and neuronal death.

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References: Abramoff, M.D., P.J. Magelhaes, and S.J. Ram 2004, Biophotonics Intl. 11: 36-42; Auluck, P.K., H.Y.E. Chan, J.Q. Trojanowski, V.M-Y. Lee, and N.M. Bonini 2002, Science 295: 865-868; Brand, A.H., and N. Perrimon 1993, Development 118: 401-415; Cauchi, R.J., and M. Heuvel 2006, Neurodeg. Dis. 3: 338-356; Celotto, A.M., and M.J. Palladino 2005, Mol. Interv. 5: 292-303; Chan, H.Y.E., and N.M. Bonini 2000, Cell Death Differ. 7: 1075-1080; Chinta, S.J., J.K. Mallajosyula, A. Rane, and J.K. Andersen 2010, Neuroscience Let. 486: 235–239; Feany, M.B., and W.W. Bender 2000, Nature 404: 394-398; Freeman, M., 1996, Cell 87: 651–660; Forno, L.S., 1996, J. Neuropathol. Exp. Neurol. 5: 259-272; Hardaway, J.A., 2010, Vanderbilt Rev. Neurosci. 2: 55-61; Haywood, A.F.M., and B.E. Staveley 2004, BMC Neurosci. 5: 14; Haywood, A.F.M., and B.E. Staveley 2006, Genome 49: 505-510; Jeibmann, A., and W. Paulus 2009, Int. J. Mol. Sci. 10: 407-440; Leroy, E., R. Boyer, G. Auburger, B. Leube, G. Ulm, E. Mezey, G. Harta, M.J. Brownstein, S. Jonnalagada, T. Chernova, A. Dehejia, C. Lavedan, T. Gasser, P.J. Steinbach, K.D. Wilkinson, and M.H. Polymeropoulos 1998, Nature 395: 451-452; Martinez-Vicente, M., Z. Talloczy, S. Kaushik, A.C. Massey, J. Mazzulli, E.V. Mosharov, R. Hodara, R. Fredenburg, D-C. Wu, A. Follenzi, W. Dauer, S. Przedborski, H. Ischiropoulos, P.T. Lansbury, D. Sulzer, and A.M. Cuervo 2008, J. Clin. Invest. 118: 777-788; Polymeropoulos, M.H., C. Lavedan, E. Leroy, S.E. Ide, A. Dehejia, A. Dutra, B. Pike, H. Root, J. Rubenstein, R. Boyer, E.S. Stenroos, S. Chandrasekharappa, A. Athanassiadou, T. Papapetropoulos, W.G. Johnson, A.M. Lazzarini, R.C. Duvoisin, G.D. Lorio, L.I. Golbe, and R.L. Nussbaum 1997, Science 276: 2045-2047; Staveley, B.E., J.P. Phillips, and A.J. Hilliker 1990, Genome 33: 867-872; Thomas, B.J., and D.A. Wassarman 1999, Trends in Gen. 15(5): 184-190; Todd, A.M., and B.E. Staveley 2008, Genome 51: 1040-1046; Winslow, A.R., C-W. Chen, S. Corrochano, A. Acevedo-Arozena, D.E. Gordon, A.A. Peden, M. Lichtenberg, F.M. Menzies, B. Ravikumar, S. Imarisio, S. Brown, C.J. O'Kane, and D.C. Rubinszteinet 2010, J. Cell Biol. 190: 1023-1037; Wolf, F.W., and U. Heberlein 2003, J. Neurobiol. 54: 161–178; Xilouri, M., and L. Stefanis 2011, Expert Rev. Mol. Med. 13: e8.

Exposure to heat stress modulates DNA methyltransferase activity in the embryonic S2 cell line of *Drosophila melanogaster*.

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Summary

D. melanogaster is a dipteran model system for many diverse phenomena including animal development. The first report on the presence of 5-methylcytosine in the genomic DNA was by Deobagkar nee' Achwal (Achwal *et al*, 1984), where by use of sensitive and specific immunochemical staining and photoacoustic spectroscopy, the amount of 5mC was shown to be of

the order of 0.008mol%. Along with 5mC other methylated nucleotides like 6-methylAdenine and 7-methylGuanine were also shown to be present in genomic DNA of *D. melanogaster* using immunochemical methods (Achwal *et al*, 1983). Epigenetic changes in eukaryotic biology regulate diverse processes predominantly due to the cross-talk between DNA methylation and histone modifications. An appropriate environmental stimulus can reprogram the development of an organism (Felsenfeld, 2007; Allis *et al.*, 2007). The amount of methylation varies with different stages of life cycle in all organisms (Hendrich *et al*., 2003). It is known that there is differential expression of genes in response to external stimulus, and the stimuli for this change could be temperature, medium condition, or presence of oxidative stress (Gonsalves *et al*., 2011). *Drosophila* has been shown to possess a single known methyltransferase, dDNMT2 (Schaefer *et al*., 2008), which belongs to the enigmatic DNMT2 family. The DNMT2 family of proteins is found to be conserved, but their functions are still elusive. Molecular and biochemical experiments show that Dnmt2 can localize in the cytoplasm as well as nucleus (Lyko *et al*., 2000) and is supposed to help the fly against transposable elements and retroviral infections (Schaefer *et al*., 2010).

Prior studies indicate change in the expression of the genes in response to change in temperature (Sorensen *et al*., 2005; Kristensen *et al*., 2003). The cellular heat stress response is well studied in *Drosophila* with respect to the role of heat shock proteins (HSP), molecular chaperones that are highly expressed during and after exposure to numerous stress types. All HSPs appear to be regulated by a common transcription factor, the heat shock factor (HSF), which may also regulate uncharacterized heat-responsive genes (Jensen *et al*., 2008) besides Hsp genes. It has been suggested that HSPs also play a direct role in the extended longevity and stress resistance of flies exposed to non-lethal stress at a young age (Tatar *et al.*, 1997).

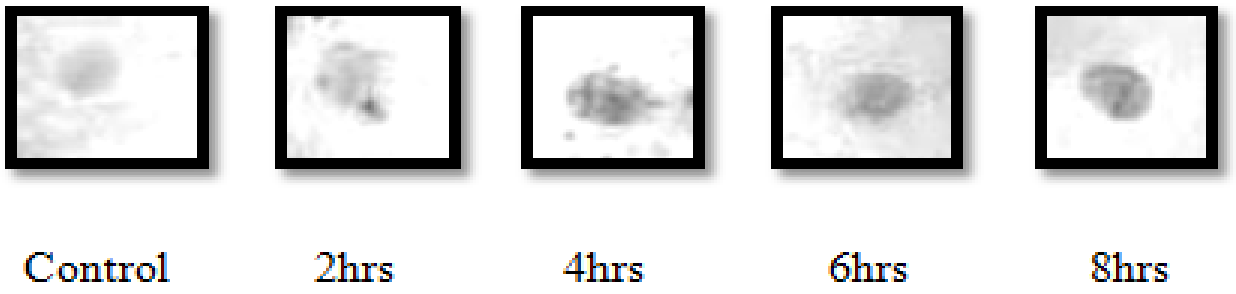

Figure 1. Immunochemical staining of unmethylated DNA substrate with 5 methylcytosine antibodies after incubation with protein extract from S2 cells under stress conditions.

Results

In this work we have studied the effect of heat stress on the 5 methyltransferase activity at the various time points in S2 embryonic cell line of *Drosophila melanogaster*. The S2 cell line was derived from a primary culture of late stage (20-24 hours old) *Drosophila melanogaster* embryos. This versatile cell line grows rapidly at temperature of 22ºC without CO_2 and is easily adapted to suspension culture. S2 cells can be grown in both serum-containing (Schneider's *Drosophila* Medium) or serum-free medium (*Drosophila* SFM). These are used represent a studies on early embryos. We have exposed the S2 cell line to a higher temperature for different time intervals and assayed for DNA methyltransferase activity. The S2 cell line grown in Schneider's medium was exposed to 37ºC for 0 hrs (control), 2 hrs, 4 hrs, 6 hrs, and 8 hrs, respectively. Cellular protein was

extracted and used for *in vitro* methyltransferase assay followed by immunochemical staining using 5-methyl cytosine antibodies (Paniker *et al.*, 2008). The concentration of the unmethylated substrate and cell protein used for the assay was 4 µg and 400 µg, respectively. The technique used to detect the activity of DNA methyltransferase and its effect on DNA methylation has been published by our lab (Deobagkar *et al*., 2012).

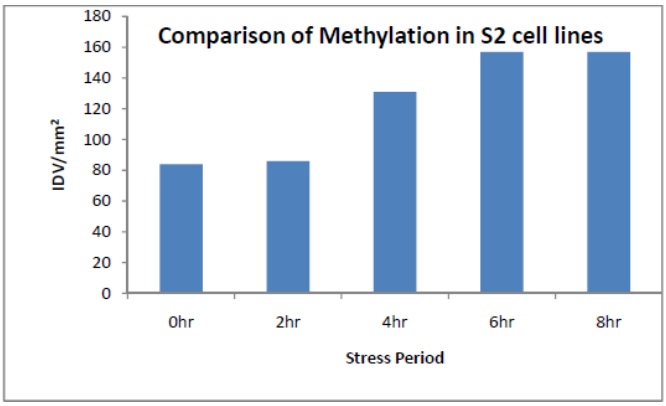

Figure 2. Graphical representation of DNA Methylation Assay for S2 Cell Lines of *Drosophila melanogaster* after exposure to higher temperatures (Deobagkar *et al*., 2012).

This result clearly documents an increase in cytosine methylation upon exposure to temperature stress. Although the exact role of DNA methylation in *Drosophila* is unclear, if involved in chromatin remodeling, physiological changes could result from epigenetic modifications due to change in the activity of methyltransferase. Different genotypes in the fly may respond differentially to the same environmental stress. The variation in these norms of reaction may be due to the genetic variation in their metabolic traits. The role of individual nutrients in stress resistance and longevity are largely unknown. Despite these variable parameters it can be affirmed that a progressive change in the activity of methyltransferase is observed under stress conditions in the embryonic stages of *Drosophila melanogaster*. The activity goes on increasing in case of S2 cell line as the time of exposure to stress is increased. The enigmatic role of DNA methylation in *Drosophila* physiology provides a model to integrate and analyze molecular and genetic mechanisms which are governed by methylation changes.

References: Achwal, C.W., and H.S. Chandra 1982, FEBS Lett. 150, 469-472; Achwal,C.W., P Ganguly, and H.S. Chandra 1984, EMBO J. 1984: 263–266; Allis, C.D., T. Jenuwein, and D. Reinberg 2007, *Overview and Concepts, Epigenetics*. Cold Spring Harbor Laboratory Press, 23-62; Deobagkar, D.D., C. Panikar, S. Iyer; 2008, Dros. Inf. Serv. 91: 101; Deobagkar, D.D., C. Panikar, S. Rajpathak, N. Shaiwale, S. Mukherjee 2012, Methods 56: 260-267; Felsenfeld, G., 2007, *A Brief History of Epigenetics*, *Epigenetics*, Cold Spring Harbor Laboratory Press, 15-22; Gonsalves, S.E., A.M. Moses, Z. Razak, F. Robert, and J.T. Westwood 2011, PLoS ONE, Volume 6; Hendrich, B., and S. Tweedie 2003, TRENDS in Genetics 19(5): 269–277; Jensen, L.T, M.M. Nielsen, and V. Loeschcke 2008, *Cell Stress and Chaperones*, 177–182; Kristensen, T.G., J.G. Sorensen, and V. Loeschcke 2003, IAAS, Journal of Genetics 82(3): 89-94; Lyko, F, B.H. Ramsahoye, and R. Jaenisch 2000, Nature 408: 538–540; Schaefer, M., and F. Lyko 2010, Nature Genetics 2010: 920–921; Schaefer, M., J.P. Steringer, and F. Lyko 2008, PLoS ONE; Sorensen, J.G., M.M. Nielsen, M. Kruhoffer, J. Justesen, and V. Loeschcke 2005, Cell Stress Society International 10(4): 312–328; Tatar, M., A.A. Khazaeli, and J.W. Curtsinger 1997, Nature 390: 30.