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Nuclear-mitochondrial interactions in the regulation of protein synthesis in Drosophila hydei salivary gland cells

J.F.J.G. Koninkx

NUCLEAR-MITOCHONDRIAL INTERACTIONS IN THE REGULATION OF PROTEIN SYNTHESIS IN DROSOPHILA HYDEI SALIVARY GLAND CELLS

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TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE WISKUNDE EN NATUURWETENSCHAPPEN AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN, OP GEZAG VAN DE RECTOR MAGNIFICUS PROF.DR. A.J.H. VENDRIK, VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN IN HET OPENBAAR TE VERDEDIGEN OP DONDERDAG 1 JUNI 1978 DES NAMIDDAGS TE 4.00 UUR

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Summary

Introduction

In larval and in some adult tissues of a variety of Drosophila species containing polytene chromosomes the activity of certain chromosome loci is visualised by the appearance of puffs. The activation of previously inactive chromosome loci is always preceded by a local accumulation of nonhistone proteins in a restricted area of the presumptive puffing site (Swift, 1964; Berendes, 1968; Helmsing and Berendes, 1971; Berendes and Helmsing, 1974; Mitchell and Lipps, 1975). The accumulation of nonhistone proteins results in an increase of the chromosome diameter in combination with a dispersed banding pattern. Autoradiographs prepared from Drosophila polytene cells, which have been exposed to radioactive RNA precursors, reveal that these genome loci are active in RNA syntheses (Berendes, 1968; Berendes, Alonso, Helmsing, Leenders and Derksen, 1973). As soon as the protein accumulation becomes manifest, by the time that the active loci have attained their final size, the appearance of ribonucleo-protein particles can be observed at the submicroscopic level (Leenders, Derksen, Maas and Berendes, 1973; Derksen, Berendes and Willart, 1973; Derksen, 1975[a]; Derksen, 1975[b]).

Puffs can thus be considered to represent visible changes in gene activity in terms of production of mRNA and do occur during ontogeny of Drosophila species (Pelling, 1964; Clever, 1967; Berendes, 1968). The variety of puffs that can be observed in tissues of Drosophila larvae can be divided into two different inducible classes. The first group of puffs is related to the cellular metabolism. These puffs fluctuate in their activity and are apparently activated during the life-time of the organism, whenever their activity is needed (Ritossa, 1962, 1963, 1964; Berendes, van Breugel and Holt, 1965; van Breugel, 1966; Ashburner, 1970; Leenders and Berendes, 1972; Leenders, Derksen, Maas and Berendes, 1973). The second group of puffs is involved in the differentiation of the organism. These puffs, the "hormone"-puffs are only active at certain stages in the development such as moulting or puparium formation.

(Clever and Karlson, 1960; Berendes, 1967; Crouse, 1968; Poels, 1970; Berendes and Thijssen, 1971; Poels, de Loof and Berendes, 1971; Asburner, 1971; Poels, 1972; Ashburner, 1973). Moreover, the "hormone"-puffs are tissue specific.

Both the puffs related to the cellular metabolism and the puffs related to differentiation do occur in normally developing larvae, but they can also be induced experimentally by treatments interfering with the cellular metabolism or by administration of the moulting hormone ecdysone, respectively.

Major puffs, which normally develop following treatments interfering with the cellular respiratory metabolism in the polytene salivary gland cells of Drosophila hydei, are 2-32 A, 2-36 A, 2-48 BC and 4-81 B (Leenders and Berendes, 1972; Leenders and Beckers, 1972).

The actual factor(s) responsible for the induction of these specific puffs is still unknown. As a working hypothesis, however, the following has been suggested. Treatments interfering with the cellular respiratory metabolism may result in decreasing substrate levels. Such deficiencies in respiratory substrate supplies might decrease the substrate affinity of allosteric mitochondrial enzymes. This decrease in substrate affinity, resulting in a reduced enzyme protection by its substrate, could give rise to macromolecules, which, following their release into the cytoplasm, could associate with specific proteins, migrate into the nucleus and induce the activity of specific chromosome loci (Leenders, Berendes, Helmsing, Derksen and Koninkx, 1974; Sin, 1975). The active genes could be responsible in turn for changes in the activity of mitochondrial enzymes (see fig. 1).

It seems reasonable to suppose that the nuclear genome has a role in the correction of a deficiency in the mitochondrial metabolism, since during mitochondrial biogenesis most of the mitochondrial proteins are coded for by the nuclear genome and translated by the cytoplasmic protein synthesis machinery (Beattie, 1971; Tzagoloff, Rubin and Sierra, 1973; Avadhani, Lewis and Rutman, 1975).

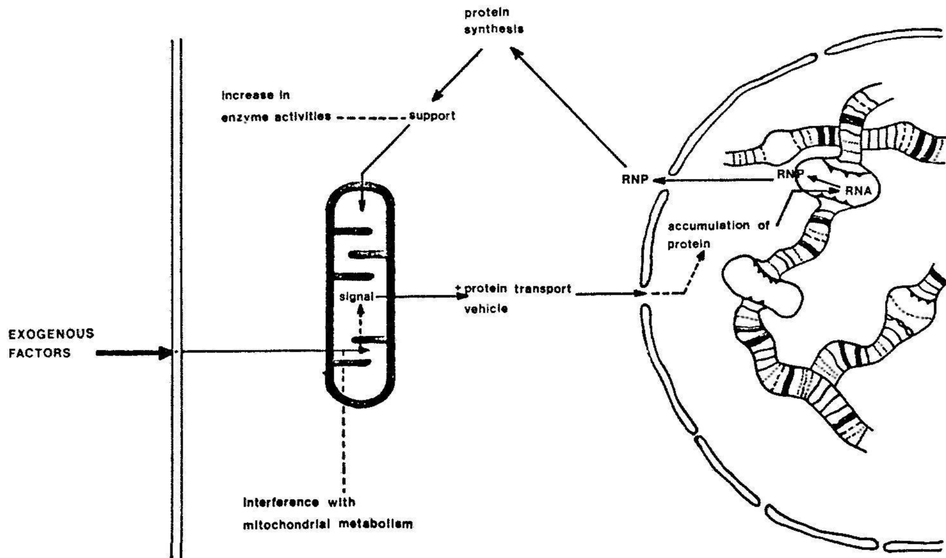

Figure 1. Schematic drawing of the hypothetical nuclear mitochondrial interactions in the control of the respiratory metabolism of Drosophila salivary gland cells.

The first indications suggesting a relationship between changes in the cellular respiratory metabolism and a particular genome response at the genome level, was the observation that addition in vitro to polytene tissues of Drosophila hydei of 2,4-dinitrophenol, salicylate or dicumarol, uncouplers of the oxidative phosphorylation, resulted in an almost immediate appearance of chromosome puffs at previously inactive chromosome loci (Ritossa, 1962, 1964; Berendes, van Breugel and Holt, 1965; van Breugel, 1966; Ashburner, 1970).
An induction of changes in the activity of the same loci which respond to an experimental uncoupling of oxidative phosphorylation, can also be observed following the application of inhibitors of the respiratory chain, such as azide, amytal, rotenone, antimycine A and 2-heptyl-4-hydroxy quinoline-N-oxide (Leenders and Berendes, 1972). A sudden rise in environmental temperature or the release from anaerobiosis, treatments which affect the cellular respiratory metabolism, are also able to evoke the specific chromosomal response. Moreover, the availability of substrates of the citric acid cycle (malate, succinate) or substrates supporting the mitochondrial respiration (glutamine, α-glycerolphosphate) during the temperature treatment or during the recovery from anaerobiosis retard or even completely prevent the genome response. This finding makes it highly likely, that there exists a relationship between the newly appearing puffs and definite changes in the mitochondrial metabolism (Leenders and Berendes, 1972; Leenders and Beckers, 1972; Berendes, 1972).

A variety of experimental treatments interfering with the cellular respiratory metabolism is thus very effective in evoking gene activity of previously inactive chromosome loci. Although the actual mechanism(s), by which gene activity is regulated quantitatively as well as qualitatively, has not been elucidated, it has been suggested that, since these treatments and substances interfere with the cellular metabolism, a decrease in the cellular ATP level could serve as a possible trigger for the activation of particular chromosome loci (Ritossa, 1962, 1963; Ellgaard, 1972). Whether a change in the cellular ATP level serves as a signal for the induction of puffs 2-32 A, 2-36 A, 2-48 BC and 4-81 B,

has been investigated in the first chapter of this thesis.

The other chapters of this thesis deal with the effects of induced gene activity upon cellular metabolism.

In general, the activation of genes always precedes changes in RNA-synthesis, protein-synthesis and/or changes in enzyme activity (Baudisch and Panitz, 1968; Daneholt, Edström, Egyhazy, Lambert and Ringborg, 1969; Leenders and Beckers, 1972; Tissières, Mitchell and Tracy, 1974; Lewis, Helmsing and Ashburner, 1975; Bisseling, Berendes and Lubsen, 1976). The effects of induced gene activity of the chromosome loci 2-32 A, 2-36 A, 2-48 BC and 4-81 B by treatments interfering with the cellular respiratory metabolism could possibly be expressed in changes in mitochondrial enzyme activities.

Measurements of the effect of various substrates on the oxygen consumption of glands recovering from anaerobiosis indicated that isocitrate and tyrosine stimulated the respiration of recovering glands to a much greater extent than the respiration of the control glands (Leenders and Knoppien, 1973). Therefore, the activity of the mitochondrial tyrosine aminotransferase was investigated (Leenders and Beckers, 1972; Leenders, Berendes, Helmsing, Derksen and Koninkx, 1974; Sin and Leenders, 1975).

Squashes, made from salivary glands treated with rotenone or amytal, display puffs at loci 2-32 A, 2-36 A, 2-48 BC and 4-81 B. Puff 4-81 B is not induced when antimycin A is present in the incubation medium, whereas the other mentioned loci are activated (Leenders and Berendes, 1972). This consistent difference initiated the study of the activity of the mitochondrial NADH dehydrogenase, which may be considered as one of the rate limiting enzymes in respiratory chain (Hemmerich, Nagelschneider and Veeger, 1970).

In homogenates of salivary glands the activity of NADH dehydrogenase had increased significantly after recovery from anaerobiosis of the salivary glands (Leenders and Beckers, 1972). This increases in NADH dehydrogenase activity has evoked several questions, which are dealt with in this thesis, for example:

Does the increased enzyme activity of NADH dehydrogenase originate

from an intramitochondrial or extramitochondrial enzyme ? Is the increased enzyme activity of NADH dehydrogenase caused by synthesis *de novo* of this enzyme after experimentally induced gene activity ? Does increased gene activity always precede changes in NADH dehydrogenase activity and are these changes dependent upon transcription of puffed chromosome loci and translation of new mRNA species ? Is the increase in NADH dehydrogenase activity due to mitochondrial biogenesis ?

By electrophoresis in sodium dodecyl sulphate polyacrylamide gels Tissières, Mitchell and Tracy (1974) demonstrated that puff induction by a temperature shock in Drosophila melanogaster changes the pattern of protein synthesis, namely, six new strongly labelled polypeptides appeared. A similar set of polypeptides could be induced by a temperature shock in salivary glands of Drosophila hydei (Lewis, Helmsing and Ashburner, 1975).

To test whether or not the appearance of the polypeptides is causally related to the presence of the puffs 2-32 A, 2-36 A, 2-48 BC and 4-81 B, salivary glands of Drosophila hydei larvae have been submitted to a variety of treatments interfering with the cellular respiratory metabolism.

Both the increase in enzyme activity of NADH dehydrogenase and the appearance of the strongly labelled polypeptides as a result of gene activity occur with very similar kinetics of induction. This finding could indicate that the strongly labelled polypeptides might be regarded as precursors of NADH dehydrogenase. This possibility is investigated in this thesis.

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CHANGES IN CELLULAR ATP, ADP AND AMP LEVELS FOLLOWING TREATMENTS AFFECTING CELLULAR RESPIRATION AND THE ACTIVITY OF CERTAIN NUCLEAR GENES IN *DROSOPHILA* SALIVARY GLANDS

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SUMMARY

Changes in the cellular adenine nucleotide contents of larval salivary glands of *Drosophila hydei* as a result of treatments affecting the respiratory metabolism were established and correlated with changes in the activity of four genome loci The results demonstrate that the activation of the genome loci is not a necessary consequence of a reduction in the ATP level or changes in ADP or AMP levels Other regulatory mechanisms for the activation of these genome loci are discussed

A variety of experimental treatments interfering directly or indirectly with cellular respiration is effective in the activation of a group of specific genome loci in various dipteran species The activity of these loci, visualized by the presence of chromosome puffs active in RNA synthesis can be brought about by a sudden increase in environmental temperature [1–3], a recovery from an anaerobic treatment [4, 5], the administration of uncouplers of oxidative phosphorylation [5, 6], and by specific inhibition of certain mitochondrial processes [5]

Although the actual mechanism by which these gene activities are monitored has not been elucidated, it has been suggested on account of the treatments causing the activation of these genes, that the cellular ATP level might play a decisive role in the activation mechanism [3, 5, 6] It could be argued that a drop in cellular ATP or a rise in ADP or AMP serves as a signal for the activation of the particular genome loci

In order to explore this possibility, cellular nucleotide levels were measured following treatments which have as a consequence the activation of the particular puffs

MATERIALS AND METHODS

All experiments were performed with salivary glands of mid third instar larvae of *Drosophila hydei* isolated according to the method of Boyd et al [7] using ice-cold *Drosophila* Ringer (for composition see [8]) The larvae were obtained from mass cultures maintained under standardized conditions with respect to food supply, temperature, relative humidity and light regimen Each culture was started with eggs laid by 3-week-old females during a 1 h period

Isolated salivary glands were submitted to one of the following treatments which were all performed in the complex incubation medium described by Poels [9]

(*a*) a temperature treatment in which the medium temperature was raised from 25 to 37°C and kept at

Table 1. *ATP, ADP and AMP levels and puff size of the regions 2–32A, 2–36A, 2–48C and 4–81B following an in vitro temperature treatment or N_2-anaerobiosis and subsequent recovery in air applied to isolated larval salivary glands of Drosophila hydei*

Incubation conditions	ATP (nmol/ mg prot.)	ADP (nmol/ mg prot.)	AMP (nmol/ mg prot.)	Total nucleotide content (nmol/ mg prot.)	~P (nmol/ mg prot.)	~P / nucleotide content	Relative puff size[a]			
							32A	36A	48C	81B
25 min medium at 25°C	5 80	0 92	0 18	6 90	12 52	1.82	−	−	−	−
25 min medium at 37°C (temp. treatment)	1 12	2 04	1.40	4 56	4.28	0.94	− +	− ⊤	− −	− −
2 h medium	6.44	1.43	0.57	8.44	14.31	1 70	−	−	−	−
2 h medium saturated with N_2 (N_2-anaerobiosis)	0 00	1.09	2.43	3.52	1.09	0.31	−	−	−	−
145 min medium	9.86	1 67	0.22	11.75	21 39	1 82	−	−	−	−
120 min N_2-anaerobiosis + 25 min recovery in air	5.21	1 90	0.71	7.82	12 32	1.57	− −	-- −	− ⊥	− −

[a] Estimated puff sizes.—, no puff present. − −, large puff present.

37°C for 25 min; (*b*) an anaerobic treatment in which the glands were incubated in an N_2-saturated medium under N_2 atmosphere for 2 h followed by 25 min recovery in air, (*c*) 2 h incubation of the glands in medium supplied with 7.5×10^{-5} M Na-arsenite, (*d*) 75 min incubation of the glands in medium saturated with rotenone (Sigma) (0 5 mg/ml) and containing 3.4×10^{-3} M amytal (Merck) (see [5]), (*e*) 75 min incubation of the glands in medium saturated with oligomycin (Sigma) (0 5 mg/ml) and containing either 2×10^{-3} M KCN (Merck) or 2.5×10^{-4} M atractyloside (Sigma) All incubations, except the temperature treatment, were carried out at 25 ± 1°C.

Following treatment, the glands were thoroughly washed with ice-cold *Drosophila* Ringer, pelleted and homogenized (3×10 sec, Branson sonifier step 2) in 1 ml 4 % citric acid in Ringer per 100 mg of glands. Subsequently, 0.1 ml 40 % ice-cold perchloric acid (PCA) per ml of homogenate was added The resulting precipitate was pelleted and used for protein determination according to Lowry et al [10]. The supernate was adjusted to pH 7.4 with 0.1 M Tris-KOH and after freezing and removing of excess salt, aliquots were used for determinations of ATP, ADP and AMP

ATP was determined with hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase (EC 1 1 1 49) ADP with pyruvate kinase (EC 2 7.1 40) and lactate dehydrogenase (EC 1.1 1 27) as described by Bergmeyer [11]. Since the adenine nucleotide concentrations were low an Aminco Chance dual wavelength spectrophotometer was used.

All nucleotide values are expressed in nmoles/mg protein From each batch of salivary glands used for measurements of the nucleotide levels, 5 glands were fixed, stained and squashed and the relative puff size of the chromosome regions 2–32A, 2–36A, 2–48C and 4–81B were estimated [12].

RESULTS

As can be seen from table 1, a temperature treatment of the isolated glands for 25 min results in an increase in puff size at all loci studied, a result which confirms earlier findings [2, 5]. In these experiments, as well as after the anaerobic treatments, the levels of ATP, ADP and AMP were measured in homogenates of glands which had been treated with citric acid immediately after the incubation. Washing was omitted in order to avoid a possible change in distribution and content of nucleotides. Following the temperature treatment, the level of ATP was reduced, and ADP and AMP levels were increased as compared with the control glands.

After an anaerobic treatment of 2 h, ATP

Table 2. *ATP, ADP and AMP levels and puff size of the regions 2–32 A, 2–36 A, 2–48 C and 4–81 B following in vitro incubation of larval salivary glands of Drosophila hydei with arsenite, rotenone, amytal, oligomycin, cyanide and atractyloside*

Incubation contitions	ATP (nmol/ mg prot.)	ADP (nmol/ mg prot.)	AMP (nmol/ mg prot.)	Total nucleotide level in (nmol/ mg prot.)	~P (nmol/ mg prot.)	~P / nucleotide content	Relative puff size[a]			
							32A	36A	48C	81B
2 h medium	7.47	1.85	0.19	9.51	16.79	1.77	–	–	–	–
2 h medium 7.5 × 10⁻⁵ M arsenite	7.18	1.52	0.20	8.90	15.88	1.78	–	+	–	+ –
75 min medium	7.22	2.58	—	—	17.02		–	–	–	–
75 min medium saturated with rotenone 3.4 × 10⁻³ M amytal	1.11	3.32	—	—	5.54		+	– –	– –	+ –
75 min medium saturated with oligomycin and rotenone 3.4 × 10⁻³ M amytal	0.33	2.92	—	—	3.58		–	–	+ +	–
2 h medium	4.58	1.43	0.28	6.29	10.59	1.68	–	–	–	–
2 h medium saturated with oligomycin	0.81	1.89	1.50	4.20	3.51	0.84	–	–	–	–
2 h medium saturated with oligomycin 2 × 10⁻³ M KCN	0.43	3.00	—	—	3.86		–	+	– +	–
45 min medium	7.14	3.11	0.21	10.46	17.39	1.66	–	–	–	–
45 min medium 10⁻³ M KCN	3.93	3.12	1.30	8.35	10.98	1.31	–	–	–	–
2 h medium	7.22	2.58	—	—	17.02		–	–	–	–
2 h medium 2.5 × 10⁻⁴ M atractyloside	10.63	3.25	—	—	24.51		–	–	–	–
2 h medium saturated with oligomycin 2.5 × 10⁻⁴ M atractyloside	1.64	4.38	—	—	7.66		+	– –	+ +	–

[a] Estimated puff sizes: –, no puff present. +, small puff present. –, large puff present

can no longer be detected, the ADP content is slightly decreased and the AMP content is increased significantly. However, no increase in puff size was observed under these conditions. Complete depletion of the ATP pool could be responsible for the failure of the puffs to respond to this treatment. As has been reported previously [13], the development of a puff is dependent on the availability of sufficient energy. This suggestion finds some support from the data obtained after a recovery from anaerobiosis in which the ATP level has increased again and the puffs do appear (table 1). In con-

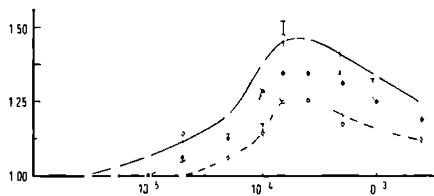

Fig 1 Abscissa M atractyloside in the presence of oligomycin, *ordinate* rel puff size Response of chromosome region ⊐ ⊐, 2–48C, ●----●, 2–32A, ○- - -○, 2 36A

The effect of incubation of salivary glands for 75 min in various concentrations of atractyloside supplied to medium saturated with oligomycin Puff diameters are recorded as relative values They represent the ratio of the measured width of the puff region and a non-puffed neighboring region (see [5]) Each value is an average of 5 measurements S E are indicated

trast, it was found that a significant reduction in the ATP level during a 30 min period of anaerobiosis failed to result in the appearance of the puffs During this period the ATP level was reduced from 11 23 to 0 79 nM/mg protein This level should be sufficient, however, to allow puff formation (see table 2) Moreover, the period of treatment is sufficiently long to permit the development of a puffed morphology at the loci studied [14] Table 1 further reveals that the temperature treatment, anaerobiosis and anaerobiosis followed by recovery in air, all result in a decrease in total nucleotide content and ~P

Both table 1 and table 2 indicate that the adenine nucleotide levels in the controls vary greatly This may be due to variations in the age of the larvae from which the glands were isolated In all instances, therefore, the changes in nucleotide contents should be assessed by a comparison of the results from the treated glands with those of the control glands, which in all instances are aliquots of the same batch of glands

The energy content of the system as expressed by ~P(2 × ATP + ADP)/mg protein varies to the same extent as the total quantity of adenine nucleotides Because of the variation between various preparations, the ratios of ~P/total adenine nucleotides were compared As can be seen in tables 1 and 2, this ratio (1 82–1 66) is very similar in different control experiments

In table 2, the results of treatments interfering with the respiratory metabolism are presented with regard to their effect on the adenine nucleotide levels and puff size. Incubation of the isolated glands in medium containing arsenite, which effectively inhibits the lipoic acid dehydrogenase [15], results in the appearance of small puffs at the loci 2–32A and 2–36A and large puffs at 2 48C and 4–81B The ATP and ADP levels are only slightly reduced as are the total nucleotide level and ~P

Incubation of the glands in medium containing the mitochondrial respiratory inhibitors rotenone and amytal, either in the presence of or without oligomycin (inhibitor of ATP formation) results in an almost complete depletion of the ATP pool and in the appearance of all four puffs when oligomycin is absent and of three of them when oligomycin is present in the medium

Oligomycin alone (saturated solution), though causing a significant reduction in the ATP and an increase in AMP level, does not cause the induction of the specific puffs In the presence of KCN, however, a saturated solution of oligomycin does induce the appearance of three of the puffs, even though the ATP level is reduced to almost the same level as after incubation with oligomycin alone KCN alone, which also reduces the ATP level, does not stimulate puff formation The observations confirm earlier results which revealed that incubation of the glands neither with oligomycin nor with KCN result in the induction of the puffs [5]

Incubation of the glands in medium saturated with oligomycin and containing

atractyloside (inhibitor of nucleotide transport across the mitochondrial membrane) resulted in significant reduction of the ATP level and in the appearance of three of the puffs A dose-response curve for the three puffs (fig 1) revealed that the response of region 36A was less than that of 32A and region 48C showed the strongest reaction Comparison of these results with those obtained with oligomycin in combination with KCN reveals that the response of the puffs 32A and 36A under the latter conditions is higher for 36A than for 32A [5] This comparison indicates that, although in both treatments the level of ATP is substantially reduced, the two loci 32A and 36A respond differently to the two treatments

DISCUSSION

It has been suggested that the characteristic response of particular genome loci in *Drosophila* to the addition of agents affecting the cellular respiratory metabolism is mediated by changes in the levels of adenine nucleotides In particular, a decrease in cellular ATP was proposed as a possible trigger for the activation of the particular genome loci [3, 5, 6]

The results presented in this paper do not favor the idea that changes in adenine nucleotide levels should be considered as primary triggers for the activation of the specific genes Anaerobiosis, which depletes the ATP pool, does not result in the induction of puffs, neither does treatment with oligomycin or KCN, both of which cause a significant reduction in the ATP content Conversely, an arsenite treatment which hardly changes the cellular ATP concentration does result in the activation of the genome loci studied

Examination of the ADP and AMP levels after the various treatments failed to reveal a correlation between quantitative changes in the levels of these nucleotides and the activation of the specific genome loci

It could be argued that a drop in cellular ATP will produce an activation of the respiratory metabolism and, thus, an increased demand for either—exogenous—substrates or certain enzymes However, the treatment with arsenite revealed that the specific puffs may become active even when neither the ATP level, nor the respiratory activity changes significantly (unpublished results) It seems possible that either the inhibition of the lipoic dehydrogenase by arsenite or a general activation of the respiratory metabolism may result in the release of one or more triggering substances, which induce the activation of the particular genome loci The release of these triggering substances may be performed by steps in the respiratory metabolism other than those which are directly affected by the treatments used For instance some mitochondrial entrance pathways, which perhaps function as monitors, may be responsible for the release of the triggering substances The resulting gene activities may produce information for the synthesis of respiratory components which although corresponding to the particular triggering substances are not, however, always able to release the inhibitions or activation caused by the treatments applied Consequently the triggering mechanism would then continue to operate and the puffs would continue synthesis of their products

The described control mechanism finds support from recent findings on the action of vitamin B_6 and the effect of cycloheximide on the activation and persistent activity of the puffs studied [16, 19]

A good example to illustrate this control mechanism might be provided by puff 4–81 B Whenever oligomycin is present in the in-

cubation medium, in addition to other substances which interfere with respiration, this puff will not become active, in spite of a drastic decrease in ATP level (table 2) *Following inhibition of terminal respiration* by rotenone and/or amytal, puff 4–81 B will become active This inhibition may cause an increase in the oxidation and thus a fall in the level of α-glycerophosphate (α-GP), leading to an increased demand for α-GP This decrease in level of α-GP may be abolished in the presence of oligomycin or antimycin A under which conditions puff 4–81 B is not induced (see also [5]) An indication that possibly the α-GP oxidase is the monitor in this feed back system is found in the definite change in stimulatory action of α-GP in respiration, if puff induction is performed. α-GP does stimulate respiration in 'non-puffed', but not in 'puffed' glands [17] The hypothesis that the activity of puff 4–81 **B** might be related to the activity of the enzyme NADH-dehydrogenase-enzyme activity increases following the activity of puff 4–81 **B** [19] fits the aforementioned observation, because an increased dehydrogenation of NADH will reduce the increased oxidation of α-GP

So far, the data available do not disagree with the idea that the induction of a particular group of puffs as a result of treatments interfering with respiration seem rather a consequence of an increased demand for either substrates or certain enzymes than of a decrease in cellular ATP

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A CORRELATION BETWEEN NEWLY INDUCED GENE ACTIVITY AND AN ENHANCEMENT OF MITOCHONDRIAL ENZYME ACTIVITY IN THE SALIVARY GLANDS OF *DROSOPHILA*

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SUMMARY

A comparison was made between some respiratory characteristics of mitochondria isolated from larval salivary glands of *Drosophila hydei* displaying chromosome puffs induced by anaerobiosis and mitochondria from non treated glands Mitochondria from anaerobically treated glands displayed a K_m of the respiration in the presence of isocitrate (2 4 mM) which is half that of the K_m found in control glands (5 6 mM) The V_{max} of respiratory activity in the presence of isocitrate is similar for mitochondria of treated and non treated glands The apparent V_{max} of the NADH dehydrogenase (E C 1 6 99 3) activity in mitochondria isolated from treated glands was 70 higher than in the control glands Neither the change in K_m of the respiratory activity in the presence of isocitrate nor the change in app V_{max} of the NADH dehydrogenase in the anaerobically treated glands was apparent when puff induction occurred in the presence of actinomycin D or cycloheximide in the incubation medium The present results indicate that the changes in the pattern of active genes (the occurrence of new puffs) may be related with a change in the respiration of isocitrate and a change in NADH dehydrogenase activity

A variety of treatments interfering with the cellular respiratory metabolism have as a consequence definite changes in genome activity at the nuclear level [1] As indicated from studies on isolated larval salivary glands of *Drosophila*, a particular group of genome loci becomes active whenever the cellular respiratory metabolism is submitted to a stress situation On account of these observations, it was suggested that the activated genome loci may provide information which, in some way or another, can stimulate the respiratory activity in order to meet the higher demands of the cellular metabolism under the artificial conditions applied [2 3]

As has been demonstrated, the appearance of a set of new puffs in the polytene chromosomes, following treatments affecting the respiratory metabolism, is correlated with an increase in activity of the enzyme NADH-dehydrogenase (E C 1 6 99 3) as established in homogenates of whole salivary glands [2] In addition, a significant difference in substrate-dependent stimulation was found when glands with the particular group of gene loci active were compared with glands in which these loci were inactive Glands in which the particular set of gene loci were active displayed a significantly higher utilization of isocitrate and tyrosine as substrates for cellular respiration [4] So far, however, it was uncertain as to whether the enhanced iso-

citrate and tyrosine respiration and the increase in NADH-dehydrogenase activity are consequences of enhanced intra-, rather than extra mitochondrial processes.

In order to distinguish between those possibilities, isocitrate respiration and NADH-dehydrogenase were measured using purified mitochondria isolated from salivary glands with and without the particular group of puffs activated.

Before this aspect of the mitochondrial metabolism was investigated, the respiratory activity of mitochondria isolated from the salivary glands in the presence of various substrates was characterized.

MATERIALS AND METHODS

All experiments were performed with salivary glands of mid-third instar larvae of *Drosophila hydei*. Isolation of the glands was done essentially as described by Boyd et al. [5]. Mitochondria were isolated from freshly prepared glands or from glands preincubated in a complex medium described by Poels [6]. The induction of the particular set of gene activities, revealed by the occurrence of chromosome puffs at previously inactive chromosome sites, was performed in vivo by submitting the larvae to a period of CO_2 anaerobiosis followed by a period in air. Subsequently, the salivary glands were isolated. Puff induction in isolated salivary glands was performed by N_2 anaerobiosis followed by a recovery in aerated medium. Both treatments result in the induction of the same group of genome loci [7].

Inhibition of nuclear RNA synthesis or cytoplasmic protein synthesis was performed by administration of 5 μg/ml actinomycin D (Merck, Sharp & Dohme) or 10 μg/ml cycloheximide (Serva) respectively, to the incubation medium. These concentrations inhibit RNA or protein synthesis in isolated salivary glands of *Drosophila* to a level of less than 5% of that of non-treated glands [6, 8].

Preparation of mitochondria

Mitochondria were prepared by homogenization of 200–400 mg salivary glands in 3 ml of a medium containing 0.154 M KCl, 1 mM EDTA and 1% bovine serum albumin (BSA, Calbiochem) pH 7.2 at 25°C [9, 10]. As will be indicated, BSA was omitted from the medium in some preparations. Following homogenization and twice repeated centrifugation at 750 g for 1 min to remove nuclei and mucopolysaccharide containing secretion droplets, the resulting supernatants were centrifuged at 6 000 g for 10 min. The pellet was homogenized gently with 0.5 ml isolation medium when respiratory activity was measured or with 3 ml 0.1 M Tris-HCl containing 1 M KCl (pH 7.2) when NADH-dehydrogenase was prepared.

Measurement of respiratory activity was carried out in a medium composed of 120 mM KCl, 10 mM K_2HPO_4, 10 mM Tris HCl, 5 mM $MgCl_2$, 1 mM EDTA and 5 mM ADP (pH 7.1) at 25°C in 5 ml Warburg flasks [11, 12]. When mitochondria isolated in the presence of BSA were used, BSA was included in the medium with a final concentration of 2%. ADP was omitted from the incubation medium when the dependence of respiration upon oxidative phosphorylation was tested. The following substrates were added at the concentrations as indicated in the text: succinate, isocitrate, proline, glutamate, glutamine, pyruvate, malate and α-glycerophosphate. In some experiments 2 mM NAD^+ was administered to the incubation medium. Oxygen consumption/h was computed by extrapolation of the data obtained by measuring oxygen consumption over a period of 30 min.

Measurements of P/O ratios in isolated mitochondria were performed after 30 min incubation followed by the addition of 0.1 ml 40% cold perchloric acid/ml mitochondria suspension and sonication of this mixture (3 × 10 sec, Branson sonifier, step 2). ATP, ADP and AMP concentrations were measured spectrophotometrically as described previously [16].

Preparation and assay of NADH-dehydrogenase was performed as described previously, but in the absence of Na-deoxycholate [2]. The protein content was measured according to Lowry et al. [13].

RESULTS

Respiratory properties of mitochondria from isolated salivary glands

As indicated by the results presented in table 1 the various substrates tested have different effects on respiratory activity in intact glands as compared with isolated mitochondria. From the first column it is evident that pyruvate and isocitrate do not stimulate respiration in intact glands, whereas all other substrates have a stimulatory effect.

Isolated mitochondria did not display endogenous respiratory activity. All substrates, except pyruvate, support respiration in isolated mitochondria. However, the respiratory activity attained varies for the different substrates. When the effect of the substrates upon respiration of isolated mitochondria is compared with their effect on respiration of intact glands certain differences become evi-

Table 1 *A comparison of the effect of various substrates on oxygen consumption of freshly prepared intact salivary glands and salivary gland mitochondria isolated without and in the presence of BSA*

Oxygen consumption is expressed in μl O_2/h/mg salivary glands[a]

			Salivary gland mitochondria prepared without BSA		Salivary gland mitochondria prepared in the presence of BSA		
Substrate	mM	Isolated glands	+5 mM ADP	−ADP	5 mM ADP	−ADP	P/O ratio in the presence of 0 5 mM ADP
No substrate (endogenous respiration)		0 16	0 00	—	0 00	0 00	
Succinate	50	—	0 64	0 35	0 79	0 08[c]	1 80
	10	0 34[b]	0 49	0 17	0 56	0 08[c]	
αGP	25	—	0 12	0 14	0 25	0 00	
	10	0 30	0 10	—	0 19	—	
Pyruvate	50	—	0 00	—	0 00	—	
	10	0 16[b]	0 00	—	—	—	
Pyruvate plus malate	10 10	—	0 34	0 00	0 52	0 00	
Isocitrate	50	—	0 57	0 02	0 76	0 00	3 54
	10	0 18[b]	0 46	—	0 54	—	
Malate	50	—	0 15	—	0 28	—	
	10	0 32[b]	0 07	—	0 21	—	
glu-NH_2	25	—	0 22	0 00	0 55	0 00	
	10	0 33[b]	0 12	—	0 47	—	
glu	50	—	0 39	—	0 53	—	
	10	0 22[b]	0 27	—	0 43	—	
pro	50	—	0 40	—	0 46	—	
	10	0 35[b]	0 31	—	0 42	—	

— No measurements performed
[a] 124 mg salivary glands is equivalent to 1 mg mitochondrial protein
[b] Inclusive endogenous respiration
[c] No oxygen consumption detectable during first 15 min of measurement

dent Whereas α-glycerophosphate and malate stimulate respiration in intact glands to a similar extent as succinate, glutamine and proline, both substrates support the respiration of isolated mitochondria to a significantly lower degree than succinate, glutamine and proline Isocitrate and glutamate, on the other hand, having a weak stimulatory effect on respiration of intact glands as compared with succinate, glutamine and proline, support respiration of isolated mitochondria to a similar degree as succinate, glutamine and proline

The presence of BSA in all media during the isolation and subsequent incubation of salivary gland mitochondria not only improves the support of respiration by all substrates, but also coupling of respiration and oxidative phosphorylation This is also indicated by the P/O ratios measured in these mitochondria preparations (table 1), as well as by the values for oxygen consumption in the presence and absence of ADP Whereas mitochondria isolated without BSA display respiration when incubated without ADP in the presence of succinate or α-glycerophosphate, mitochondria isolated in the presence of BSA fail to do so (in the presence of

Table 2 *Respiration of mitochondria isolated in the presence of BSA from salivary glands incubated for 3 25 h in Poels' medium*

The effect of ADP and NAD^+ was determined in the presence of various substrates Oxygen consumption is expressed in $\mu l\ O_2$ h/mg salivary glands

Substrate	mM	−5 mM ADP		ADP	P/O ratio in the presence of 5 mM ADP
		2 mM NAD^+	NAD^+	$-NAD^+$	2 mM NAD^+
No substrate (endogenous respiration)		0 00	0 00	—	
Succinate	50	0 07	0 34	0 00	1 52
αGP	25	0 00	0 12	—	
Pyruvate plus Malate	10, 10	0 09	0 03	0 00	
Isocitrate	50	0 42	0 32	0 00	2 33
glu-NH_2	25	0 17	0 12	0 00	
glu	50	0 25	0 21	—	
pro	50	0 30	0 25	—	

α-glycerophosphate), or have a significantly reduced oxygen consumption (in the presence of succinate)

Because the study of the relationship between the mitochondrial metabolism and nuclear gene activity has to be performed in many instances with salivary glands incubated for relatively long periods (up to 3¼ h) in vitro, a necessary requirement was the determination of the effect of these incubations upon the mitochondrial respiration As can be seen in table 2 the respiration of mitochondria isolated from salivary glands incubated for 3¼ h is significantly reduced as compared with the respiration of mitochondria isolated from freshly prepared glands (table 1, col 4 and 5) Addition of 2 mM NAD^+ to the mitochondrial incubation medium caused an increase in respiration of 20–40 % (compare col 1 and 2, table 2) in the presence of isocitrate, glutamine, glutamate and proline In the presence of succinate and α-glycerophosphate the presence of NAD^+ caused a strong reduction in oxygen consumption The latter effect may be a consequence of a partially reversed electron flow

As indicated by the P/O ratios of mitochondria isolated from incubated glands, the coupling between respiration and oxidative phosphorylation is lower than in mitochondria isolated from fresh glands

Isocitrate respiratory activity in mitochondria of NP and P glands

As has been demonstrated previously [4] aerobic recovery of isolated salivary glands from a 2 h anaerobiosis (N_2) is accompanied by an increase of 40 % in oxygen consumption as determined 1 h after the onset of the recovery period (see also table 3) During the recovery period a number of specific genes became active in the salivary gland nuclei (specific puffs in the polytene chromosomes) The increase in respiratory activity in the presence of isocitrate during the recovery period is abolished when cycloheximide (10 μg/ml) or actinomycin D (5 μg/ml) are added to the medium (table 3) Because the antibiotics used in these concentrations diminish de novo protein and RNA synthesis to less than 5 % of the level in control glands, it seems that the increase in isocitrate-supported respiration is dependent upon an intact RNA

Table 3 *Isocitrate-supported oxygen consumption in salivary glands and salivary gland mitochondria*

Measurements performed on preparations of salivary glands incubated in vitro (Poels medium) for 3.25 h

		Control	Recovering glands	Recovering glands in presence of actinomycin D	Recovering glands in presence of cycloheximide	Recovering glands KCN (1 mM)
Whole glands[a]	10 mM isocitrate	0.28	0.40	0.30	0.28	0.00
Mitochondria[b]	50 mM isocitrate NAD	18.7	19.9	17.2	19.7	0.0

[a] Oxygen consumption expressed in µl O/h/mg salivary glands
[b] Oxygen consumption expressed in µl O/h/mg mitochondrial protein

and cytoplasmic protein synthesizing system. The increase in isocitrate-supported respiration, however, should be primarily based upon the intramitochondrial metabolism because (as can be seen in table 3) 1 mM KCN does suppress this respiration completely.

In order to seek further support for the suggestion that the isocitrate-supported respiration is of intra-mitochondrial origin, the oxygen consumption of mitochondria isolated from glands recovering from anaerobiosis was compared with that of mitochondria of control glands incubated for the same period of time. This comparison, carried out in the presence of 50 mM isocitrate, did not reveal a clear-cut difference in oxygen consumption between the two mitochondrial preparations. The respiration of mitochondria isolated from glands recovered from anaerobiosis in the presence of the antibiotics actinomycin D or cycloheximide was similar to that of the control glands (table 3).

It was expected that the comparison performed would have revealed an increased activity in mitochondria of recovering glands as well as an inhibition of this effect by the antibiotics. The similar values for oxygen consumption in mitochondria of control glands and glands recovering from anaerobiosis could be explained, however, if at the isocitrate concentration used (50 mM) the V_{max} of the isocitrate respiration is reached and that the V_{max} is not significantly different in the two conditions compared. In order to test this suggestion the V_{max} and K_m of the respiration were established (fig. 1). As can be seen from fig. 1, mitochondria of control glands have a lower substrate (isocitrate) affinity (K_m 5.6 mM) than those of glands recovering from anaerobiosis (K_m 2.4 mM), whereas the V_{max} is essentially identical.

The increase in substrate affinity in mitochondria of glands recovering from anaerobiosis is not observed when the glands were incubated in medium containing actinomycin D or cycloheximide during the period of recovery (fig. 1).

NADH-dehydrogenase activity

Whereas in the case of isocitrate-supported respiration the V_{max} did not differ in mitochondria of control glands and those of glands recovering from anaerobiosis, it has been established previously that the app. V_{max} of the NADH-dehydrogenase is increased by 30–40% in homogenates of whole glands recovering from anaerobiosis as compared with homogenates of control glands

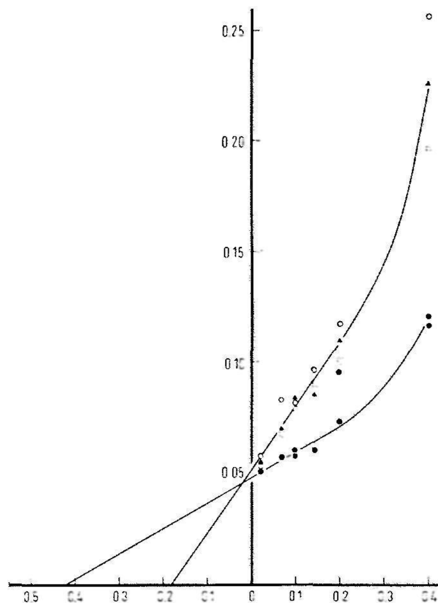

Fig. 1. Abscissa: (mM isocitrate)$^{-1}$; *ordinate:* (μl O_2/h mg protein)$^{-1}$.
Lineweaver-Burk plot of oxygen consumption by mitochondria isolated from (● ●) puffed (P) and (□—) non-puffed (NP) salivary glands in relation to different isocitrate concentrations in the presence of NAD^+. Addition of (○—○) cycloheximide and (▲—▲) actinomycin D as described under Materials and Methods.

So far, however, it was not determined to what extent this increase in the app. V_{max} of NADH-dehydrogenase in the homogenates was of mitochondrial origin.

A determination of the app. V_{max} of NADH-dehydrogenase in isolated mitochondria of control glands and glands recovering from anaerobiosis also revealed a clear-cut increase of the app. V_{max} in the latter. In this case the increase was approx. 70% (fig. 2*a*, *b*). The change in app. V_{max} is inhibited when the glands are placed in an actinomycin D or cycloheximide-containing medium during the recovery period (fig. 2*b*). Because a comparison of the mitochondria isolated without and in the presence of BSA did not reveal significant differences in the app. V_{max} of NADH-dehydrogenase and only a very little difference in app. K_m (compare fig. 2*a* and *b*), it seems that the presence of BSA in the isolation medium is not essential with respect to the activity of the NADH-dehydrogenase.

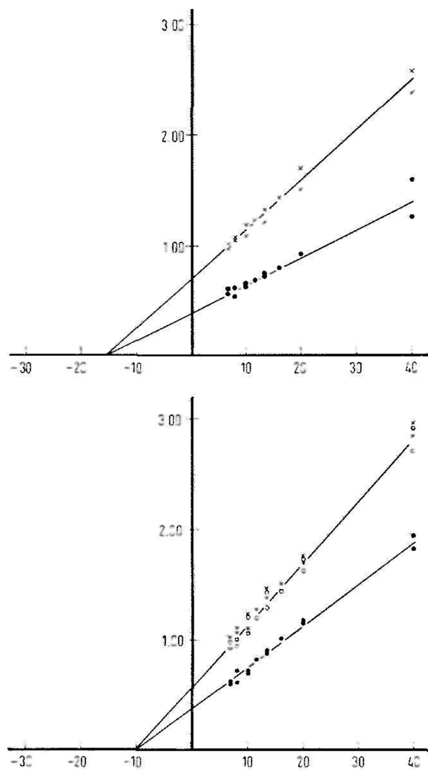

Fig. 2. Abscissa: (mM NADH)$^{-1}$; *ordinate:* (μM NADH/min mg protein)$^{-1}$.
Lineweaver-Burk plots of the NADH dehydrogenase activity isolated from (●—●) puffed (P) and (×—×) non-puffed (NP) salivary gland mitochondria in relation to different concentrations NADH. (*a*) Plot of NADH dehydrogenase activity from BSA-isolated mitochondria from fresh glands; (*b*) plot of NADH dehydrogenase activity from mitochondria isolated without BSA from incubated glands. Addition of (○—○) cycloheximide and (△—△) actinomycin D as described under Materials and Methods.

DISCUSSION

The characterization of mitochondrial respiration of salivary gland mitochondria prepared from freshly isolated glands and from glands incubated in Poels' medium for a period of 3½ h revealed that in both cases the relative support of respiration by various substrates is similar Although mitochondria from incubated glands have a lower oxygen consumption and lower P/O ratios than those isolated from freshly prepared glands, both preparations display essentially the same respiration characteristics The results of these comparisons of mitochondrial oxygen consumption in the presence of various exogenous substrates are also similar to the results obtained with mitochondria from other insect sources [10, 11, 14]

Because mitochondria prepared from glands which were incubated for 3½ h in vitro were, in their respiration characteristics, similar to mitochondria prepared from freshly isolated glands, it seems reasonable to assume that the enzymatic pattern remains essentially intact during the long term in vitro incubation of the salivary glands

From this point of view, it was worthwhile to investigate variations in isocitrate support of respiratory activity under different metabolic circumstances, comparing the isocitrate supported oxygen consumption in mitochondria from glands recovering from an anaerobic treatment in vitro with that of mitochondria from control glands kept in vitro for the same period of time A comparison of V_{max} and K_m of isocitrate respiration indicated that in mitochondria from recovering glands the V_{max} is the same, whereas the K_m is lower than in control glands

It may be pointed out that in glands recovering from an anaerobic treatment certain 'genes' are activated which are in the control glands inactive or active at a very low level [1, 4] These genes become active in all instances in which the respiratory metabolism is experimentally disturbed [1]

Because inhibition of nuclear RNA synthesis and cytoplasmic protein synthesis do exert an effect at the level of mitochondrial isocitrate respiration, it could be suggested that the regulation of this part of the respiratory metabolism is controlled by the nuclear genome

So far, however, it is unclear how the isocitrate respiration is regulated via specific gene action It appears that the V_{max} is the same in mitochondria of recovering glands and control glands irrespective of the presence or absence of the antibiotics in the incubation medium during the recovery period The K_m, on the other hand, is lower in mitochondria of recovering glands than in those of control glands and this decrease in K_m is prevented when the antibiotics are present in the medium It appears that the change in K_m is dependent upon nuclear RNA and, probably subsequent, cytoplasmic protein synthesis

It could be suggested that the protein synthesis dependent change in K_m reflects a change in the relative quantities of different subunits of the allosteric enzyme isocitrate dehydrogenase in such a way that the production of α-ketoglutarate is enhanced A change as such could require de novo synthesis of an isocitrate dehydrogenase isozyme with a lower K_m The suggestion that the decrease in K_m may result in an increased availability of α-ketoglutarate could not only be a clue for the regulation of the increase in substrate respiration as shown in fig 1, but also for transamination and substrate transport [15] These increased activities would all support the restoration of the mitochondrial metabolism which became partly deficient as a consequence of the anaerobic treatment The restoration of the mitochon-

drial metabolism after such a treatment seems to be, at least partly, dependent upon the activity of certain nuclear genes

This idea is also supported by the finding that the mitochondrial NADH-dehydrogenase displays a protein synthesis dependent increase in app V_{max} during the recovery from anaerobiosis

As has been shown previously [1, 16], the activity of one particular 'gene' (chromosome puff) is correlated with the increase in app. V_{max} of NADH-dehydrogenase in whole gland homogenates

The present data indicate that this increase is mainly due to an increase in the intramitochondrial enzyme, suggesting a regulatory system for the activity of this enzyme based upon previous action at the genome level (RNA synthesis) and at the cytoplasmic level (protein synthesis)

The nature of this regulatory mechanism remains to be elucidated

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Induced Transcription-Dependent Synthesis of Mitochondrial Reduced Nicotinamide–Adenine Dinucleotide Dehydrogenase in *Drosophila*

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Salivary glands of *Drosophila hydei* recovering from an anaerobic treatment show a significant increase in apparent V_{max} of mitochondrial NADH dehydrogenase This increase in V_{max} is based on an increase in enzyme molecules resulting from synthesis *de novo* in the cytoplasm, as indicated by the inhibition by cycloheximide of both the increase in apparent V_{max} and the increase in amino acid incorporation into enzyme fractions The increase in enzyme activity is also inhibited by actinomycin D, which is in support of previous data indicating a causal relationship between transcription in puff 4-81B in the polytene chromosomes and an increase in apparent V_{max} of the enzyme Gel electrophoresis of mitochondrial protein extracts revealed three protein fractions with NADH dehydrogenase activity All three fractions showed increased activity as well as increased amino acid labelling in glands recovering from anaerobiosis compared with control glands The data suggest that the increase in mitochondrial NADH dehydrogenase activity in salivary glands recovering from an anaerobic treatment depends on increased gene transcription

A variety of treatments which interfere with the cellular respiratory metabolism results in the activation of a particular set of genes In the polytene chromosomes this gene response is manifested by the occurrence of new chromosome puffs at loci which were inactive before the treatment (Ritossa, 1962, Berendes *et al*, 1965, Ashburner, 1970, Leenders & Berendes, 1972)

In *Drosophila hydei*, the genome response after the release from anaerobiosis or incubation of salivary glands in media containing inhibitors of the terminal respiratory chain consists of the formation of four major puffs (Leenders & Berendes, 1972)

It has been previously shown that the appearance of these new puffs is followed by increases in apparent V_{max} of both mitochondrial NADH dehydrogenase (EC 1 6 99 3) and tyrosine aminotransferase (EC 2 6 1 5) (Leenders *et al*, 1974, Koninkx *et al*, 1975) No change in the apparent K_m of these enzymes was observed Moreover, it was established that the increase in apparent V_{max} of the mitochondrial enzymes was dependent on synthesis *de novo* of RNA and cytoplasmic protein (Leenders & Beckers, 1972; Leenders *et al*, 1974, Koninkx *et al*, 1975)

The increase in NADH dehydrogenase activity could be correlated with the increase in activity of puff 4-81B, since treatment with antimycin A, which induces only three of the four puffs, did not lead to elevated NADH dehydrogenase activity If the activity of locus 4-81B does indeed result in an increase in mRNA coding for NADH dehydrogenase this increase should be reflected in the synthesis of the enzyme *de novo*.

The aim of the present study was to demonstrate that synthesis *de novo* of this mitochondrial enzyme does indeed increase after puff induction

Materials and Methods

Materials

Bovine serum albumin and actinomycin D were obtained from Calbiochem, Los Angeles, Calif, U S A , 3-(4,5-dimethylthiazol-2 yl)-2,5-tetrazolium bromide was obtained from Sigma Chemical Co., St Louis, Mo , U S A , cycloheximide was from Serva, Heidelberg, Germany, β-NADH (disodium salt) and chloramphenicol were from Boehringer G m b H , Mannheim, Germany, acrylamide and bisacrylamide were from BDH Chemicals, Poole, Dorset, U K , [^{35}S]methionine (sp radioactivity 325Ci/mmol) was from The Radiochemical Centre, Amersham, Bucks , U K , and mixtures of ^{3}H-labelled amino acids (lot no 787-172) and ^{14}C-labelled amino acids (lot no 828-021) were from New England Nuclear Corp , Boston, Mass , U S A All other chemicals were of analytical grade from E Merck A G , Darmstadt, Germany

In all experiments salivary glands of mid-third-instar larvae of *Drosophila hydei*, raised under standardized conditions in mass culture (Mitchell & Mitchell, 1964), were used Salivary glands were isolated as described by Boyd *et al* (1968)

Puff induction

Puffs at the chromosome loci 2-32A, 2-36A, 2-48BC and 4-81B were induced by incubating salivary

glands for 120min in Poels's (1972) medium flushed with N_2 under an N_2 atmosphere. The glands were allowed to recover for 75min by exposure to air. Glands used as controls were kept in the same medium (well aerated) for 195min. Before the glands were used for isolation of the mitochondria the chromosomal puffing pattern was analysed to ascertain that anaerobically treated glands had developed the specific puffs and that they were absent from the controls.

Preparation of mitochondria

After incubation, 150–250mg of glands was washed three times with 5ml of Mg Ringer medium (see Leenders & Berendes, 1972) and homogenized in 3.5ml of a solution containing 0.154M-KCl, 1mM-EDTA and 1% (w/v) bovine serum albumin, pH 7.2. The homogenate was centrifuged for 10min at 1000g, the pellet was resuspended in 1.5ml of the same solution and re-centrifuged for 10min at 1000g. The supernatants were combined and centrifuged for 10min at 6000g. After resuspension of the resulting mitochondrial pellet in 5ml of 0.154M KCl 1mM-EDTA, pH 7.2, and re-centrifugation for 10min at 6000g, the pellet was suspended in 0.35ml of the same solution and sonicated with a Branson sonifier (step 2, 3×10s). The sonicate was used for protein determination (Lowry *et al.* 1951, bovine serum albumin as standard) and for gel electrophoresis.

Gel electrophoresis and enzyme activity assay

Gel electrophoresis was performed as described by Davis (1964) and Ornstein (1964) on 10% (w/v) polyacrylamide gels for 3h. Bromophenol Blue was used as a marker. The current applied per gel was 2mA. The inner diameter of the Pyrex glass tube was 5mm, the outer diameter was 7mm, the length of the tube was 115mm. Three different buffers were used. The small pore gel buffer was composed of 48ml of 1M-HCl, 36.6g of Tris and water added to 100ml, pH 8.9. The large-pore gel buffer was composed of 48ml of 1M-HCl, 5.98g of Tris and water added to 100ml, pH 6.7. The reservoir buffer was composed of 3.0g of Tris, 14.4g of glycine and water added to 1 litre, pH 8.3. The protein quantity applied varied for different experiments between 50 and 150μg/gel. In all instances in which mitochondrial proteins from treated and non-treated glands were compared, equal protein quantities were applied to the gels.

NADH dehydrogenase activity was determined after gel electrophoresis of the mitochondrial protein preparations by incubation of the gels overnight at 0°C in 0.1M-Tris HCl (pH 7.2) containing 0.5mM-NADH and 0.5mM-oxidized 3-(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium bromide. NADH dehydrogenase activity was detected on the basis of the formation of formazan [reduced 3-(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium bromide] which has a blue purple colour with a peak absorbance at 560nm (Sin & Leenders, 1975). The quantity of reduced 3-(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium bromide formed was measured densitometrically at 560nm with a Zeiss PMQ II spectrophotometer, equipped with an integrating recorder. Control experiments revealed a linear relationship between the densitometrically established peak area of staining bands and the quantity of mitochondrial protein in the range from 20 to 150μg gel.

After incubation of the gels in buffer with neither NADH nor oxidized 3-(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium bromide, no stained bands were observed.

Incorporation of [^{35}S]methionine and ^{3}H- and ^{14}C-labelled amino acids

The incorporation of [^{35}S]methionine in salivary glands or in mitochondria of these glands was determined after incubation in 200μl of Poels's (1972) medium devoid of methionine sources other than the [^{35}S]methionine. After incubation, the glands were washed six times for 5min each with 5ml of ice-cold Mg–Ringer (pH 7.2) containing 10mM-methionine (unlabelled).

To establish the time of maximal protein synthesis in glands recovering from an anaerobic treatment, protein was extracted by the procedure described by Tissières *et al.* (1974) and the radioactivity per μg of protein determined by liquid-scintillation counting in 10ml of scintillant containing 20ml of methoxyethanol (E. Merck A.G.), 5.5g of Permablend III (Packard Instrument Co., Downers Grove, Ill., U.S.A.) and 30ml of Soluene-100 (Packard Instrument Co.) per litre of toluene. Liquid-scintillation counting was performed with a Philips liquid-scintillation analyser PW 4510/01 which was equipped with a computer program for calculation of the number of d.p.m. from the number of c.p.m. and the external-standard ratio.

A comparison of the radioactivity incorporated by 30mg of glands incubated in 100μl of medium containing 10μCi of [^{35}S]methionine during 20min incubation periods beginning immediately after the release from anaerobiosis or 15min, 30min, 45min or 60min after the release from anaerobiosis revealed a higher rate of incorporation of radioactivity per μg of protein in the treated glands than in the control glands if the incorporation period begins at 45 or 60min after the release from anaerobiosis (Table 1). If the incorporation period of 20min begins at an earlier time, the incorporation per μg of protein in the

Table 1 *Rate of [^{35}S]methionine incorporation into total salivary-gland protein after various periods of recovery from anaerobiosis*

The treated salivary glands were recovering from a 2 h (N_2) anaerobic treatment in a well-aerated medium at 25 C The control salivary glands were kept in a well aerated medium for the same period of time at 25°C The salivary glands were pulse-labelled for 20 min

Time after release from anaerobiosis (min)	Radioactivity incorporated (d p m /μg of protein) Control glands	Treated glands	Treated glands/control glands
0	5610	1611	0 29
15	5839	4305	0 74
30	5902	6012	1 02
45	6021	7230	1 20
60	5727	7322	1 28

treated glands is lower than in control glands (Table 1) On account of these data comparisons between pulse labelled proteins of treated and non-treated glands or mitochondria prepared from them are always based on experiments in which the glands were pulse labelled for a 30 min period beginning at 45 min after the release from anaerobiosis

The patterns of newly synthesized pulse-labelled proteins were studied after electrophoresis on 10% (w v) polyacrylamide gels which were either cut into 1 mm slices and counted for radioactivity or radioautographed with Kodak medical X ray films, RP/R14 (exposure time 5 days)

Double-labelling experiments were performed in which anaerobically treated glands were incubated for a 30 min period beginning 45 min after the release from anaerobiosis, in 75 μl of medium containing 40 μCi of a mixture of ^{3}H-labelled amino acids and non treated glands were incubated in 75 μl of medium containing 15 μCi of a mixture of ^{14}C-labelled amino acids Equal amounts of the protein extracts from treated and non treated glands were mixed and submitted to electrophoresis The gels were cut into 1 mm slices and the ^{3}H and ^{14}C labelling was determined by liquid scintillation counting

Inhibition experiments

To test whether or not the changes in the pattern of newly synthesized proteins after the release of salivary glands from anaerobiosis is dependent on synthesis *de novo* of RNA and protein inhibition experiments were performed RNA synthesis was inhibited during the recovery period by 20 μg of actinomycin D/ml, cytoplasmic protein synthesis by 5 μg of cycloheximide ml and mitochondrial protein synthesis by 100 μg of chloramphenicol, ml

Results

Electrophoretic separation of proteins with NADH dehydrogenase activity

After electrophoresis of sonicates of mitochondria and incubation of the gels with NADH and oxidized 3-(4,5-dimethylthiazol-2 yl) 2,5-tetrazolium bromide overnight, three blue-purple bands [reduced 3-(4,5-dimethylthiazol-2 yl)-2,5-tetrazolium bromide] were detected These bands, designated A, B and C, with relative mobilities of 0 157 0 177 and 0 201 (Table 2) differed in E_{560} as measured by densitometry (Fig 1) Measurements of the peak areas of densitograms of the three bands obtained from mitochondria of control glands revealed the proportion of A B C as 52 3 33 8 13 9

Electrophoresis of sonicates of mitochondria isolated from glands 75 min after the onset of recovery from an anaerobic treatment also revealed three bands The R_F values of these bands as well as the relative absorbances were identical with those of the control glands (Table 2 Fig 1) These findings demonstrate that the number and electrophoretic behaviour of protein fractions with NADH dehydrogenase activity are the same in mitochondria from control as in those from anaerobically treated glands

However, the absolute values of the measured peak areas of all bands differed significantly when each band in the mitochondrial protein extract of treated glands was compared with the corresponding band of non treated (control) glands (Table 2) In all protein fractions with NADH dehydrogenase activity, the activity was higher in mitochondria obtained from anaerobically treated glands than in control glands This result agrees with previous data indicating an increase in the apparent V_{max} of the enzyme during the recovery of glands from anaerobiosis (Koninkx *et al*, 1975)

Table 2. *Relative electrophoretic mobilities, relative absorbance values and increase in peak areas of mitochondrial protein fractions with NADH dehydrogenase activity*

The relative mobility of a protein fraction is defined as the ratio between the migration distance in polyacrylamide gels of the protein fraction and of the tracking dye Bromophenol Blue. The relative absorbance value is defined as the ratio between the peak areas of the individual fractions in densitograms made at 560nm and the total peak area of all fractions with NADH dehydrogenase activity in the same densitograms. The increase in peak areas of each mitochondrial protein fraction with NADH dehydrogenase activity from treated glands is expressed as percentage increase over the corresponding fractions in the controls. Each value is a mean of five experiments (±S.D.).

	Relative mobilities		Relative absorbance values		
	Untreated	Treated	Untreated	Treated	Increase in peak area
Band A	0.157±0.002	0.156±0.004	52.3±3.2	50.5±8.3	38.8±1.4
Band B	0.177±0.003	0.177±0.004	33.8±1.3	34.2±3.7	17.3±0.7
Band C	0.201±0.002	0.200±0.003	13.9±2.4	15.3±5.0	9.7±0.6

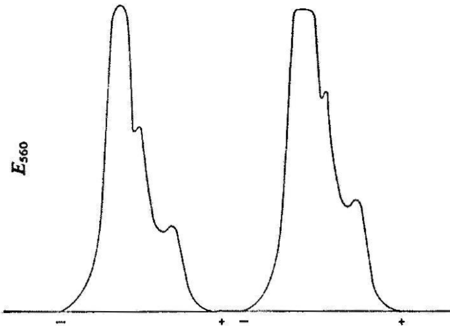

Fig. 1. *Densitometer tracings of polyacrylamide gels showing the protein fractions with NADH dehydrogenase activity*

The gels were measured at 560nm which is the peak absorbance of reduced 3-(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium bromide. The densitometer tracing at the left is from gels of controls, the tracing at the right from gels of mitochondrial proteins from recovering glands.

To establish whether or not this increase in enzyme activity is based on an increase in enzyme molecules, both the rate of protein synthesis and the pattern of incorporation of labelled precursors in mitochondrial protein were determined.

Electrophoresis of [^{35}S]methionine-labelled mitochondrial proteins

The rate of protein synthesis is very low in glands which are just released from anaerobiosis (see Table 1 in the Materials and Methods section). At 50min after the onset of the recovery period the rate of protein synthesis is higher than in the control glands. The chromosome puffs attained their maximum size at

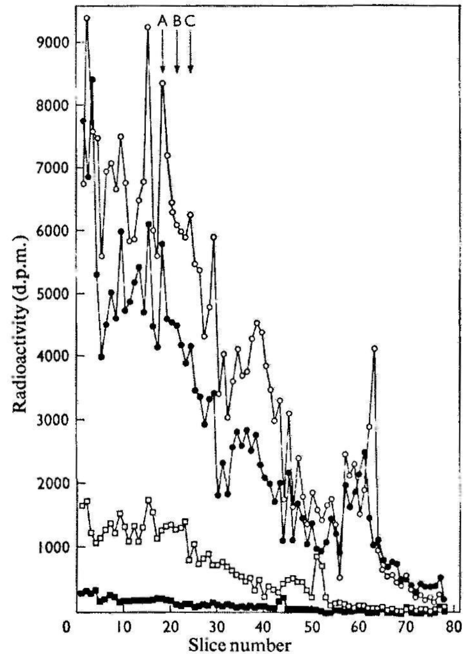

Fig. 2. *Radioactivity profiles of [^{35}S]methionine-pulse-labelled mitochondrial proteins isolated from salivary glands*

●, Non-treated glands; ○, glands after 75min recovery from anaerobiosis (identical with that of glands recovering in the presence of 100μg of chloramphenicol/ml); ■, glands after 75min recovery from anaerobiosis in the presence of 5μg of cycloheximide/ml; □, glands after 75min recovery from anaerobiosis in the presence of 20μg of actinomycin D/ml. The [^{35}S]methionine pulse was given from 45min until 75min after the onset of recovery. The inhibitors were present during the entire recovery period. Arrows indicate the position of protein fractions (A–C) with NADH dehydrogenase activity.

20min, whereas the greatest rate of increase in the apparent V_{max} of the NADH dehydrogenase occurs between 30 and 75 min after the onset of the recovery period (Leenders & Beckers, 1972)

Fig 2 shows the electrophoretic analysis of labelled mitochondrial proteins obtained after 30 min labelling with [^{35}S]methionine beginning at 45 min after the onset of recovery from anaerobiosis This analysis demonstrated that, among others, the protein fractions with NADH dehydrogenase activity displayed a significantly higher incorporation in treated than in non-treated glands (arrows) As expected, no incorporation was seen in mitochondrial proteins of glands recovering from anaerobiosis in the presence of cycloheximide Little incorporation was found when the glands recovered in medium with actinomycin D (Fig 2) Chloramphenicol had no effect on the labelling of the protein fractions These data suggest that the increase in label also depends on nuclear RNA synthesis *de novo*

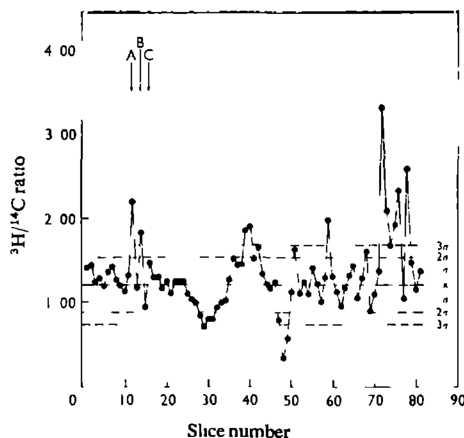

Fig 3 $^3H/^{14}C$ ratios for individual fractions of gels in which a mixture of equal quantities of ^3H-labelled mitochondrial proteins from recovering glands and ^{14}C-labelled mitochondrial proteins from control glands were electrophoretically separated

$\bar{x}$ is the mean of all ^{3}H/^{14}C ratios σ is the standard deviation of these ^{3}H/^{14}C ratios which are indicated in the figure Arrows show the positions of the fractions (A–C) with NADH dehydrogenase activity

Double-labelling experiments

To exclude the possibility that the higher [^{35}S]-methionine incorporation in mitochondria of anaerobically treated glands merely reflects changes in amino acid pools, equal quantities of ^{14}C-labelled mitochondrial proteins from control glands and ^{3}H-labelled proteins from treated glands were mixed and run on polyacrylamide gels After electrophoresis the ^{3}H ^{14}C ratio of each 1 mm slice was determined The ratios found for slices containing proteins with NADH dehydrogenase activity were significantly higher in mitochondrial extracts from treated than from non-treated glands (Fig 3)

Inhibition experiments

Measurements of enzyme activity in gels of mitochondrial proteins extracted from glands recovering from anaerobiosis in the presence of cycloheximide revealed that the peak area of each of the three bands with enzyme activity is approximately the same as in extracts of control glands This finding indicates that the increase in NADH dehydrogenase activity found after release from anaerobiosis is due to a net increase in enzyme molecules in the mitochondria Since the activity of a mitochondrial enzyme synthesized in the cytoplasm can be increased in three different ways, i e decreased enzyme turnover in the mitochondria, increased uptake or increased synthesis *de novo* accompanied by increased uptake, the effect of cycloheximide on the apparent V_{max} of the enzyme was tested An increase in enzyme activity owing to either a decreased turnover or an increase in uptake without an increase in synthesis *de novo* would be insensitive to cycloheximide inhibition of protein

Table 3 *Apparent V_{max} of the mitochondrial NADH dehydrogenase*

The apparent V_{max} was measured in sonicates of mitochondria isolated from salivary glands (see the Materials and Methods section) after incubation of the control salivary glands in the absence (–) or presence (+) of cycloheximide (5 μg/ml) and of salivary glands in the absence (–) or presence (+) of cycloheximide during the 75 min recovery from anaerobiosis The enzyme assay and the calculation of the apparent V_{max} were done as described by Leenders & Beckers (1972)

	Apparent V_{max} (μmol of NADH/min per mg of protein)
Control glands – cycloheximide	4 12
Control glands + cycloheximide	3 84
Glands recovering from anaerobiosis for 75 min – cycloheximide	7 61
Glands recovering from anaerobiosis for 75 min + cycloheximide	4 24

synthesis Table 3 shows that the apparent V_{max} of mitochondrial NADH dehydrogenase in control glands incubated in medium without and with cycloheximide is slightly decreased when cycloheximide is present in the medium The apparent V_{max} of the enzyme in mitochondria of glands recovering in medium without cycloheximide is significantly higher than in the controls (see Koninkx *et al*, 1975), whereas this increase is almost completely absent from glands recovering in the presence of the protein-synthesis inhibitor Thus the increase in apparent V_{max} of the enzyme during recovery from anaerobiosis depends on protein synthesis *de novo*

Discussion

The present results demonstrate that the previously established increase in apparent V_{max} of the mitochondrial NADH dehydrogenase after experimental gene activation (Koninkx *et al*, 1975) is correlated with an increased enzyme activity of three mitochondrial protein fractions as well as with an increased labelling of these proteins The results of the inhibition experiments further suggest that the increase in apparent V_{max} depends on synthesis *de novo* of nuclear RNA and cytoplasmic protein rather than on changes in turnover rate or uptake of enzyme molecules without increased synthesis *de novo* The apparent lack of labelled protein fractions in mitochondria of glands recovering in the presence of cycloheximide indicates that the synthesis of cycloheximide-insensitive mitochondrial proteins is very low or completely absent As such, this finding is in accord with a previous suggestion that the increase in mitochondrial activity after recovery from anaerobiosis is not due to mitochondrial biogenesis

The present data show a definite relationship between the induced activity of nuclear genes and increase in the activity of a mitochondrial enzyme which is known to occupy a key position in the terminal respiration (Hemmerich *et al*, 1970)

It is tempting to suggest that one of the induced puffs, in particular puff 4-81B, is responsible for the production of mRNA molecules coding for the enzyme on the basis of the fact that this puff is not activated when the glands are treated with antimycin A and that under those conditions no increase in mitochondrial NADH dehydrogenase activity occurs

An analysis of the pattern of newly synthesized cytoplasmic proteins in glands recovering from anaerobiosis revealed six strongly labelled polypeptide fractions in *Drosophila hydei* glands (Lewis *et al*, 1975) Preliminary experiments indicated that the labelled mitochondrial protein fractions with NADH dehydrogenase activity separated by polyacrylamide-gel electrophoresis migrate in sodium dodecyl sulphate–polyacrylamide gels in the range of proteins with mol wts 67000 75000 In this region two or three heavily pulse labelled protein fractions occur in cytoplasmic protein extracts of glands recovering from anaerobiosis Since the NADH dehydrogenase isolated from rat heart mitochondria has approx mol wt 300000 (King *et al*, 1966), the mitochondrial protein migrating in the range 67000–75000 mol wt may be a subunit of the enzyme

The relationship between the transcriptional activity of one chromosome locus (4 81B) and the increased synthesis of proteins with NADH dehydrogenase activity which are incorporated in the mitochondria supports Beermann's hypothesis (1952) that puffs are sites of mRNA synthesis

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Protein Synthesis in Salivary Glands of *Drosophila hydei* after Experimental Gene Induction

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Several treatments, namely incubation at 37°C, in the presence of arsenite, 2,4-dinitrophenol or vitamin B-6, or release from anaerobiosis induce the same set of puffs in the polythene chromosomes of salivary glands of *Drosophila hydei* Analysis of changes in protein-synthetic patterns (as determined by radioautography of sodium dodecyl sulphate-gel electrophoretograms of extracts from [^{35}S]methionine-labelled salivary glands) showed that concomitant with puff induction by these various treatments the same six strongly labelled polypeptide bands appeared The amount of radioactive label in these peptides accounted for 25% of the total incorporation of [^{35}S]methionine, except during incubation at 37°C when it accounted for about 50% The rate of synthesis of these peptides was maximal 1 h after the start of the puff-inducing treatment The rate of decay of the rate of synthesis showed first-order kinetics both after removal of the puff-inducing stimulus or in the presence of actinomycin, with a half-life of approx 4h

Interference with the respiratory metabolism of salivary glands of *Drosophila hydei* (or *Drosophila melanogaster*) results in the appearance of a specific set of puffs, the 'heat shock' puffs (Ritossa, 1962, 1964, Berendes *et al*, 1965, Ashburner, 1970, Leenders & Berendes, 1972, Leenders *et al* 1973, 1974*a*, *b*) About 10 to 20min after these puffs have reached their maximum size in *D hydei*, the activities of several mitochondrial enzymes start to increase (Leenders & Beckers, 1972; Leenders *et al*, 1974 *a*, *b*, Koninkx *et al*, 1975, Koninkx, 1975, Sin & Leenders, 1975) These increases are dependent on transcription and translation *de novo* Moreover, the increase in activity of mitochondrial NADH dehydrogenase (EC 1 6 99 3) could be correlated with the presence of a puff at locus 4-81B, whereas the increase in mitochondrial tyrosine transaminase activity (EC 2 6 1 5) appeared to be related to the presence of a puff at locus 2-48BC (Leenders & Beckers, 1972, Leenders *et al*, 1973) It was therefore suggested that the RNA product of these puffs might code for at least part of these enzymes

On the other hand, Tissieres *et al* (1974) have shown that puff induction by a temperature shock in *D melanogaster* is followed by a change in the pattern of protein synthesis six new strongly labelled bands appear These findings were extended by Lewis *et al* (1975) who found that a similar set of bands could be induced by a temperature shock in salivary glands of *D hydei* or *D simulans* If the appearance of these bands is indeed causally related to the presence of the 'heat-shock' puffs, as has been suggested, then all treatments which induce these puffs should also lead to the appearance of the 'heat-shock' bands However, Lewis *et al* (1975) found that puff induction by release from anaerobiosis induced the 'heat-shock' bands only in salivary glands from *D melanogaster*, but not in those from *D hydei*, where a partial induction was found, whereas after treatment of *D melanogaster* glands with 2,4-dinitrophenol, which also induces the 'heat-shock' puffs, only one of the 'heat-shock' bands could be detected

I have therefore re-investigated the induction of the 'heat-shock' bands under various conditions Further, preliminary to a study of a possible correlation between the induction of one or more of the 'heat-shock' bands and the observed increases in enzyme activity, an effort has been made to study the kinetics of induction and de-induction of these bands quantitatively

Experimental

Materials

NNN N-Tetramethylethylenediamine, riboflavin, acrylamide, bisacrylamide and sodium arsenite were from BDH Chemicals, Poole, Dorset, U K, sodium dodecyl sulphate and cycloheximide were from Serva, Heidelberg, Germany, 2,4-dinitrophenol and oligomycin were from Sigma Chemical Co, St Louis, MO, U S A, vitamin B-6 was from Eastman Kodak Co, Rochester NY, U S A, actinomycin D was from Calbiochem, Los Angeles, CA, U S A, and chloramphenicol from Boehringer G m b H, Mannheim, Germany, Permablend III and Soluene-100 were from Packard Instrument Co, Downers Grove, IL,

U S A ; [^{35}S]methionine (specific radioactivity 330Ci/mmol) and [^{3}H]uridine (specific radioactivity 53 5Ci/mmol) were from The Radiochemical Centre, Amersham, Bucks , U K All other chemicals were of analytical grade from E Merck A G , Darmstadt, Germany

Incubation and labelling of salivary glands with [^{35}S]methionine

Salivary glands were hand-isolated from late-third-instar larvae of *Drosophila hydei*, reared as a mass culture under standard conditions (Mitchell & Mitchell, 1964) Five pairs of glands, freed of the adhering fat-body as much as possible, were incubated in 50μl of incomplete Poels' (1972) medium (modified to remove exogenous methionine) at 25°C (except for 'heat-shock' experiments) under the various conditions and for the length of time necessary (as detailed in the legends to the Figures) to induce the puffs In all experiments, the effectiveness of puff induction was checked by examining a squash preparation of one of the glands Glands were labelled at 25°C for 15min in 5μl of incomplete Poels' (1972) medium containing 10μCi [^{35}S]methionine, except after treatment with 2,4-dinitrophenol or vitamin B-6 In those cases, the labelling period was 30min and 50μCi [^{35}S]methionine was used Samples were prepared for electrophoresis as described by Tissieres *et al* (1974), except that the dried glands were dissolved by boiling them for 2min in 35μl of sample buffer (for composition see below) A sample (2μl) was then counted directly in 10ml of scintillant containing 20ml of methoxyethanol, 5 5g of Permablend III and 30ml of Soluene-100/litre of toluene Liquid-scintillation counting was performed with a Packard Tri-Carb liquid-scintillation spectrometer, model 3004 A sample of the remainder was applied to a slab gel such that all slots contained an equal amount of radioactivity (this usually varied between 150000 and 1850000c p m)

Gel electrophoresis and radioautography

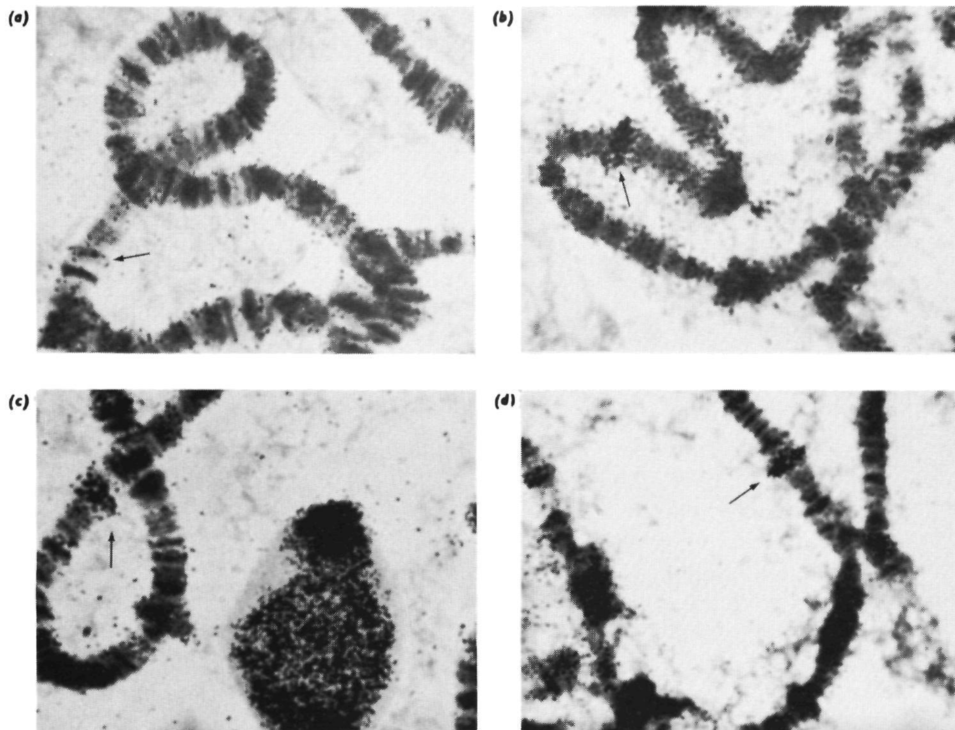
The protein samples were run for 3h at 16mA on 10% (w/v) polyacrylamide-slab gels, 1 5mm thick in a discontinuous sodium dodecyl sulphate system at room temperature (23°C) The system used was slightly modified from that described by Tissieres *et al* (1974) The small pore gel buffer was composed of 375mM-Tris/HCl, pH8 9, 4M-urea and 0 1% sodium dodecyl sulphate The large-pore buffer was composed of 62 5mM-Tris/HCl, pH6 7, 4M-urea and 0 1% sodium dodecyl sulphate The sample buffer contained 0 01M-sodium phosphate, pH7 6, 1% sodium dodecyl sulphate, 4M-urea, 1% 2-mercaptoethanol and 0 001% Bromophenol Blue The reservoir buffer was composed of 3 0g of Tris, 14 4g of glycine, 0 1% of sodium dodecyl sulphate and water added to 1 litre, pH8 3 After drying, the slab gels were exposed to Kodak RP/R 14 X-ray film for 4–72h The radioautographs were quantified by scanning with a Vitatron densitometer From this densitogram, the areas under the various peaks were then determined

Results

Correlation between puffing patterns induced by interference with the cellular respiratory metabolism and changes in protein-synthetic patterns

As mentioned above, several treatments that interfere with the respiratory metabolism all induce the same set of puffs namely 2-32A, 2-36A, 2-48BC and 4-81B in *D hydei* (Leenders & Berendes, 1972; Leenders *et al* , 1974*a*, *b*) The size of the puff induced is virtually the same irrespective of the puff-inducing stimulus, except for the puff at locus 2 48BC, which becomes very much larger during treatment with vitamin B-6 (Leenders *et al* , 1973) Not only are puff sizes the same, the amount of uridine incorporated in the puff area, as assayed radioautographically, is also very similar (Plates 1 and 2) In order to determine whether the induction of similar puffs is accompanied by similar changes in protein-synthetic patterns, salivary glands were pulse-labelled with [^{35}S]-methionine at various times after the start of the puff-inducing treatment The gland extracts were then electrophoresed on polyacrylamide gels in the presence of sodium dodecyl sulphate and the pattern of labelled protein was determined by radioautography Plate 3 shows such an experiment with temperature as the puff-inducing stimulus In agreement with Lewis *et al* (1975), new bands appear during the temperature treatment, whereas some of the bands present in the untreated control sample disappear The same set of bands appear during recovery from anaerobiosis, although the bands of lower molecular weight are less prominent than after a temperature shock (Plate 4*a*) In contrast with the pattern obtained after temperature treatment, however, the bands also present in control glands continue to be synthesized The pattern obtained during incubation of the glands in arsenite (Plate 4*b*) is very similar to that obtained after release of the glands from anaerobiosis the extra bands are added to the normal pattern of protein synthesis and do not replace it

Glands incubated in medium containing 2,4-dinitrophenol or vitamin B-6 did not incorporate enough [^{35}S]methionine to allow a radioautographic analysis of the protein-synthetic pattern This failure is presumably due to the very low cellular concentration of ATP under these conditions Therefore glands were pre incubated with either 2,4-dinitrophenol or vitamin B-6 to induce the puffs, then rinsed with and further incubated in normal medium and pulse labelled with [^{35}S]methionine



EXPLANATION OF PLATES 1 AND 2

Radioautographs of salivary-gland chromosomes displaying region* 4-81*B

After incubation of the glands in Poel's (1972) medium under various conditions to induce the puffs (see below), the glands were labelled for 5 min with [^{3}H]uridine (1 mCi/ml) and prepared for radioautography as described by Berendes (1966). Exposure time was 3 days. (Plate 1*a*) Control (untreated glands); (Plate 1*b*) 2 h incubation at 37°C; (Plate 1*c*) 2 h N_2 anaerobiosis followed by 45 min recovery in air; (Plate 1*d*) incubation for 2 h in the presence of 0.1 mM-sodium arsenite; (Plate 2*a*) incubation for 2 h in the presence of 1 mM-2,4-dinitrophenol; (Plate 2*b*) incubation for 2 h in the presence of 50 mM-vitamin B-6 (Plate 2*c*) the banding pattern of the salivary-gland chromosomes after aceto-orcein staining (incubated as in Plate 2*a*).

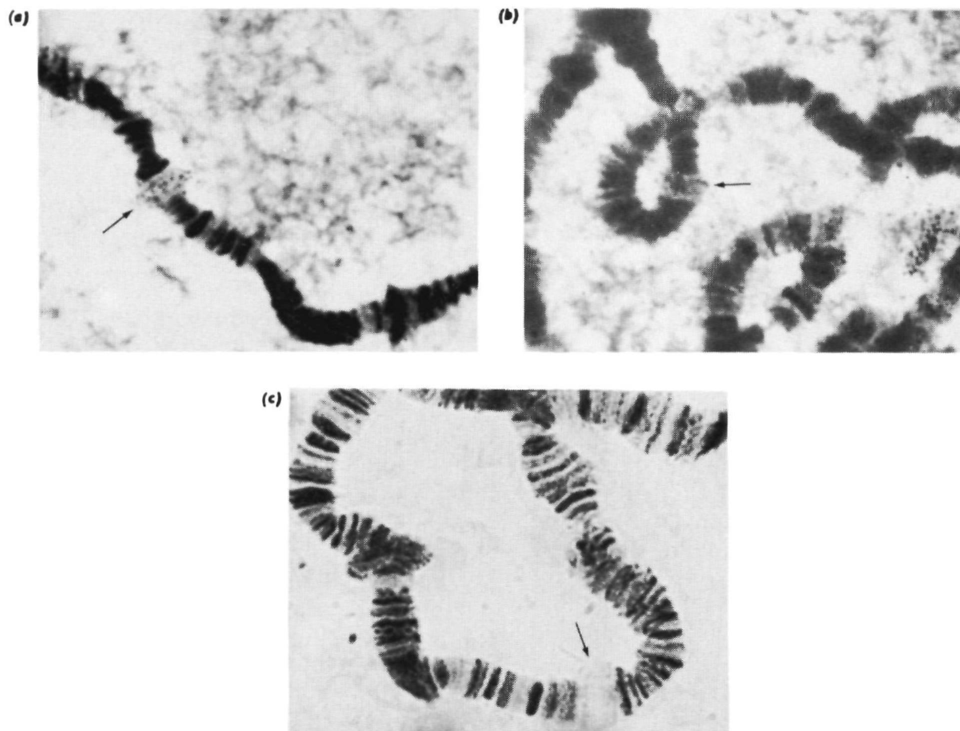


PLATE 2

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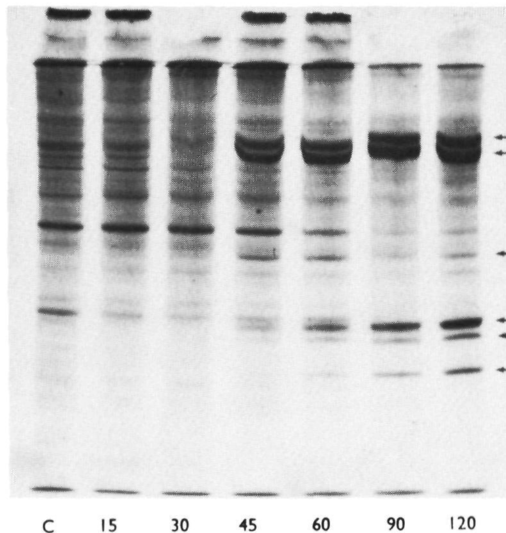
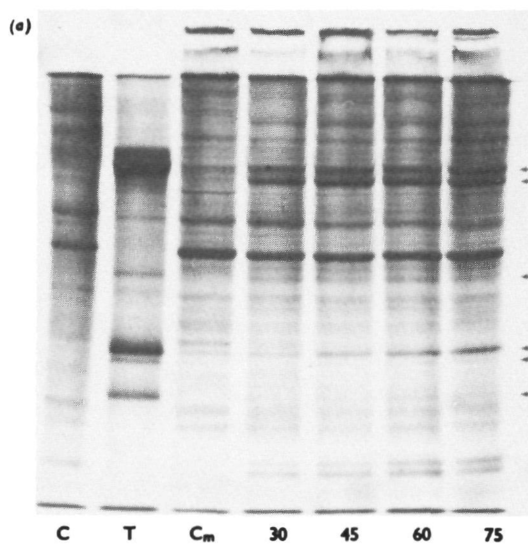


EXPLANATION OF PLATES 3, 4 AND 5

Radioautographs of [^{35}S]methionine pulse-labelled proteins, separated by sodium dodecyl sulphate-gel electrophoresis, after puff induction by various treatments

In all radioautographs the pattern of protein synthesis in control glands (C) and in 2h heat-treated glands (T) is shown for comparison. The numbers indicate the length (in min) of the incubation or the recovery period before the addition of label. (Plate 3) Incubation at 37°C (heat shock); (Plate 4*a*) recovery from 2h N_2 anaerobiosis [in this case, glands were mass-isolated as described by Boyd *et al.* (1968), C_m is the pattern obtained after labelling of control mass-isolated glands]; (Plate 4*b*) incubation in 0.1mM-sodium arsenite; (Plate 5*a*) recovery from a 2h incubation in 1mM-2,4-dinitrophenol; (Plate 5*b*) recovery from a 2h incubation in 50mM-vitamin B-6.



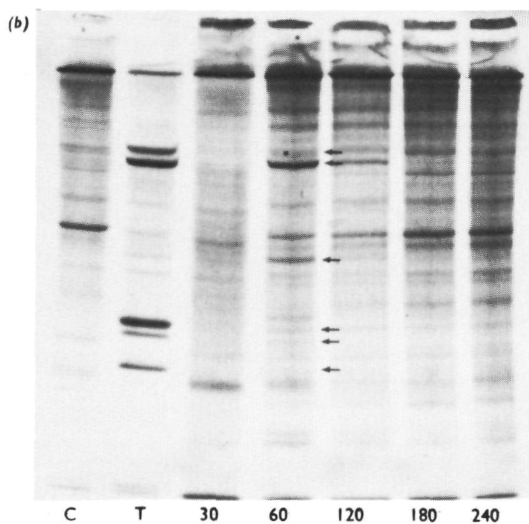


PLATE 4

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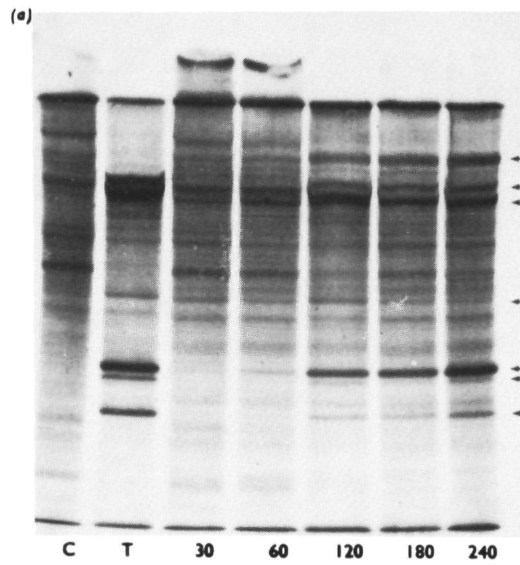

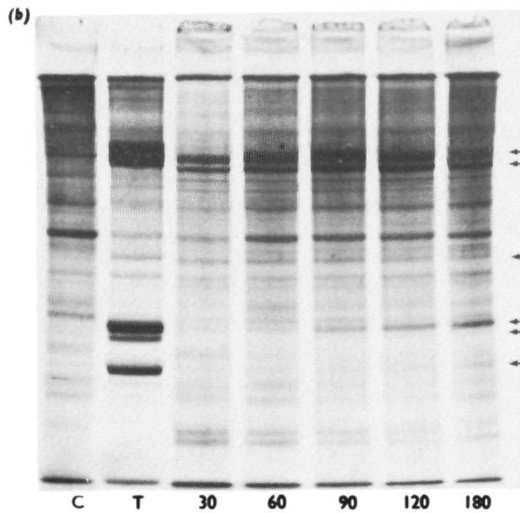

PLATE 5

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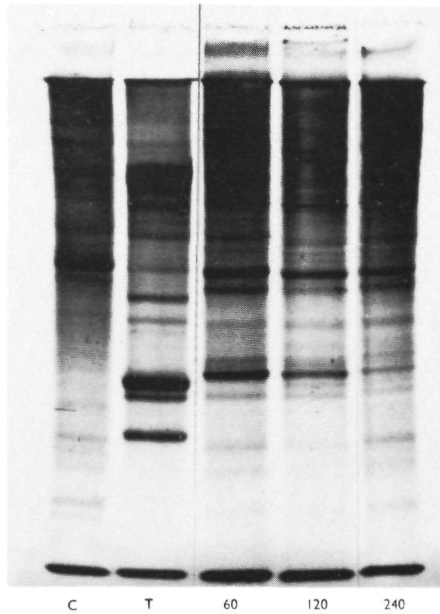

EXPLANATION OF PLATE 6

Radioautographs of [^{35}S]methionine labelled proteins from extracts of glands incubated with oligomycin

Glands were labelled as described in the Experimental section after the following incubation conditions: Control (C), 2h at 25°C; heat-treated (T), 2h at 37°C; 60, 120 and 240, after 1, 2 and 4h in the presence of 0.25mg of oligomycin/ml respectively.

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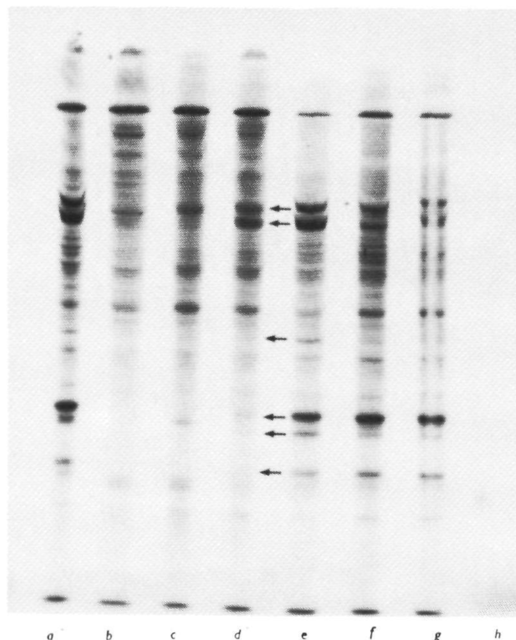

EXPLANATION OF PLATE 7

Radioautographs of [^{35}S]*methionine pulse-labelled proteins extracted from glands incubated with actinomycin D, chloramphenicol or cycloheximide*

(*a*) Glands were labelled after 2h incubation at 37°C; (*b*) glands were labelled after 2h incubation at 37°C in the presence of 20μg of actinomycin D/ml, actinomycin D was added before the glands were transferred to 37°C; (*c*) as in (*b*), except that actinomycin D was added after a 15min preincubation at 37°C; (*d*) as in (*b*), except that actinomycin D was added after a 30min preincubation at 37°C; (*e*) glands were labelled after a 2h incubation at 37°C in the presence of 100μg of chloramphenicol/ml; (*f*) as in (*e*), except that chloramphenicol was also present during the labelling period; (*g*) as in (*e*), except that chloramphenicol was present only during the labelling period; (*h*) glands were labelled after a 2h incubation at 37°C in the presence of 5μg/ml of cycloheximide.

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Under these conditions, if the mRNA coding for the 'heat-shock' bands is unstable, one would expect a transient appearance of the 'heat-shock' bands. This is indeed seen after puff induction with 2,4-dinitrophenol (Plate 5*a*): the six extra bands are clearly seen 60 min after the removal of 2,4-dinitrophenol from the medium, but only faintly after 120 min. However, in the case of vitamin B-6, a slow increase in the amount of additional bands made is seen, even though the puff-inducing stimulus was removed (Plate 5*b*). The reason for this phenomenon is not clear, but it may be due to a slow rate of transport of RNA from the puff area to the cytoplasm. It is clear, however, that also during vitamin B-6 treatment, as well as during all other treatments used here, six additional (as compared with untreated glands) peptide chains are synthesized.

The majority of treatments that interfere with the respiratory mechanism also cause a lowering of the ATP concentration in the cell (Leenders *et al.*, 1974*a*, *b*). This decrease could affect the rate of initiation and thus change the pattern of protein synthesis, independent of any new transcription. To determine whether such a pattern shift does indeed occur, salivary glands were incubated in the presence of oligomycin, which lowers the ATP concentration but does not induce puffs. A change in pattern of protein synthesis does indeed occur, but the typical six bands are not visible (Plate 6). Further, as shown in Plate 7, and in agreement with others (Lewis *et al.*, 1975), the appearance of these bands is strictly dependent on RNA synthesis *de novo* (since it is inhibited by actinomycin D) and cytoplasmic protein synthesis *de novo* (since it is blocked by cycloheximide, but not by chloramphenicol).

Kinetics of induction of the 'heat-shock' bands

From radioautograms such as shown in Plates 3, 4 and 5 it is possible to quantify the relative amount of radioactivity incorporated in each band by measuring the darkness of the film. Clearly such a quantitation cannot yield absolute rates of synthesis: only the relative amount of incorporation of methionine in the 'heat-shock' bands with respect to the total incorporation can be determined. The quantitative changes observed in protein-synthesis patterns under the various puff-inductive conditions are very similar: the rate of synthesis of the 'heat-shock' bands increases rapidly within the first hour. In contrast with previous work (Lewis *et al.*, 1975) no indications were found for a sequential appearance of the bands, rather, all bands appear at the same time

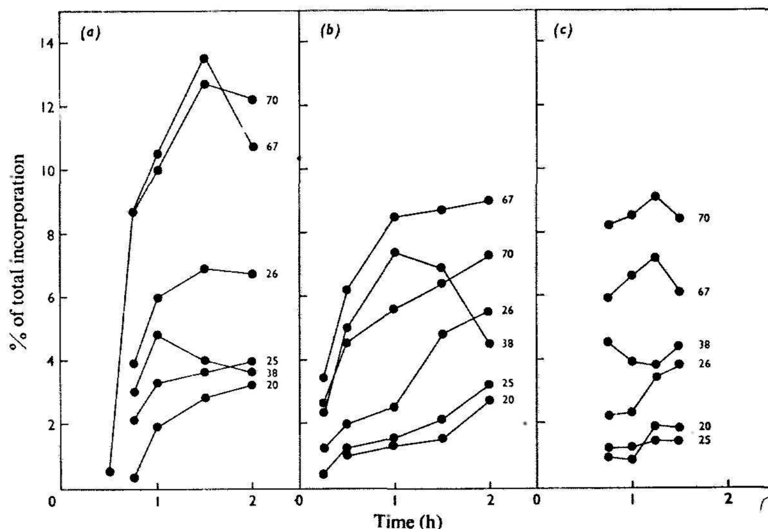

Fig. 1. *Kinetics of induction of the 'heat-shock' bands during various treatments*

The relative amount of label (expressed as % of the total darkening of the film) in the 'heat-shock' bands was calculated as described in the Experimental section from radioautograms as shown in Plates 3, 4 and 5. (*a*) At 37°C; (*b*) in the presence of 0.1 mM-sodium arsenite; (*c*) after release from 2 h of N_2 anaerobiosis. The numbers given in the Figure indicate the molecular weights ($\times 10^{-3}$) of the various peptides.

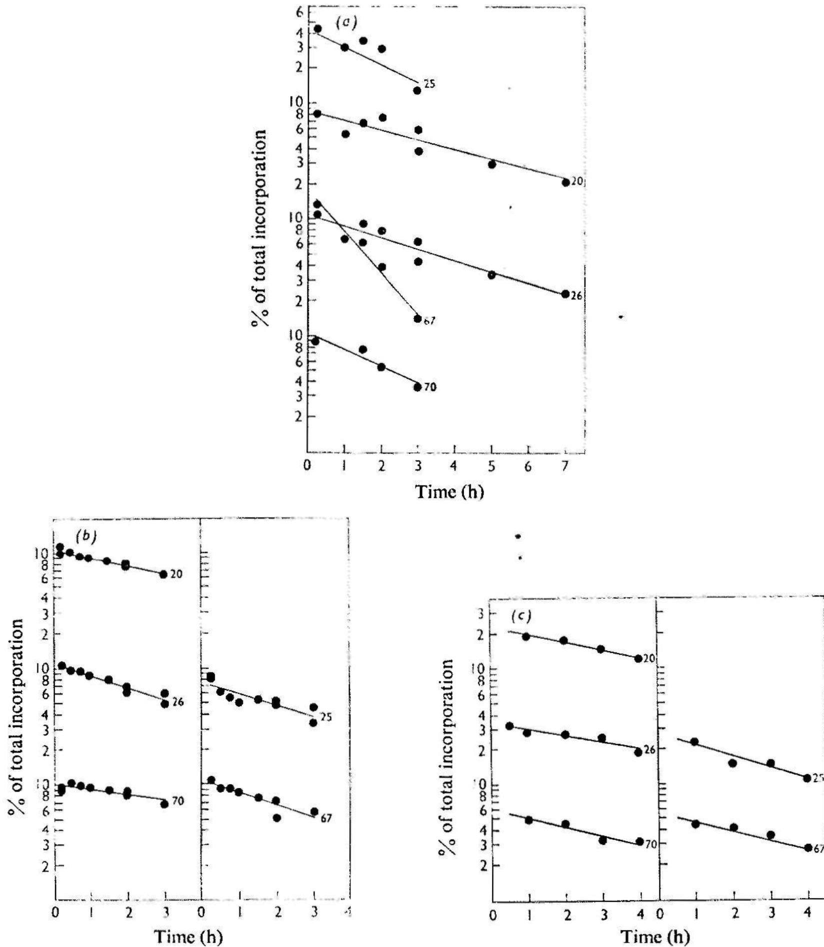

Fig. 2. *Kinetics of decay of the rate of synthesis of 'heat-shock' bands*

The relative amount of label in the 'heat-shock' bands was calculated as described in the Experimental section. (*a*) Glands were incubated for 2h at 37°C and then further incubated at 25°C; (*b*) glands were preincubated for 2h at 37°C, then actinomycin D (20μg/ml) was added and incubation was continued at 37°C; (*c*) glands were preincubated for 2h in 1mM-2,4-dinitrophenol. The numbers given in the Figure indicate the molecular weights (× 10^{-3}) of the various peptides.

(Fig. 1). The rate of synthesis of the bands remains relatively constant after the first hour of induction, except for the 38000mol.wt. band, whose rate of synthesis decreases again after 1h, at least at 37°C or in the presence of arsenite. The relative rate of synthesis of this protein at 37°C appears to be lower than during arsenite treatment or during recovery from N_2 anaerobiosis. On the other hand, the two high mol.wt. proteins (70000 and 67000) are synthesized at a relatively greater rate at 37°C as compared with the other treatments used. The 'heat-shock' proteins account for about 50% of the total protein synthesis at 37°C, but for only 25% during arsenite treatment or after release from N_2 anaerobiosis.

Kinetics of decay of the synthesis of the 'heat-shock' bands

If the incubation temperature of the glands is lowered from 37° to 25°C the temperature puffs rapidly regress A similar response is seen in the rate of synthesis of the 'heat-shock' bands (Fig 2*a*) the rate of synthesis of all bands decreases rapidly and with apparent first-order kinetics The rate of decay of most bands has a half-life of 3h Only the 67000 mol wt band decays faster, with a half-life of about 45min A decrease in the rate of synthesis is also found after addition of actinomycin D to glands incubated at 37 C (Fig 2*b*) again the rate of synthesis of the 'heat-shock' bands decreases with first-order kinetics and with a half-life of 3h After puff induction with 2,4-dinitrophenol the rate of synthesis of the 'heat-shock' proteins decreased with the same apparent half-life (Fig 2*c*) The mRNA coding for the 'heat-shock' proteins thus appear to be unstable with a half-life of roughly 4h

Discussion

The data presented in this paper confirm and extend the observations of Lewis *et al* (1975) namely, all treatments that induce the full set of 'heat-shock' puffs also lead to similar qualitative changes in the pattern of protein synthesis, thus strengthening the hypothesis that these puffed loci contain the genetic information for the protein of these 'heat-shock' bands Other changes in the protein-synthesis pattern are also observed, for example, an additional high-molecular-weight protein appears during arsenite treatment, but such changes appear to be treatment specific, and do not accompany puff induction in all cases

As expected, since the size and activity in RNA synthesis of the puffs induced by the various treatments are very similar, there are no large quantitative differences in either the kinetics of induction or the rate of synthesis of the various 'heat-shock' bands between the various treatments, with the possible exception of the 38000mol wt band The decrease in the rate of synthesis of this latter band during either temperature or arsenite treatment is not accompanied by obvious changes in the puffing patterns From the data presented here, no correlation can be made between the presence of any one puff and the appearance of any one 'heat-shock' band Not only can a precursor–product relationship between any two bands not be excluded, but a further difficulty is that there are only four major 'heat-shock' puffs in *D hydei* and at least three small puffs (Berendes *et al*, 1965) These latter puffs have not been studied intensely, but two of these might also be involved in the synthesis of the 'heat-shock' bands In *D melanogaster*-locus 3-87B is the largest puff and the 67000 mol wt band the most prominently labelled one (Tissieres *et al*, 1974). Further, RNA isolated from 'heat-shocked' tissue-culture cells hybridized most heavily to this locus (McKenzie *et al*, 1975, Spradling *et al*, 1975*a, b*) It has thus been suggested that in *D melanogaster* locus 3-87B codes for the 67000 mol wt protein Such a correlation between protein and puff would be strengthened, if the protein patterns are analysed after treatments, which induce only a few of the 'heat-shock' puffs, for example after treatment with antimycin A, which induces the loci 2-32A, 2 36A and 2-48BC, but not 4-81B in *D hydei.* Unfortunately, antimycin A also inhibits [^{35}S]-methionine incorporation severely and apparently irreversibly

At 37°C the relative rate of synthesis of the protein in these bands is about twice as high as during the other treatments used here The data presented by McKenzie *et al* (1975) suggest that the high rate of synthesis of the 'heat-shock' proteins at 37°C is due to a translational rather than transcriptional control of protein synthesis If so, the translational control must act negatively on the pre-existing mRNA species rather than positively on mRNA species coding for the 'heat-shock' proteins since the rate of decay of the synthesis of these bands is the same at 25° and at 37 C in the presence of actinomycin D (with the possible exception of the 67000-mol wt band) Under the latter conditions, the rate of decrease of synthesis of the bands presumably reflects the rate of decay of the mRNA Moreover, it cannot be excluded that this translational effect is non-specific, it could be the result of changes in the net rate of initiation of protein synthesis due to an increase in temperature and a decrease in the cellular ATP concentration Such changes in net initiation rate are expected to result in a change in the protein-synthetic pattern, both theoretically (Lodish, 1974) and also experimentally (Ayuso-Parilla & Parilla, 1975, Plate 6) The lack of suppression of background synthesis at 25°C during arsenite treatment or after N_2 anaerobiosis also suggests that any translational effect at 37°C is correlated with the specific inductive stimulus rather than with the presence of the set of puffs

Spradling *et al* (1975*a, b*) have found that the half-life of unstable mRNA in an *Aedes albopictus* cell line is 1 2h A significantly longer functional half-life was found here for the mRNA species coding for the 'heat-shock' bands, namely about 4h A similar decay rate of the synthesis of the 'heat-shock' proteins was found after removal of the puff-inductive stimulus or in the presence of actinomycin D, except after puff induction with vitamin B-6 In this case the rate of synthesis of the 'heat-shock' proteins increased up to 3h after removal of vitamin B-6 from the medium. The reason for this phenomenon is not clear, but it may be due to a combination of a slow regression of the puffs and a slow rate of transport of the puff RNA from the nucleus Cytological studies do show a slow

regression of the puff 2-48BC and a slow rate of transport of material from this puff after induction with vitamin B-6 (J. Derksen, personal communication).

The kinetics of induction of the 'heat-shock' bands are very similar to the previously found rate of increase of some mitochondrial enzyme activities (Leenders & Beckers, 1972; Leenders *et al.*, 1974*a*,*b*; Koninkx *et al*, 1975; Koninkx, 1975; Sin & Leenders, 1975). The data presented here do not therefore exclude the possibility that the 'heat-shock' peptides are part of such enzymes, nor do they offer further support for this suggestion. Further evidence for such a function of the 'heat-shock' proteins must come from a characterization of the peptide chains of these enzymes.

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Summary

A variety of treatments, all of which interfere with the cellular respiratory metabolism and most of which cause a drop in the cellular ATP level, evoke the induction of puffs at the loci 2-32 A, 2-36 A, 2-48 BC and 4-81 B. By means of the experiments, described in the first part of the thesis, it has been verified whether changes in the cellular adenosine nucleotide levels are responsible for the release of the primary signal for the activation of these specific gene loci. However, there is no positive correlation between the reduction in the ATP level and the response at the genome level during all treatments applied. A temperature shock reduces the cellular ATP level and induces gene activity. Anaerobiosis, which depletes the ATP pool, does not result in the activation of the chromosome loci unless the salivary glands are exposed to oxygen again. Oligomycine or cyanide are not able to induce puffs, although the ATP level is significantly reduced. On the other hand, exposure to arsenite, which hardly changes the cellular ATP level, is very effective in evoking the appearance of the specific puffs. Therefore the initiation of gene activity following treatments causing a deficiency in mitochondrial respiratory metabolism, cannot be a direct consequence of changes in cellular adenosine nucleotide levels. Other regulatory mechanisms are proposed, which favor the idea that the signals evoking the induction of gene activity originate from deficiencies in respiratory substrate supplies or certain mitochondrial enzymes.

Previously it has been demonstrated that following puff induction the isocitrate respiration is enhanced as well as the enzyme activity of NADH dehydrogenase. In part two of the thesis data are presented, which indicate that both the increased isocitrate respiration and the increased NADH dehydrogenase activity are from intramitochondrial rather than from extramitochondrial origin. The isocitrate respiration of mitochondria isolated from salivary glands recovering from anaerobiosis shows a significant

decrease in app. Km (control glands 5,6 mM; glands recovering from anaerobiosis 2,4 mM), whereas the app. Vmax of the mitochondrial NADH dehydrogenase is increased by about 70%.
Inhibition of RNA synthesis or protein synthesis during puff induction by actinomycin D or cycloheximide inhibits the decrease in app. Km of the mitochondrial isocitrate respiration and the increase in app. Vmax of the mitochondrial NADH dehydrogenase. This inhibitory effect and the fact that gene activity always precedes these changes suggest that these changes may be dependent upon transcription of new mRNA species in one of the newly activated chromosome loci and its subsequent translation.

In the next part of the thesis polyacrylamide gelelectrophoresis reveals that the increase in app. Vmax of the mitochondrial NADH dehydrogenase after experimentally induced gene activity is correlated with an increase of NADH dehydrogenase activity in three mitochondrial protein fractions as well as with an increased amino acid incorporation into these proteins. Inhibition of nuclear RNA synthesis or cytoplasmic protein synthesis inhibits the increase in app. Vmax and the increase in amino acid incorporation. The results of several experiments indicate that these changes cannot be accounted for merely by changes in amino acid pools, enzyme turnover rate or uptake of enzyme molecules by the mitochondria from the cytoplasm without increased synthesis de novo of NADH dehydrogenase. Moreover, the increase in mitochondrial NADH dehydrogenase activity after recovery from anaerobiosis cannot be ascribed to mitochondrial biogenesis, because the synthesis of cycloheximide insensitive mitochondrial proteins is very low or completely absent as can be judged from the radioactive profiles of pulse labelled mitochondrial proteins. Thus it is reasonable to suggest that the increase in mitochondrial NADH dehydrogenase activity in salivary glands recovering from anaerobiosis depends on increased gene transcription.

In the last part of the thesis the protein synthesis in salivary glands of Drosophila hydei after experimental gene induction has been analysed by electrophoresis on sodium dodecyl sulphate polyacrylamide

gels of labelled salivary gland proteins and subsequent autoradiography. Gene activation by treatments interfering with the cellular respiratory metabolism, which induce the same set of puffs in Drosophila hydei, namely 2-32 A, 2-36 A, 2-48 BC and 4-81 B, is always followed by the appearance of six strongly labelled polypeptides in the pattern of protein synthesis. Oligomycine, an inhibitor of the ATP formation, does not cause the induction of the specific puffs. Although oligomycine changes the pattern of protein synthesis, most probably as a result of a decreasing ATP concentration in the cell, the changes do not include the appearance of the six polypeptides. These data clearly indicate, that the presence of the same set of puffs leads to similar qualitative changes in the pattern of protein synthesis. The kinetics of induction of the six polypeptides are very similar to the previously found rate of increase in app. Vmax of mitochondrial NADH dehydrogenase and mitochondrial tyrosine amino transferase and the decrease in app. Km of mitochondrial isocitrate dehydrogenase. The change in activity of the mitochondrial enzymes occurs about 30 minutes after the loci 2-32 A, 2-36 A, 2-48 BC and 4-81 B have started their puff formation. The six strongly labelled polypeptides also appear about 30 minutes after puff induction.

However, whether the polypeptides are part of the mitochondrial enzymes increasing in activity following treatments interfering with the cellular respiratory metabolism needs further investigation. It is tempting to suggest that the puffs, which are induced by treatments interfering with the cellular respiratory metabolism, represent regions of the genome which are derepressed through the action of the puff inducing stimuli, in such a way that they actively transcribe mRNA's which are immediately translated, thus altering the pattern of protein synthesis in Drosophila hydei salivary glands. In this thesis data are presented demonstrating that the temperature sensitive genes of the genome respond to fluctuations in mitochondrial metabolism and that these genes hasten the synthesis of gene products, which following translation, effect a return to normal metabolism. This involves probably the de novo synthesis of at least one mitochondrial protein as has been demonstrated.

Samenvatting

Behandelingen, die inwerken op het cellulair ademhalingsmetabolisme en waarvan de meeste een significante daling van het cellulaire ATP niveau veroorzaken, induceren specifieke aktiviteit in de genloci 2-32 A, 2-36 A, 2-48 BC en 4-81 B. Door middel van de experimenten, die beschreven zijn in het eerste gedeelte van het proefschrift, is nagegaan of veranderingen in de cellulaire adenosine nucleotide concentraties verantwoordelijk zijn voor het vrijkomen van het primaire signaal, dat specifiek aktiviteit in deze genloci induceert. Een positieve correlatie tussen deze ATP daling ongeacht de toegepaste behandeling en een reaktie op genoomniveau is echter niet gevonden. Een temperatuurshock verlaagt de cellulaire ATP concentratie en induceert genaktiviteit. Het ontbreken van ATP tijdens anaerobiose resulteert alleen dan in de aktivering van chromosoomloci, als de anaerobiose van speekselklieren wordt opgeheven. Oligomycine of cyanide zijn niet in staat puffs te induceren, terwijl de ATP concentratie toch significant gereduceerd is. Arseniet daarentegen verandert de cellulaire ATP concentratie nauwelijks, maar is zeer effektief in het laten verschijnen van de specifieke puffs. Om deze reden kan de initiatie van genaktiviteit door behandelingen, die een deficiëntie veroorzaken in het mitochondriaal ademhalingsmetabolisme, niet een direct gevolg zijn van veranderingen in de cellulaire adenosine nucleotide concentraties.
Andere regelmechanismen worden voorgesteld, die er van uitgaan, dat de signalen, die inductie van genaktiviteit oproepen, veroorzaakt worden door tekorten in de substraatvoorziening van het ademhalingsmetabolisme of in bepaalde mitochondriale enzymen.

In eerste instantie werd aangetoond, dat na puff-inductie zowel de isocitraat ademhaling als de enzymatische aktiviteit van NADH dehydrogenase toegenomen is. De experimenten beschreven in het tweede gedeelte van het proefschrift wijzen er op, dat zowel de toegenomen isocitraat ademhaling als de toegenomen NADH dehydrogenase aktiviteit veeleer intramitochondriaal dan extramitochondriaal is. De isocitraat ademhaling van mitochondriën, geïsoleerd uit speekselkliercellen na herstel van anaerobiose, laat een significante daling in app. Km zien (controle klieren

5,6 mM; klieren hersteld van anaerobiose 2,4 mM), terwijl de app. Vmax van de mitochondriale NADH dehydrogenase met ongeveer 70% is toegenomen. Remming van de RNA-synthese of eiwitsynthese door respectievelijk actinomycine D of cycloheximide gedurende de puff-inductie remt de afname in app. Km van de mitochondriale isocitraat ademhaling en de toename in app. Vmax van de mitochondriale NADH dehydrogenase. Deze remmende werking en het feit, dat genaktiviteit altijd voorafgaat aan de toename in enzymaktiviteit, doen vermoeden, dat deze veranderingen afhankelijk zouden kunnen zijn van transcriptie van nieuwe mRNA soorten in één van de nieuw geaktiveerde chromosoomloci en translatie van dit mRNA.

In het volgende deel van het proefschrift is door middel van polyacrylamide gelelectroforese aangetoond, dat de toename in app. Vmax van de mitochondriale NADH dehydrogenase ten gevolge van experimenteel geinduceerde genaktiviteit gecorreleerd is met een toename van de NADH dehydrogenase aktiviteit in drie mitochondriale eiwitfrakties en een verhoogde aminozuur incorporatie in deze eiwitten. Inhibitie van de nucleaire RNA-synthese of cytoplasmatische eiwitsynthese remt de toename in app. Vmax en de toename in aminozuur incorporatie. De resultaten van meerdere experimenten wijzen er op, dat deze veranderingen niet enkel en alleen verklaard kunnen worden door wijzigingen in aminozuurpools, in turnover snelheid van enzymen of opname van enzymmoleculen zonder toegenomen _de novo_ synthese van NADH dehydrogenase. Bovendien kan de toename van de mitochondriale NADH dehydrogenase aktiviteit na herstel van anaerobiose niet toegeschreven worden aan mitochondriale biogenese, omdat de synthese van cycloheximide-ongevoelige mitochondriale eiwitten zeer gering of volledig afwezig is, zoals op te maken valt uit de radioaktieve profielen van de pulse-gelabelde mitochondriale eiwitten. Op grond van deze gegevens is het redelijk te veronderstellen, dat de toename van de mitochondriale NADH dehydrogenase aktiviteit in speekselklieren, die zich van anaerobiose herstellen, afhankelijk is van toegenomen gen-transcriptie en cytoplasmatische eiwitsynthese.

In het laatste deel van het proefschrift is de eiwitsynthese na experimentele gen-inductie in speekselklieren van Drosophila hydei geanalyseerd met behulp van SDS polyacrylamide gelelectroforese van

gelabelde speekselklier eiwitten en autoradiografie. Behandelingen, die inwerken op het cellulaire ademhalingsmetabolisme induceren altijd dezelfde puffs in Drosophila hydei, namelijk 2-32 A, 2-36 A, 2-48 BC en 4-81 B. Inductie van genaktiviteit wordt altijd gevolgd door het verschijnen van zes sterk gelabelde polypeptiden in het eiwitsynthese patroon. Oligomycine, een inhibitor van de ATP synthese, is niet in staat de specifieke puffs te induceren. Hoewel oligomycine het eiwitsynthese patroon verandert, hoogstwaarschijnlijk als gevolg van een dalende ATP concentratie in de cel, behelzen deze veranderingen niet het verschijnen van de zes polypeptiden. Deze gegevens tonen duidelijk aan, dat de aanwezigheid van dezelfde puffs leidt tot gelijke kwalitatieve veranderingen in het patroon van de eiwitsynthese. De inductie-kinetiek van de zes polypeptiden vertoont veel overeenkomst met de voorheen gevonden toename in de app. Vmax van de mitochondriale NADH dehydrogenase en mitochondriale tyrosine aminotransferase en de afname in app. Km van de mitochondriale isocitraat dehydrogenase.
De verandering in aktiviteit van de mitochondriale enzymen begint ongeveer 30 minuten nadat de puffs 2-32 A, 2-36 A, 2-48 BC en 4-81 B aktief worden. Ook de zes sterk gelabelde polypeptiden verschijnen ongeveer 30 minuten na puff-inductie. Of deze polypeptiden deel uitmaken van de mitochondriale enzymen, die in aktiviteit toenemen na behandelingen welke inwerken op het cellulaire ademhalingsmetabolisme, vereist nader onderzoek. Het is verleidelijk te veronderstellen, dat de puffs, die geïnduceerd worden door behandelingen welke inwerken op het cellulaire ademhalingsmetabolisme, gebieden van het genoom vertegenwoordigen, die door inwerking van de puff inducerende stimuli vrijkomen voor een snelle transcriptie van mRNA's, welke onmiddellijk vertaald worden en aldus het eiwitsynthese-patroon veranderen van de speekselklieren van Drosophila hydei.

In dit proefschrift worden gegevens gepresenteerd, die aantonen, dat een bepaalde groep genen, n.l. de temperatuur gevoelige loci van het genoom,reageert op schommelingen in het mitochondriaal metabolisme en dat deze loci de synthese van gen producten versnellen, die na transcriptie een terugkeer naar een normaal metabolisme zouden bewerkstelligen. Dit betekent dat zoals in dit proefschrift beschreven werd minstens één mitochondriaal enzymen de novo gesynthetiseerd zou worden.

Curriculum vitae

De auteur van dit proefschrift, Joseph Frans Jan Gerard Koninkx werd geboren op 2 april 1944 te 's-Gravenhage. De lagere en middelbare school heeft hij doorlopen in Roermond. Van september 1950 tot en met juli 1956 werd Lager Onderwijs gevolgd op de Don Boscoschool, van september 1956 tot en met juli 1957 Uitgebreid Lager Onderwijs op de Lindanusschool en van september 1957 tot en met juli 1962 Middelbaar Onderwijs op het Bisschoppelijk College. Het Middelbaar Onderwijs werd afgesloten met het behalen van het diploma H.B.S.-b.
Na als octrooi-analist werkzaam te zijn geweest in het Centraal Laboratorium van de Staatsmijnen te Geleen van september 1962 tot en met januari 1964 en na vervulling van de militaire dienstplicht van februari 1964 tot en met juli 1965, is hij in september 1965 begonnen met de studie biologie aan de faculteit Wiskunde en Natuurwetenschappen van de Katholieke Universiteit te Nijmegen. Op 6 januari 1970 werd het kandidaats examen biologie afgelegd en op 6 februari 1973 het doctoraal examen biologie. Adaptatie van micro-organismen aan extreme omstandigheden was het onderwerp van zijn hoofdvak en werd op de afdeling Exobiologie bestudeerd onder leiding van dr. W. Heinen. Op de afdeling Submicroscopische Morfologie werd onder leiding van dr. M.M.A. Sassen onderzoek gedaan naar de synthese van de celwand bij Allomyces arbuscula en op de afdeling Genetica werd hij begeleid door dr. H.J. Leenders en prof. dr. H.D. Berendes in een onderzoek naar de relatie tussen genactiviteit en veranderingen in mitochondriale enzymactiviteiten in speekselkliercellen van Drosophila hydei.
Van 1 maart 1973 tot en met 31 december 1975 volgde een aanstelling als wetenschappelijk medewerker aan het Genetisch Laboratorium van de Faculteit Wiskunde en Natuurwetenschappen van de Katholieke Universiteit te Nijmegen onder leiding van prof. dr. H.D. Berendes en dr. H.J. Leenders. Tijdens deze periode was hij van 1 maart 1973 tot en met 31 augustus 1975 in dienst van de Nederlandse Organisatie voor zuiver-wetenschappelijk Onderzoek (Z.W.O.) en van 1 september 1975 tot en met 31 december 1975 in dienst van de Katholieke Universiteit te Nijmegen.
Hij heeft gedurende zijn promotie-studie op de afdeling Genetica van 1 - 14 augustus 1974 te Erice, Sicilië, Italië, deelgenomen aan een

Summer School on Molecular and Developmental Biology.
Van 1 januari 1976 tot heden is hij werkzaam als wetenschappelijk medewerker in het Centrum voor Electronenmicroscopie, Medische Faculteit van de Rijksuniversiteit Utrecht.

Stellingen

1. Het is niet waarschijnlijk, dat de toegenomen enzym aktiviteit van de mitochondriale NADH-dehydrogenase, zoals die optreedt na induktie van de "temperatuur-puffs", veroorzaakt wordt door een verhoogde biogenese van mitochondriën.

   Dit proefschrift.

2. De regulatie van de "temperatuur-puffs" is onafhankelijk van de ATP concentratie in de cel.

   Vossen, J.G.H.M., Leenders, H.J., Derksen, J. en Jeucken, G. Exp. Cell Res. 109: 227-283 (1977).
   Ashburner, M. Chromosoma 31: 356-376 (1970).

3. De verschillende vormen van DNA-endoreduplicatie zijn op te vatten als een evolutionair alternatief voor een hoog nucleair DNA-gehalte, zoals dat bereikt kan worden door tandem duplicatie van bepaalde DNA sequenties.

   Nagl, W. Nature 261: 614-615 (1976).

4. Voor een juiste beschrijving van het transport van de (glyko)proteïnen, onder verschillende fysiologische omstandigheden, in secernerende cellen, m.b.v. elektronenmikroskopische autoradiografie, is het noodzakelijk de verschillen in eiwitsynthese-patroon te kennen.

   Kent, C. en Williams, M.A. J. Cell Biol. 60: 554-570 (1974).

5. De konklusie van Völkl, Bieger en Kern, dat de gelijktijdige sekretie door de pancreascellen van verschillende soorten eiwitten tot stand komt, doordat het Golgi-komplex werkt als een soort mengkamer van deze eiwitten, is onjuist.

   Völkl, A., Bieger, W. en Kern, H.F. Cell Tiss. Res. 175: 227-243 (1976).

6. Het is onjuist te veronderstellen, dat de opslag van sekretiegranula in mukeuze en sereuze kliercellen, voor zover die voor hun sekretie afhankelijk zijn van een bepaalde stimulus, verschillend zou zijn.

   Neutra, M.R. en Schaeffer, S.F. J. Cell Biol. 74: 983-991 (1977).
   Poels, C.L.M., de Loof, A. en Berendes, H.D. J. Insect Physiol. 17: 1717-1729 (1971).
   Poels, C.L.M. Cell Differentiation 1: 63-78 (1972).

7. Het is onwaarschijnlijk, dat de door Olańczuk-Neyman en Vosjan beschreven experimenteel oecologische methodiek bruikbaar is voor de veldsituatie.

Olańczuk-Neyman, O. en Vosjan, J.H. Neth. J. Sea Res. 11: 1-13 (1977).
Vosjan, J.H. en Olańczuk-Neyman, O. Neth. J. Sea Res. 11: 14-23 (1977).

8. De trage ontwikkeling van alternatieve schone energiebronnen is hoofdzakelijk te wijten aan de grootschaligheid, die men beoogt bij de toepassing ervan.

9. Energiebesparing wordt vaak ten onrechte als argument aangehaald teneinde tot aanschaffing van producten over te gaan, waarvan het gebruik niet energiebesparend behoeft te zijn.