Molecular Mechanisms Controlling Tubulin Synthesis

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1. Introduction

Microtubules, which are comprised principally of dimeric subunits of one $\alpha$- and one $\beta$-tubulin polypeptide, participate in a diverse spectrum of cellular events. This repertoire of microtubule-associated functions includes formation of mitotic and meiotic spindles, establishment of some forms of intercellular and intracellular motility, establishment of programmed modifications of cell shape during morphogenesis (such as neurite outgrowth), and, in concert with actin filaments and intermediate filaments, establishment of internal cytoarchitecture.

The realization that microtubules were integral participants in this wide variety of processes led quickly to the expectation that expression of tubulin polypeptides would be a closely regulated process. Indeed, with the demonstration of the presence of multiple tubulin genes within the genomes of most eukaryotes, it initially became clear that the requisite regulatory mechanisms must control two separate aspects of tubulin gene expression. First is the selection of which among a family of similar genes are to be expressed at specific points during development and differentiation. Second is establishment of the appropriate quantitative level of expression. With regard to the first of these regulatory problems, I shall not attempt to review here the considerable work that has led to the demonstration through the efforts of a variety of investigators that the genomes of multicellular organisms contain multiple genes that are expressed in characteristic developmental and tissue-
specific patterns. Rather, I refer the interested reader to other recent reviews (Cowan, 1983; Cleveland, 1983; Raff, 1984; Cleveland and Sullivan, 1985). In the present work, I have chosen instead to dwell at some length on reviewing the results derived from several experimental systems which have yielded provocative findings concerning the diverse mechanisms utilized for establishing the proper level of tubulin gene expression.

2. Apparent Autoregulatory Control of Tubulin Synthesis in Animal Cells

2.1. Tubulin Synthesis in Animal Cells Is Sensitive to the Apparent Pool of Unpolymerized Subunits

Even in the absence of direct evidence, it seemed obvious at the outset (at least to this observer) that animal cells must possess a sensitive mechanism(s) for regulation of their tubulin contents. Since the unpolymerized tubulin subunit concentration is generally in rapid equilibrium with the polymer (albeit through a complicated interaction of many factors), this postulated regulatory system for establishing tubulin subunit and polymer content could act by maintenance of a specified level of total tubulin content, of microtubule polymer, or of unpolymerized tubulin subunits.

But it was not until the pioneering work of Ben Ze'ev, Farmer, and Penman (1979) that some initial insight emerged as to what kind of regulatory mechanism might actually be utilized. Although many investigators had previously recorded the effects of antimicrotubule drugs on cellular microtubule arrays, it was Ben Ze'ev et al. who first noted that the marked alterations in the morphology of cultured animal cells following colchicine-induced microtubule depolymerization were accompanied by specific repression of new tubulin synthesis. For this demonstration, these investigators used a combination of pulse radiolabelling of newly synthesized proteins followed by two-dimensional gel electrophoresis to resolve the pattern of new protein synthesis in 3T6 cells, a mouse fibroblastic cell line. When the patterns of newly made proteins in normal cells or in colchicine-treated cells (which had lost all of their microtubule polymers) were compared, it was found that, although the overall pattern of protein synthesis was not notably affected by microtubule depolymerization, there was a dramatic repression of synthesis of new α- and new β-tubulin polypeptides. As later investigators were to show, this specific repression of tubulin synthesis in response to colchicine treatment is a general response of animal cells and has been found in all types of cultured animal cells investigated including a spectrum of different types of mouse cells (Cleveland et al., 1981) and cells from organisms as diverse as humans (Fellous et al., 1982; Cleveland and Havercroft, 1983), chickens (Lau et al., 1985), and mosquitos (Cleveland et al., 1981).

An example illustrating a two-dimensional gel analysis of protein synthetic patterns in control and colchicine-treated Chinese hamster ovary cells is displayed in Fig. 1A,B. Inspection of the patterns reveals that they are re-