

The *Drosophila* transcription factor *tramtrack* (TTK) interacts with *Trithorax-like* (GAGA) and represses GAGA-mediated activation

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ABSTRACT

In this study, we report the interaction of the *Drosophila* transcription factors *Trithorax-like* (GAGA) and *tramtrack* (TTK). This interaction is documented both *in vitro*, through GST pull-down assays, as well as *in vivo*, in yeast and Schneider S2 cells. GAGA and TTK share in common the presence of an N-terminal POZ/BTB domain that was found to be necessary and sufficient for GAGA–TTK interaction. Structural models that could account for this interaction are discussed. GAGA is known to activate the expression of many genes in *Drosophila*. On the other hand, TTK was proposed to act as a maternally provided repressor of several pair-rule genes, such as *even-skipped* (*eve*). As with many *Drosophila* genes, *eve* contains at its promoter region binding sites for GAGA and TTK. Here, in transient expression experiments, we showed that GAGA activates transcription from the *eve* stripe 2 promoter element and that TTK inhibits this GAGA-dependent activation. Repression by TTK of the *eve* promoter requires its activation by GAGA and depends on the presence of the POZ/BTB domains of TTK and GAGA. These results indicate that GAGA–TTK interaction contributes to the regulation of gene expression in *Drosophila*.

INTRODUCTION

Many genes in *Drosophila* contain, at their regulatory regions, binding sites for the transcription factors *Trithorax-like* (GAGA) and *tramtrack* (TTK). GAGA and TTK share in common the presence of an N-terminal POZ/BTB domain (1,2). POZ/BTB is a highly conserved structural motif that has been found in proteins participating in a variety of biological processes from the formation of ion-selective channels to the organisation of the cytoskeleton and the nuclear matrix, or the regulation of transcription (reviewed in 3). Typical of a

structurally independent domain that plays specific functions, the POZ/BTB domain is most frequently found in combination with other domains such as kelch repeats, ankryn repeats, transmembrane domains or zinc-finger domains (3). The POZ/BTB domain mediates protein–protein interactions. In several proteins, the POZ/BTB domain has been found to participate in the formation of homo-oligomers (4–7) and, based on *in vitro* co-expression experiments, it was proposed that POZ/BTB domains can also form hetero-oligomers (4). In addition, in several transcription factors, the POZ/BTB domain was also shown to be involved in interactions with various co-repressor proteins (8–14). The POZ/BTB domain of the human promyelocytic leukaemia zinc finger (PLZF) protein was crystallised and its structure was solved (15,16). The crystal structure shows a tightly interwound dimer and, therefore, provides a general structural basis for the participation of POZ/BTB domains in homomeric interactions. Some features of the PLZF dimer are strongly reminiscent of an obligate homo-dimer. Dimerisation occurs through an extensive hydrophobic interface that involves approximately one quarter of the monomer accessible surface (15,16) and unfolding of the POZ/BTB dimer of PLZF occurs as a single coupled two-state transition that is irreversible (7). These considerations raise the question as to what extent POZ/BTB domains can actually support the formation of hetero-oligomers *in vivo*. On the other hand, heterophilic interactions with co-repressors were proposed to occur through a cluster of conserved residues that lie at a surface-exposed groove formed at the dimer interface (16).

In this study, we report that GAGA519 and TTK69 isoforms interact *in vitro* as well as *in vivo*, both in yeast and Schneider S2 cells, and that their POZ/BTB domains are necessary and sufficient for this interaction to occur. Structural models that could account for this interaction are discussed. The various GAGA and TTK isoforms all share the corresponding POZ/BTB domain (17,18) and, therefore, are likely to support similar GAGA–TTK interactions. The potential contribution of this interaction to the regulation of transcription is also discussed. As with many *Drosophila* promoters, the *even-skipped* (*eve*) stripe 2 regulatory region contains several binding sites for GAGA and TTK which are in relatively close

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proximity (2,19,20), suggesting that the GAGA–TTK interaction described here might participate in its functional regulation. GAGA and TTK appear to have opposite effects on transcription. The expression of many genes in *Drosophila* is positively regulated by GAGA (21) and it has been shown that GAGA acts as a transcription activator *in vitro*, or upon transient transfection in cultured S2 cells (2,18,22). On the other hand, during early embryo development, TTK was shown to function as a transcription repressor of several pair-rule genes, including *eve* (23–25). Here, we show that GAGA activates transcription from the *eve* stripe 2 promoter and that TTK inhibits this GAGA-dependent activation. Repression by TTK of the *eve* promoter depends on the presence of GAGA at the promoter and requires the contribution of the POZ/BTB domain.

MATERIALS AND METHODS

Proteins

The proteins used in these experiments correspond to GAGA519 (2,18) and TTK69 forms (17,26). Several truncated peptides derived from them were constructed: Δ POZ_{GAGA} and Δ POZ_{TTK}, which are missing the first 122 and 115 residues of GAGA519 and TTK69, respectively, and POZ_{GAGA} and POZ_{TTK} which carry the first 122 and 114 residues of GAGA519 and TTK69, respectively. Proteins were expressed in BL21LysS or BL21 *Escherichia coli* cells as 6×His-tagged proteins, using pET14 expression vectors (Novagen), and/or as GST-fusions proteins, using pGEX-KG vectors (Amersham).

Rabbit polyclonal antibodies raised against bacterially expressed GAGA519 and TTK69 proteins were obtained and purified by conventional methods.

Immunoprecipitation experiments

For co-immunoprecipitation experiments, Schneider S2 cells were co-transfected by the calcium phosphate method. For each transfection, $7\text{--}8 \times 10^6$ cells were plated onto 100 mm diameter tissue culture dishes 1 day before transfection. Cells were co-transfected with 15 μ g of pAct5CPPA expression vectors carrying the full-length cDNAs encoding GAGA519 and TTK69. The total amount of transfected DNA was adjusted to 30 μ g by the addition of pUC19. Cell extracts were prepared 48 h after transfection. Cells were washed with 1 ml of ice-cold PBS and lysed with 0.5 ml of IPH buffer [50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 0.1 mM PMSF, 0.1 mM aprotinin and 0.1 mM leupeptin] for 30 min on ice. Extracts were cleared by centrifugation. Immunoprecipitation was carried out at 300 mM NaCl by the addition of affinity-purified α GAGA or α TTK rabbit polyclonal antibodies. When the preimmune serum was used, the IgG fraction was prepared. Incubation was performed at 4°C for 2 h. Twenty microlitres of Protein A–Sepharose beads (Amersham) were then added and incubated at 4°C for 2 h. Complexes were pelleted by centrifugation, washed four times with IPH buffer and analysed by western blotting with affinity-purified α GAGA or α TTK antibodies and detected by ECL (Amersham).

Yeast two-hybrid experiments

For the yeast two-hybrid assays, the various constructs used were cloned into plasmids pGBT9 and pGAD424 (Clontech) to express fusion proteins containing either the GAL4 DNA-binding domain or the GAL4 activation domain, respectively. Appropriate plasmids were then transformed into yeast HF7c strain (*MATa*, *ura3-52*, *his3-200*, *ade2-101*, *lys2-801*, *trp1-901*, *leu2-3,112*, *gal4-542*, *gal80-538*, *LYS2::GAL1_{UAS}-GAL1_{TATA}-HIS3*, *URA3::GAL4_{17mers(3x)}}-C_YC1_{TATA}-HIS3) and the transformants were tested for their ability to grow on selective medium lacking histidine. For quantitative analysis, transformants were tested for activation of the GAL4-dependent *LacZ* reporter gene.*

GST pull-down assays

GST-fusion proteins expressed in *E.coli* were bound to glutathione–Sepharose-4B beads. Protein concentrations, determined by gel electrophoresis, were adjusted by dilution with unbound beads. Agarose-bound GST fusion proteins were equilibrated in 25 mM HEPES (pH 7.5), 150 mM KCl, 12.5 mM MgCl₂, 20% glycerol, 0.1% NP-40 and incubated for 1 h at room temperature with ³⁵S-labelled full-length TTK or Δ POZ_{TTK} proteins that were produced *in vitro*, using the TNT-coupled reticulocyte lysate system (Promega). Beads were washed four times with 1 ml of 20 mM Tris–HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 0.5% NP-40. Bound proteins were analysed on SDS–PAGE gels by autoradiography.

Transcription assays in S2 cells

The effects of GAGA and TTK on the transcription activity of the *eve* stripe 2 promoter were analysed by transient expression experiments in cultured S2 cells using a pGL3 reporter plasmid (Promega) that carries a luciferase reporter gene fused to a 1.8 kb fragment of the *eve* promoter (from position +102 to position –1759), which was found to drive transcription within the limits of stripe 2 (19). The structure of the reporter plasmid carrying the 5×GAL4-*hsp70* promoter was described earlier (22). Seven micrograms of each reporter plasmid was co-transfected to S2 cells together with increasing amounts of pAct5CPPA vectors expressing GAGA519, TTK69, Δ POZ_{TTK}, Δ POZ_{GAGA}, GAL4Q or POZ_{GAGA}GAL4Q as indicated in each case. GAL4Q was described earlier (22). POZ_{GAGA}GAL4Q was constructed by fusing the POZ/BTB domain of GAGA (122 amino acids) to the N-terminus of GAL4Q using a 6×His-tag as linker. Three micrograms of pCMV β Gal plasmid (Stratagene) was added to the transfection mixture to allow correction for variations in transfection efficiencies. The total amount of plasmid added was adjusted to 20 μ g by the addition of pAct5CPPA. Transfection was carried out by the calcium phosphate method. Cells were recovered 48 h after transfection and lysed according to the β -galactosidase gene reporter kit (Roche Molecular Biochemicals). Luciferase activity was determined according to the luciferase activity assay kit (Promega) and normalised with respect to β -galactosidase activity.

Model building

The models of the POZ/BTB domain complexes analysed in this study were built using the program MODELLER v.4, kindly provided by Dr Sali, which models protein structures

by satisfying spatial restraints, starting from the extended polypeptide chain conformation (27). For comparative modelling of the POZ/BTB domains, these restraints were derived from the aligned sequences of the modelled domains with that of PLZF, whose crystal structure (15,16) was taken as a template (PDB no. 1buo).

Four kinds of models were built. For the POZ/BTB domains of GAGA and TTK, homo-dimers were built using the whole crystallographic PLZF homo-dimer as template. No symmetry restraints were applied, so that each monomer was solely constrained to keep in the dimer, but not to have exactly the same conformation as its partner. A model of a putative GAGA–TTK hetero-dimer was obtained following the same principles as for the homo-dimers. The models of GAGA and TTK homo-tetramers were built on the basis of the crystallographic PLZF tetramer, as observed in the same PDB entry indicated above. Again, no symmetry restraints were imposed to the monomers. Finally, a GAGA–TTK hetero-tetramer was constructed by the same procedure, but using POZ_{GAGA} and POZ_{TTK} homo-dimers. The flexible loops that for GAGA and TTK lie in the exposed groove at the dimer interface, were generated by using internal co-ordinates from the CHARMM topology library included in MODELLER. All the models were refined by the program using thorough simulated annealing with molecular dynamics. During the modelling process the target structures were internally checked for their consistency with the restraints used to calculate them. Five models were generated in each round, and that with the best self-consistency score was selected.

Global analysis of the models was performed with the programs ProsaIIv.3 (28), to assess the compatibility of residues with their environment, and WHATCHECK (29), to elucidate its stereochemical correctness. Only the WHATCHECK packing quality scores were found to be significantly worse in the models, which was also reflected in slightly higher ProsaII z -scores. These values were, however, in the expected range for theoretical models. The sc program (30) from the CCP4 suite was used to calculate the ‘surface complementarity’ statistics, as well as to incorporate their values to the solvent accessible surface generated by GRASP (31).

RESULTS

GAGA and TTK interact *in vivo*

The question of whether GAGA and TTK interact *in vivo* was addressed by co-immunoprecipitation experiments performed with total extracts prepared from Schneider S2 cells that were co-transfected with plasmids expressing GAGA519 and TTK69 forms (Fig. 1). Immunoprecipitation with α GAGA antibodies brings down a significant amount of TTK (Fig. 1A, lane 3). Under these conditions, GAGA complexes are efficiently immunoprecipitated (Fig. 1B, lane 2). In S2 cells, the majority of GAGA appears as two doublets of roughly the same intensity (13,18). The doublet of slower electrophoretic migration corresponds to the endogenous GAGA581 form, while the species of fast mobility correspond to the GAGA519 form that was selectively overexpressed in these experiments. This co-immunoprecipitation is specific since no TTK was observed when immunoprecipitation was

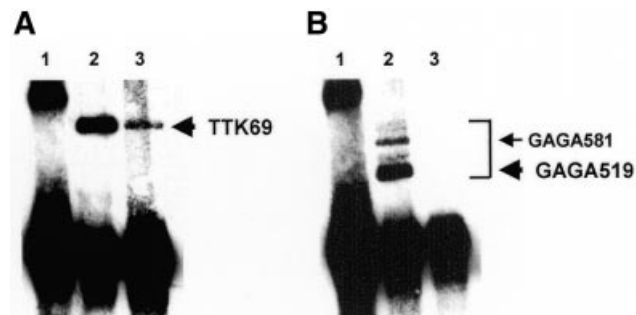


Figure 1. Co-immunoprecipitation of GAGA and TTK. Extracts from S2 cells overexpressing GAGA519 and TTK69 forms were immunoprecipitated with affinity-purified α TTK (A, lane 2 and B, lane 3) and α GAGA (A, lane 3 and B, lane 2) antibodies, or with the IgG fraction of a preimmune serum (lanes 1). Immunoprecipitates were analysed by western blotting with α TTK antibodies (A) or α GAGA antibodies (B). The positions corresponding to TTK69, GAGA519 and GAGA581 forms are indicated.

performed with an excess of preimmune serum (Fig. 1A, lane 1). On the other hand, no significant amount of GAGA is detected when immunoprecipitation is carried out with α TTK antibodies (Fig. 1B, lane 3) though, under these conditions, TTK is efficiently immunoprecipitated (Fig. 1A, lane 2). The lack of co-immunoprecipitation observed with α TTK antibodies might arise from their inability to recognise GAGA–TTK complexes or the distortion of such complexes in their presence. As described below, the interaction of GAGA and TTK was corroborated by yeast two-hybrid assays (Fig. 2) and, *in vitro*, through GST pull-down assays (Fig. 3).

The POZ/BTB domains of GAGA and TTK are necessary and sufficient for GAGA–TTK interaction

The POZ/BTB domain defines a conserved structural motif of approximately 110–120 residues that, in GAGA and TTK, is located at the N-terminus. The region of homology between the POZ/BTB domains of GAGA and TTK extends up to residue 118 of the GAGA sequence (115 of the TTK sequence), showing an overall identity of 40% (16). The contribution of the POZ/BTB domain to GAGA–TTK interaction was analysed with truncated proteins missing such a domain. The various constructs used in these experiments were designed according to the sequence comparison described above. Δ POZ_{GAGA} and Δ POZ_{TTK} are missing the first 122 and 115 residues of GAGA and TTK, respectively. Similarly, POZ_{GAGA} and POZ_{TTK} correspond to polypeptides containing the first 122 and 114 residues of GAGA and TTK.

The interaction between GAGA and TTK was analysed by yeast two-hybrid assays (Fig. 2). Significant growth on selective medium lacking histidine is observed when GAGA–BD_{GAL4} and TTK–AD_{GAL4} fusions are used as baits (Fig. 2A, row GAGA–TTK), confirming the interaction between GAGA and TTK also in yeast cells. This interaction is strictly dependent on the presence of the POZ/BTB domains of GAGA and TTK. Deletion of the POZ/BTB domain of GAGA fully abolishes its interaction with TTK (Fig. 2A, row Δ POZ_{GAGA}–TTK) as, vice versa, deletion of the POZ/BTB domain of TTK does (Fig. 2A, row GAGA– Δ POZ_{TTK}). On the other hand, the POZ/BTB domains of GAGA and TTK are, by

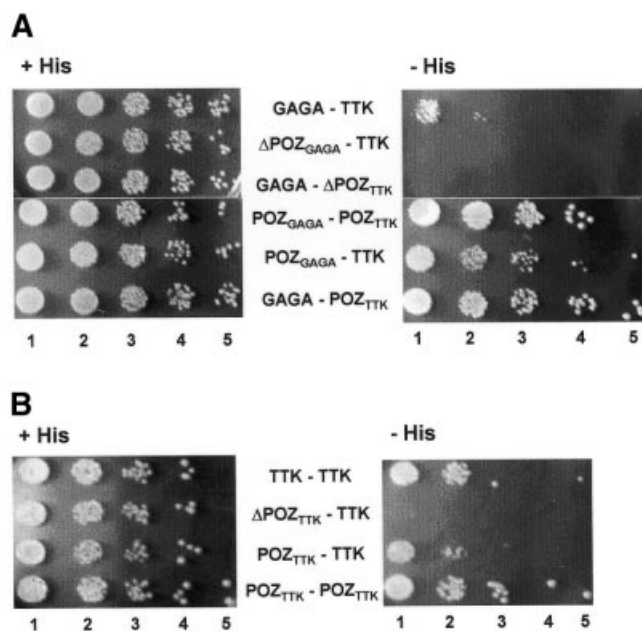


Figure 2. Yeast two-hybrid assay of GAGA-TTK (A) and TTK-TTK (B) interaction. Twenty microlitres of cultures containing the constructs indicated in each case were plated on His⁺ (left) and His⁻ (right) plates at a cell density of 2×10^6 cells/ml (lanes 1) and serial 10-fold dilutions (lanes 2–5).

themselves, capable of interacting with TTK and GAGA, respectively (Fig. 2A, rows POZ_{GAGA}-TTK and GAGA-POZ_{TTK}). Moreover, POZ_{GAGA} and POZ_{TTK} interact strongly (Fig. 2A, row POZ_{GAGA}-POZ_{TTK}). These results were corroborated when the interactions were analysed quantitatively through β -galactosidase assay (data not shown).

Similar results were obtained *in vitro* through GST pull-down assays (Fig. 3). Recombinant TTK was specifically bound by GST-GAGA or GST-POZ_{GAGA} but not by GST- Δ POZ_{GAGA} (Fig. 3A) fusion proteins. On the other hand, GST-GAGA does not bind Δ POZ_{TTK} efficiently (Fig. 3B).

It was shown earlier that the POZ/BTB domain also mediates the formation of GAGA-GAGA homo-oligomers (6,32). As judged by yeast two-hybrid assays, TTK also forms homo-oligomers (Fig. 2B, row TTK-TTK). Homomeric TTK-TTK interaction is also dependent on the POZ/BTB domain, which is necessary (Fig. 2B, row Δ POZ_{TTK}-TTK) and sufficient (Fig. 2B, rows POZ_{TTK}-TTK and POZ_{TTK}-POZ_{TTK}) for the interaction to occur. Altogether, these results indicate that the POZ/BTB domain, that mediates the formation of GAGA-GAGA and TTK-TTK homo-oligomers, is also responsible for GAGA-TTK interaction, both *in vitro* and *in vivo*.

TTK inhibits GAGA-dependent activation of the *eve* stripe 2 promoter element

The functional consequences of GAGA-TTK interaction were evaluated in the context of the *eve* promoter. In the cellular blastoderm, the pair-rule *eve* gene is expressed in seven defined stripes, each spanning five to six cells. It was shown

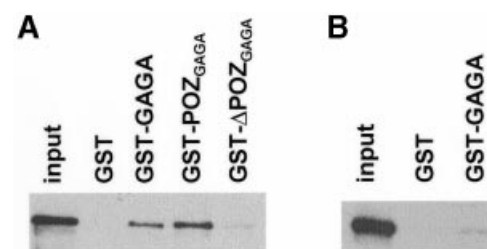


Figure 3. GST pull-down assays of the interaction of recombinant TTK (A) and Δ POZ_{TTK} (B) with the GST-fusion proteins indicated in each case. Lanes labelled input show 10% of the input protein.

that a truncated *eve* promoter spanning 1.7 kb of the region immediately upstream from the transcription start site carries the basal promoter elements, being sufficient to drive expression within the limits of stripe 2 (19). This *cis*-regulatory element was used to analyse the potential contribution of GAGA-TTK interaction to the regulation of transcription since it contains several binding sites for both GAGA and TTK (20). For this purpose, a plasmid carrying the *eve* stripe 2 promoter fused to a luciferase reporter gene was co-transfected into S2 cells with plasmids expressing GAGA and/or TTK. As shown in Figure 4A, overexpression of GAGA results in a significant activation of the *eve* stripe 2 promoter. Luciferase activity increases upon increasing GAGA overexpression reaching a maximum activation of ~15-fold when 4–6 μ g of the GAGA expressing vector is co-transfected. Activation of the *eve* stripe 2 promoter by GAGA is efficiently repressed by overexpressing TTK (Fig. 4B). Luciferase activity is drastically reduced in the presence of 0.5 μ g of the TTK-expressing plasmid, reaching background levels with just 2 μ g of vector. As judged by western analysis (data not shown), the level of GAGA overexpression is not significantly reduced by overexpressing TTK, indicating that the decrease in luciferase activity observed in the presence of TTK reflects repression of the *eve* stripe 2 promoter rather than a reduced expression of the activator, GAGA. TTK overexpression by itself does not affect the basal activity of the *eve* stripe 2 promoter since no significant changes in luciferase activity are detected when TTK is overexpressed in the absence of GAGA overexpression (Fig. 4C). Note that, in Figure 4C, luciferase activities are normalised with respect to the basal activity of the *eve* stripe 2 promoter determined in the absence of any overexpressed protein. Actually, in S2 cells, the *eve* stripe 2 promoter shows significant basal transcription activity. Luciferase activity produced by the pGL3 reporter plasmid is 250-fold higher in the presence of the *eve* stripe 2 promoter than in its absence being, therefore, sufficiently high to eventually detect repression.

Repression by TTK of GAGA-dependent activation requires the contribution of the POZ/BTB domains of TTK and GAGA

The results reported above indicate that, in the experimental system described here, repression by TTK of the *eve* promoter depends on its activation by GAGA, suggesting that it actually involves direct interaction between the two proteins. In good agreement with this hypothesis, TTK repression of the GAGA-activated *eve* stripe 2 promoter depends on the

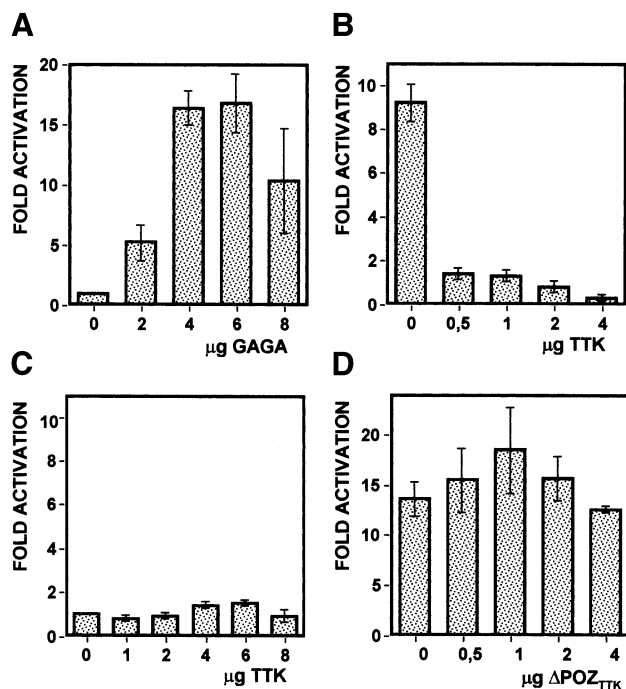


Figure 4. TTK represses GAGA-dependent activation of the *eve* stripe 2 promoter element. S2 cells were co-transfected with a pGL3 reporter plasmid carrying a luciferase gene fused to the *eve* stripe 2 promoter element and the amounts indicated of pAct5CPPA plasmids overexpressing GAGA (A) or TTK (C), or with the same reporter plasmid and 4 μ g of the plasmid overexpressing GAGA and the amounts indicated of a plasmid overexpressing TTK (B) or Δ POZ_{TTK} (D). Luciferase activities were measured as indicated in Materials and Methods, and normalised with respect to the basal activity of the *eve* stripe 2 promoter determined when only empty pAct5CPPA overexpression plasmids were co-transfected and, therefore, no proteins were overexpressed. The fold of activation is presented as a function of increasing amounts of the relevant overexpression plasmid. The data correspond to the average of four independent experiments.

presence of the POZ/BTB domain that, as shown above, mediates GAGA–TTK interaction. As shown in Figure 4D, overexpression of Δ POZ_{TTK} does not interfere with GAGA-dependent activation. In these experiments, Δ POZ_{TTK} was expressed at equivalent levels as TTK, as judged by western blotting (data not shown). Reciprocal experiments were performed to analyse the contribution of the POZ/BTB domain of GAGA but it turned out that Δ POZ_{GAGA} was not efficiently expressed in S2 cells (data not shown). The contribution of the POZ/BTB domain of GAGA to repression by TTK was, however, demonstrated in a heterologous system. The C-terminal glutamine-rich domain of GAGA (Q domain) is known to be required for transcription activation both *in vitro* as well as *in vivo* (22). Moreover, a heterologous protein, GAL4Q, carrying the DNA-binding domain of the yeast activator GAL4 fused to the Q domain of GAGA, was found to drive transcription from a 5 \times GAL4-*hsp70* promoter that contains five GAL4 binding sites inserted upstream of the minimal *hsp70* promoter of *Drosophila* (22). As shown in Figure 5A, maximal activation is obtained in the presence of 1–4 μ g of the GAL4Q-expressing vector. A similar fusion protein, POZ_{GAGA}GAL4Q, carrying the POZ/BTB domain of GAGA, activates transcription from the 5 \times GAL4-*hsp70* promoter to equivalent levels as GAL4Q, though, in this

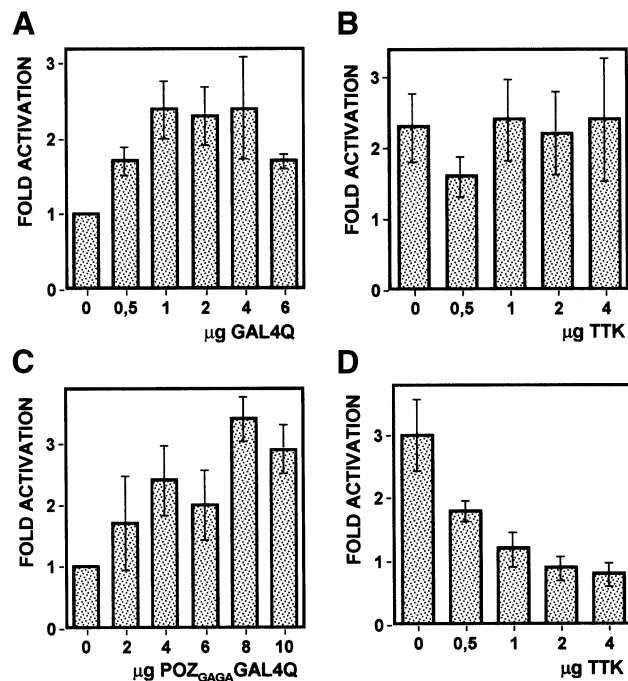


Figure 5. Repression by TTK of GAGA-dependent activation requires the POZ/BTB domain of GAGA. S2 cells were co-transfected with a reporter plasmid carrying a luciferase gene fused to the 5 \times GAL4-*hsp70* promoter (see Materials and Methods) and the amounts indicated of plasmids overexpressing GAL4Q (A) or POZ_{GAGA}GAL4Q (C), or with the same reporter plasmid and either 2 μ g of the plasmid overexpressing GAL4Q (B) or 8 μ g of the plasmid overexpressing POZ_{GAGA}GAL4Q (D) and the amounts indicated of the plasmid overexpressing TTK. The data correspond to the average of six independent experiments.

case, maximal activation occurs at a higher concentration of vector (Fig. 5C). In this context, TTK represses transcription from the 5 \times GAL4-*hsp70* promoter when it is activated by POZ_{GAGA}GAL4Q (Fig. 5D). On the contrary, no repression is observed when the promoter is activated by GAL4Q (Fig. 5B). TTK represses POZ_{GAGA}GAL4Q as efficiently as GAGA; full repression is observed in the presence of 1–2 μ g of the TTK-expressing vector but a significant effect is detected with just 0.5 μ g of vector (Fig. 5D). Similar results were obtained when the ability of TTK to repress activation by GAL4VP16, which carries the acidic transactivation domain of VP16, was determined. Also in this case, TTK was unable to efficiently repress activation of the 5 \times GAL4-*hsp70* promoter by GAL4VP16 in the absence of the POZ/BTB domain (data not shown).

Altogether these results indicate that repression by TTK of GAGA-dependent activation requires the contribution of the POZ/BTB domain of both TTK as well as GAGA and, therefore, strongly argue in favour of mechanisms involving direct GAGA–TTK interaction.

DISCUSSION

Here, we have reported the interaction of the *Drosophila* transcription factors GAGA and TTK. Based on *in vitro* co-transformation, performed in a cell-free system, and co-immunoprecipitation experiments, it was shown earlier (4) that a

strongly relies on the $\beta 1/\beta 5'$ -sheets which, in PLZF, involve the formation of five main chain H-bonds between the $\beta 1$ chain of one monomer and the $\beta 5'$ chain of the second (15,16). The only other contact that contributes to dimer formation involves the symmetry related $\alpha 1/\alpha 1'$ helices of each monomer (15,16). Therefore, the $\beta 1$ residues that could mediate dimer-dimer interactions are themselves essential for dimerisation as it was found by mutational analysis of the POZ/BTB domain of PLZF (33).

GAGA-TTK interaction might play a role in the regulation of transcription. In the early *Drosophila* embryo, TTK was proposed to act as a maternally provided repressor of several pair-rule genes (23). Ectopic expression of TTK69 in blastoderm-stage embryos results in repression of several pair-rule genes, such as *eve* and *fushi tarazu* (*ftz*) (24,25). GAGA, which is also maternally provided, activates the expression of *ftz* (34) and, as indicated by our results, it could also activate *eve* expression. We have shown here that TTK represses GAGA-dependent activation, but not basal activity, of the *eve* stripe 2 promoter. Repression by TTK of GAGA-dependent activation requires the contribution of the POZ/BTB domain of TTK as well as of GAGA, indicating that it actually involves direct GAGA-TTK interaction. Several models could account for these results; GAGA-TTK hetero-oligomers might not be capable of binding DNA or, alternatively, they might either actively participate in the recruitment of co-repressor complexes or interfere with loading, and/or assembly, of the activation complex at the promoter. Additional experiments are required to discriminate between these possibilities. Our results, however, indicate that neutralising the action of transcription activators, such as GAGA, might be a major determinant of the contribution of TTK to transcription repression.

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