

with colchicine, chloro-isopropyl-*n*-phenylcarbamate, 50 mM potassium phosphate (pH 6.8) and other agents that prevent flagellar regeneration, provide nearly identical results to those obtained with IBMX (not shown). These findings provide clear evidence that utilisation of tubulin reserves for flagellar outgrowth cannot serve as a signal for the initiation of tubulin synthesis.

Finally, it may be noted that although intracellular tubulin concentrations do not seem to control the early phases of tubulin synthesis, indirect evidence indicates that increasing reserve levels may govern tubulin production during the later stages of flagellar regeneration. A comparison of data in Figs 1 and 3 shows that relatively high levels of tubulin production continue well beyond the time when flagellar outgrowth is essentially complete and therefore suggests that tubulin reserves accumulate during the late stages of regeneration.



Fig. 2 Autoradiographs of SDS-polyacrylamide gels containing *in vivo* labelled proteins from deflagellated *Chlamydomonas* gametes. Cells were labelled with $^{35}\text{S}\text{-H}_2\text{SO}_4$ using modifications of procedures developed by Lefebvre and Rosenbaum (personal communication) and other laboratories⁹⁻⁸. Approximately 10^7 sulphate-starved cells in 0.5 ml were labelled for 30 min in 100 μCi carrier-free $^{35}\text{S}\text{-H}_2\text{SO}_4$. Non-deflagellated cells were incubated without protein synthesis inhibitors (track 1); with 100 $\mu\text{g ml}^{-1}$ chloramphenicol to inhibit chloroplast protein synthesis (track 2); with 10 $\mu\text{g ml}^{-1}$ cycloheximide (track 3) or with 10^{-4} M 2-(4-methyl-2,6-dinitroanilino)-*N*-methylpropionamide (ref. 9, MDMP) (track 4) to inhibit protein synthesis by 80S cytoplasmic ribosomes. Cells deflagellated by the pH shock method were incubated for 15-45 min after deflagellation in the absence (track 5) or presence of chloramphenicol (track 6). After incubation, a 5- μl aliquot of each sample was taken for hot TCA determination of ^{35}S incorporation into protein¹. The remainder of each sample was precipitated with 5 ml acetone. For analysis on SDS-polyacrylamide gels, the acetone powder from each sample (which can be stored indefinitely) was dissolved in 2% SDS, 5% mercaptoethanol, 10% glycerol, 60 mM tris (pH 7.6), and an aliquot with 2×10^6 c.p.m. (except for tracks 3 and 4) electrophoresed on 9% SDS-polyacrylamide gels as described previously¹. Autoradiographic exposure of the dried gel was for 12 h.

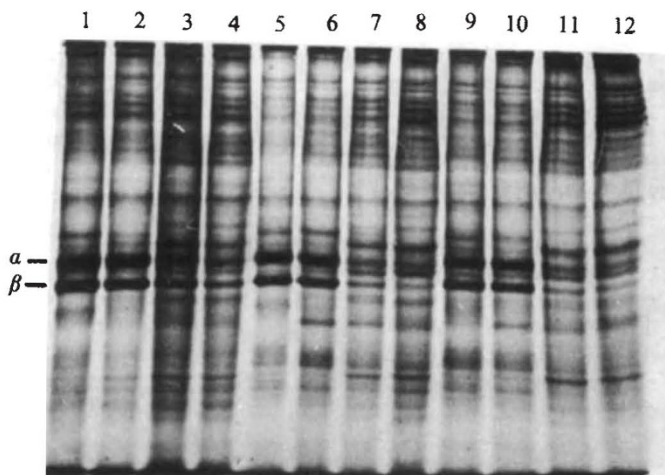


Fig. 3 Tubulin synthesis following deflagellation of control or IBMX-treated cells. Deflagellated cells were labelled for 30-min intervals at 15-45, 45-75, 105-135 and 165-195 min after deflagellation in the absence (tracks 1-4) and presence of 10^{-3} M IBMX given either 15 min before deflagellation (tracks 5-8) or immediately after deflagellation (tracks 9-12). Labelling with $^{35}\text{S}\text{-H}_2\text{SO}_4$ in the presence of chloramphenicol was as described in Fig. 2.

Thus, the earlier than normal shut off of tubulin synthesis in IBMX-treated cells (Fig. 3, tracks 5-12 compared with 1-4) suggests that a feedback mechanism responsive to increasing tubulin concentrations, could be responsible for the ultimate repression of tubulin synthesis during flagellar regeneration.

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Erratum

In 'Stability of Lotka-Volterra systems' (*Nature*, 264, 381; 1976) the name of the co-author, J. S. Semura, was omitted.

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