Kinetics similar to those for temperature-induced bleaching were observed by SCHIFF *et al.*[6] for the loss of photoreactivability of ultraviolet-inhibited chloroplast replication in Euglena. Their calculations indicate the loss by dilution of 10–12 particles which happens to coincide with the number of chloroplasts. We have noted a precipitous drop in the number of targets responsible for ultraviolet-induced bleaching during growth in the light at 32° as well as a proportional drop in photoreactivability. We are investigating the possible relation between the loss of ultraviolet-sensitive sites and the sites of light-dependent temperature-induced bleaching. Cells derived from heat-bleached cultures have been shown to lack the characteristic satellite DNA that has been attributed to the chloroplast[7]. On the other hand, it has been shown[8] that heat-bleached cells can incorporate δ-aminolevulinic acid and form fluorescent sites. Whether these sites represent proplastid-like structures remains to be shown.

We are continuing to examine the effects of elevated temperature on the ability of Euglena to form green colonies and the role of light in these effects. A detailed action spectrum of the light effect is in progress coupled with biochemical analyses to identify the cellular sites involved in temperature-induced bleaching. An electron and fluorescence microscopic examination of cells during temperature-induced bleaching is also in progress.

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The identity of X in $F_1 \cdot X$ with F_c

Recently VALLEJOS *et al.*[1] described the isolation and the properties of a coupling factor which they called $F_1 \cdot X^*$. This soluble factor, which contains a Mg^{2+}-dependent, oligomycin-insensitive and cold-labile ATPase, resembles very much the soluble ATPase F_1, described by PULLMAN *et al.*[2]. The major difference between the two

* VALLEJOS *et al.*[1] called the factor F_1–X. Since, however, this might imply a covalent bond between F_1 and X, we prefer to refer to it in this paper as $F_1 \cdot X$, the central dot implying an association between F_1 and X.

factors is that $F_1 \cdot X$ has a much higher coupling-factor activity than F_1. VALLEJOS *et al.*[1] concluded that in addition to F_1 there was another cold-stable factor present in their $F_1 \cdot X$ preparation. We have studied the nature of this additional factor X, specially in relation to the reconstruction of the oligomycin-sensitive mitochondrial ATPase.

When F_1 or $F_1 \cdot X$ is incubated with ASU-particles[3] the ATPase activity of both factors is rendered sensitive to oligomycin. However, when the particles are depleted of F_0 activity* by treatment with ammonia[5], yielding ASUA-particles, F_1 no longer recombines (Table I). The low (10%) oligomycin sensitivity probably represents incomplete removal of the F_0 activity from the particles. $F_1 \cdot X$, however, does recombine with these particles as can be seen from the partial restoration of the oligomycin sensitivity. Storage of $F_1 \cdot X$ at 0° leads to an inactivation of the ATPase activity, but the activity of the additional factor X is not affected[1]. As can be seen from Table I, the restoration of the oligomycin sensitivity of F_1 is increased, when increasing amounts of cold-treated $F_1 \cdot X$ are added.

TABLE I

RECONSTRUCTION OF THE OLIGOMYCIN-SENSITIVE ATPase

F_1 (8 μg) or $F_1 \cdot X$ (7 μg), both prepared as described by VALLEJOS *et al.*[1], was preincubated with 200 μg ASUA-particles at 30° in 0.8 ml medium containing 25 μmoles sucrose, 5 μmoles Tris–HCl (pH 7.5) and 1 μmole $MgCl_2$. The reaction was started by the addition of 0.2 ml containing 5 μmoles ATP, 5 μmoles phosphoenolpyruvate, 6 μg pyruvate kinase, 20 μmoles Tris–HCl (pH 7.5) and 2 μmoles $MgCl_2$. After 10-min incubation the reaction was stopped by the addition of 1.0 ml 10% trichloroaceticacid. P_i released was determined according to Fiske and SubbaRow, as described by SUMNER[9]. Cold-treated (2 h at 0°) $F_1 \cdot X$ and oligomycin (5 μg) were added during the preincubation.

Additions	*P_i released (μmoles/10 min)*		*Inhibition (%)*
	− Oligomycin	*+ Oligomycin*	
F_1	1.26	1.14	10
$F_1 \cdot X$	1.47	0.73	50
F_1 + cold-treated $F_1 \cdot X$ (5 μg)	1.28	0.98	24
F_1 + cold-treated $F_1 \cdot X$ (7.5 μg)	1.27	0.89	30
F_1 + cold-treated $F_1 \cdot X$ (15 μg)	1.26	0.65	49

In Fig. 1 the effect of cold-treated $F_1 \cdot X$ on the cold lability of F_1 is shown. F_1 is inactivated during storage at 0° (ref. 6), and incubation of F_1 with ASUA-particles does not protect F_1 against cold treatment. Only the small part of the ATPase that is sensitive to oligomycin is unaffected by the cold treatment. However, when cold-inactivated $F_1 \cdot X$ is present, the ATPase of F_1 becomes sensitive to oligomycin and cold-stable.

From these experiments it is clear that the unknown factor, present in $F_1 \cdot X$, is necessary for the restoration of oligomycin sensitivity and cold stability of F_1 in the presence of particles depleted of F_0 activity. It can be concluded that X is identical

* F_0 activity is defined as the ability to confer oligomycin sensitivity on the ATPase activity of F_1 (ref. 4).

with F_c, as described by BULOS AND RACKER[4], or with the oligomycin sensitivity conferring protein, as described by TZAGOLOFF *et al.*[5], MACLENNAN AND TZAGOLOFF[7] and MACLENNAN AND ASAI[8].

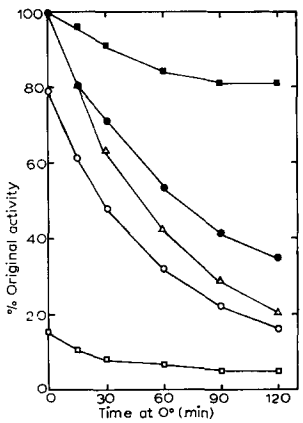

Fig. 1. Restoration by cold-treated $F_1 \cdot X$ of cold stability and oligomycin sensitivity of F_1. In a volume of 1.5 ml 120 μg F_1 and 3 mg ASUA-particles were mixed in the presence or absence of 225 μg cold-treated $F_1 \cdot X$ as described in Table I. After a preincubation of 5 min at 30°, these mixtures were chilled to 0° and at the times indicated a sample of 0.1 ml was removed to determine the ATPase activity, as described in Table I. △—△, F_1; ●—●, ASUA-particles + F_1; ○—○, ASUA-particles + F_1 + oligomycin (5 μg); ■—■, ASUA-particles + F_1 + cold-treated $F_1 \cdot X$; □—□, ASUA-particles + F_1 + cold-treated $F_1 \cdot X$ + oligomycin (5 μg).

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