Ziegler, R. and H. Emmerich*. University of Cologne, Germany. Phosphorylation of chromosomal proteins in Drosophila hydei.

It is generally believed that chromosomal proteins are involved in regulation of the genome in eukaryotes. Histones can be acetylated (Allfrey 1970), phosphorylated (Langan 1969) or varied in their thiol/disulphide ratio (Ord and Stockem 1969). The more tissue specific non-histone chromosomal proteins (NHP) show a correlation between their phosphorylation and the derepression of genes (Allfrey 1970), the electrophoretic pattern of phosphorylated NHP shows specificity for different tissues (Platz et al. 1970, Rickwood et al. 1972). In dipteran giant chromosomes, however, Benjamin and Goodman (1969) could not find a correlation between ^{32}-P-incorporation and puff sites. Their results seemed to us to be worth a re-examination: Those authors used a normal thin layer autoradiography, which has for ^{32}P a resolution of 5 - 10 µm only; they examined only already existing, but not newly induced, puffs and they didn't include biochemical examinations of the chromosomal proteins in the giant chromosomes. We therefore investigated the phosphorylation of chromosomal proteins in D. hydei during puff induction by track autoradiography of ^{32}phosphorous and by electrophoretic separation of the NHP.

Manually isolated salivary glands were incubated in Gehring's balanced saline for 45' with 100 µCi/1.5 ml ^{32}-orthophosphate either in the presence of 10^{-6}M β-ecdysone or, in order to induce temperature puffs, at 37°C. cAMP was used at 10^{-3}M, puromycin at 100 µg/ml. One gland of each isolated pair was used as a control. Mass preparations of salivary glands were made after Boyd et al. (1968). To examine the phosphorylation of NHP in the chromosomes, the glands were squashed in orcein-acetic acid; for the study of the phosphorylation of histones they were fixed in 2.5% glutaraldehyde. After postfixation with 10% formaldehyde in 0.1 M phosphate, the slides were treated with ribonuclease. Track auto radiography was performed with Ilford G5 emulsion after Rogers (1967). NHP from the incubated mass preparations of glands were isolated after Helmsing and Berendes (1971) and separated by iso-electric focussing after Gronow and Griffith (1971). The polyacrylamide gels were sliced and counted in a Packard Liquid Scintillation Counter.

Phosphorylation of NHP: Fixation of the chromosomes with alcohol-acetic acid extracts part of the histones, especially the phosphorylated ones, so in the chromosomes mainly NHP remained. As shown the chromosomes are phosphorylated. We do not think that the label is

Figure 1: Early ecdysone puffs (78B) are induced; temperature puffs (e.g. 48B) already existed. Note low label of the nucleolus.

Figure 2: Early ecdysone puffs are only poorly labeled.

RNA phosphorous as the squashes had been treated with RNase and the nucleolus retained only slight labeling (Figure 1; in looking at the autoradiographs one must keep in mind that this is a track autoradiography and the grains must be followed back to their source). The cytoplasm shows also some label. The label of the chromosomes is randomly distributed and their is no preferential incorporation in pre-existing or newly-formed puffs, either after ecdysone or after temperature treatment (Figures 2 and 3).

Figure 3: Induced temperature puff (48B).

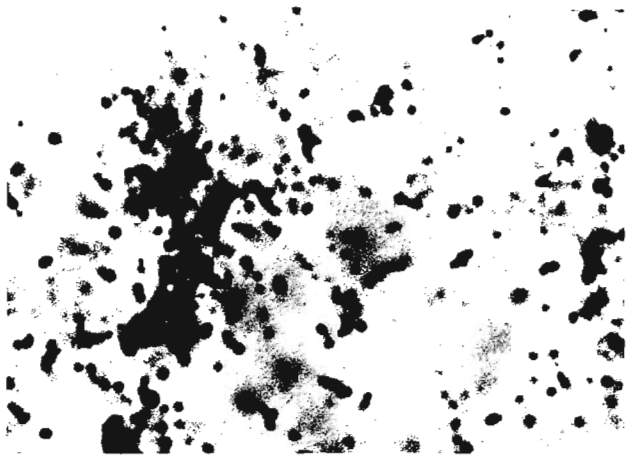

Figure 4: Induced early ecdysone puff (78B), glands fixed with glutaraldehyde.

Phosphorylation of histones and NHP: When the salivary glands were fixed with glutaraldehyde, which does not extract histones, we found a higher incorporation of ^{32}P, but the distribution was essentially like that observed for NHP (Figure 4). There were reports about a histone specific protease which might preferentially attack phosphorylated histones (Marushige and Dixon 1971, Elgin and Bonner 1972). The label could therefore be absent from puffs after prolonged induction, so we incubated the salivary glands for only 10' with ecdysone. The chromosomes just started to develop small ecdysone puffs, but the incorporation of phosphate was very low and not preferential at the sites of puff formation. The suppression of protein synthesis by puromycin did not diminish the ^{32}P-labeling, so it seems to be improbable that newly synthesized proteins would be phosphorylated to a higher extent than pre-existing ones.

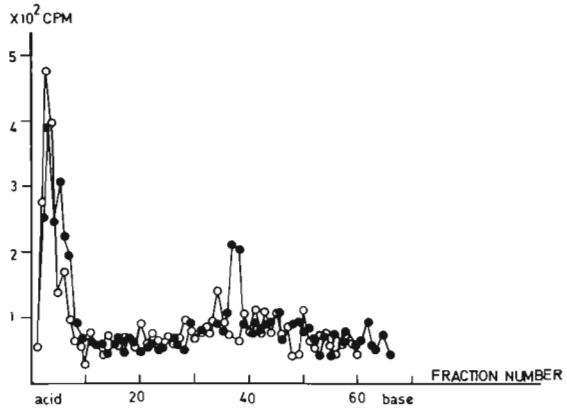

Figure 5: Isoelectric focussing of chromosomal NHP in 10% acrylamide containing 8M urea, 30 min. after temperature shock in the presence of 100 µCi ^{32}P.

Isoelectric focussing of phosphorylated NHP: The NHP of the chromatin from 350 mg salivary glands which were kept at 37°C for 45' in the presence of 200 µCi ^{32}-orthophosphate showed one additional phosphorylated protein band with an isoelectric point of 6.5, whereas the controls kept at 21°C only showed ^{32}P in the very acid part of the gel. The addition of cAMP enhanced the incorporation of ^{32}P into glutaraldehyde fixed chromosomes as compared with cAMP-free controls, but the peak in the isoelectric focussing could not be enlarged by that treatment.

Despite the fact that we could not find a histological correlation between puff induction and chromosomal phosphorylation, we were able to demonstrate a specifically phosphorylated NHP

after temperature shock of mass prepared salivary glands. Since the chromatin was carefully washed and no cholesterol was demonstrable in it by the Liebermann'Burchard reaction, we do not think that this newly phosphorylated protein is a cytoplasmic contamination or deriving from membranes. We have no explanation for the origin or function of that phosphoprotein. It might have a cellular transport or disposal function as Sheperd et al. (1971) have suggested for phosphorylated histones.

References: Allfrey, V.G. 1970, Fed. Proc. 29:1447-1460; Benjamin, W.B. and R.M. Goodman 1969, Science 166:629-631; Boyd, J.B., H.D. Berendes and H. Boyd 1968, J. Cell Biol. 38:367-376; Elgin, S.C.R. and J. Bonner 1972, Proc. Austr. biochem. Soc. 5:18; Gronow, M. and J. Griffith 1971, FEBS Letters 15:340-344; Langan, T.A. 1969, PNAS 64:1276-1283; Marushige, K. and G.H. Dixon 1971, J. Biol. Chem. 246:5799-5805; Ord, M.G. and L.A. Stocken 1969, Biochem. J. 112:81-89; Platz, R.D., V.M. Kish and L. Kleinsmith 1970, FEBS Letters 12:38-40; Rickwood, D., G. Threlfall, A.J. MacGillavary, J. Paul and P. Rickes 1972, Biochem. J. 129:50p; Rogers, A.W. 1967, Techniques in autoradiography, Elseviers Amsterdam; Sheperd, G.R., B.J. Noland and J.M. Hardin 1971, Arch. Biochem. Biophys. 142:299-302.

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Erratum in previous note.

DIS 49:63 (1972), Temporal properties of the ERG on-transient recorded in the retina and lamina. Erratum: In the electrical network (figure, part b) certain components were inadvertantly interchanged. The signal source should be located where the monopolar neuron membrane resistance (r) and capacitance (c) were and vice versa. The polarity of the relocated signal source should be positive outside the monopolar neuron.

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Studies on antigens of wild type strains of Drosophila melanogaster.

Extracts of eggs, third instar larvae and flies from 75 different wild type strains either collected locally (4) or at Strömsvreten (10) in Sweden or obtained from different laboratories around the world were analysed on immunoelectrophoresis plates using antisera prepared against extracts of eggs, third instar larvae or flies of the strain Oregon-R. 24 different antigens were studied and in only one case was there an obvious difference between strains. Small changes in electrophoretic mobility which could have been detected by other techniques such as polyacrylamide gels, would not have been detected here. The striking difference was an antigen present in 72 of the 75 strains but not present in Bacup, Bannerdale or Berlin. This antigen (No. 2, Roberts, Nature 1971, 233:394) was present in unfertilized Oregon-R eggs and persisted throughout embryogenesis finally disappearing soon after hatching. In reciprocal crosses between Oregon-R and Bacup the antigen was found in eggs when Oregon-R was the female parent but not when Bacup was the female parent. This together with the disappearance of the antigen soon after hatching suggests that the antigen is synthesised during oogenesis and is diluted out during embryogenesis. The eggs of Oregon-R/Bacup heterozygotes possess the antigen.

To locate the gene responsible for the synthesis of this antigen Berlin flies were crossed with strains carrying marked 1st, 2nd or 3rd chromosomes which also carried inversions. The marked strains all possessed the antigen. Eggs from the F_2 females were tested for the presence of the antigen. Females homozygous for the Berlin 2nd chromosome layed eggs which did not possess the antigen while flies homozygous for Berlin 1st or 3rd chromosome layed eggs which possessed the antigen. This suggests that the gene responsible for the synthesis of this antigen is on chromosome II. No further location studies have been carried out.