Deoxyribonucleoside Triphosphate Pools in Vitamin B-12-Deficient *Euglena gracilis*

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The size of the deoxyribonucleoside triphosphate pools of vitamin B-12-deficient cells of *Euglena gracilis*, and of vitamin B-12-deficient cells repleted with the vitamin, were measured. We found that the pools were very small, if they exist at all, in deficient cells but expand rapidly with the addition of the vitamin. The sizes of the pools decrease when DNA synthesis is completed, and are very small when the cells begin to divide.

Euglena gracilis when grown in sub-optimal amounts of vitamin B-12 undergoes morphological and biochemical alterations (Carell, 1969; Carell *et al.*, 1970) that are characteristic of cells in which the growth cycle and the DNA synthesis–cell division cycle have been uncoupled (Mitchison, 1971). The amounts of RNA and protein increase severalfold, the cells become enlarged, and the S phase of the cell cycle becomes extended (Carell *et al.*, 1970). This unbalanced growth condition is reversed by the replenishment of the vitamin B-12-deficient culture (Johnston & Carell, 1973) with the vitamin (reversion). DNA synthesis resumes at normal rates, and the cells undergo two relatively synchronous cell divisions (Johnston & Carell, 1973; Goetz *et al.*, 1974).

Studies *in vitro* have shown that *Euglena gracilis* Z strain has a vitamin B-12-dependent ribonucleotide reductase, though with extremely low activity (Gleason & Hogenkamp, 1972). Nevertheless, we reasoned that the diminishing rate of DNA synthesis and the lengthening of the S phase when the cultures progress into deficiency (Christopher *et al.*, 1974) could be due to a shortage or absence of the deoxyribonucleoside triphosphates (dNTP). In the present paper we provide evidence that the dNTP pools are very small in vitamin B-12-deficient cells, but expand rapidly on the addition of vitamin B-12.

Materials and Methods

Euglena gracilis Klebs strain Z (Pringsheim) cells were grown in sub-optimal amounts of vitamin B-12 (25 ng/l) until they reached advanced deficiency, as described previously (Carell, 1969). Vitamin B-12 was added to the cultures (10 µg/l) at zero time, and samples were taken at indicated times for the different determinations. DNA was determined by the diphenylamine method of Burton (1955). Cell number was determined with a Coulter counter. The dNTP

pools were extracted by using 0.8 M-KOH and 0.16 M-KH₂PO₄, rather than 0.8 M-KOH and 0.12 M-NaHCO₃ as described by Bagnara & Finch (1972). The pool sizes were determined by using ³H-labelled deoxyribonucleoside triphosphates (New England Nuclear Corp., Boston, MA, U.S.A.) in the assay rather than ¹⁴C-labelled deoxyribonucleoside triphosphates as described by Solter & Handschumacher (1969). The DNA polymerase was purchased from Miles Laboratories (Elkhart, IN, U.S.A.).

Discussion

Table 1 shows that the pools of the dNTP are very small (if they exist at all) in deficient cells, and that they appear rapidly on the addition of vitamin B-12, reaching high values by the end of the first hour. The dATP, dCTP and dGTP reach their peak values at this time. The dTTP reaches its peak value after 3 h, which corresponds to the completion of the S phase (Goetz *et al.*, 1974). The dNTP pool sizes increase in an almost linear manner from 20 min to 1 h after vitamin B-12 replenishment. After the first hour they fluctuate, as they do in other systems (Nexo, 1975; Skoog *et al.*, 1973).

The pool of dTTP is always the largest in reverting *Euglena*. This is also the case in *Tetrahymena pyriformis* (Nexo, 1975). The pool sizes we obtain during the completion of the S phase in reverting cells are larger than those of *T. pyriformis* (Nexo, 1975) or Chinese-hamster cells (Skoog *et al.*, 1973). However, in *Euglena* the dGTP pool, as in Chinese-hamster cells (Skoog *et al.*, 1973) and human bone-marrow cells (Hoffbrand *et al.*, 1974), is always the smallest.

Table 1 also shows that the amount of DNA/ml of culture increases after the addition of vitamin B-12 and then levels off before the end of the third hour. At the end of S phase (3 h) the pool sizes remain large, a phenomenon which was also reported to occur in

Table 1. Deoxyribonucleoside triphosphate pools in reverting *Euglena gracilis* cells

Experimental conditions were as described in the text.

Time (min)	Deoxyribonucleoside triphosphate pool size (pm/10 ⁶ cells)				DNA/ml (μ g)	10 ⁻⁶ × Cells/ml
	dATP	dCTP	dGTP	dTTP		
0	0	0	0	3	52	1.04
5	0	0	0	10	—	—
10	0	0	0	8	—	—
20	16	6	5	38	—	—
30	38	47	20	92	—	—
45	78	81	35	140	—	—
60	126	99	50	154	58	—
120	96	90	27	150	68	1.04
180	125	95	14	194	71	1.04
240	45	47	11	164	71	1.16
300	0	0	0	11	75	1.35

Chinese-hamster cells (Skoog *et al.*, 1973) and in *T. pyriformis* (Nexo, 1975). However, after the completion of S phase, the pool sizes decrease and reach a minimum as the cells start to divide at 4h. By the fifth hour the dATP, dCTP and dGTP pools become undetectable, although with our method we could detect pool sizes as small as 2pm/10⁶ cells. On the other hand there is a measurable pool of dTTP at that time. Such a rapid disappearance of the dNTP pools does not occur in *T. pyriformis* (Nexo, 1975) or in the Chinese-hamster cells of Skoog *et al.* (1973), although Walters *et al.* (1973) have observed such an event in their Chinese-hamster cells. They report that degradation begins in phase G₂ and that cells in early phase G₁ have very small or no dNTP pools.

Vitamin B-12 deficiency in *Euglena gracilis* is similar in many ways to megaloblastic anaemia in man. Megaloblasts have an extended S phase (Yoshida *et al.*, 1968), as do vitamin B-12-deficient *Euglena* cells. Megaloblasts are large and have increased amounts of RNA and protein but less than the 4C (double) amount of DNA (Lajitha & Oliver, 1960; Davidson *et al.*, 1948). Vitamin B-12-deficient *Euglena gracilis* shows the same characteristics (Carell *et al.*, 1970). The chromosomes are abnormally elongated and diffuse in both cases. Vitamin B-12 replenishment corrects this condition.

The ribonucleotide reductase of humans is vitamin B-12-independent (Millard, 1972), whereas the ribonucleotide reductase of *Euglena* is vitamin B-12-dependent (Gleason & Hogenkamp, 1972). The pools of the dNTP in megaloblasts are at least of normal size (Hoffbrand *et al.*, 1974), whereas the pools of dNTP in vitamin B-12-deficient *Euglena* are essentially non-existent, but appear within 20min on the

replenishment with the vitamin. These results suggest that under the experimental conditions described here there may be no vitamin B-12-independent ribonucleotide reductase in *Euglena* Z strain. The apparent absence of the pools of dNTP from the vitamin B-12-deficient cells, and their appearance on replenishing the cells with the vitamin, suggest that the ribonucleotide reduction may be the limiting step in DNA synthesis.

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