The Effects of Low Temperature and Colchicine on Chromosome Structure in Urodele Developmental Stages

I. A comparison of euchromatic and heterochromatic condensation patterns

Rosalyn M. Patterson and Ernest L. Hunt

Emory University, Atlanta, Georgia, U.S.A.

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Chromosome of *Trillium* and certain other species of plants have demonstrated less intense reaction of the heterochromatin to the Feulgen staining procedure than does the euchromatin when maintained at temperatures below 5°C (Darlington and LaCour 1941, Dyer 1963). This reaction of the heterochromatic DNA to low temperature has been demonstrated in a few urodele organisms, including *Triton* and *Ambystoma* (Callan 1941, Dyer 1963, Callan 1966). The temperature dependent heterochromatic reaction was originally interpreted as representing DNA deficiency in the Feulgen negative areas (Darlington and LaCour 1941). Recently this conclusion has been challenged through studies with autoradiography and microspectrophotometry; through these techniques it has been suggested that the Feulgen negative areas of the chromosomes are under spiralized areas rather than deficient in DNA (Woodard and Swift 1965, Woodard *et al.* 1966).

This investigation was initiated to evaluate the low temperature reaction of chromosomes in premetamorphic stages of urodele development. Evaluations were made of condensation patterns for euchromatin and heterochromatin following low temperature and colchicine exposure.

Materials and methods

The animals used in this investigation were premetamorphic stages of the urodele amphibians, *Ambystoma maculatum* and *Triturus viridescens*. *Ambystoma* embryos were collected from a local pond, *Triturus* eggs were obtained from females induced to ovulate by two injections of 0.3 cc of Antuitrin "S" (Parke, Davis and Co., Detroit, Michigan), two days apart. Embryonic and larval stages used for experimental study were maintained at 1°C to 5°C for periods of 3 to 7 days. Generally, the developmental stages were treated with 10^{-4} M colchicine solution for 1 to 2 days at low temperature and prepared for observation. Some stages were exposed to colchicine

concentrations up to 0.1%. Comparable stages of development were maintained at temperatures from 12°C to 22°C for control purposes, and some of these were treated with comparable concentration of colchicine. The period of exposure to colchicine was 12 to 24 hours for embryos maintained above 12°C. The jelly coats of the embryos were removed mechanically with curved needles.

Hypotonic pretreatment procedures were adapted to enhance the scattering of chromosomes for structural analysis. The procedures used were in some instances modifications of previously reported methods for amphibian chromosomes (DiBerardino 1962, Hennen 1963, 1964, Witschi and Laguens 1963). Blastula stages of *T. viridescens* were pretreated in deionized water for 6 to 8 minutes prior to fixation. Other stages were pretreated in 40% calcium and magnesium free Niu-Twitty solution followed by distilled or deionized water. The length of pretreatment time was gradually increased from 10 to 15 minutes for the gastrula stages, from 30 to 60 minutes for later stages. Fragments of the pretreated experimental and control stages were fixed in 45% acetic acid or in acetic alcohol (1:3); the fragments were prepared by the aceto-orcein and Feulgen squash methods (Darlington and LaCour 1962).

Observation and results

In this investigation the developmental stages of *A. maculatum* and *T. viridescens* were subjected to low temperature for periods sufficient to allow some of the cells to enter mitosis prior to fixation. A comparative analysis was then made for chromosomes from experimental stages and those maintained above 12°C. The chromosomes of *A. maculatum* produced better results than those of *T. viridescens* mainly because of larger chromosomes and more specific chromosome reaction to low temperature. The major advantage of *T. viridescens* was the readily available supply of eggs for experimental purposes.

In *A. maculatum* the neurula was the earliest stage investigated. Chromosomes in neurulae maintained above 12°C revealed metaphase chromosomes with no indication of understained areas in cells pretreated in colchicine (Fig. 1) and cells fixed without colchicine treatment (Fig. 2). *A. maculatum* neurula stages maintained at low temperature for more than three days contained obvious deficiently stained heterochromatic areas (Fig. 3). This reaction of the heterochromatin to low temperature formed a consistent pattern for most neurula chromosome complements observed. Some chromosomes within experimental cells lacked the heterochromatic regions, particularly the smallest within a complement. The larger chromosomes typically contained one to three understained regions. An increase in the colchicine exposure time resulted in a super contraction of the metaphase chromosomes, which considerably reduced the length of euchromatic and heterochromatic areas (Fig. 4). However, this increased contraction was not usually sufficient to

completely obscure the areas which react to low temperature. Similar results were obtained with chromosomes from tailbud stages as with neurulae (Fig. 5). In experimental tailbud stages some of the chromosome complements subjected to prolonged colchicine treatment contained the heterochromatic cold reactive areas, while other chromosome complements did not show the understained regions. The latter observations could have been made on chromosome complements which had not passed through DNA synthesis at the low temperature level. The supercontraction was noted in cold treated chromosomes which received prolonged colchicine exposure or intensified dosage of colchicine up to 0.1%. The extreme contraction phenomenon was not noted in the chromosomes maintained above 12°C when given comparable colchicine exposure. This suggests a synergistic relationship between low temperature and colchicine on chromosome condensation patterns.

A. maculatum tailfin and larval stages demonstrated cold reactive heterochromatic areas. A comparison of the amount and distribution of the reactive areas of these two stages with neurulae and tailbud stages suggested a progressive decrease in the

Figs. 1-5. 1, *Ambystoma maculatum* neurula chromosomes maintained above 12°C in colchicine for 12 hrs. Differentially stained areas are not noted. 2, *A. maculatum* neurula chromosomes maintained above 12°C without colchicine pretreatment. Note absence of differentially stained areas. 3, *A. maculatum* neurula stage chromosomes maintained below 5°C and treated with colchicine 2 days at the low temperature. Numerous differentially stained regions are present throughout the complement. 4, chromosomes of *A. maculatum* neurula stage exhibiting supercoiling reaction to prolonged colchicine treatment below 5°C. 5, tailbud stage of *A. maculatum*; chromosomes showing supercontraction reaction to conditions described for Fig. 4.

number of areas which respond to low temperature. In order to investigate the reaction to low temperature of early developmental stages, *T. viridescens* blastula through tailbud stages were subjected to the experimental procedures described for *A. maculatum*. The results of this investigation on heterochromatin quantitative distribution during development will be reported in a subsequent publication. Chromosomes from developmental stages of *T. viridescens* did show the super coiling effect of prolonged colchicine treatment and low temperature exposure as observed in *A. maculatum*.

Discussion

The major objective of this investigation involved observations on the reaction of chromosomes in amphibian developmental stages to low temperature and colchicine. The reaction of specific chromosomal areas to low temperature has been demonstrated in all premetamorphic stages studied for *A. maculatum* and *T. viridescens*. The genome complements of *A. maculatum* were used mainly for interpretation of the significance of the appearance of under stained segments after low temperature.

In *A. maculatum* an increase in the amount of colchicine treatment to 0.1% or prolonged colchicine exposure of two to three days at low temperature produced super contraction of the metaphase chromosomes. This resulted in a decrease in the relative length of the chromosome reducing the euchromatic areas and in some instances obscuring the heterochromatin. However, with nearly maximum euchromatic condensation, areas of apparent segmentation could be observed. These observations indicated reconsideration of previous interpretations on heterochromatic condensation patterns of low temperature maintained cells. Originally Darlington and LaCour (1941) had designated the cold reactive chromosomal areas of *T. erectum* as deficient in DNA. This interpretation was not challenged for several years, and other qualitative and quantitative data tended to support this conclusion (Callan 1941, LaCour *et al.* 1956, Evans 1956, Haque 1963). Further evaluation of these conclusions were made following the proposal of the DNA constancy hypothesis (Swift 1953). Recent evaluation of DNA quantities in *T. erectum* chromosomes revealed comparable amounts before and after cold treatment (Woodard and Swift 1964, Woodard *et al.* 1966). It was suggested that cold reactive segments were under spiralized areas within the chromosome rather than areas of DNA deficiency. An explanation has not been offered for the chemical and physical factors responsible for the uncoiled areas. If the areas are merely underspiralized, increased exposure to colchicine should eliminate the segments which remained visible.

Although the cold reactive chromosomal segments were reduced in length after prolonged colchicine exposure, this could have been due to euchromatic coiling which obscured the heterochromatic segmentation. Since quantitative measurements were not used in this investigation, an interpretation of results

cannot be offered on the basis of DNA quantity. However, comparison of these results with observations which have been made on late replication of heterochromatic DNA in mammalian cells (Lima-de-Faria 1959, Taylor 1960, Brown *et al.* 1966), has suggested a reinterpretation in regards to the behavior of heterochromatin in amphibian cells. It is postulated from data obtained in this investigation that replication of heterochromatin within the urodele chromosome is asynchronous and this asynchrony is accentuated by low temperature to the extent that its condensation is delayed into metaphase. Whether this delayed condensation is due to insufficient DNA quantity in the segmented area or related to the lack of other chemical or physical factors, e.g. histone bridge formation, could not be ascertained with present results. Smith (1965) has found in *Chilocorus* that various concentrations of colchicine produced differential coiling of euchromatic and heterochromatic areas of the chromosome. He noted that the contracted chromosome regions appeared double after colchicine treatment, while the heterochromatic areas had only one coiled axis within the same chromosome. This suggested either a delay in replication within the heterochromatin or a failure of separation of the replicated products.

Late replicating patterns of heterochromatin, particularly sex chromatin, are wide spread among mammals and have been reported for some avian organisms (Taylor 1960, Galton and Bredbury 1966). Although sexual dimorphism of nuclei has not been demonstrated in amphibians, this does not negate the comparison of allocyclic chromosome behavior between different vertebrate groups. The fact that allocyclic areas of chromosomes from amphibians and mammals may be described as relatively genetically inert, suggests a physical and biochemical relationship between the heterochromatin from these two groups.

Summary

An investigation has been made of the differential reactivity of chromosomes in urodele developmental stages after exposure to colchicine and low temperature. Embryonic stages of *Ambystoma maculatum* and *Triturus viridescens*, and larvae of *A. maculatum* were maintained at low temperature for specific periods of time. Colchicine was added to the experimental stages one to two days prior to fixation. All stages studied demonstrated a differential reactivity or differential staining of the heterochromatin after maintenance at the low temperature. Prolonged treatment of the chromosomes in colchicine at low temperature produced a supercoiling phenomenon which reduced or obscured the heterochromatic areas, but did not completely eliminate the segmentation. These observations are discussed in relationship to asynchronous replication of heterochromatin in mammalian cells.

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