more, the 30s ribosomes from spheroplasts resemble those from intact cells in being able to adsorb RNase (Table 5).

Summary.—Most of the so-called "latent" ribonuclease of *E. coli* can be released from spheroplasts in 5 min although the spheroplast ribosomes remain intact as demonstrated by RNA content, sucrose-density gradient profiles, and ability to adsorb soluble RNase. Ribosomes are able to adsorb up to a 12-fold excess of soluble RNase even under dissociated conditions. All of the RNase binds to 30s particles. Spheroplasts prepared by lysozyme alone release neither alkaline phosphatase nor RNase, but nearly half of the alkaline phosphatase can be removed by washing EDTA-treated *E. coli*, although other enzymes are not released.

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ROLE OF FERREDOXIN IN PHOTOSYNTHETIC PRODUCTION OF OXYGEN AND PHOSPHORYLATION BY CHLOROPLASTS

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It was previously reported from this laboratory[1, 2] that: (1) ferredoxin, the red, iron-containing protein native to chloroplasts, has a redox potential about 100 mV more electronegative than pyridine nucleotide and is, therefore, the strongest,

chemically defined reductant isolated from the photosynthetic apparatus of plants; (2) ferredoxin is photochemically reduced by grana; and (3) ferredoxin catalyzes photosynthetic phosphorylation by chloroplasts, without the addition of other cofactors.

We have concluded from these findings that photoreduction of ferredoxin may be regarded as the terminal photochemical event in the conversion of radiant energy into chemical energy by chloroplasts. All subsequent photosynthetic reactions in chloroplasts would, accordingly, be thermochemical events independent of light. Thus, what was formerly described as the photoreduction of TPN by isolated chloroplasts has now been experimentally separated into a photoreduction of ferredoxin followed by two dark reactions: the reduction of a flavoprotein (ferredoxin-TPN reductase) by reduced ferredoxin and the subsequent reduction of TPN by the reduced flavoprotein.[3, 4]

If photoreduction of ferredoxin is indeed the terminal photochemical event in chloroplasts, then it must be linked not only to photophosphorylation but also to oxygen evolution. The evolution of oxygen by chloroplasts is uniquely dependent on light, and it occurs only in the presence of a proper electron acceptor. Thus, photoproduction of oxygen by chloroplasts should accompany the photoreduction of ferredoxin. Such direct demonstration, however, has not been sought until now because it did not seem experimentally feasible. Reduced ferredoxin is readily auto-oxidizable, and hence a rapid back reaction between reduced ferredoxin and evolved oxygen was expected.[1, 2]

In the experiments which we now report, the photoproduction of oxygen by isolated chloroplasts was found to be linked to the photoreduction of ferredoxin. With "substrate" amounts of added ferredoxin, and in the absence of Hill reagents or TPN, the stoichiometry between the ferredoxin reduced and the O_2 produced was 4 to 1.

Photoreduction of ferredoxin was coupled with oxygen evolution at wavelengths of light shorter than 700 mμ, i.e., in light that is absorbed by both chlorophylls *a* and *b*. At 714 mμ, a wavelength of light which is not absorbed by chlorophyll *b* and which cannot support oxygen evolution by chloroplasts,[5-7] complete photoreduction of added ferredoxin still occurred, but only in the presence of an artificial electron donor system. Thus, the photoreduction of ferredoxin itself, without oxygen evolution, depends only on the photoactivity of the chlorophyll *a* pigment system. But the photoactivity of both chlorophyll *a* and *b* pigment systems is required if the photoreduction of ferredoxin is to be accompanied by oxygen evolution.

Further evidence will be reported on the role of ferredoxin in the two main types of photosynthetic phosphorylation which occur in chloroplasts: (1) cyclic photophosphorylation, in which catalytic amounts of ferredoxin bring about, anaerobically, an accumulation of ATP, and (2) noncyclic photophosphorylation, in which ATP formation is stoichiometric with the reduction of "substrate" amounts of ferredoxin. Evidence will also be presented that ferredoxin catalyzes a pseudocyclic photophosphorylation,[8] i.e., a variant of noncyclic photophosphorylation in which oxygen is being continuously evolved as ferredoxin is reduced but is immediately consumed as the reduced ferredoxin is reoxidized.

The present experiments strengthen the view that, under physiological conditions, the energy conversion process in chloroplasts terminates with the photore-

duction of ferredoxin and yields, aside from reduced ferredoxin, ATP, and molecular oxygen. The widely used cofactors of photophosphorylation (such as menadione, FMN, phenazine methosulfate) and the electron acceptors (Hill reagents such as ferricyanide and benzoquinone) needed to demonstrate oxygen production by isolated chloroplasts now appear to be nonphysiological substitutes for ferredoxin.

Methods.—Oxygen evolution was measured polarigraphically by Hagihara's closed cell method.[9] The polarizing voltage was 0.7 or 0.8 V. The recorder was first calibrated by tracing the oxygen removed from the solution by a dilute suspension of yeast. In the experiments concerned with the reduction of ferredoxin and the evolution of oxygen, all vessels were flushed and solutions gassed with argon prior to use. All measurements were carried out under conditions approaching anaerobicity. Gassing and flushing with argon were also used in the photophosphorylation experiments when anaerobic conditions were required.

Illumination was as described previously.[5] When monochromatic light was not used, illumination was provided by a wide band of red light which was obtained by passing white light through a filter (Jena RG-1) that blocked out radiation below 600 mμ.

The photoreduction of ferredoxin was determined by measuring the decrease in optical density at 420 mμ, in a Cary model 14 spectrophotometer equipped with an expanded scale slidewire and a scattered transmission attachment. The reaction mixture was placed in a cuvette with a 1.0-mm light path. The cuvette was illuminated by the actinic light through an aperture in the side of the sample compartment. An interference filter with peak transmission at 420 mμ was placed in front of the photodetector to block out stray actinic radiation. The photoreduction of ferredoxin by chloroplasts was in good agreement with the chemical reduction of ferredoxin by sodium dithionite.

Other experimental procedures were the same as those previously described.[5]

Results.—Photoreduction of ferredoxin coupled with photoproduction of O_2: Figure 1 shows the photoreduction of added ferredoxin by washed chloroplasts. On turning off the light, ferredoxin was reoxidized. The amount of ferredoxin reduced in the light was equal to the amount of ferredoxin reoxidized in the dark. The sequence of photoreduction followed by dark reoxidation was reproducible at least three consecutive times. As shown in Table 1, the different amounts of added ferredoxin were completely photoreduced.

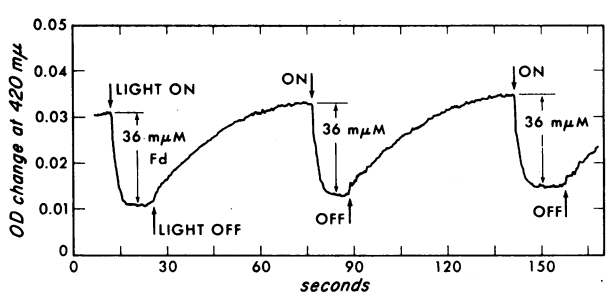

FIG. 1.—Photoreduction of ferredoxin (Fd) by isolated chloroplasts and its reoxidation in the dark. Experimental conditions as in Table 1 (Expt. A).

TABLE 1

PHOTOREDUCTION OF ADDED FERREDOXIN (Fd) BY ISOLATED CHLOROPLASTS (mμmoles)

Expt.	Test no.	Fd added	Fd photoreduced	Fd photoreduced/ Fd added
A	1	36	36	1.00
	2	36	36	1.00
	3	36	36	1.00
B	1	54	55	1.02
	2	54	52	0.97
	3	54	55	1.02
C	1	71	66	0.93
	2	71	67	0.95
	3	71	68	0.96

The reaction mixture contained per ml: chloroplasts (equivalent to 0.2 mg chlorophyll); tris buffer, pH 8.3, 45 μmoles; $MgCl_2$, 4.5 μmoles; and ferredoxin as indicated. Temperature, 20°C.

The photoproduction of oxygen, dependent on added ferredoxin, is shown in Figure 2. No oxygen was evolved without the addition of ferredoxin. On turning off the light, the oxygen evolved during the preceding illumination period was consumed. The successive evolution and consumption of oxygen parallels the photoreduction and reoxidation of ferredoxin shown in Figure 1. The sequence of oxygen production in the light and consumption in the dark was also reproducible several times in succession.

Table 2 shows the observed 4 to 1 stoichiometry between ferredoxin added and O_2 produced. The 4 to 1 stoichiometry was obtained with different amounts of added ferredoxin.

Photoreduction of ferredoxin independent of photoproduction of O_2: In the experiments described so far, illumination was provided by a broad band of incandescent light from which only light of a wavelength shorter than 600 mμ was filtered out. Such illumination is known to support oxygen evolution by isolated chloroplasts. But, as previously reported,[5] illumination above 700 mμ, which cannot support oxygen evolution, will still support not only cyclic photophosphorylation but also noncyclic photophosphorylation with an artificial electron donor system. It seemed desirable, therefore, to measure directly the photoreduction of ferredoxin with monochromatic light above 700 mμ, in the presence of ascorbate-DPIP as an electron donor system.

Figure 3 shows the photoreduction of ferredoxin at 714 mμ. Since no oxygen is produced by chloroplasts at this wavelength,[5, 6] very little reduced ferredoxin was reoxidized when light was turned off. The photoreduction of ferredoxin at 714

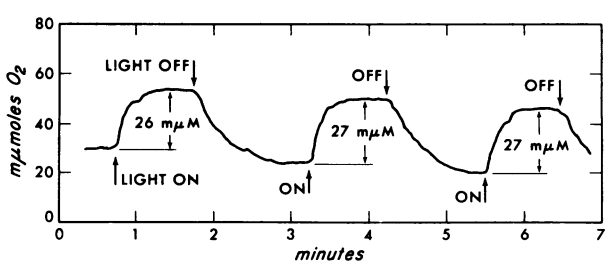

FIG. 2.—Ferredoxin-dependent oxygen evolution in the light and oxygen consumption in the dark. Experimental conditions as in Table 2 (Expt. D).

TABLE 2

STOICHIOMETRY BETWEEN OXYGEN EVOLUTION AND PHOTOREDUCTION OF FERREDOXIN (mμmoles)

Expt.	Test no.	Fd added	O_2 produced	Fd added/ O_2 produced
D	1	110	26	4.2
	2	110	27	4.1
	3	110	27	4.1
E	1	128	32	4.0
	2	128	33	3.9
	3	128	32	4.0
F	1	154	37	4.2
	2	154	42	3.7
	3	154	39	3.9

The reaction mixture (final volume 2.7 ml) included chloroplasts (equivalent to 400 μg chlorophyll), and the following in μmoles: tris buffer, pH 8.3, 125; and $MgCl_2$, 5; and ferredoxin (Fd) as indicated. Temperature, 17°C. The initial concentration of O_2, dissolved in the reaction mixture, was not greater than 3.6×10^{-5} *M*.

TABLE 3

PHOTOREDUCTION OF FERREDOXIN (Fd) WITH MONOCHROMATIC LIGHT (714 mμ) (mμmoles)

Expt.	Fd added	Fd photoreduced	Fd photoreduced/ Fd added
G	36	35	0.97
H	54	52	0.97
I	71	69	0.97

The reaction mixture was as given for Table 1, but in addition, each ml contained: antimycin A, 10 μg; Na ascorbate, 2 μm; and DPIP, 0.04 μm.

mμ was complete with the different amounts of ferredoxin added (Table 3). Complete photoreduction of ferredoxin at 714 mμ was obtained under anaerobic conditions in the presence of antimycin A. Antimycin A was used to inhibit the light-induced cyclic electron flow[2] which reoxidizes reduced ferredoxin under anaerobic conditions.

Pseudocyclic and cyclic photophosphorylation with ferredoxin: As already mentioned, ferredoxin catalyzes an anaerobic cyclic photophosphorylation by chloroplasts.[2] The ferredoxin-dependent cyclic photophosphorylation can be observed anaerobically when experimental conditions are arranged to inhibit the evolution of oxygen. This is done either by the use of far-red monochromatic light which cannot support oxygen evolution ($\lambda > 700$ mμ) or by the use of specific inhibitors of oxygen evolution, when white light or illumination by shorter wavelength is supplied.[5, 7]

Table 4 shows that at 663 mμ and in an atmosphere of argon, the ferredoxin-dependent cyclic photophosphorylation was markedly increased by the presence of CMU—a powerful inhibitor of oxygen evolution by chloroplasts. On the

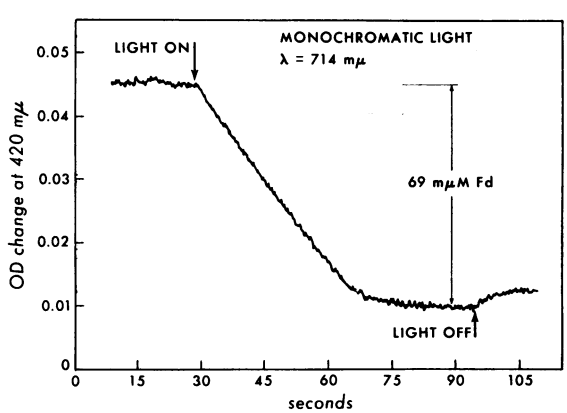

FIG. 3.—Photoreduction of ferredoxin (Fd) by isolated chloroplasts supplied with monochromatic light (714 mμ). Experimental conditions as in Table 3 (Expt. I).

TABLE 4

EFFECT OF p-CHLOROPHENYLDIMETHYL UREA (CMU) ON FERREDOXIN-DEPENDENT PHOTOPHOSPHORYLATION IN MONOCHROMATIC LIGHT (μmoles ATP FORMED)

	663 mμ	714 mμ
Gas phase: argon		
Control	0.22	1.08
CMU	1.09	0.79
Gas phase: air		
Control	1.48	0.14
CMU	0.31	0.03

The reaction mixture (final volume 1.5 ml) included chloroplasts (equivalent to 400 μg of chlorophyll), 1.5 mg of spinach ferredoxin, and the following in μmoles: tris buffer, pH 8.0, 50; $MgCl_2$, 2.5; ADP, 2.5; $K_2HP^{32}O_4$, 2. 2.0 μg of CMU was added where indicated. Temperature, 15°C; illumination, 15 min.

TABLE 5

EFFECT OF ANTIMYCIN A ON FERREDOXIN-DEPENDENT PHOTOPHOSPHORYLATION IN MONOCHROMATIC LIGHT (μmoles ATP FORMED)

	663 mμ	714 mμ
Gas phase: argon		
Control	1.09	0.79
Antimycin A	0.16	0.06
Gas phase: air		
Control	1.48	0.14
Antimycin A	1.33	0.03

The experimental conditions were as given for Table 4, except that under argon 2.0 μg of CMU was always present in the reaction mixture. 10 μg antimycin A was added where indicated.

other hand, at 714 mμ where no oxygen is produced, the presence of CMU gave no increase and, in fact, slightly inhibited phosphorylation.

Different results were obtained under air. Here the ferredoxin-dependent phosphorylation was strongly inhibited by CMU (Table 4). This suggests that under aerobic conditions ferredoxin catalyzes a type of photophosphorylation which is dependent on oxygen evolution (pseudocyclic photophosphorylation[8]). Inhibition of oxygen evolution by CMU, or by unfavorable illumination (714 mμ), resulted in a sharply decreased photophosphorylation. These results are in agreement with those of Forti and Jagendorf[10] and Black *et al.*[11] who observed that under aerobic conditions ferredoxin ("PPNR") stimulates an endogenous photophosphorylation in chloroplasts which is dependent on, and consumes, molecular oxygen.

A characteristic feature of the ferredoxin-catalyzed cyclic photophosphorylation, which is used to distinguish it from other types of photophosphorylation, is a sensitivity to inhibition by antimycin A.[2] Table 5 shows that photophosphorylations under argon, both at 663 and at 714 mμ, were indeed strongly inhibited by antimycin A. On the other hand, when significant pseudocyclic photophosphorylation occurred under air (at 663 mμ), it was little affected by the presence of antimycin A.

Noncyclic photophosphorylation with ferredoxin: In experiments which are not reported here, we found that cysteine prevents the reoxidation of reduced ferredoxin by oxygen and may, therefore, be used as an inhibitor of pseudocyclic photophosphorylation. In the experiments represented by Table 6, cysteine and antimycin A were added to inhibit the ferredoxin-dependent cyclic and pseudocyclic photophosphorylations, respectively. Under these conditions, illuminated chloroplasts exhibited a noncyclic photophosphorylation in which the amount of ATP formed was proportional to the amount of ferredoxin added in a molar ratio of approximately 1 ATP to 2 ferredoxins (P:2e = 1). This ratio is consistent with other evidence that the oxidation-reduction of ferredoxin involves a transfer of one electron.

Concluding Remarks.—The stoichiometry of 4 to 1 between ferredoxin and O_2 is in agreement with earlier evidence,[12] confirmed by San Pietro and associates,[13] that reduction or oxidation of ferredoxin involves transfer of 1 electron per molecule. This property of ferredoxin is in accord with its postulated role as the terminal

TABLE 6

STOICHIOMETRY OF NONCYCLIC PHOTOPHOSPHORYLATION WITH FERREDOXIN
(μmoles/ml)

Minutes	Fd→	0	0.20	0.40	0.60
2.5		0.00 ATP	0.13 ATP	0.13 ATP	0.08 ATP
5		0.00 "	0.13 "	0.21 "	0.16 "
10		0.00 "	0.12 "	0.21 "	0.27 "
15		0.00 "	0.11 "	0.21 "	0.26 "
Fd:ATP*		—	1.5	1.9	2.2

* Based on the highest values of ATP for each concentration of ferredoxin.
The reaction mixture (final volume 3 ml) contained chloroplasts (150 μg chlorophyll) preincubated at 0° for 30 min with 120 μmoles of cysteine, 30 μg antimycin", A, and the following in μmoles: Tris buffer, pH 8.3, 150; $MgCl_2$, 15; ADP, 3; $K_2HP^{32}O_4$, 3; and spinach ferredoxin (Fd) as indicated. Gas phase, argon; temperature, 15°; illumination, monochromatic light, 663 mμ. At the indicated times, 0.5-ml aliquots were withdrawn for ATP determination (0.1 μmole ATP≡3147 c/m).

electron acceptor in the photochemical apparatus of chloroplasts where the capture of a photon is likely to result in the transfer of one electron to ferredoxin.

The present evidence and that presented earlier[2, 5, 7] indicates that the conversion of radiant energy into chemical energy in chloroplasts yields three products simultaneously: a strong reductant, ATP, and O_2. It should be stressed that, contrary to some speculations, the formation of a strong reductant by illuminated chloroplasts is accompanied by the formation and not by the consumption of ATP. The concurrent formation of ATP, reductant, and oxygen was first noted when noncyclic photophosphorylation was discovered.[14] The only change now concerns the identity of the reductant. Instead of reduced TPN, the strongest, photochemically formed reductant is now known to be reduced ferredoxin, whose reducing power is no less than that of molecular hydrogen.

Of the products of noncyclic photophosphorylation, oxygen escapes, whereas the ATP and the reduced ferredoxin which remain jointly constitute the assimilatory power that drives photosynthetic carbon assimilation. Noncyclic photophosphorylation appears to be the main pathway of photosynthetic energy conversion in chloroplasts but, under special conditions, when oxygen evolution is suppressed, reduced ferredoxin is used to catalyze cyclic photophosphorylation. ATP then becomes the sole product of the energy conversion process. The experimental conditions under which cyclic photophosphorylation can be observed have been described here for isolated chloroplasts and by Forti and Parisi[16] for intact leaves.

The role of ferredoxin as the terminal electron acceptor in the photochemical reactions of chloroplasts is diagrammatically summarized in Figure 4. The unidirectional or noncyclic electron transfer from water to ferredoxin proceeds against the thermodynamic gradient at the expense of radiant energy and is accompanied by oxygen evolution and photophosphorylation. With water as the electron donor, the photoactivity of both chlorophylls *a* and *b* is required to supply the necessary energy (Fig. 4, photoreactions *A* and *B*). But ferredoxin itself can be reduced by photoreaction *A* alone, i.e., by monochromatic light which is absorbed only by chlorophyll *a*. This is the case in cyclic photophosphorylation or when an artificial electron donor (ascorbate-DPIP) donates electrons at a site past PQ in Figure 4 (see Tables 3 and 4). The position assigned to plastoquinone (PQ) as the electron acceptor for photoreaction *B* is based on evidence from this and other laboratories, discussed elsewhere.[17]

Of the two cytochromes present in chloroplasts[18] (b_6 and *f*), cytochrome b_6 is

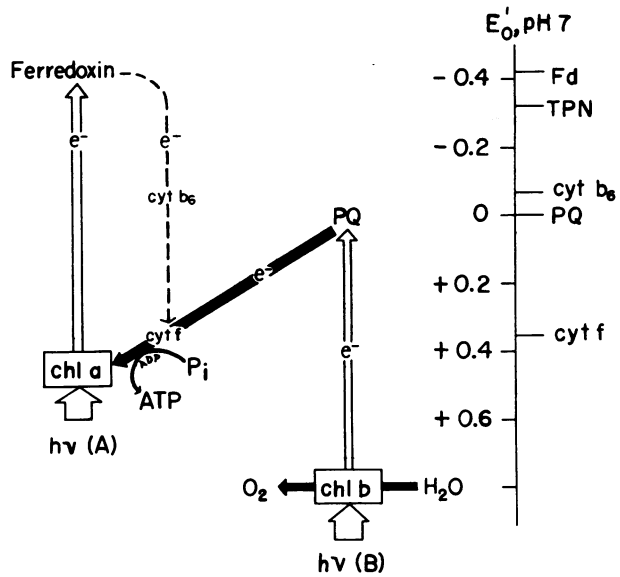

FIG. 4.—Diagrammatic summary of photochemical reactions in chloroplasts. Details in the text.

envisaged as participating only in cyclic photophosphorylation (Fig. 4, dotted line) since only this type is sensitive to inhibition by antimycin A. A primary phosphorylation site common to all pathways is envisaged between cytochrome *f* and chlorophyll *a* (cf. ref. 19). However, cyclic photophosphorylation may have additional phosphorylation sites in the sectors between ferredoxin, cytochrome b_6, and cytochrome *f*.

It is now believed that reduced ferredoxin contributes its reducing power to carbon assimilation by way of TPN—a step that entails a loss of 0.1 eV in reducing potential. However, in view of the recent evidence on the direct role of ferredoxin in reductive carbon assimilation by bacteria,[15] a re-examination of the mode of action of ferredoxin in photosynthetic carbon assimilation becomes timely.

Note added in proof: A net synthesis of pyruvate[15] from acetyl-CoA, CO_2, and reduced ferredoxin, leading to amino acid synthesis, has now been obtained with a cell-free extract of the photosynthetic bacterium, *Chromatium* (to be reported in these PROCEEDINGS by B. B. Buchanan, R. Bachofen, and D. I. Arnon).

We thank William Ufert for excellent technical assistance.

Abbreviations: TPN, triphosphopyridine nucleotide; ATP, adenosine triphosphate; DPIP, 2,6-dichlorophenol indophenol; CMU, p-chlorophenyldimethyl urea.

* Aided by grants from the U.S. Public Health Service and the Office of Naval Research.

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FUNDAMENTAL CHROMOSOME STRUCTURE

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Read before the Academy April 29, 1964

Currently, there are two concepts of the relation of the hereditary material, DNA, to chromosomes. One view is that each chromosome has an axis, or core, of presumably nongenetic proteins to which the genes (DNA) are attached locally. The other view is that DNA forms the axis of the chromosome so that the latter may be thought of as a huge DNA molecule or a series of DNA molecules linked end to end.

Unquestionably, in some microorganisms, what have been interpreted as "chromosomes" consist of a double helix of DNA as envisioned by Watson and Crick. And the current interpretation of the origin of the loops of lampbrush chromosomes, given by Callan[1] and by Gall,[2] lends support to the DNA axis hypothesis. But, as will appear below, there are serious objections to such a concept.

During the growth period of meiosis, in many vertebrates, oöcytes show for a time during the diplotene stage processes arising from the surface of homologous chromomeres. Similar processes, but on a much reduced scale, have been often reported for this stage in spermatogenesis. It is now generally agreed that these processes are expressions of gene activation which results in the formation of messenger RNA and the synthesis of proteins within the nucleus. Thus, the loop processes are strictly comparable to the puffs which appear in salivary chromosomes.

Our immediate concern is the question: How are the loops and other processes formed along diplotene chromosomes? Gall and Callan have accepted the DNA axis hypothesis and explain the loops as being due to the local unwinding of these meiotic chromosomes so there may be a transcription of the base-sequences of the genes (DNA) in messenger RNA. Callan and MacGregor[3] have shown that when lampbrush chromosomes are treated with DNase, the loops fragment indicating that there is an axis of DNA in the loops.