Dd-STATa. In the parental strain, most of these genes are expressed in the cone shaped mass of pstAB cells which is located within the prestalk region. These cDNAs show little or no expression in the *Dd-STATa*-null strain. This contrasts markedly with the paradigmatic *ecmB* gene which is expressed in pstAB cells in parental cells, but which is expressed *throughout* the prestalk zone in the *Dd-STATa*-null strain. We also identified several genes which are normally expressed in pstA cells, or throughout the prestalk region, but whose expression is markedly down-regulated in the null mutant. Again, this contrasts with markers derived from the paradigmatic, *ecmA* gene which are expressed normally in the *Dd-STATa*-null strain. The identification of these novel genes provides valuable tools to investigate the role of Dd-STATa.

KEY WORDS: *in situ hybridisation, transcriptional regulation, Dictyostelium, STAT transcription factor*

STATs are transcription factors which lie at the end of cytokine or growth factor signal transduction pathway. They play a role in many important biological phenomena and are evolutionarily conserved among multicellular eukaryotic organisms (Darnell, 1997; O'Shea *et al.,* 2002). Recently, it has also been reported that STATs are involving in regulating morphogenetic cell movement (Hou *et al.,* 2002). Thus, it is of importance to know which gene classes are regulated by STATs. For this purpose we used the cellular slime mould *Dictyostelium*, the simplest eukaryote having STAT proteins, as a model organism.

In *Dictyostelium*, four STAT genes, *Dd-STATa, b, c* and *d* have been identified (Kawata *et al.,* 1997; Mohanty *et al.,* 1999; Fukuzawa *et al.,* 2001; Zhukovskaya *et al.,* 2003; Abe and Williams, unpublished). Dd-STATa is necessary for efficient chemotactic cell movement at the aggregation stage and morphogenesis after the slug stage (Mohanty *et al.,* 1999), suggesting that Dd-STATa is involved in aggregation and multicellular development by regulating chemotactic cell movement. In the slug tip, Dd-STATa is phosphorylated on a tyrosine residue in response to cAMP signaling and then translocates into nuclei (Kawata *et al.,* 1997; Araki *et al.,* 1998). Therefore, Dd-STATa seems likely to regulate gene expression in the tip region of the slug. In support of this hypothesis, the *Dd-STATa*-null mutant lacks a subset of the tip cells, does not express the *cudA* gene (Fukuzawa and Williams, 2000) in the tip and fails to culminate (Mohanty *et al.,* 1999). Therefore, genes regulated by Dd-STATa presumably have important functions during culmination. As STAT signalling networks are known to be evolutionarily conserved, hunting for such genes is of considerable importance. However, *ecmB* and *cudA* are the only target genes of Dd-STATa thus far known (Mohanty *et al.,* 1999; Fukuzawa and Williams, 2000).

Abbreviations used in this paper: ALC, anterior-like cells; Dd, Dictyostelium discoideum; psp, prespore; pst, prestalk; STATs, signal transducers and activators of transcription.

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In order to understand the role of Dd-STATa in *Dictyostelium*, we have searched for Dd-STATa target genes, which are expressed in the anterior tip cells and positively regulated by Dd-STATa protein. *In situ* hybridisation analyses identified 13 new candidate Dd-STATa target genes. Most of these genes are expressed in the cone shaped mass of prestalk AB cells in the wild-type strain. In addition, several Dd-STATa dependent genes are expressed in the pstA (cells that express the proximal, pstA-specific part of the *ecmA* promoter) and pstAO cells (cells which express the distal, pstO-specific part and the proximal, pstA-specific part of the *ecmA* promoter). The role of Dd-STATa is discussed in terms of morphogenetic cell movement via the newly identified target candidate genes.

Results & Discussion

Dd-STATa can act as either transcriptional activator or repressor in *Dictyostelium* (Mohanty *et al.,* 1999; Fukuzawa and Williams, 2000). In order to search for the Dd-STATa target genes, we focused on the prestalk-specific genes whose expressions seemed likely to be positively regulated by Dd-STATa; because the *Dd-STATa* gene is expressed in the pstA cells at the early slug stage (Fig. 1; Shimada *et al.,* 2004) and Dd-STATa protein is activated by cAMP through the cyclic AMP receptor (cAR1) and then translocates into nucleus of pstA cells (Araki *et al.,* 1998).

Among the 104 ESTs previously analysed by *in situ* hybridisation, we chose 37 pstA or pstAO, 8 pstAB and 8 late prestalk genes whose expression patterns are very clear (Maeda *et al.,* 2003). Expression patterns of all these genes were analyzed and compared between wild-type (Ax2) and *Dd-STATa*-null strains (Tables I, II). Most of pstA/AO genes were expressed even in the absence of Dd-STATa (Table II), suggesting that Dd-STATa is unlikely to act as a transcriptional activator for these genes. Also, all 8 late "prestalk" genes, which are not expressed at the slug stage but that become expressed during culmination, showed indistinguishable patterns in wild type and *Dd-STATa*-null strains, This suggests that Dd-STATa is unlikely to be a repressor for these genes at the slug stage.

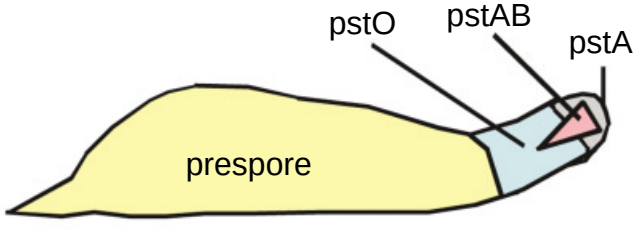

Fig. 1. Schematic illustration of cell types in a *Dictyostelium* slug. *The slugs of* Dictyostelium *are composed of two basic cell types, prespore (psp) and prestalk (pst) cells. Prestalk cells are further divided into several subtypes, pstA, pstO and pstAB cells, based on the expression of promoter subfragment of* ecmA *and* ecmB *genes (Jermyn* et al., *1996). The pstA cells occupy the front half of the anterior prestalk cells, while the pstO cells occupy the rear half of the pst cells. There are additional prestalk subpopulations, anterior like cells (ALC) and rearguard cells, which are not indicated in this illustration.*

TABLE 1

Dd-STATa-INDUCED PRESTALK-SPECIFIC GENES IN *DICTYOSTELIUM*

Accession no.	cDNA	Product	E value
PstA/AO genes			
AU052911	*SLF308*	Extracellular matrix protein	1e-05
AU038606	*SSL238*	Extracellular matrix protein St15	0
AU060824	*SLB609*	Acid phosphatase	5e-22
C90144	*SSI141*	base-nonspecific/acid RNase	1e-123
C91531	*SSK395*	Homologous to Adducin 1 (alpha) head	1e-52
PstAB genes			
AU060958	*SLC388*	*Dictyostelium* steroid isomerase	5e-45
AU060722	*SLB233*	*Arabidopsis* dynamin-like protein	2e-42
AU060076	*SLA128*	Homologous to expansin	5e-10
AU061846	*SLG322*	Carboxypeptidase	5e-62
AU038850	*SSL558*	Homologous to endo-1,4-b-glucanase	2e-76
AU062236	*SLI604*	Aconitate hydratase	0.0
C91965	*SSC596*	Unknown	
AU074317	*SSK348*	Homologous to Acetyl-CoA synthetase	6e-82

Interestingly, however, we were able to identify 13 new candidate Dd-STATa induced genes. Expressions of five pstA/AO genes, *SLF308, SSL238*, *SLB609, SSI141* and *SSK395* were greatly reduced or totally absent in the *Dd-STATa*-null strain (Fig. 2A,B, Table I). As shown by these results, cells expressing these genes almost overlapped the cell-types of wild type slugs where Dd-STATa is activated (Figs. 1 and 2 A,B and Araki *et al.,* 1998). Thus, the loss of such gene expression suggests that Dd-STATa could serve as a direct activator of these genes. Alternatively, Dd-STATa may indirectly activate expressions of the five genes. The fact that the expressions of other prestalk-specific genes, as listed in Table 2, were all expressed even in the absence of Dd-STATa is very striking; thus Dd-STATa appears to be involved in transcriptional regulation in different manners, gene by gene, even in the same cell types.

TABLE 2

Dd-STATa-INDEPENDENT PRESTALK-SPECIFIC GENES USED IN THIS STUDY

PstA/AO genes	*SSM194*	*SSF823*	*SSB559*	*SSG767*	*SLF535*
	SSA854	*SSM416*	*SSD873*	*SLA387*	*SSA210*
	SSJ246	*SSJ814*	*SLG775*	*SSL349*	*SLF774*
	SSA358	*SLK182*	*SSH630*	*SSL853*	*SSL550*
	SSB337	*SLE474*	*SSF295*	*SSM642*	*SSK232*
	SSJ314	*SSG805*	*SSK159*	*SSM184*	*SSK861*
	SSF509	*SSH475*			
Not expressed at slug stage	*SSE634*	*SSD492*	*SSG357*	*SSG721*	*SLH173*
	SSD123	*SLH752*	*SLI271*		

We also found that all pstAB genes, which we identified previously, are not expressed in the pstAB cells in the mutant slugs (Fig. 2 C,D, Table I). These results again suggest that Dd-STATa might act as a transcriptional activator for these pstAB genes. However, we cannot rule out the possibility that pstAB cells are simply absent from the *Dd-STATa*-null, because the expression of the *ST::lacZ* marker gene, the paradigmatic pstAB marker, is absent in the *Dd-STATa*-null mutant (Mohanty *et al.,* 1999). Among these pstAB genes, several such as *SSL558* are expressed in pstA and pstO cells at the earlier, tipped mounds and standing slug stages (see Fig. 2D yellow arrowheads). Expressions of these genes in both pstA and pstO cells was not affected by the absence of Dd-STATa. Therefore, these results indicate that the regulatory mechanism necessary for the expression of these genes in pstA and pstO cells differs from that in pstAB cells.

In conclusion, we have identified 13 new candidate Dd-STATa-regulated genes by *in situ* hybridisation. Eight are pstAB genes and five are pstA/AO genes. It remains to be determined whether Dd-STATa directs their expression directly or indirectly. A more extensive analysis of these genes should provide useful information with which to understand the molecular mechanism whereby Dd-STATa to controls *Dictyostelium* pattern formation.

Experimental Procedures

Cells and growth conditions

D. discoideum Ax2 and *Dd-STATa*-null cells were cultured axenically in HL5 medium at 22ºC. Cells of the *Dd-STATa*-null strain were kept growing in HL5 supplemented 10 μg/ml blasticidine S (Kaken Pharmaceutical, Tokyo, Japan).

In situ *hybridisation*

Whole-mount *in situ* hybridisation analyses were performed as described previously (Escalante and Loomis, 1995; Maeda *et al.,* 2000; 2003). In this study, Ax2 and *Dd-STATa*-null cells in an Ax2 background were allowed to develop on filter paper (FILTER PAPER 4A, ADVANTEC, Tokyo, Japan) placed on non-nutrient agar at 22ºC. After fixation, samples were hybridised in microtubes. RNA probes were synthesized using DIG RNA labeling kit (Roche, Germany) using T7 or SP6 RNA polymerase according to the manufacturer's instruction.

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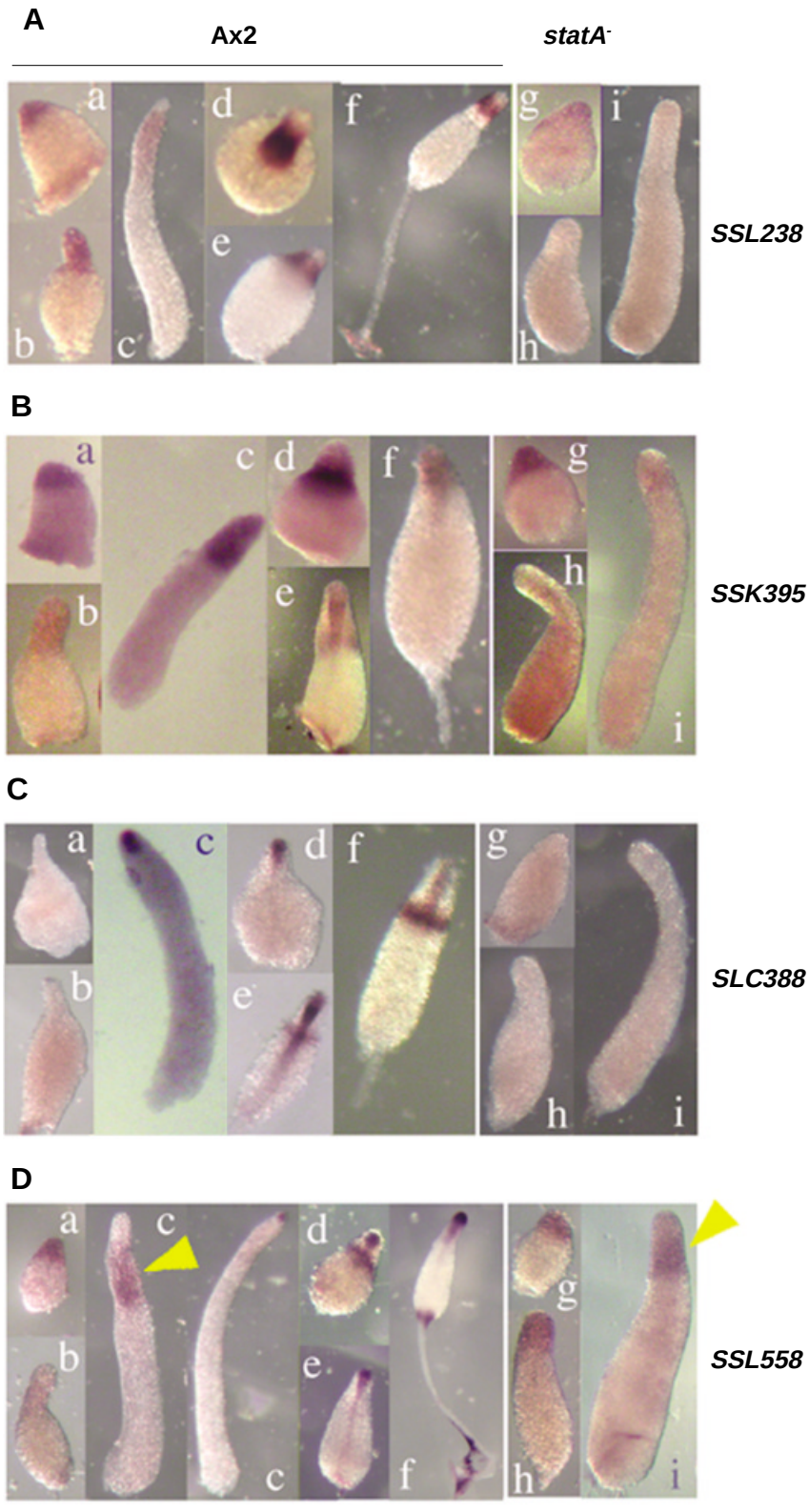

Fig. 2. Expression profiles of several prestalk-specific genes in both wild-type (Ax2) and *Dd-STATa*-null mutant. (a-d) *Spatial expression of* **(A)** SSL238, **(B)** SSK395, **(C)** SLC388 *and* **(D)** SSL558 *genes detected by* in situ *hybridisation. Photographs show individual developmental stages of Ax2* **(a-f)**, *for* **(a)** *tipped mound,* **(b)** *first finger,* **(c)** *slug,* **(d)** *Mexican hat,* **(e,f)** *early culminants and* Dd-STATa*-null mutant* **(g-i)**, *for* **(g)** *tipped mound,* **(h)** *first finger and* **(i)** *slug.*

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