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Effects of Temperature on Xenopus Cell Cycle

Abdul Aziz A. Al-Saleh
Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia.

An analysis of the duration of the cell cycle stages obtained from pulse/chase labelling experiments gave the following results: (1) at 18 °C G_1 lasts for 31 h, S for 29.5 h, G_2 for 8.5 h, M for 3 h and the total generation time is 72 h; (2) at 23 °C G_1 lasts for 14.3 h, S for 15.5 h, G_2 for 5.7 h, M for 0.5 h and the total generation time is 36 h; and (3) at 28 °C G_1 lasts for 11.3 h, S for 13.5 h, G_2 for 4.8 h, M for 0.4 h, and the total generation time is 30 h.

In eukaryotic cells DNA replicates during a particular period of interphase (Swift 1950; Walker and Yates 1952; Howard and Pelc 1953; Lajtha *et al.* 1954). This synthesis period, or S-phase, is usually preceded by a presynthetic gap (G_1) and succeeded by a postsynthetic gap (G_2) before the cell goes through its mitotic division (M). The above nomenclature was originally introduced by Howard and Pelc (1953).

The duration of G_1, S, G_2, and M vary during the development of an organism and also vary from one type of cell to another (Defendi and Manson 1963; Graham 1966; Graham and Morgan 1966; Callan 1973).

Changing the pH or altering the growth medium causes alterations of cell cycle duration mainly by changing the post mitotic period (Sisken and Kinosilta 1961; Sisken and Moraska, 1965; Tobey *et al.* 1967). The temperature at which cells are grown plays an important part in determining the distribution of the various parts of the cell cycle. An important general point is that all phases of the cycle change, the variation not being restricted to G_1 (Sisken 1963; Sisken *et al.* 1963; Rao and Endelberg 1966; Watanabe and Okada 1967; Chibon 1973).

There is not much information in the literature concerning the relationship between cell cycle duration and temperature in Amphibia. However, in the case of 34 embryo of *Plurodeles waltlii* it has been shown, for example, that in forelimb mesenchyme cells the S-phase lasts for 79 h at 12 °C and for 14 h at 26 °C (Chibon 1973).

The aim of the present study has been to determine the duration of all parts of the *Xenopus* somatic cell cycle in tissue culture cells at three different temperatures by means of the labelled mitoses method.

Materials and Methods

The experiments were carried out on an established cell line (A-6) of *Xenopus laevis*. The cells were grown at 18, 23, and 28 °C in a complete amphibian medium. Subcultures were started with 5×10^5 cells/ml of medium in 25 cm^2 plastic tissue culture flasks. Replicate subcultures at 23 and 28 °C were left for 48 h to reach logarithmic growth, while subcultures grown at 18 °C were left for 4 days. Cells in the log phase at 23 and 28 °C were labelled with 2 μCi/ml of ^{3}H-TdR (5 Ci/mM) for 30 min, while those at 18 °C were labelled for 1 h. After labelling, the radioactive medium was poured off, cells washed twice with nonradioactive medium, and then 5 ml of fresh medium containing TdR at 100 times the molarity of the previous ^{3}H-TdR medium was added to each flask and incubation continued.

At 3-h intervals (6 h in the case of cultures at 18 °C) one flask was removed, the cells were trypsinized, centrifuged and the pellet of cells resuspended in 0.076 M KCl. The cells were fixed directly on slides with 3 parts of absolute alcohol to 1 part glacial acetic acid, and air dried. The slides were filmed with NTB2, exposed for 5 to 6 days, developed, and stained for 1 h with Giemsa. Using these preparations, labelled and unlabelled metaphases were scored and the percentage of labelled metaphases plotted against time.

Table 1. The durations of cell cycles and their component phases as determined from pulse-labelled preparation obtained from *Xenopus* in culture at 18, 23, and 28 °C.

Temperature (°C)	$G_1+\frac{1}{2}M$ (h)	S (h)	$G_2+\frac{1}{2}M$ (h)	Tg (h)
18	32.5	29.5	10	72
23	14.5	15.5	6	36
28	11.5	13.5	5	30

Table 2. The durations of mitosis in *Xenopus* cells cultures at 18, 23, and 28 °C.

Temperature (°C)	No. of mitoses: Total no. of cells	Mitotic index MI	Generation time Tg (h)	Mitotic time M = Tg × MI × 1.44 (h)
18	29/1000	0.029	72	3.00
23	26/2667	0.010	36	0.50
28	13/1264	0.010	30	0.40

Results

Figure 1 shows the percentages of labelled metaphases in *Xenopus* kidney cells in culture at three different culture temperatures, 18, 23, and 28 °C as a function of time. These curves show the proportions of labelled metaphases, rise to a peak, then descend through a trough, and rise to a peak again. The ascending portion of the curve is a consequence of cells that were labelled at progressively earlier stages at the S-phase passing through G_2 and reaching mitosis. Theoretically the labelled frequency should reach 100%, but in practice this is not observed because of some cell-to-cell variability in cycle durations. The descending portion of the curve is a conscequence of cells that where in progressively earlier stages of G_1 at the time of labelling passing through the S and G_2 phases and reaching mitosis.

The time interval between the ordinate and midpoint or the ascending curve represents the duration of $G_2 + \frac{1}{2}M$ (where M = time spent in mitosis). Half mitotic time is added to G_2 because the mitoses are scored only in metaphase, which means that cells have to be through not only G_2 but also prophase. The width of the first wave from the midpoint of the ascending curve to midpoint of the descending curve measures the duration of the S-phase. The duration of the cell cycle, or generation time (Tg) is measured by the time interval between the first and second ascending curves, both taken at their midpoints. The duration of G_1 and the other half of the mitotic time may be determined by subtracting the total duration of $G_2 + \frac{1}{2}M$ plus S from the generation time (Mitchison 1971).

Table 1 has been constructed from the curves shown in Fig. 1. As can be seen in Table 1, durations of $G_1 + \frac{1}{2}M$, S, $G_2 + \frac{1}{2}M$, and Tg response to the change in the incubation temperature and increase as the temperature decrease.

Mitotic indices were determined for the cells growing at all three temperatures and these are shown in Table 2. One can calculate the approximate duration of mitosis

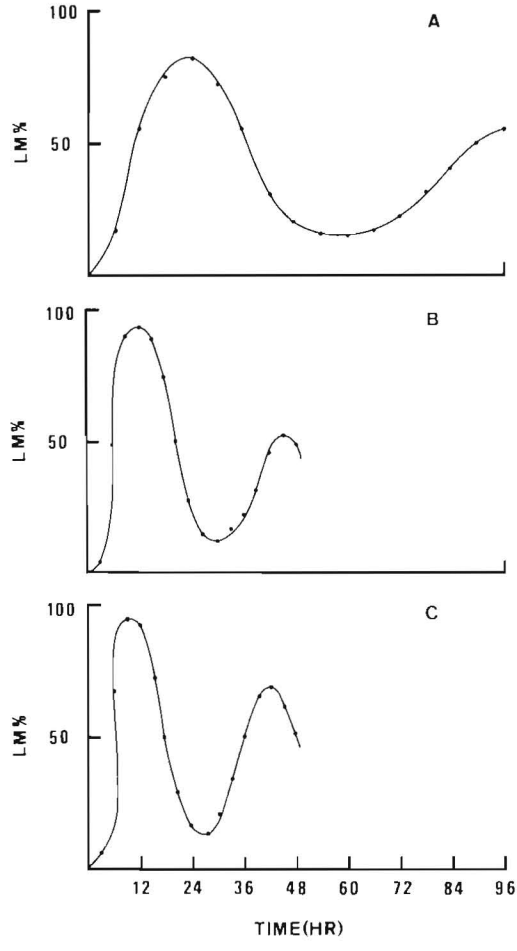

Fig. 1. Shows the percentages of labelled metaphases as a function of time, obtained from whole-cell autoradiographic preparations of *Xenopus* cell cultures at (A) 18, (B) 23, and (C) 28 °C.

from the following formula: time in mitosis = generation time × mitotic index × 1.44, provided mitosis is only a small fraction of generation time (Hughes 1952).

It will be seen from the mitotic indices that mitosis takes roughly 3 h at 18 °C, but only half an hour at 23 and 28 °C.

With the above information one can assess the duration of G_1 and G_2 more precisely by subtracting half the mitotic time from each; these values are given in Table 3. The G_1 takes 31, 14.3, and 11.3 h while G takes 8.5, 5.7, and 4.8 h at 18, 23, and 28 °C, respectively.

Table 3. The absolute durations of the component phases of *Xenopus* tissue culture cell cycles at 18, 23 and 28 °C after adjustment of the G_1 and G_2 phases for the time spent in mitosis.

Cell cycle parts	18 °C (h)	23 °C (h)	28 °C (h)
M	3.0	0.5	0.9
$G_1+\frac{1}{2}M$	32.5	14.5	11.5
G_1	31.0	14.3	11.3
$G_2+\frac{1}{2}M$	10.0	6.0	5.0
G_2	8.5	5.7	4.8
S	29.5	15.5	13.5
Tg	72.0	36.0	30.0

There is no firm information regarding an optimum culture temperature for amphibian cells in culture; Freed and Mezger-Freed (1970) have noted that amphibian cells (*Rana pipiens*) in culture grow rapidly at 25 °C but that temperatures above 28 °C are injurious, although this effect may only become apparent after a delay. Seto and Rounds (1968) point out that culture of frog (*Rana nigromaculata*) kidney cell strain and newt (*Taricha torosa*) lung cells grow best at 26 °C, are retarded below 22 °C, and inhibited at 37 °C. J.H. Priest and J.A.M. Cooper [unpublished, quoted in Callan (1972)] give an S-phase duration on the order of 13 h at 25 °C. These data, coupled with that of Priest and Cooper, would suggest that optimum temperature of the *Xenopus* kidney cells line must be in the neighbourhood of 25 °C.

Discussion

Mammals and birds have constant body temperatures and mammalian cells in culture grow best at 37 °C. Below or above this optimum temperature cell growth is impaired. The metabolic enzymes in mammals and birds have optimal temperatures in the range of 30–40 °C at which they are relatively stable (Hardy 1972). Although amphibia do not regulate their body temperature, it is likely that amphibian cells have growth temperature optima in tissue culture. Seto and Rounds (1968) mention that frog kidney cells (*Rana nigromaculata*) and newt lung cells (*Taricha torosa*) in tissue culture grow best at 26 °C, are retarded below 22 °C, and inhibited at 37 °C. It cannot be stated with assurance that *Xenopus* cells in culture do have an optimum temperature; it should be emphasized, however, that temperature influences the relative durations of the cell cycle. Chibon (1973) found

that the cell cycles of *Pleurodeles waltlii* embryonic cells are temperature dependent, and that the synthetic period decreases sharply between 12 and 17 °C but less so above 17 °C. In the experiments described here using *Xenopus* cells, a sharp decrease in the durations of G_1, S, G_2, and M is observed over a temperature increase from 18 to 23 °C, whereas from 23 to 28 °C only a slight decrease in durations of the cell cycle stages is observed. The increasing durations of the cell cycle as temperatures are reduced must in part be ascribed to the general property of chemical reactions that their rates are directly dependent on temperature. However, what must also be taken into account are the temperature optima of the various enzymes involved in cell "housekeeping" functions such as the synthesis of DNA, RNA, proteins, and their precursors. The disproportionate increase in mitotic time at 18 °C may well be due to an inhibition of spindle assembly (see Barber and Callan 1943). Rao and Englberg (1966) have similarly shown that the increase in the duration of mitosis in mammalian cells (HeLa cells) at low temperature is disproportionately greater than that of the other phases of the cell cycle.

The lengthening of G_2 as a consequence of low temperature may be due to a reduced rate of nonhistone protein synthesis, including those proteins that are necessary for chromosomal condensation (Anderson 1956; Swift 1964; 1965; Frenster 1965). Several studies indicate that such proteins associated with mitotic chromosomes are synthesized continuously throughout interphase (Prescott and Bender 1963; Prensky and Smith 1964; Chernick and Davidson 1968; Gerner and Humphrey 1973). Similarly, the lengthening of the G_1 period could be due to a reduction in the rate of synthesis of high-molecular-weight proteins (above 45,000 daltons); these latter are known to be synthesised at a particularly high rate in the late G_1 (Gerner and Humphrey 1973).

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تأثير درجة الحرارة على دورة الخلية لحيوان الزنوبس

عبد العزيز عبد الرحمن الصالح
قسم علم الحيوان ـ كلية العلوم ـ جامعة الملك سعود

قامت الدراسة باجراء تجارب عديدة لتحليل المراحل المختلفة لدورة الخلايا بطريقة ترقيم النبض الاشعاعى/المخفف وقد نتج عن الدراسة ما يلى :

ـ عند درجة الحرارة ١٨ درجة مئوية استمرت جـ ١ لمدة ١٤ر٣ ساعة ، س لمدة ٢٩ر٥ ساعة كما وأن جـ ٢ استغرقت ٨ر٥ ساعة ٠ وكذلك م فقد استغرقت ٣ ساعات وقد نتج عن كل ذلك دورة خلية كاملة فى ٧٢ ساعة ٠

ـ عند درجة الحرارة ٢٣ درجة مئوية استمرت جـ ١ لمدة ١٤ر٣ ساعة ، س لمدة ١٥ر٥ ساعة كما وأن جـ ٢ استغرقت ٧ر٥ ساعة وكذلك م فقد استغرقت ٥ر٠ ساعة وقد نتج عن كل ذلك دورة خلية كاملة فى ٣٦ ساعة ٠

ـ عند درجة الحرارة ٢٨ درجة مئوية استمرت جـ ١ لمدة ١١ر٣ ساعة ، س لمدة ١٣ر٥ ساعة كما وأن جـ ٢ استغرقت ٤ر٨ ساعة وكذلك م فقد استغرقت ٤ر٠ ساعة وقد نتج عن كل ذلك دورة خلية كاملة فى ٣٠ ساعة ٠