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Observational and Experimental Studies of Meiosis with Special Reference to the Bouquet Stage

XII. Experimental study of the nucleolar movement in *Salvinia*

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A remarkable movement of the nucleolus is observed in the bouquet stage in *Salvinia* spore mother cells (Hiraoka, 1948). In the present paper, the results of an experimental analysis of the factors necessary for the occurence of the movement will be briefly reported.

Material and Method

Intact spore mother cells of *Salvinia natans* in the bouquet stage, lying *in situ* in the sporangium were used as the material. The nucleolar movement was experimentally studied under the influence of various physical and chemical external agencies. The determinations of intracellular hydrogen ion concentration and redox potential were also carried out to know the physiological condition of the cells.

Observation

The nucleolar movement in the intact state: In the bouquet stage, the nucleus of the spore mother cell is hyaline except for the nucleolus, no chromosome threads being visible at all. The nucleolus, which is ellipsoidal in shape, locomotes smoothly in the nuclear cavity without changing its original shape. This locomotory movement may be interrupted for a while but is recovered again. Sometimes, the nucleolus makes a rotatory movement not more than one round round its axis and a transformation movement into shapes deviated from the original one (Hiraoka, 1948).

Experiments

When the spore mother cells in the bouquet stage, lying *in situ* in

the sporangium, are subjected to the influence of some physical and chemical agencies, the appearance of the nucleus, which is originally hyaline except for the nucleolus, may be changed reversibly from the original one. Thus, three types are discriminable as to the appearance assumed by the nucleus. In the first type, the nuclear cavity remains hyaline as in the original state, in the second type, the chromosome threads are visible occupying the whole nuclear cavity, and in the third type, the chromosome threads, which first become visible occupying the whole nuclear cavity, contract to the bouquet base to form a mass (cf. HIRAOKA, 1941). The nucleus of type II or III may become hyaline again, when it is set free from the influence of these external agencies.

Effect of hypertonic saccharose solutions: When 0.8, 1.0 and 1.2 M saccharose solutions are used as mounting mediums, the spore mother cells shrink. The nucleus at first assumes the appearance of type II, and then that of type III owing to dehydration. In the nucleus of the former appearance, the nucleolus continues to locomote in the nuclear cavity, but in the nucleus of the latter appearance, the nucleolus becomes motionless, that is, the nucleolar movement is inhibited completely. The inhibition of the movement takes place about 40 minutes after the mounting with the solutions (latent time). This inhibition is cancelled reversibly when the solutions are replaced with pond water and the nucleus comes to assume the appearance of type II or I.

Effect of di-distilled water: Di-distilled water does not affect the nucleolar movement at all.

Effect of high and low temperatures: When the sporangia mounted with a drop of liquid paraffin are heated at the constant temperatures of 30.3 and 32.5° C,[1] the nucleus of the spore mother cell takes the appearance of type II and the nucleolar movement is inhibited completely. The nucleolus becomes motionless in about 50—60 minutes after the heating. The nucleolar movement is recovered reversibly when the sporangia are kept again at the room temperature (19.5—21.6° C). At any temperatures lower than 28.5 and higher than 7.0° C, the nuclear cavity is hyaline (type I) and the nucleolus continues its active movement in the cavity. At 6.5° C,[2] the nucleus takes the appearance of type I or II and the nucleolus becomes motionless. This inhibition of the movement is cancelled reversibly when the sporangia are kept again at the room temperature.

1) "Heizbarer Objekttisch" of E. Leitz was used.

2) Cooling apparatus for microscopic use after MIYOSHI (1932) was used.

Effect of acetic acid: 1×10^{-2} M (pH 3.5), 2×10^{-3} M (pH 4.0), 1×10^{-3} M (pH 4.2) and 1×10^{-4} M (pH 4.6) acetic acid solutions were used as mounting mediums. The nucleus of the spore mother cell, at first, assumes the appearance of type II and then that of type III. In the nucleus of type II, the nucleolus continues to locomote in the nuclear cavity. In the cases where 1×10^{-2} M, 2×10^{-3} M and 1×10^{-3} M solutions are used as the mediums, the nucleolar movement is inhibited completely in the nucleus of type III, that is, the nucleolus is motionless when it is found in the mass of chromosome threads, and shows only a vibratory movement of small amplitude when it is free from the chromosome mass. In the case where the 1×10^{-4} M solution is used as the medium, the nucleolar movement is inhibited incompletely in the nucleus of type III, that is, the nucleolus does no longer make a smooth locomotory movement but locomotes vibrating irregularly in the nuclear cavity. The latent time of the inhibition of the movement to acetic acid is about 6 minutes for 1×10^{-2} M, 30 minutes for 2×10^{-3} M, 60 minutes for 1×10^{-3} M and 360 minutes for 1×10^{-4} M solution. The inhibition of the movement due to acetic acid is reversibly cancelled when the acetic acid solutions are replaced with pond water (pH 6.8), and the nucleus assumes the appearance of type II or I.

Effect of ammonium hydroxide: When 2×10^{-2} M (pH 11.0) and 1×10^{-2} M (pH 10.4) ammonium hydroxide solutions are used as mounting mediums, the nucleus of the spore mother cell assumes the appearance of type I or II and the nucleolar movement is inhibited completely. The inhibition takes place about 20 minutes after the mounting with the former solution and 40 minutes after the mounting with the latter solution. The inhibition is cancelled reversibly when the solutions are replaced with pond water. In a 2×10^{-3} M solution, the nucleolar movement is no longer inhibited by ammonium hydroxide.

Effect of hydrogen gas atmosphere: When the sporangia mounted with a drop of pond water are kept in a hydrogen gas atmosphere, the nucleus of the spore mother cell takes the appearance of type I. The nucleolus becomes completely motionless within 5 minutes after the air is replaced by hydrogen gas. This inhibition of the nucleolar movement is reversibly cancelled when the hydrogen gas is replaced with air.

Hydrogen ion concentration and redox potential of cell interior:

The hydrogen ion concentration of the cytoplasm was studied by a method of staining the fresh spore mother cells in the bouquet stage

with indicator dyestuffs. The results obtained are given in the following table (Table 1). The hydrogen ion concentration of the cytoplasm of the spore mother cells is determined to be pH 5.6—5.8. This value is quite in accord with that described by SMALL (1929) as the reaction value of the plant cytoplasm.

Table 1

Dyestuffs	Coloration of cytoplasm	pH range	
Methyl orange	Yellow		>4.4
Brom phenol blue	Blue		>4.6
Brom cresol green	Blue		>5.2[1]
2-6-dichlorphenol indophenol	Blue		>5.6
Methyl red	Orange yellow	5.8>	>5.6
Brom cresol purple	Yellowish brown	6.0>	>5.2
Brom thymol blue	Greenish yellow	6.0>	
Phenol red	Yellowish brown	6.6>	
Neutral red	Red	6.8>	

The aerobic redox potential of the cytoplasm was also studied by a method of staining the fresh spore mother cells with several redox dyestuffs. The results obtained are summerized in Table 2. As shown in this table, the redox potential of cell interior is more positive than −0.072 volt and more negative than +0.056 volt provided that the pH of cell interior is 5.8 and that the temperature is 30° C (WURMSER, 1930). The pink coloration of Janus green gives more accurate information about the rH value of cell interior. In view of the fact that Janus green turns pink at +0.024 volt at pH 5.8, the redox potential in question is inferred to be approximately +0.024 volt, that is, rH 12.6. This rH value coincides with that obtained by CHAMBERS, POLLACK and COHEN (1929) in *Amoeba* and in some marine ova (cited from MICHAELIS, 1933).

Table 2

Dyestuffs	Coloration of cytoplasm	
	Oxidized colour	Reduced colour
Bindschedler's green		Pale yellow or colourless
2-6-dichlorphenol indophenol		Colourless
New blue		Colourless
Thionin		Colourless

1) The nucleus is stained in somewhat alkaline colour than the cytoplasm (cf. YAMAHA, 1938).

Brillant cresyl blue			Colourless
Toluidine blue			Colourless
Methylen blue			Colourless
Azur I			Colourless
Nile blue	Blue		
Indigocarmine	Blue		
Cresyl echt violet	Violet blue		
Phenosafranin	Red		
Janus green [1]		Pink	
Neutral red	Red		
Neutral violet extra	Red		

Effect of low oxygen tension of the medium: Mediums of known oxygen tension were prepared by an addition of some amount of a 2×10^{-3} M sodium hydrosulfite solution [2] to pond water containing a small amount of redox dyestuffs. The addition was continued till each dyestuff became just bleached (PANTIN, 1930). The medium in which brillant cresyl blue is used as redox dyestuff has a redox potential of ca. +0.040, that in which methylen blue is used a redox potential of ca. +0.017 and that in which neutral red is used a redox potential of ca. −0.329 volt at pH 6.8. When the sporangia are mounted with these mediums, the nucleus of the spore mother cell remains hyaline except for the nucleolus (type I). In the first medium, the nucleolus continues to move smoothly in the nuclear cavity, but in the second and the third mediums, the nucleolar movement is completely inhibited. The latent time of the inhibition to low oxygen tension is 20 minutes in the second medium and only 10 minutes in the third. The nucleolar movement is reversibly recovered when the second and the third mediums are replaced with pond water.

Conclusion

Under the influence of hypertonic sugar solutions and acetic acid, the nucleolar movement is inhibited reversibly when the chromosome threads, which have first become visible occupying the whole nuclear cavity, begin to contract to the bouquet base to form a mass (type III). The latent time of the inhibition of the movement to these external agencies is about 40—60 minutes, when the solution of the

1) Two step reduction occurs in this dyestuff.

2) In a 2×10^{-3} M sodium hydrosulfite solution, whose reaction is adjusted at pH 6.8, the nucleus of the spore mother cell assumes the appearance of type I and the nucleolar movement is reversibly inhibited within 5 minutes after the mounting with the solution.

lowest critical concentration is used to induce the inhibition. These two points may imply that these external agencies induce the inhibition by changing the colloidal state of the karyoplasm from the normal one.[1)]

Hydrogen gas atmosphere and mediums of low oxygen tension bring about the inhibition of the movement while the nuclear cavity remains hyaline as in the normal state (type I). The inhibition of the movement occurs very rapidly (within 5 minutes) in a hydrogen gas atmosphere and rapidly (within 20 minutes) after the sporangia are mounted with the medium of the highest critical oxygen tension to induce the inhibition. In view of the facts that there exists a negative limit of redox potential of the medium beyond which the nucleolar movement is no longer carried on and that the limit nearly coincides with the potential of cell interior, these external agencies induce the inhibition of the nucleolar movement by inhibiting some oxidation process in the cell. Though no conclusive evidence to decide whether the inhibition of the nucleolar movement induced by high and low temperatures and ammonium hydroxide is due to the colloidal change in the karyoplasm or to the inhibition of the oxidation process was obtained, we may say that there exist at least two separate and independent factors indispensable for the occurence of the nucleolar movement. One is a hydrated state of the karyoplasm and the other is the oxidation process in the cell, which may liberate the energy necessary for the occurence of the movement.

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1) That the nucleus in the bouquet stage is in a highly hydrated state is inferable from the facts that the nuclear volume increases remarkably in this stage (BEASLEY, 1938) and that "vitale Entmischung" of the karyoplasm tends to occur in this stage (BELAR, 1930).