Dosage Compensation of X-linked Heat-shock Puffs
in Drosophila pseudoobscura

Dorothy A. Pierce and John C. Lucchesi

Department of Zoology, and Genetics Curriculum, University of North Carolina,
Chapel Hill, North Carolina 27514, U.S.A.

Abstract. Four major puffs are inducible by heat shock in the larval salivary
gland chromosomes of D. pseudoobscura. Two of these puffs are present
at 23 and 39–40 on the right arm of the X chromosome and two are present
at 53 and 58 on chromosome 2. By means of in situ hybridization, residual
homologies were demonstrated between the puffs at 23 in D. pseudoobscura
and at 63C in D. melanogaster, and between the two chromosome 2 puffs
of D. pseudoobscura and 87A and 87C of D. melanogaster. RNA synthesis
was monitored as a function of 3H-uridine incorporation in the major heat-
induced puffs of D. pseudoobscura and was found to be equivalent in males
and females indicating dosage compensation of the two X-linked loci. The
evolution of the regulatory controls of these genes is discussed.

Introduction

Dosage compensation and heat shock have been actively investigated in Drosophila
as model systems for studying the control of gene activity in eukaryotes
(see Lucchesi, 1977; Ashburner and Bonner, 1979). As a result of dosage compen-
sation, the activity of a single X chromosome in the male is equal to that
of both Xs in the female. This equalization has been detected, for all Drosophila
species studied, at the level of translated gene products or at the level of RNA
synthesis (Mukherjee and Beermann, 1965; Seecof et al., 1969; Abraham and
Lucchesi, 1974; Mukherjee and Chatterjee, 1975; Strobel et al., 1978). Since
compensation involves the whole X chromosome, it is a mechanism which
regulates the activity of 20 to 40% of the haploid genome. In contrast, the
heat shock response comprises a limited number of loci. During a brief exposure
to elevated temperatures (29–37°C), RNA synthesis is rapidly activated at a few
specific sites while a sharp reduction in transcription occurs along all other
chromosomal regions (Ritossa, 1964; Berendes et al., 1965; Ashburner, 1970).
This phenomenon has been observed in numerous Drosophila species (Lewis
et al., 1975), and seems to occur in every tissue, at every developmental stage,
and even in cells cultured in vitro (Tissieres et al., 1974; Spradling et al., 1975; McKenzie et al., 1975).

No X-linked heat shock loci have been identified in any of the *Drosophila* species studied to date, although, in some of these species, only heat-induced proteins were compared and the existence of X-linked loci has not been ruled out. We sought to enhance our chances of finding X-linked heat shock loci by selecting a species where an ancestral autosomal arm bearing such loci has become part of the X chromosome. In *D. pseudoobscura*, XL and XR are homologous to the telocentric X and to 3L of *melanogaster*, respectively (Lancefield, 1922; Sturtevant and Tan, 1937; Sturtevant and Novitski, 1941) and are fully compensated (Abraham and Lucchesi, 1974; Mukherjee and Chatterjee, 1975). In *melanogaster*, 3L contains four heat-inducible puffs, at 63C, 64F, 67B and 70A. The other major heat shock puffs of *melanogaster* are on 3R (87A, 87C, 93D, 95D), an arm which is homologous to chromosome 2 of *pseudoobscura* (Sturtevant and Tan, 1937; Sturtevant and Novitski, 1941).

Four major puffs are generated by heat shock in the larval salivary gland chromosomes of *D. pseudoobscura*. Two of these puffs are present on chromosome 2 and, as expected, two are present on XR. Using cloned cDNA fragments homologous to transcribed *D. melanogaster* heat shock sequences (Livak et al., 1978; R. Holmgren, pers. comm.) we have confirmed the homology of the *melanogaster* puff at 63C with the proximal puff on XR of *pseudoobscura*; the homology of sequences in both major heat-shock puffs on chromosome 2 of *pseudoobscura* and at 87A and 87C of *melanogaster* was also established.

We monitored RNA synthesis as a function of 3H-uridine incorporation in the four major heat-shock puffs of *D. pseudoobscura* and found it to be equivalent in males and females indicating dosage compensation of the two X-linked loci. The purpose of this paper is to report these results and to discuss their implications regarding the evolution of regulatory systems for genes which are coordinately controlled.

**Materials and Methods**

*Drosophila pseudoobscura* eggs were collected over an 8–12 h period, transferred to a thick yeast paste and allowed to develop at 18°. Third instar larvae were selected prior to spiracle eversion and classified by sex.

For studies on sequence homologies, in situ hybridizations to heat-shocked polytene chromosomes of *D. pseudoobscura* were performed, using 3H-cRNA transcribed from cloned *D. melanogaster* DNA fragments complementary to heat-shock mRNAs. These clones, pPW244 homologous to 63C and pPW 229 homologous to 87A and 87C sequences, were made available to us through the kindness of Drs. K.F. Livak, R. Holmgren, and M. Meselson. pPW 229 is characterized in Livak et al. (1978).

3H-cRNA was transcribed in vitro according to a procedure modified from Gall and Pardue (1971) and Wensink et al. (1974). The standard transcription mixture contained 5–10 units of *E. coli* RNA polymerase (Miles, or provided by Dr. D. Sittman) and 5 μg of DNA in a volume of 100 μl. Following incubation for 90 min at 37°, the reaction volume was brought to 0.5 ml with 40 mM Tris, pH 7.5, 5 mM MgCl₂, 50 μg of *E. coli* tRNA (Boehringer-Mannheim), 20 μg of RNase-free DNase (Worthington), and incubated for 30 min at 25°. The mixture was made 1.0% SDS, 50 mM EDTA and extracted twice with phenol. The aqueous phase was run onto a Sephadex G-50 column. The peak fractions of trichloroacetic acid – precipitable radioactivity