

Changes in nuclear proteins induced by heat shock in *Drosophila* culture cells

T. Rabilloud, P. Vincens, M. Hubert, J.L. Penetier and P. Tarroux

ERA 1048, Ecole Normale Supérieure, Laboratoire de Zoologie, 46 rue d'Ulm, 75005 Paris Cédex, France

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Nuclear proteins of normal and heat-shocked *Drosophila* cells were analysed by two-dimensional electrophoresis. The computerized processing of the gels allowed us to detect 6 proteins strongly induced by the heat treatment, but which were different from the usually described heat-shock proteins. The possible role of these proteins in genetic regulation is discussed, as is the value of this type of approach for the study of other genetic regulation phenomena.

Nuclear protein Heat-shock Genetic regulation Drosophila cell

1. INTRODUCTION

One of the best known model systems for the study of genetic regulation is the system of heat-inducible genes of *Drosophila melanogaster* [1]. Upon transfer from 25°C (normal growth temperature) to 37°C, activation of a few defined genes occurs rapidly, with production of defined sets of polypeptides [2]. Much work has been carried out concerning both the heat-shock genes and their protein products [3–7], but little is known about the factors controlling these genes [8]. However, this topic is under active study, and a transcription factor specific for some hsp genes has been discovered recently [9]. We therefore decided to analyse the nuclear proteins of normal and heat-shocked *Drosophila* cells to detect differences which may be correlated with the expression of the heat-shock genes.

Abbreviations: hsp, heat-shock proteins; NP-40, Nonidet P-40; PMSF, phenylmethylsulfonyl fluoride; BSA, bovine serum albumin; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)propanesulfonate

2. MATERIALS AND METHODS

2.1. Cell culture

D. melanogaster Kc cell line, subclone 8-9K, kindly provided by Drs Echalié and Bestbel-pomme [10], was grown as a monolayer in Echalié D22 medium at 23°C. Heat shock was induced by floating the culture flasks in a 37°C water bath for 30 min. The nuclei were then isolated immediately.

2.2. Preparation of nuclei

All operations were performed at 4°C with cold solutions. The pelleted cells (800 × g, 5 min) were suspended in 2 ml of buffer A by vortex-mixing (buffer A: 100 mM sucrose, 10 mM MgCl₂, 5 mM Hepes-NaOH, pH 7.5). 10% NP-40 was then diluted 20-fold in the sample, and the cells were lysed by vigorous vortex-mixing. The suspension was then transferred into an SW-60 Beckman tube and was underlayered by a 2 ml cushion of buffer B (as A but containing 1.8 M sucrose). Centrifugation at 50000 × g for 20 min pelleted the nuclei.

2.3. Preparation of the nuclear proteins

The pellet of nuclei was suspended in 200 µl of buffer C (0.35 M NaCl, 25 mM Hepes-NaOH, pH 7.5). The suspension was divided into two por-

tions, for duplicate experiments, and was stored at -80°C until use. A $100\ \mu\text{l}$ aliquot was then digested with $10\ \mu\text{g}$ RNase A at 30°C for 30 min in the presence of $0.5\ \text{mM}$ PMSF. 100% trichloroacetic acid was then added to a final concentration

of 10% and the proteins were precipitated and collected as described in [11]. The protein pellet was then suspended in $50\ \mu\text{l}$ of $9\ \text{M}$ urea, $0.1\ \text{M}$ sodium borate, and the proteins were labelled by reductive methylation as described [11], but using a high

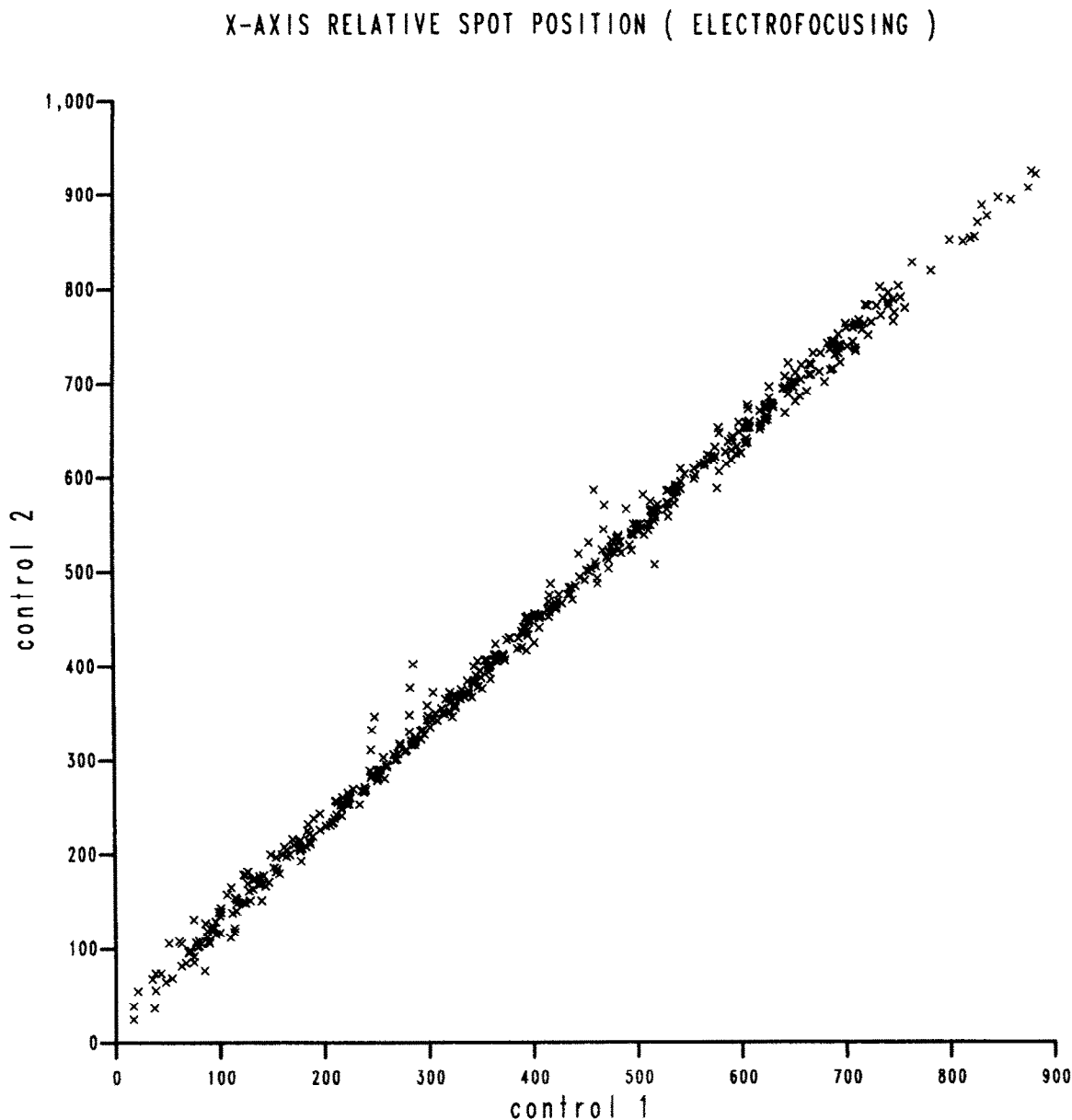


Fig.1. Positional reproducibility of the gels along the x -axis (electrofocusing). In this diagram, the spots that are common to two gels are determined and plotted. The abscissa defines their position along the x -axis in the first gel, and the ordinate their position along the x -axis in the second gel. A comparison between two control gels is represented here.

specific activity tritiated sodium borohydride (NEN, 50–75 Ci/mmol). The pellet was then resuspended in 100 μ l of O'Farrell lysis buffer containing CHAPS instead of NP-40 [12], and the DNA was pelleted by ultracentrifugation as in [13].

2.4. Electrophoresis

Two-dimensional electrophoresis was performed essentially as described by O'Farrell [14] with the modifications suggested by Perdew et al. [12] for the electrofocusing, but the sample was applied at the anodic end of the gel. The gels were treated and exposed as in [15]. A calibration curve for the fluorography was constructed as described [16], using BSA labelled by the same method as the nuclear proteins.

2.5. Gel analysis

A complete description of the computerized

system used for two-dimensional electrophoretograms will be published elsewhere (P. Tarroux et al., in preparation). Briefly, fluorograms were digitalized using a video camera coupled to a graphic system built at the Ecole Normale Supérieure by the Laboratoire d'Informatique Expérimentale. The spots were then detected, quantified, and the spot lists corresponding to two different gels were matched using home-made programs and an IBM 4341 computer. A data base was then constructed, and the results were extracted from this base.

3. RESULTS

The first results concern the tests made to verify that our approach was not plagued by artefacts. We compared a gel with itself and we found that over 99% of the spots were correctly matched. We



Fig.2. Two-dimensional gel electrophoresis of nuclear proteins from control cells. The acidic end is on the left of the figure, and the high molecular mass proteins are at the top.

also tested the reproducibility of the experiments by performing two electrophoresis runs with a sample prepared from the same batch of nuclei.

More than 95% of the spots were found to be correctly matched and of comparable intensities. We also tested the positional reproducibility by plot-

RECIPROCAL INTENSITY PLOT

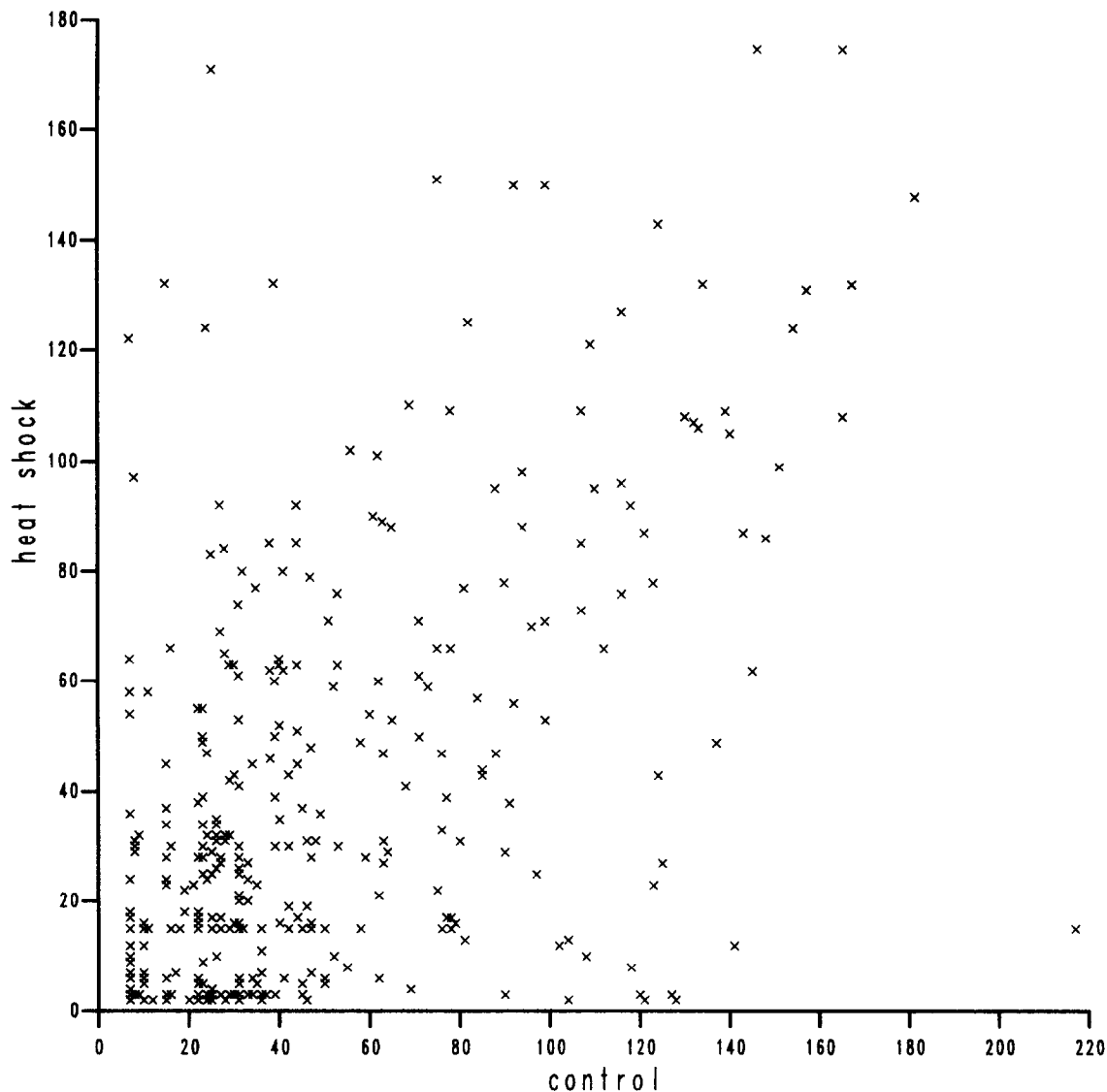


Fig.3. Reciprocal intensity diagram. One control and one heat-shock sample are compared by two-dimensional electrophoresis. The spots common to the two gels are determined and their intensities are plotted. In this diagram, the abscissa shows the intensity of the spot in the control experiment, the ordinate showing its intensity in the heat-shock experiment. Note the strongly induced spots at the left of the figure.

ting the coordinates of the matched spots in each of the matched gels. The results are shown in fig.1. Most of the spots are located along the diagonal, indicating that their coordinates are the same in the two gels, and that the matching program does not produce gross artefacts. An example of the gel patterns we have obtained is shown in fig.2. More than 800 spots were detected by the computerized system, and the calibration experiments show that our set of techniques is able to detect proteins which are present at the level of 500–1000 copies per nucleus. The quality of the gels having been established, it was then necessary to build a tool allowing us to extract meaningful information from the gels. We therefore plotted the intensity of the spots present in both gels on a reciprocal diagram (fig.3) as described in [17]. From this diagram, we can detect the spots that vary with heat treatment as being spots located far from the diagonal. By using the data base constituted from the spots, we can select the spots that are induced or repressed by the heat-shock. The histograms displayed in fig.4 show the dramatic variation which can be observed for some spots. It should be noted that we did not observe spots that are deeply repressed by the heat treatment. To verify that these findings were not artefactual (spots detected in the streaking or precipitation zones) we located the spots on the gels, as shown in fig.5. It can be seen that the induced spots are found over a wide range of *pI* and molecular mass, indicating that they are not chemically related.

4. DISCUSSION

Our aim here is to present a system which allows precise study of nuclear proteins, including minor ones. To avoid the low-quality gels which can be obtained [18], we increased the solubilizing power of the electrofocusing medium and lowered the protein loadings by labelling the proteins to high specific activity ($\sim 1 \mu\text{Ci}/\mu\text{g}$). The computerized system which we have developed ensures full and quantitative use of the data that can be extracted from such electrophoretograms. However, it must be kept in mind that the nuclear proteins are very likely to change even with minimal variations in the cell physiology. With the great sensitivity of our system, such variations will be detected. So, the greatest care must be taken to ensure optimal

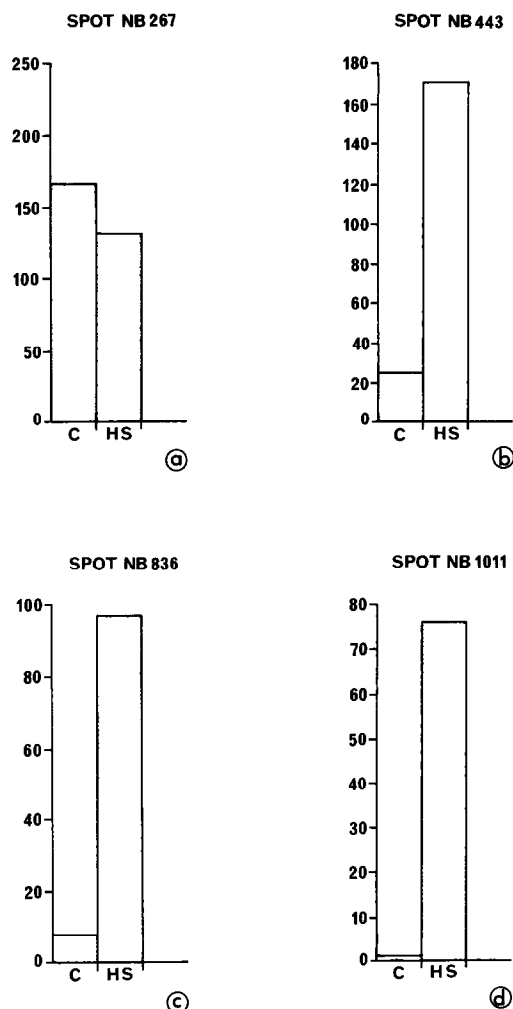


Fig.4. Intensity histograms for a non-induced: (a) spot 267; and for induced spots: (b) spot 443, (c) spot 836 and (d) spot 1011.

reproducibility, and many control or duplicate experiments must be performed.

We have used this system to study the heat-shock response of eukaryotic cells. Although further study is evidently necessary, our results demonstrate some of the acute nuclear changes accompanying the heat response. It should be remembered that our methods are not restricted to the detection of newly synthesised proteins, and that we are able to detect any translocation of proteins from the cytoplasm to the nucleus, which may occur at the onset of the heat-shock response,

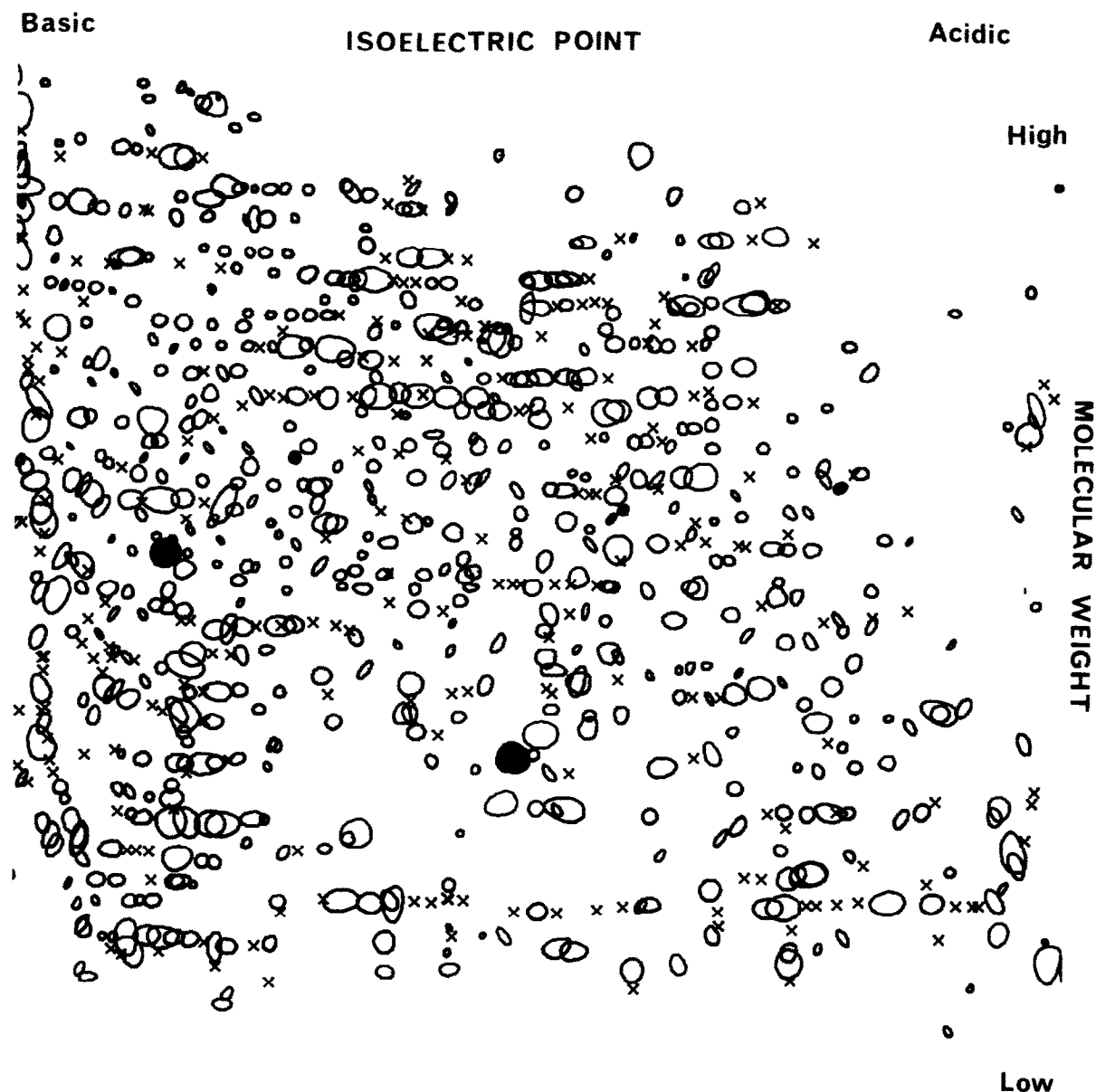


Fig.5. Computerized representation of a model two-dimensional gel. The heat-shock induced proteins are represented as dark circles, the other spots being represented as open contours.

before any gene activation. In conclusion, much work is still needed to specify the biological characteristics and functions of these newly described proteins, and to establish whether they are neosynthesised during the heat shock or are

simply translocated from the cytoplasm into the nucleus. Some of them might be the DNA-binding proteins described by Falkner and Biessmann [8], as they are located in the correct 40-kDa range.

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