

Rappailles, A., L. Landemarre, D. Locker, and M. Decoville. 2002. DSP1 is part of a new large multiprotein complex. *Dros. Inf. Serv.* 85: 7-8.



DSP1 is part of a new large multiprotein complex.

Rappailles, A¹, L. Landemarre², D. Locker¹, and M. Decoville¹. ¹Centre de Biophysique Moléculaire, CNRS UPR 4301, conventionnée avec l'Université d'Orléans, 45071 Orléans, France. ²Agro-Bio, S.A.S., rue D. Papin, 45240 La ferté St Aubin, France.

Introduction

Drosophila Polycomb Group or trithorax Group proteins are responsible for the maintenance of the repressed or activated state of several target genes. The best known are the homeotic genes which are first activated in the early embryo by the products of segmentation genes. After gastrulation, epigenetic mechanisms mediated by PcG and trxG proteins maintain the segmental pattern of expression during the rest of development. In general PcG proteins maintain a repressed state while trxG proteins maintain an activated state. This set of regulators work into multimeric complexes to locally remodel the chromatin structure (Simon and Tamkun, 2002). Two PcG proteins complexes have been identified: Polycomb repressive complex 1 (PRC1) and the ESC-E(Z) complex. PRC1 complex (2-6 MDa) contains the PcG proteins Polycomb (Pc), Polyhomeotic (PH), Posterior Sex Combs (PSC), dRING1 and additional polypeptides (Shao *et al.* 1999; Saurin *et al.* 2001). The ESC-E(Z) complex (600 kDa) contains the PcG proteins Extra Sex Combs (ESC), Enhancer of Zeste (E(Z)) and NURF-55 a fly homolog of RbAp48 which is associated with a histone deacetylase complex (HDAC) (Tie *et al.*, 2001). Several lines of evidences suggest that *Drosophila* embryos contain at least three distinct protein complexes containing trx-G proteins: the 2 MDa BRM complex, a 2 MDa ASH1 complex and a 500 kDa ASH2 complex (Papoulas *et al.*, 1998). The BRM complex, which is related to SWI/SNF complexes, contains seven major polypeptides, four of which (BRM, BAP155, BAP60 and BAP45/SNR1) are conserved in the yeast and human SWI/SNF complexes. It contains also BAP111, an HMGB protein which binds non specifically the minor groove of DNA. By using a genetic assay it was demonstrated that BAP111 functionally interacts with BRM *in vivo* (Papoulas *et al.*, 2001).

DSP1, an HMGB-like protein, has been shown to work as a chromatin remodelling factor (Decoville *et al.*, 2001). It acts as a trx-G or Pc-G protein, depending of the considered locus. Genetic interaction studies between a *dsp1* nul mutation and trx-G mutations has revealed that DSP1 interacts with several trx-G proteins such as trx and ASH1. To determine whether this reflects a physical interaction, we were interested in identifying a stable complex containing DSP1.

Results

Native protein extracts were prepared from 0 - 12 hour embryos as described in Elfring *et al.* (1998). Approximately 3 mg of protein extract were loaded on a 1 × 60 cm Sephacryl S-400 gel filtration column (Amersham, Pharmacia Biotech). Molecular mass standards (Blue Dextran [2Mda], thyroglobulin [670 kDa], catalase [232 kDa]) were used to calibrate the column. Fractions were eluted in column buffer (50 mM NaP pH 7.8, 400 mM NaCl, 0.05% Tween-20, 0.1mM EGTA, 1 mM

MgCl₂, 0.1 mM DTT, 10% glycerol, 1 mM PMSF, 1 µg/ml of protease inhibitors leupeptinin, aprotinin and pepstatin), and 0.4 ml fractions were collected. 16 µl of each fraction was run on an SDS-PAGE. The DSP1 protein was detected on western blot using rabbit polyclonal anti- DSP1 (Mosrin-Huaman *et al.*, 1998).

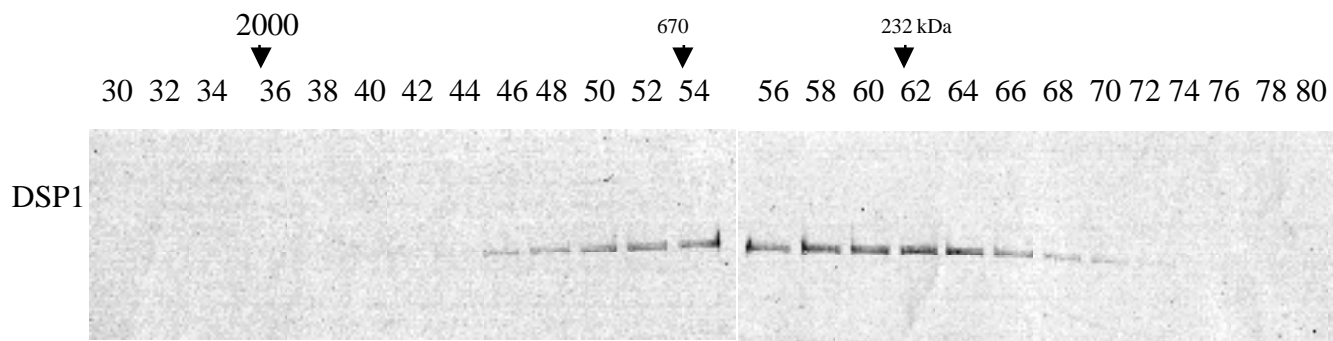


Figure 1. Gel filtration analysis of DSP1 protein in *Drosophila* embryo extracts.

Figure 1 showed that DSP1 eluted with a peak between fractions 56 and 64, indicating that DSP1 is incorporated into a complex approximately 300 kDa in size. This complex seems to be distinct from Pc-G or trx-G protein complexes already described. However, DSP1 was also detected, in a lesser extent, in fractions corresponding to the range of 1MDa. These results suggest that DSP1 could be associated with at least two distinct complexes, one of which constituting a new one. Our aim now, is to identify the proteins incorporated into the DSP1 complex.

References: Decoville, M., E. Giacomello, M. Leng, and D. Locker 2001, *Genetics* 157: 237-244; Elfring, L.K., C. Daniel, O. Papoulas, R. Deuring, M. Sarte, S. Moseley, S.J. Beek, W.R. Waldrip, G. Daubresse, A. DePace, J.A. Kennison, and J.W. Tamkun 1998, *Genetics* 148: 251-265; Mosrin-Huaman, C., L. Canaple, D. Locker, and M. Decoville 1998, *Dev. Genet.* 23: 324-334; Papoulas, O., S.J. Beek, S.L. Moseley, C.M. McCallum, M. Sarte, A. Shearn, and J.W. Tamkun 1998, *Development* 125: 3955-3966; Papoulas, O., G. Daubresse, J.A. Armstrong, J.J. Matthew, P. Scott, and J.W. Tamkun 2001, *Proc. Natl. Acad. Sci. USA* 98: 5728-5733; Saurin, A.J., Z. Shao, H. Erdjument-Bromage, P. Tempst, and R. Kingston 2001, *Nature* 412: 655-659; Shao, Z., F. Raible, R. Mollaaghababa, J.R. Guyon, C.T. Wu, W. Bender, and E. Kingston 1999, *Cell* 98: 37-46; Simon, J., and J.W. Tamkun 2002, *Curr. Opin. Genet. Dev.* 12: 210-218; Tie F., T. Furuyama, J. Prasad-Sinha, E. Jane and P.J. Harte 2001, *Development* 128: 275-286.