Differentiation and regeneration (claw formation) of remainder pieces after culture in starved adult hosts

Culture time in starved adults (days)	Age of larval hosts (h)	n	Coxa	Trochanter	Femur	Claw formation (%)
0	105	18	14.9 ± 2.3	10.1 ± 1.9	101.7 ± 11.5	0
2	105	9	10.9 ± 4.2	6.0 ± 2.8	53.0 ± 23.2	0
4	105	9	10.7 ± 4.8	6.4 ± 3.1	62.5 ± 20.9	0
8	105	9	4.4 ± 3.9	2.3 ± 2.7	17.7 ± 13.6	0
8	72	9	10.7 ± 6.3	7.8 ± 5.1	78.4 ± 35.4	33

Average number of bristles in Coxa, Trochanter and Femur.

of the cases the tissue had regenerated including the claw and the bristle number in the proximal segments was the same as in controls.

We can conclude that regeneration is dependent on proliferation which is inhibited in sugar-fed flies. In such hosts the implants become smaller but the damage is repairable[3].

Zusammenfassung. Fragmente von Beinimaginalscheiben regenerieren nicht, wenn sie in restriktiv ernährten Wirten kultiviert werden, wo Proliferation unterdrückt wird. Die Regenerationshemmung ist reversibel, da solche Fragmente in gut ernährten Wirtslarven normal regenerieren.

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Histones from the Red Alga, *Rhodymenia palmata*?

While it is clear that histones of the type most fully characterized in calf thymus are found only in eucaryotes, it is becoming apparent that not all encaryotes possess such histones[1–5]. This raises a number of questions. At which stage in the development of living organisms did histones appear? Prior to this stage, did other proteins fulfil their role in relation to the structure and properties of chromatin? If so, what is the nature of these proteins?

It is thought that development of a typical nuclear envelope occurred in the transition from the blue-green algae to the red algae[5]. Therefore, it seemed advisable to examine a member of the red algae in an initial attempt to answer these questions. Because of its ready availability, *Rhodymenia palmata* (L.) Grev.[6] was chosen.

Materials and methods. Calf thymus histones, standard proteins and 2-deoxy-adenosine were obtained from the Sigma London Chemical Co. Ltd. All other reagents used were of Analar grade where-ever possible.

Fresh samples of the alga were collected from the coast of East Lothian and homogenized in distilled water by two passages through a 25 ml capacity X-press (Biotec Ltd.). Following this, proteins were extracted from the homogenate by 3 techniques normally used to obtain histones, viz. serial pH titration[7], direct acid extraction with 0.25 M sulphuric acid following removal of ribosomal proteins at pH 2.8, and differential salt extraction[8].

DNA was estimated by the diphenylamine reaction[9] following extraction by the method of SCHMIDT and THANNHAUSER[10]. Protein was measured in extracts by the method of WARBURG and CHRISTIAN[11]. Protein bound phosphate was determined by the method of AMES[12]. Amino-acid analysis was carried out on a Technicon Auto-Analyser following hydrolysis of the proteins in 5.65 M hydrochloric acid for 24 h at 105 °C.

Polyacrylamide gel electrophoresis was either as described by JOHNS[13] for histones, or as described by WEBER

Table I. Relative amounts of protein obtained by pH titration

pH	Protein obtained (mg)
2.8	93.4
2.1	9.3
1.8	16.0
1.3	10.5
1.0	6.9

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and OSBORN[14] using sodium dodecyl sulphate, to permit separation solely on the basis of molecular size.

Results. Table I shows the weights of the fractions obtained by serial extraction. It is apparent that the fractions which should contain histones, i.e. at pH 2.1 and below, collectively contain almost half as much protein as is found in the putative ribosomal and soluble fraction obtained at pH 2.8. It was found that none of these fractions would enter acrylamide gels under the conditions suggested by JOHNS[13] for analyzing histones, even when the acrylamide concentration was reduced to 5% and pH of the buffer lowered to 1.5. However, sodium dodecyl sulphate electrophoresis indicated that all the fractions contained one protein or closely related group of proteins. One somewhat diffuse band was obtained with a mobility corresponding to a molecular weight of 18,000. Total 'histone' extracts by both the acid extraction and salt extraction techniques gave the same result. In addition, the ratio of 'histone' to DNA (w/w) in both these cases was almost the same, 11.1 to 1 for the acid extract, 11.5 to 1 for the salt extract.

Table II. Amino-acid analysis of total 'histone' fraction

Amino	% of total
Lys	3.8
Arg	3.1
His	3.1
Asp	7.6
Glu	12.2
Ser	5.9
Thr	3.8
Ala	10.6
Val	10.4
Ilu	7.3
Leu	2.0
Met	0.0
Phe	8.4
Tyr	3.5
Try	5.7
Pro	0.0
Gly	12.6
Cys	0.0

Amounts of amino-acids are expressed as moles per 100 moles of total recoverd amino-acids; no corrections are applied for hydrolytic losses of any of the amino-acids.

Table III. Correlation in amount of 'histone' and degree of phosphorylation with activity of tissues

	'Histone': DNA	ng Phosphate/mg 'histone'
Non-meristematic tissue	12.0:1	2.1
Meristematic tissue	6.4:1	4.9

The amino-acid analysis of the total 'histone' fraction obtained by salt extraction is shown in Table II. The content of lysine and arginine is much lower than in calf thymus histones while that of phenylalanine and tyrosine is higher. Tryptophane is present in appreciable amount. The absence of methionine and cysteine is common to most histones. The absence of proline may also be noted. The calculated minimum molecular wight from this analysis is 6,662 roughly a third of that indicated by sodium dodecyl sulphate electrophoresis.

Since there is evidence that chromatin of high template activity has a lower histone to DNA ratio than chromatin of low template activity[15], the 'histone' to DNA ratio was determined in meristematic and non-meristematic tissues of *R. palmata*. The results are shown in Table III together with the protein-bound phosphate content of the 2 'histone' preparations.

Discussion. The extraction methods used are standard methods for obtaining histones. Otherwise, the proteins obtained resemble histones of higher eucaryotes chemically only in the absence of methionine and cysteine and in their correlation with DNA in both the acid and salt extractions, though the amount relative to DNA is much higher than is the case with the well defined histones[15]. Functionally, these proteins resemble histones in being present in larger amounts relative to DNA in non-meristematic tissue, where template activity is small, than in meristematic tissue, where template activity is greater. The lower phosphorylation of the protein from the non-meristematic tissue may also be significant in this respect since phosphorylation of histones is thought to correlate with gene unmasking[15].

Considering these results in conjunction with those of similar studies on yeast[1-3] and dinoflagellates[4], we conclude that histones of the calf thymus type evolved appreciably later than did the nucleus. If this is so, we are left with the question of the relationship of the proteins considered here to the calf thymus histones. The results suggest that there may be functional resemblances but the amino-acid analysis shows more differences than similarities. Conceivably there may be relatively small functional parts of these molecules which are indentical. Only determination of the primary structures of these proteins will reveal whether this is so or not[16].

Résumé. Protéines ont été extraites de l'algue rouge *Rhodymenia palmata*, par les méthodes normalement utilisées pour les histones. Ces protéines diffèrent des histones du thymus de veau d'une façon marquée, mais elles ont des similarités fonctionelles.

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