Specific effects of juvenile hormone on chromosome function

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Specific Effects of Juvenile Hormone on Chromosome Function

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The term "specific" used in the title of this paper can refer to different subjects. It could mean specific for a certain hormone, e.g. for juvenile hormone but not for ecdysone ; it can mean specific for a certain tissue or for a certain developmental stage, or else it can mean specific for particular genes. It is gene specificity with which we are most concerned. The reason why we stress gene specificity is because there is a tendency to take any effect on RNA synthesis as indicating specific gene activation while many of these effects are general. In principle there are two methods available today to determine gene specificity : 1. Molecular hybridization of RNA to DNA. 2. Investigation of puffing in polytene chromosomes.

The method of hybridization is rather involved and any event indirect. The interpretation of its result is difficult and sometimes even impossible, especially when hybridization is done under inappropriate conditions. The method which makes use of the phenomenon of puffing is direct and rather straightforward. However, it can only be applied to tissues with polytenic chromosomes. These chromosomes are of such enormous size that, by means of autoradiography, one can distinguish easily and precisely between those regions which are active and those which are inactive in synthesizing RNA. The active region may even be mapped along the chromosome using the characteristic banding pattern of these chromosomes as a guide. Active chromosome regions are structurally altered into so-called puffs. Puffs represent nothing else than portions of decondensed chromatin enriched in some characteristic proteins and RNA.

We chose puffing for our studies of gene specific effects of juvenile hormone. Our system is the salivary gland of the aquatic larva of *Chironomus*, a midge. It is a reasonable assumption that a chromosome region under the control of juvenile hormone should, in its course of activity, parallel the course of the juvenile hormone titer occurring naturally in the hemolymph of the animal. In *Cecropia* the juvenile hormone titer is high during all larval life but drops during the last larval instar (1). In *Chironomus* it might behave the same way. Two

possible candidates of chromosome regions under the control of juvenile hormone are regions 19A and 19B of *C. tetans* (Fig. 1). Both regions have been shown to be active during the third and the beginning of the

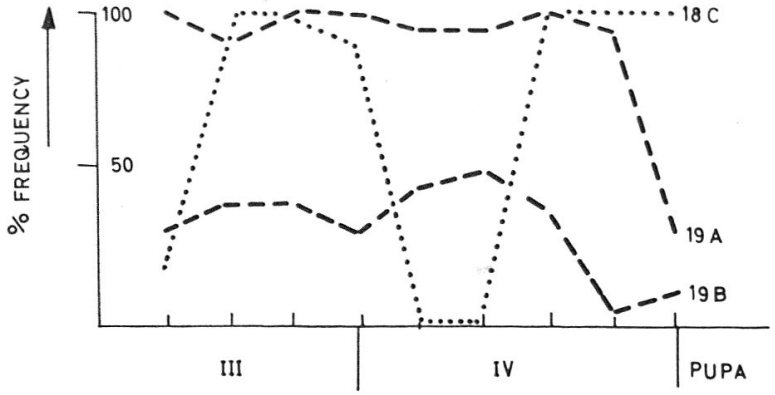

Fig. 1. — Puff frequency during normal development in salivary gland chromosomes of *C. tentans*. Regions in chromosome I are indicated on the right. III = 3rd, IV = 4th larval instar. Frequency values and developmental subdivisions are taken from Fig. 2 of ref. (16) and Figures 4 and 8 of ref. (17).

fourth instar larvae. At the end of the last instar, i.e. during the prepupal stage, their activity decreases. Because of their similar behaviour and because of their close neighbourhood we shall discuss 19A and 19B as one region. In order to be quite sure that we really are dealing with a specific juvenile hormone effect, we shall always keep an eye on the effect of ecdysone. The change of the ecdysone titer during normal development is characterized by a peak immediately before each molt (2). These peaks are paralleled by peaks in the activity of region 18C. Thus, there are two typical situations concerning the activity of 19AB and 18C : 1) both regions are active at the same time ; 2) the activity of 19AB is decreasing while that of 18C is increasing.

So far, we have only demonstrated a correlation between hormone titer and puffing activity. The causal relationship between these two phenomena has to be established by experiments. Juvenile hormone was, therefore, injected into prepupae. Prepupae have no or little activity in 19A and B and supposedly a low juvenile hormone and a high ecdysone titer. Two to four hours after juvenile hormone injection a puff is formed in region 19A or B. The pre-existing puff in 18C either persists or regresses (3).

It is evident too from the numerical evaluations of these experiments (3) that in general the effect of juvenile hormone on 19AB is opposite to that on 18C. 18C is an ecdysone sensitive region, as already discovered 10 years ago by Clever and Karlson (4). It is interesting to note that the effect of ecdysone is a sort of mirror image to the effect of juvenile hormone, in that while 18C is activated with ecdysone 19A,

though it sometimes maintains its former activity, is as a rule deactivated. In general, therefore, one may state that there exists an antagonism between the effect of juvenile hormone and that of ecdysone. This antagonism is not very strict insofar as the effects of juvenile hormone and ecdysone are not always mutually exclusive.

In the first part of this paper we discussed experiments done with *C. tentans*. In the next part we should like to switch to *C. thummi* for two reasons : 1. Since we are working with *C. thummi* for almost ten years we know more about the pecularities of this species which fact might facilitate the interpretation of our results. 2. There is a report by Laufer (5) on juvenile hormone changing the puffing activity in *C. thummi*. The results mentioned in this report appeared to us somewhat confusing.

The homologous regions to 19A, 19B and 18C of *C. tentants* are, in the case of *C. thummi*, regions d 1.1, d 1.2 and d 1, respectively (6). Besides having an evolutionary relationship, do these regions of *C. thummi* also exhibit the same activation characteristics as those of *C. tentans*? Specifically : are regions d 1.2 and d 1.1 sensitive to juvenile hormone and is region d 1 sensitive to ecdysone? From their course of activity during normal development one would assume that this is the case. For d 1 ecdysone sensitivity has already been established in experiments by Kroeger (7).

Table 1 summarizes the results we obtained by treating prepupae of *C. thummi* with juvenile hormone. The first three regions are the ones already mentioned ; they clearly fulfil our expectations in that

TABLE I. — Effect of juvenile hormone on puffing in salivary glands of *C. thummi*. Chromosome regions are from chromosome III except for BRb which is a Balbiani ring in chromosome IV. In the case of BRb "puff frequency" means frequency of active stages II or III.

Chromosome region	Number of experiments	Average puff frequency (%)		Difference (B)−(A)
		Control (A)	Juvenile hormone treated (B)	
d 1.2	22	50	79	+ 29 *
d 1.1	21	22	40	+ 18 *
d 1	21	64	43	− 21 *
a 1	13	27	47	+ 20
BRb	21	42	36	− 6

* $p < 0.01$.

d 1.2 and d 1.1 are activated and d 1 is inactivated by the action of juvenile hormone. Concerning the other two regions, although Laufer stated that a 1 becomes inactive and BRb active under the

influence of juvenile hormone (5), our observations do not support this statement. The behaviour during normal development clearly shows that a 1 is a juvenile puff in that its activity is positively correlated to the activity of d 1.2, the approved juvenile puff (regression coefficient, $b = +0.7$, $P_b = 0 < 0.01$). This is in contrast to BRb whose activity is negatively correlated to the activity of d 1.2 ($b = -0.6$, $P_b = 0 << 0.01$) but positively correlated to the activity of the prepupal puff d 1 ($b = +0.7$, $P_b = 0 \ll 0.01$). This comparison with normal development suggests that the juvenile hormone effect we observed is specific whereas Laufer's effect is not. This disagreement between our results and Laufer's might have something to do with differences in the mode of hormone application.

These differences become evident from the data compiled in Table II. In principle these differences are : 1. Laufer added the juvenile hormone to the culture water of the larvae where the hormone forms an oily

TABLE II. — Mode of hormone application and specificity of effect on puffing. Salivary gland chromosomes of *C. tentans* or *C. thummi*, T.U. = *Tenebrio* units

Author	Juvenile hormone active compound	Solvent	Application	Dose	Duration of standard experiment (h)	Puffs induced
Lezzi & Gilbert (3)	DL-juvenile hormone	olive oil	injected	2500–5000 T.U. (= 0.5–1 µg) per animal	4	juvenile
Lezzi & Frigg	juvenile hormone isomer mixture	acetone-olive oil	external	3 µg per animal	4	juvenile
Laufer & Holt (5)	farnesoic acid hydrochloride	acetone	added to larval culture water	0.03 µl per ml water	20	prepupal
Laufer & Greenwood (15)	juvenile hormone (DL?)	acetone	added to larval culture water	3000 T.U. per ml water	6–24	prepupal

film. We treated each larva individually with hormone. 2. As a rule, Laufer observed his effect only after a prolonged hormone treatment (20 hours) whereas our effect was already observable within 4 hours. 3. Laufer used lower hormone doses than we did. Assuming that the

hormone becomes evenly dispersed in the culture water he had a lower hormone concentration per ml water than we applied to a single larva.

One now might argue that the doses we used were unphysiologically high. Although in any of the above-mentioned hormone applications we do not know how much of the juvenile hormone actually arrives at the target cell, we frankly admit that the hormone concentrations used are probably higher than those occurring naturally in the animal. However, we should like to make the point that, unless the endocrine systems have been removed, the organism must be swamped with hormone. This is because the endocrine system tends to regulate the disbalanced hormonal situation introduced by the exogenous hormone. After a while, and if the doses applied were too small, the endocrine system might even overcompensate for the foreign hormone, resulting in an effect which is the reverse of that characteristic for the hormone originally administered. A similar backlash can be observed with ecdysone if one waits for a long enough time. For example, 15 to 24 hours after ecdysone injection the juvenile puff 19A reappears albeit it had disappeared in the first 4 hours of the hormone treatment (9). This reappearance can certainly not be considered as a specific effect of ecdysone, since region 19AB becomes activated after a short period of juvenile hormone treatment. In conclusion we may state that we were right in applying high doses of juvenile hormone and in studying only the early effects of juvenile hormone.

This brings up the question of whether the appearance of a specific puff is the very first effect of juvenile hormone or whether it is just the consequence of an event occurring prior to puff formation. We never actually believed that the induction of a puff is a primary action of juvenile hormone or ecdysone. This is not so much because of the lack of success with direct juvenile hormone or ecdysone application to isolated salivary gland chromosomes (8) but rather because of our general experience with isolated salivary glands (10).

As a matter of fact there are events occurring prior to puff formation which are clearly independent of any previous gene activation. These events are an increase in cell membrane permeability and, in consequence, an influx of sodium ions into the cell. This is concluded from electrophysiological measurements both on isolated salivary glands as well as on synthetic lipid membranes (11, 12).

The fact that juvenile hormone causes an increase of the intracellular sodium concentration is of great importance. This is because sodium ions if administered to isolated chromosomes are able to decondense specifically the juvenile hormone sensitive region ; this is the region 19AB of *C. tentans*. Region 18C, the ecdysone sensitive region, does not react ; it stays condensed. However, region 18C does become decondensed if isolated chromosomes are exposed to potassium instead of sodium ions. Potassium ions on the other hand, do not affect region 19AB (13). (At this point it should probably be mentioned that

electropotential measurements on isolated salivary glands tend to indicate an increase in the intracellular potassium ion concentration as a consequence to ecdysone administration (7).)

The results obtained on isolated chromosomes are in agreement with results obtained on isolated salivary gland nuclei (14). With isolated nuclei actual puffs can be induced by ions. In Table III the

TABLE III. — Classification of chromosome regions. JH = juvenile hormone. EC = Ecdysone. (See ref. 3, 4, 7, 13, 14.)

Species	Chromosome region	Hormone sensitivity *in vivo*	Ion sensitivity *in vitro* **
C. tentans	19AB	JH	Na^{+}***
	18C	EC	K^{+}***
	IV2B	EC	K^{+}
	BR 1	EC	K^{+}
C. thummi	d 1.2	JH	Na^{+}
	d 1.1	JH	Na^{+}
	a 1	JH	Na^{+}
	d 1	EC *	K^{+}

* Isolated glands.
** Isolated nuclei.
*** Isolated chromosomes.

hormonal sensitivity and the ionic sensitivity of 8 chromosome regions are compared. The point of this comparison is that all the regions which *in vivo* become activated by juvenile hormone action form a puff if isolated nuclei are incubated in a sodium-containing medium.

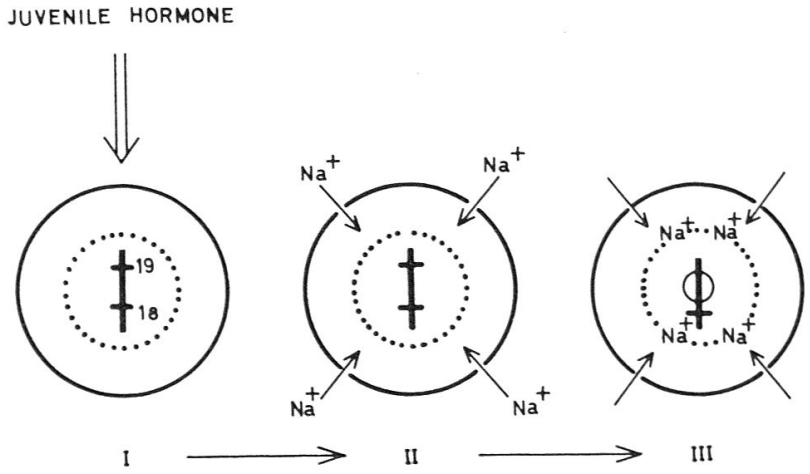

Fig. 2. — Mode of juvenile hormone action. Big solid circle = cell membrane. Stippled circle = nuclear membrane. Vertical bar = chromosome. Cross bars = inactive chromosome regions (19A and 18C of *C. tentans*). Small solid circle = active chromosome region (puff). I, II, III = sequence of event.

Whereas all the regions which *in vivo* react specifically to ecdysone respond in isolated nuclei to a potassium-rich medium. The correlation between juvenile hormone sensitivity and sodium sensitivity on one hand and ecdysone sensitivity and potassium sensitivity on the other hand is perfect and there is absolutely no reason at all to consider the ionic effects on puffing less specific than the hormonal effects. In the model shown in Figure 2 we have tried to combine all our present knowledge concerning juvenile hormone, ions and puffing. When juvenile hormone reaches the cell, the permeability of the cell membrane becomes increased. As a result, sodium ions rush into the cell. As soon as an appropriate Na^+-concentration in the cell nucleus is attained, region 19A or any other sodium sensitive region reacts by becoming active, that is by forming a puff. In this model neither a direct hormone-gene interaction nor a direct hormone-ion interaction is implied.

One last remark on the antagonism between juvenile hormone and ecdysone action : as we have already mentioned, ecdysone appears to cause an increase in the intracellular potassium ion concentration. It does so by an energy-requiring process. Our working hypothesis (Fig. 3) is that ecdysone stimulates the sodium pump which pumps potassium in and sodium out of the cell. It is obvious that juvenile hormone, by increasing the permeability of the cell membrane, antagonizes the work of ecdysone. However, since ecdysone and juvenile hormone do not attack exactly the same mechanism the antagonism between these hormones is not strict. One could easily imagine an intermediate situation where more Na^+ flows into the cell than is pumped out, but less K^+ flows out of the cell than is pumped in. This situation would lead to an accumulation of both sodium and potassium ions in the cell, resulting in the concomitant activity of both the sodium- and the potassium-sensitive chromosome regions.

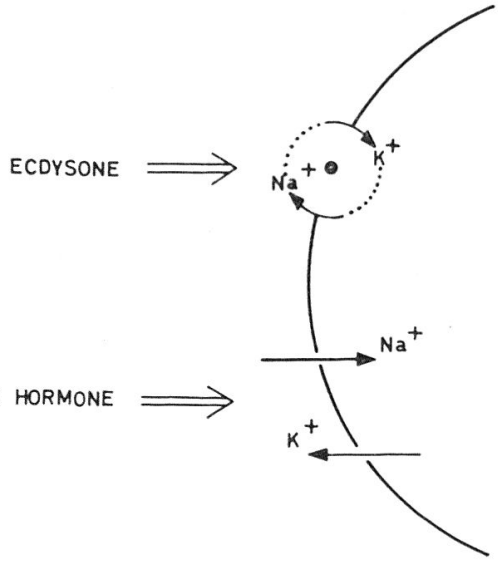

Fig. 3. — Antagonism between juvenile hormone and ecdysone action. A segment of the cell membrane is shown ; cell interior to the right. Circle with arrows = "sodium pump". Straight arrows = diffusion.

LITERATURE

1. WILLIAMS, C. M. (1961). "The juvenile hormone. II. Its role in the endocrine control of molting, pupation, and adult development in the *Cecropia* silkworm". *Biol. Bull.* **121,** 572–585.

2. SHAAYA, E. and P. KARLSON (1965). "Der Ecdysontiter während der Insektenentwicklung. IV. Die Entwicklung der Lepidopteren *Bombyx mori* L. und *Cerura vinula* L.". *Develop. Biol.* **11,** 424–432.
3. LEZZI, M. and L. I. GILBERT (1969). "Control of gene activities in the polytene chromosomes of *Chironomus tentans* by ecdysone and juvenile hormone". *Proc. Natl. Acad. Sci. U.S.* **64**, 498–503.
4. CLEVER, U. and P. KARLSON (1960). "Induktion von puff-Veränderungen in den Speicheldrüsenchromosomen von *Chironomus tentans* durch Ecdyson". *Exp. Cell Res.* **20,** 623–626.
5. LAUFER, H. and T. K. H. HOLT (1970). "Juvenile hormone effects on chromosomal puffing and development in *Chironomus thummi*". *J. Exp. Zool.* **173,** 341–352.
6. KEYL, H.-G. (1968). "Chromosomenevolution bei *Chironomus*. II. Chromosomenumbauten und phylogenetische Beziehungen der Arten". *Chromosoma* **13,** 486–514.
7. KROEGER, H. (1966). "Potentialdifferenz und puff-Muster. Elektrophysiologische und cytologische Untersuchungen an den Speicheldrüsen von *Chironomus thummi*". *Exp. Cell Res.* **41,** 64–80.
8. LEZZI, M., unpublished observation.
9. CLEVER, U. (1961). "Genaktivitäten in den Riesenchromosomen von *Chironomus tentans* und ihre Beziehungen zur Entwicklung. I. Genaktivierung durch Ecdyson". *Chromosoma* **12,** 607–675.
10. KROEGER, H. (1964). "Zellphysiologische Mechanismen bei der Regulation von Genaktivitäten in den Riesenchromosomen von *Chironomus thummi*". *Chromosoma* **15,** 36–70.
11. BAUMANN, G. (1968). "Zur Wirkung des Juvenilhormons : Elektrophysiologische Messungen an der Zellmembran der Speicheldrüse von *Galleria mellonella*". *J. Insect. Physiol.* **14,** 1459–1476.
12. BAUMANN, G. (1969). "Juvenile hormone : effect on bimolecular lipid membranes". *Nature* **223,** 316–317.
13. LEZZI, M. and L. I. GILBERT (1970). "Differential effects of K^+ and Na^+ on specific bands of isolated polytene chromosomes of *Chironomus tentans*". *J. Cell Sci.* **6,** 615–628.
14. LEZZI, M. (1967). "Spezifische Aktivitätssteigerung eines Balbianiringes durch Mg^{2+} in isolierten Zellkernen von Chironomus". *Chromosoma* **21,** 109–122.
15. LAUFER, H. and H. GREENWOOD (1969). "The effects of juvenile hormone on larvae of the dipteran, *Chironomus thummi*". *Am. Zool.* **9,** 603.
16. CLEVER, U. (1963). "Genaktivitäten in den Riesenchromosomen von *Chironomus tentans* und ihre Beziehungen zur Entwicklung. IV. Das Verhalten der puffs in der Larvenhäutung". *Chromosoma* **14,** 651–675.
17. CLEVER, U. (1962). "Genaktivitäten in den Riesenchromosomen von *Chironomus tentans* und ihre Beziehungen zur Entwicklung. II. Das Verhalten der puffs während des letzten Larvenstadiums und der Puppenhäutung". *Chromosoma* **13,** 385–436.

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